BASF
The Chemical Company

BIODEGRADATION OF SYNTHETIC POLYMERS IN THE AQUATIC ENVIRONMENT

Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften - Dr. rer. nat. - am Fachbereich II (Biologie/Chemie) der Universität Bremen

Vorgelegt von Jan P. Eubeler

Bremen im April 2010

Universität Bremen

1. Gutachter Hr. Prof. Dr. Rudolf Amann (Universität Bremen)

2. Gutachter Fr. Dr. Sabine Zok (BASF SE Ludwigshafen)

1. Prüfer Hr. Prof. Dr. Michael W. Friedrich (Universität Bremen)

2. Prüfer Hr. Prof. Dr. Thomas P. Knepper (Hochschule Fresenius, Idstein)

Termin des öffentlichen Kolloquiums:

21. Juni 2010, 13:30 Uhr, Alter Hörsaal, MPI für Marine Mikrobiologie, Celsiusstr. 1, Bremen

Eidesstattliche Erklärung

gem. § 6 (5) Nr. 1-3 Promotions-Ordnung der Universität Bremen vom 14 März 2007

Hiermit erkläre ich, das ich

die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,
keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und
die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Großkarlbach, den 01. April 2010

Jan P. Eubeler

"Bildung ist das, was übrig bleibt, wenn man alles vergessen hat, was man gelernt hat. (…)"

(Werner Heisenberg)

„… und was man dann wiederum zu erlernen bereit ist.“

Acknowledgements

First of all, I would like to thank both my supervisor Dr. Sabine Zok (BASF SE) and Prof. Dr. Thomas P. Knepper (Fresenius University of Applied Sciences) and my colleague and friend Dipl.-Ing. M. Bernhard for their help and cooperation on this project and all related topics.

Furthermore I would like Prof. Dr. Allan D. Cembella (AWI Bremerhaven) for taking me as his doctoral student and being my supervisor. I wish him all the best for recovering from his very unfortunate accident.

I also like to express my deepest gratitude to Prof. Dr. Rudolf Amann for taking over the position of Prof. Cembella and supporting me through final phase of my dissertation. Thanks also to Prof. Dr. Michael W. Friedrich for his expertise on my thesis. My gratitude goes also to Sara Kleindienst and Juliane Wippler for being part of the committee.

I would like to express my gratitude especially to Helmut Schwarz, Stephan Hammer, Juergen Bachner, Karl-Heinz Ullrich, Arnold “Lumpi” Gottwald, Helene Froehlich and Andy Haupt (GV/TC), for their support and help every day in the Lab. Special thanks for the great time with you guys and the perfect Friday morning “breakfast”. I will always remember that!

I am also most grateful to Dr. Christian Schütt and Hilke Döpke (BAH/AWI Helgoland/Bremerhaven) for the cooperation, their intensive and unlimited help and the good weeks I spent at AWI BAH.

Special thanks go to Mrs. Frauke Ernst from University of Bremen. Thank you for your fast and constant support in every administrative aspect.

I would like to thank BASF SE, especially GV/T as well as Mrs. Dr. Katrin Schwarz and her Team (E-EMV/Q) for funding my project and for their support. It was a pleasure to work with you.

I also want to thank a lot of BASF staff for various help in my project:

Jens Kampioni for sending me all Pluriol E type and Pluriol E care samples, MSc Motonori Yamamoto for supplying ECOFLEX, ECOVIO, MaterBi and PLA samples in various different versions. Thanks go as well to Dr. Joachim Fischer, Dr. Inge Langbein, Dr. Klaus Taeger and Dr. Peter Reuschenbach for inspiring talks and ideas. Thanks to Dr. Uwe Witt for supplying me with Oxo-PE samples and for discussions and literature and to Gert Asselman from BTC Specialty Chemical Distribution N.V./S.A. Belgium for sending me samples of PEG & MAPEG. Special thanks to Uwe Ruffer and Dr. Andrea Hörster from the Bioanalytical Department at BASF SE for analyzing my marine samples. I also want to thank Prof. Dr. Andreas Künkel and his team for their input on my review papers.

I want to express special gratitude also in the name of BASF to Mr. Wittner and his team at Luisenpark Mannheim for their support and interest and for allowing myself to collect many, many seawater samples &

microorganisms from their aquarium! Also, I would like to thank those involved in sending seawater samples from the North Sea near BASF Guesthouse at Sylt, Westerland to our laboratory for use in marine tests.

Thanks to Dipl.-Phil., Dipl.-Soz.-Päd. Sylvia Böttger from HS Fresenius, Idstein for her big support in my search for literature. She has been always kind and never tired of sending me publications I could not get off the Internet.

Thank you very much GDCh, SETAC, ASMS, DECHEMA e.V., ACS and all the other scientific societies. You were all being very cooperative and supportive through all my time as an undergrad and grad student! This is gratefully acknowledged and appreciated! Special Thanks to VCI/FCI for providing me with a scholarship for the SETAG/GDCh PGS-Course in Ecotoxicology. The course is done very well and I would like to thank all involved colleagues for their commitment.

All the people I have forgotten to mention here may please forgive me. You have also participated in this work and you may remind me of that whenever you wish!

Last but not least I want to thank my mom and dad and also my grandparents for always listening and being helpful and supportive. I am very grateful for many, many years of constant and unlimited support.

I would also like to thank Hendrik Jacob, Manuela Peschka, Matthias “Möcki” Möckl and Stefan Kappner, as well as Holger Rinker, Frank Hess and Jürgen Diehl for the great hours we spent together (not only in the “Promised Land”) having lots of fun! And thanks to the BOSS and all his colleagues and friends that have delivered many days of beautiful reward being my winding wheel.

Abstract

Biodegradation of synthetic polymers can be a sophisticated property for intelligent and sustainable products that offer complex benefits for specific applications. There are many entry paths for synthetic polymers that can accumulate in the aqueous and especially marine environment and little is known about their biodegradation especially in the aquatic environment. The difficulties with determining biodegradation in those environments are based on the absence of appropriate methods and also the fact that these environments often prove low biodegradation rates. It is also complicated to detect biodegradation on polymeric substances because of the high molecular weight, water insolubility and difficult molecular structure making it hard to detect biodegradation products.

This work provides an overview of the actual status of research regarding biodegradation, results and methods describing biodegradation of biodegradable polymers. The main focus of this study is to find out if standard biodegradation tests may be used for the evaluation of polymer biodegradation. Its aim is to identify difficulties and problems with these tests and to compare the biodegradation potential and biodegradation pathways in the marine and freshwater environment for a selection of polymer types.

It is also investigated whether molecular or structural properties of the polymers influence the biodegradation in different environments and the possible pathways of biodegradation. Similarities and differences are identified and on a selection of the biodegradation tests an investigation of the microorganism community is performed to evaluate the use of molecular methods to discover influences of microorganisms on the biodegradation of polymers.

Investigating available methods on how evaluation of biodegradation can be performed best in this special field is a first step in a new direction. It may be important for further development of representative test procedures. First facts on how biodegradation can be determined under the special circumstances are established based on standardized methods which are adapted to the requirements

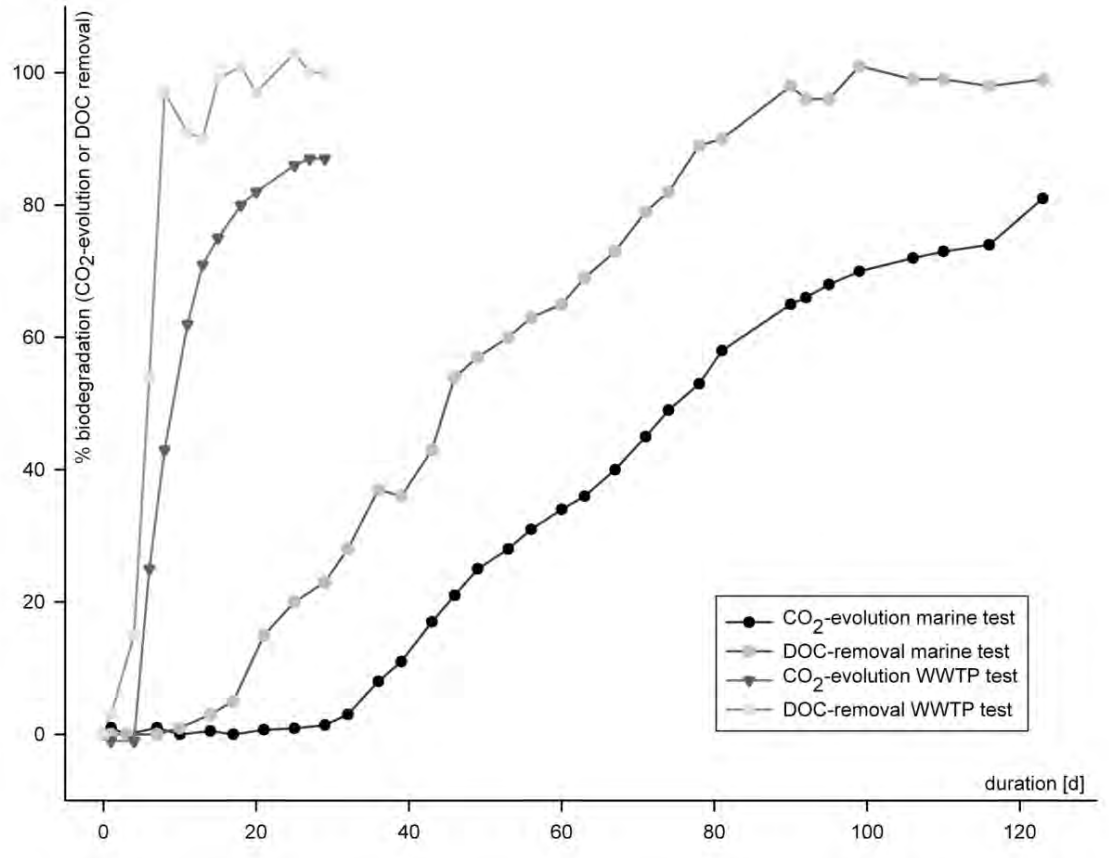
Three different types of carefully selected polymers and their degradation behaviour are evaluated based on OECD test guidelines. Compounds were selected based water solubility/insolubility and biodegradation potential:

- Poly(vinyl pyrrolidone) is selected because it is known to be recalcitrant and is investigated to observe differences and similarities in different tests and media when no biodegradation can be observed (“negative control”).
- Poly(ethylene glycol), a water soluble biodegradable polymer is investigated in broad molecular weight distribution ranging from molecular weights of 200 to almost 60’000 gmol -1. Data from different aquatic compartments are compared to establish differences systematically.

- The water insoluble biodegradable polyesters Ecoflex® and Ecovio® are investigated focusing mostly on marine environment. In these cases the biodegradation potential as well as similarities and/or differences between aqueous and compost environments (where they are known to be biodegradable) are the main aspects of the study.

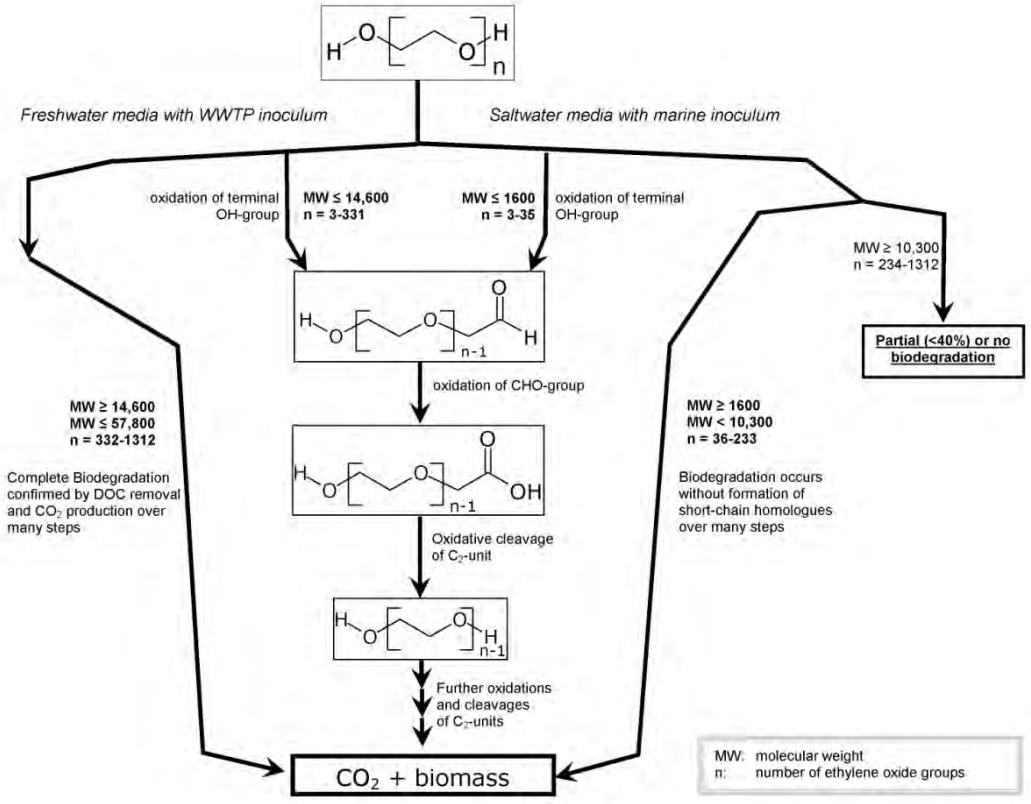
Both water soluble polymers are important mass production products that are used in manifold applications and that also have higher potential to enter the aquatic environment from their applications and products directly or indirectly.

The biodegradation of poly(ethylene glycol) especially shows the importance of systematic investigations and the possibilities in application of the available test methods. It was shown that there are major differences between freshwater (activated sludge, OECD 301) tests and those in marine (synthetic and native marine water) tests. The differences range from the time of biodegradation of the same substances to differences in the biodegradation graphs of reference substances and also to differences in the metabolic pathway as is shown with sophisticated analytical techniques. The potential of biodegradation freshwater and marine tests is shown for the first time systematically for the group of poly(ethylene glycols) ranging from 200 to almost 60'000 g·mol^{-1}. This data shows differences in the applied methods and also the applicability of the marine tests. As shown in the figure below there are significant differences of biodegradation in both test media (here: activated sludge and sea water) and between the biodegradation curves based on the different types measurement parameters (carbon dioxide evolution and dissolved organic carbon).



Biodegradation of poly(ethylene glycol) (4500 g·mol^{-1}) in activated sludge and marine biodegradation tests

It is shown in this study that poly(ethylene glycol) biodegrades up to a specific molecular weight in freshwater and marine environment after certain time to full extent (up to 60 kDa in freshwater and 15 kDa marine water). Poly(ethylene glycol) biodegradation is investigated for the first time to this extend and biodegradation pathways are postulated with the help of sophisticated analytical methods as shown in the figure below. It seems interesting, that there are obviously two different pathways in marine water for poly(ethylene glycol) of approximately <1600 g·mol^{-1} and for poly(ethylene glycol) of approximately >1600 g·mol^{-1}. It also seems interesting that some microorganisms prefer lower substrate concentration and related biodegradation degree is lower when increased substance concentrations are used. Different pathways for the biodegradation were established depending on molecular weight distribution as shown in the following figure.



Pathways for poly(ethylene glycol) degradation in freshwater and seawater

The results obtained from the complex studies in this work show that the international guidelines can be in some cases applied directly in other cases need to be adapted.

Neither the criteria from OECD 301 biodegradation tests nor those from OECD 306 tests are appropriate for biodegradation tests of synthetic polymers. The test duration is in most cases too short especially for marine biodegradation. Many substances that are degradable will fail to pass the test criteria even though they are not recalcitrant. Longer tests but below 200-300 days provide more insight with better reproducibility. It is often helpful when analytical tools are available to determine specifically the polymers tested and it is required that more than one parameter is measured to gain more reliable data. However, it seems that marine tests take

much longer than other environmental biodegradation or simulation tests. This is disadvantage for these tests in any application.

Also molecular or structural properties of the polymers do influence the biodegradation in different environments as well as number of hetero atoms in the chain, specific behaviour of groups that hydrolize but do not biodegrade etc. Possible pathways of biodegradation are confirmed and similarities and differences are identified which supports some of the known statements in published literature on other biodegradation research projects mostly in solid media.

In summary the following statements based on the biodegradation results especially with synthetic polymers, can be made:

- Biodegradation in standard test systems and marine test systems can differ in kinetics and pathway of biodegradation.
- The tests using CO_2 free air in closed systems give stable conditions and variations can be kept low during the first 200-300 days.
- Because of the immense buffer capacity of marine sea water generally higher blank control values as well as much more variation has been observed in the tests when compared with OECD 301 standard tests.
- The desired type of analytical procedure (DOC/DIC; BOD, CO_2) determines the length of the study and needs to be considered. If possible more than one parameter should be measured at the same time.
- Marine tests show mainly far lower biodegradation when compared to freshwater/WW and soil or compost.
- Marine medium can be prepared synthetically in the lab or native sea water can be used if treated carefully and water samples should be stored at constant temperature.
- Tests using native water may have more impact and are closer to natural conditions but no significant differences were observed compared to synthetic medium in this study.

Abstract (Deutsche Kurzfassung)

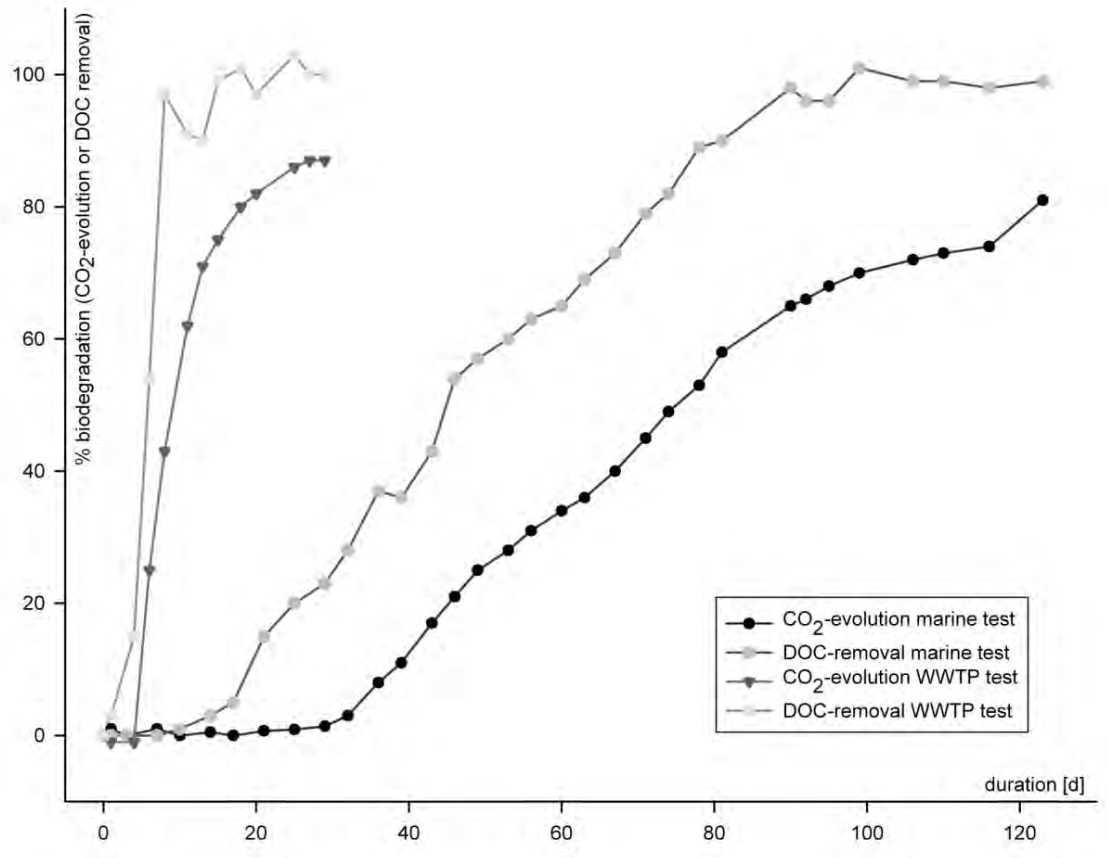
Der biologische Abbau von synthetischen Polymeren kann eine besondere Eigenschaft für intelligente und nachhaltige Produkte darstellen. Es existieren mehrere Eintragspfade für Polymere in unserer Umwelt, eine Anreicherung insbesondere im Meer kann vielerorts beobachtet werden und bisher ist über ihre biologische Abbaubarkeit in der aquatischen Umwelt relativ wenig bekannt. Schwierigkeiten bei der Bestimmung des biologischen Abbaus in den Umweltkompartimenten basieren auf dem Fehlen von geeigneten Methoden und auf den besonderen Eigenschaften des Umweltmediums welches oftmals zu niedrigen Abbauraten führt. Außerdem ist die Bestimmung biologischer Abbaubarkeit von Polymeren in Umweltmedien komplex, da diese z.T. ein sehr großes Molekulargewicht und sehr komplexe Strukturen besitzen und außerdem oftmals wasserunlöslich sind. Diese Arbeit gibt eine Übersicht über den aktuellen Stand der Forschung zum Bioabbau, Ergebnisse und Methoden welche Bioabbautests und bioabbaubare Polymere beschreiben. Die Arbeit konzentriert sich auf die Evaluation verschiedener Standardmethoden um festzustellen in welcher Weise die Bestimmung von biologischer Abbaubarkeit von synthetischen Polymeren am geeignetsten durchgeführt werden kann. Des Weiteren umfasst die Arbeit eine systematische Studie zur aquatischen Abbaubarkeit einiger ausgewählter Verbindungen in Süß- und Salzwasser-Medien.

Es wird ebenfalls untersucht ob spezielle Parameter wie z.B. die molekularen oder strukturellen Eigenschaften der Polymere die biologische Abbaubarkeit in unterschiedlichen Medien beeinflussen und ob Unterschiede oder Ähnlichkeiten in den Abbauwegen auftreten. In einem ausgewählten Teil der durchgeführten biologischen Abbautests wird zusätzlich mittels molekularbiologischer Screenings die Mikroorganismen untersucht um Einflüsse derer und deren Gemeinschaften auf die biologischen Abbauprozesse zu identifizieren. Die Untersuchung verfügbarer Methoden auf ihre bestmögliche Anwendbarkeit in biologische Abbautests unter diesen speziellen Bedingungen ist ein erster Schritt in eine neue Richtung. Dieser ist sehr wichtig um in Zukunft repräsentative Testmethoden zu entwickeln. Erste Erkenntnisse werden hier unter den besonderen Bedingungen mittels standardisierten Methoden ermittelt welche nach Bedarf angepasst werden. Konkret werden drei verschiedene Typen von Polymeren sorgfältig basierend auf deren Wasserlöslichkeit/-Unlöslichkeit sowie deren Potential biologisch abbaubar zu sein, ausgesucht:

- Poly(vinyl pyrrolidone) dient als negativ Kontrolle da die Substanz wie aus der Literatur bereits bekannt weitgehend persistent ist.
- Poly(ethylene glycol), ein wasserlösliches biologisch abbaubares Polymer wird einem breiten Molekulargewichtsspektrum von 200 bis fast 60’000 gmol^{-1} untersucht. Die erhobenen Daten zu Abbauraten aus verschiedenen aquatischen Medien werden so systematisch vergleichbar dargestellt.
- Die wasserunlöslichen biologisch abbaubaren Polyester Ecoflex® und Ecovio® werden hauptsächlich in marinen Abbautests untersucht. Hauptsächliches Augenmerk wird auf Abbauraten, Ähnlichkeiten und

Unterschiede zwischen den aquatischen Medien sowie im Vergleich mit bekannten Daten aus Boden- oder Kompostabbautests gelegt.

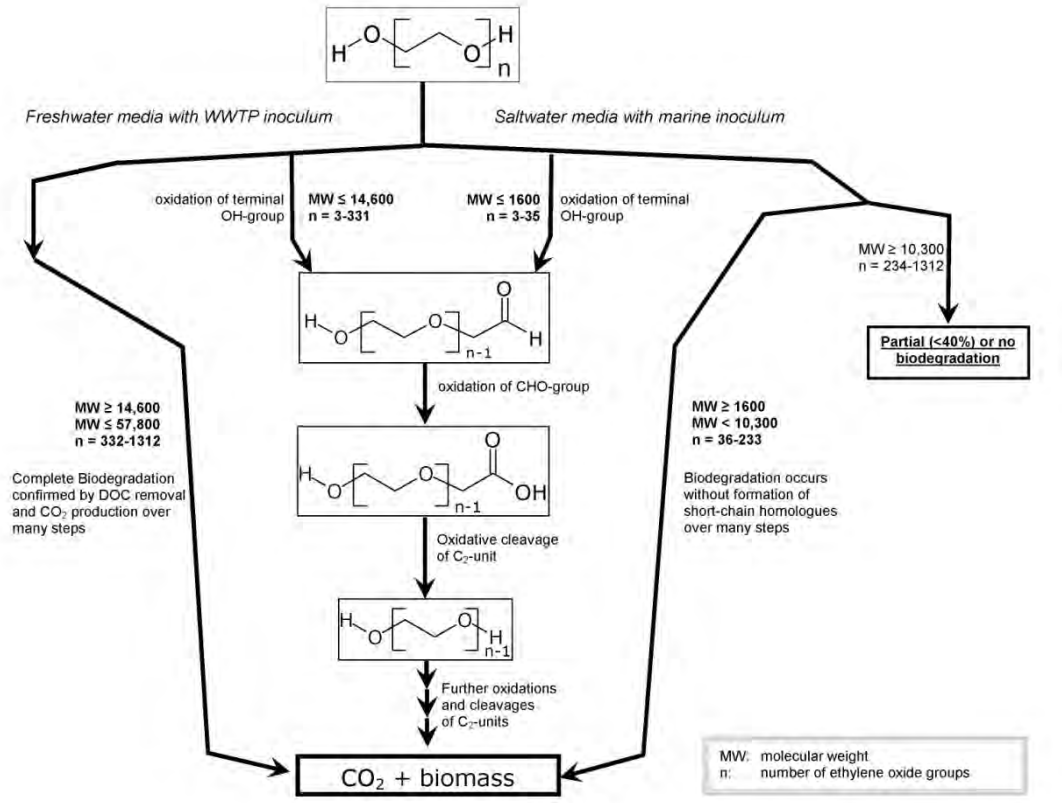
Die beiden wasserlöslichen Polymere sind bedeutende Massenproduktion die in vielfachen Gebieten Anwendung finden und die beide direkt oder indirekt aus ihren Anwendungen heraus unbeabsichtigt in die aquatische Umwelt gelangen können. Besonders die systematischen Untersuchungen zum Abbau von poly(ethylene glycol) zeigen die Wichtigkeit solcher Experimente und die Möglichkeiten der Anwendung bekannter Testmethoden. Es kann festgestellt werden, dass es signifikante Unterschiede im Abbau zwischen Süßwasser (Kläranlagen Belebtschlamm, OECD 301) und Salzwasser (OECD 306) Tests gibt. Die Unterschiede umfassen sowohl Differenzen in der Abbaurate, Geschwindigkeit und Abbaugrad im Vergleich zu Referenzmaterialien bis zu Unterschiede im Abbauweg, wie mit geeigneten analytischen Methoden gezeigt werden konnte. Das Potential geeigneter Süßwasser- und Meerwasserabbautests wird hier zum ersten Mal systematisch vergleichend in einem für PEG weiten Molekulargewichtsbereich von 200 bis annähernd 60'000 $g \cdot mol^{-1}$ ermittelt. Dies hat, wie in folgender Abbildung (Kläranlagenbelebtschlammtest vs. mariner Abbautest, sowie Messparameter Kohlenstoffdioxidentwicklung vs. gelöste Kohlenstoffbestimmung) dargestellt, Unterschiede der angewendeten Abbaumethoden sowie die speziellen Möglichkeiten in der Anwendung mariner Tests zeigen können.



Biologische Abbaubarkeit von poly(ethylene glycol) (4500 $g \cdot mol^{-1}$) in Belebtschlamm- und marinen Abbautests

Es kann ebenso in der Untersuchung mit poly(ethylen glycol) gezeigt werden, dass die Polymertypen sowohl in Süßwasser- (bis zu 60kDa) wie in Salzwassertests (bis zu 15kDa) nach einer bestimmten Zeit vollständig

abgebaut werden In diesem Ausmaß wird der Abbau von poly(ethylene glycol) zum ersten Mal untersucht und mögliche biologische Abbauwege werden, wie in der folgenden Abbildung dargestellt, ermittelt. Interessant ist die Tatsache, dass zwei unterschiedliche Wege in marinem Medium beobachtet werden. Entsprechend jeweils für solche bis zu einem Molekulargewicht von ca. <1600 $g \cdot mol^{-1}$ und für jene über einem Molekulargewicht von etwa >1600 $g \cdot mol^{-1}$. Es wird zusätzlich festgestellt, dass für manche Mikroorganismen einen niedrigere Substratkonzentration begünstigend auf den biologischen Abbau wirken kann und dass die Abbauraten sinken wenn die Substanzkonzentration im Test höher ist.



Abbauwege für poly(ethylene glycol) in Süßwasser- und Salzwasserabbautests

Besonders die systematische Untersuchung von poly(ethylene glycol) zeigt die Wichtigkeit einer solchen Herangehensweise bezogen auf die Anwendbarkeit der Methoden. Wie die Ergebnisse zeigen, können die Methoden der internationalen Richtlinien teilweise direkt oder mit leichten Anpassungen verwendet werden um valide Ergebnisse zu erzielen. Im Gegensatz erzielt die Herangehensweise der Abbaustudien mit den wasserunlöslichen Polyestern wesentlich mehr fehlerhafte und teilweise schwer einschätzbare Daten.

Weder die Kriterien aus OECD 301 Abbautests noch die aus OECD 306 Tests sind geeignet um biologische Abbaubarkeit bei synthetischen Polymeren nachzuweisen. In der Regel sind die standardisierten 30 oder 60 Tage eine oftmals zu kurze Periode, welche sogar bei biologisch abbaubaren synthetischen Polymeren nicht ausreichend sind um verwertbare Ergebnisse zu erzielen. Viele Substanzen welche ggf. biologisch Abbaubar sind, würden hier insbesondere durch diese kurzen Tests durchfallen obwohl ein Abbau zu beobachten wäre. Die Tests mit einer Laufzeit von 200-300 Tagen liefern oftmals deutlich bessere Ergebnisse bei guter

Reproduzierbarkeit. Es sollten wenn möglich immer mehrere Parameter bestimmt werden und zusätzlich sollten analytische Methoden zur Verfügung stehen um polymere und eventuelle Abbauprodukte zusätzlich zu den Standardparametern zu Bestimmen um sichere Ergebnisse zu erzielen. Trotzdem benötigen marine Tests eine deutlich längere Zeitspanne in Vergleich mit anderen biologischen Abbautests oder Simulationstests, welches eine deutliche Einschränkung für diese Tests in der Anwendung bedeutet Generell kann beobachtet werden, dass spezifische Parameter wie z.B. molekulare oder strukturelle Eigenschaften, Molekulargewicht, Anzahl an Heteroatomen oder Verzweigungsgrad der Kette der Polymere einen großen Einfluss auf die biologische Abbaubarkeit in den unterschiedlichen Medien haben. Dies bestätigt weiterhin bekannte Untersuchungen aus anderen Bereichen des biologischen Abbaus wie z.B. beim Abbau in Boden oder Kompost. Die Erkenntnisse basierend auf den biologischen Abbautests im Besonderen mit synthetischen Polymeren können wie folgt zusammengefasst werden:

- Biologischer Abbau in Standardtests und in marinen Tests kann sich deutlich sowohl in der Kinetik als auch in den Abbauwegen (Metabolismus) unterscheiden.
- Tests welche mit CO_2 freier Luft in geschlossenen Systemen durchgeführt werden liefern i.d.R. stabile Bedingungen und geringere Variabilität innerhalb der ersten 200-300 Tage
- Wegen der immensen großen Pufferkapazität von Meerwasser beobachtet man meist höhere Blindwerte und mehr Variabilität in den Messwerten als in vergleichbaren OECD 310 Standardtests.
- Die angestrebte analytische Methode (DOC/DIC; BOD, CO_2) bestimmt die Länge und Dauer der Studien ebenfalls und muss bei der Planung berücksichtigt werden. Wenn möglich sollten mehrere Parameter parallel bestimmbar sein.
- Marine Abbautests zeigen oftmals weit niedrigere Abbauraten als vergleichsweise Süßwasser bzw. Kläranlagentests oder solche in Boden oder Kompost.
- Das marine Medium für die Abbautests kann sowohl synthetisch im Labor hergestellt werden also auch aus der Umwelt als natives Medium entnommen werden sofern dieses sorgfältig behandelt und bei konstanten Temperaturen gelagert wird.
- Tests welche mit nativem Meerwasser durchgeführt werden, haben vermutlich eine größere Bedeutung weil diese näher an reellen Bedingungen sind. Allerdings werden im Rahmen dieser Studie keine signifikanten Unterschiede zwischen den Tests mit verschiedenen synthetischen oder nativen Medien beobachtet.

Abbreviations

Abbreviation	Description	Unit symbol
ABS	Acrylonitrile-butadienestyrene (styrene copolymer)	
AFM	Atomic Force Microscopy	
ASTM	American Society for Testing and Materials	
BA	Butyl acrylate	
BC	Blank control (sample or test assay)	
BOD	Biological oxygen demand	$[mg \cdot L^{-1}]$
BTA	Butyric acid, terephthalic acid & adipic acid co-polymer	
CA	Cellulose acetate	
CEN	Comitée Européenne de Normalisation, European Committee for Standardization	
CFU	Colony forming Units	$[CFU \cdot mL^{-1}]$
CMR	Cumulative measurement respirometric system	
COD	Chemical oxygen demand	$[mg \cdot L^{-1}]$
DGGE	Denaturing gradient gel electrophoresis	
DIN	Deutsches Institut für Normung (German Institute for standardization)	
DMR	Direct measurement respirometric system	
DNA	Desoxyribonucleic acid	
dNTP	Desoxyribonucleic triphosphate	
DOC	Dissolved organic carbon	
E/CO or ECO	Ethylene-carbon monoxide	
ECN	Comitée Européenne de Normalisation, European Committee for Standardization	
EDTA	Ethylenediamine tetraacetate	
EIS	Electrochemical impedance spectroscopy	
EN	European Norm	
EtOH	Ethanol	
FBBR	Fixed bed bioreactor	
FT-IR	Fourier transform-infrared spectroscopy	
GC/MS	Gas chromatography/mass spectrometry	
GMR	Gravimetric measurement respirometric system	
GPC	Gel permeation chromatography (also: SEC)	
HDPE	High density polyethylene	
HHx	Hydroxyhexanoate (hydroxy hexanoic acid)	

Abbreviation	Description	Unit symbol
HPLC	High performance liquid chromatography	
IC	Inorganic carbon	
ICP	Inductively coupled plasma (plasma emission spectroscopy)	
IEC	Ionexchange Chromatography	
IH	Inhibition control	
IR	Infrared / Infrared spectroscopy	
ISO	International organization for standardization	
JIS	Japanese industrial standards	
kb	Kilobase	
kDa	Kilo Dalton	
LAB	Lactic acid bacteria	
LALLS	Low-angle laser light-scattering	
LC/MS	Liquid chromatography/mass spectrometry	
LDPE	Low density polyethylene	
LLDPE	Linear low density polyethylene	
MALDI	Matrix assisted laser desorption ionization	
MB	Marine Broth (Difco 2216)	
M_n	Number average	
M_{Rep}	Molarmass of the repeating unit	$[g \cdot mol^{-1}]$
mRNA	Messenger RNA	
M_w	Molecular weight average	$[g \cdot mol^{-1}]$
MWD	Molecular weight distribution	
n.a. or n/a	Not analyzed or no data available	
n.d.	Not detected	
NCBI	National Center for Biotechnology information (USA)	
NMR	Nuclear magnetic resonance spectroscopy	
NTP	Ribonucleic triphosphate	
OECD	Organization for Economic Co-operation and Development	
PA	Polyamide	
PAA	Poly(aspartic acid)	
PAM	polyacrylamide	
PBAT	Poly(butylene adipate-*co*-terephthalate)	

Abbreviation	Description	Unit symbol
PBS	Poly(butylene succinate)	
PBSA	Poly(butylene succinate-co-adipate)	
PBT-PTMO	Poly(1,4-butylene terephthalate-*co*-tetramethylene oxalate)	
PCL	Poly(ε-caprolactone)	
PCR	Polymerase chain reaction	
PCU	Poly(carbonate urethane)	
PE	Polyethylene	
poly(ethylene glycol)	Poly(ethylene glycol)	
PE-PEO	Polyethylene-polyethylene oxide	
PET	Poly(ethylene terephthalate)	
PEU	Poly(ether urethan)	
PHB	Poly(hydroxy butyric acid); poly(hydroxybutyrate)	
PHBV	Poly(hydroxbutyrate-co-valerate)	
PLA	Poly(lactic acid)	
PLLA	Poly(L-lactide acid)	
PMMA	Poly(methyl methacrylate)	
POM	Particulate organic material	
PP	Polypropylene	
PPG	Poly(prolylene glycol)	
PP-ST	Polypropylene-starch blend	
PS	Polystyrene	
PTE	Polythioester	
PUR	Polyurethane	
PVA	Poly(vinyl alcohol)	
PVC	Poly(vinyl chloride)	
PxHA	Poly(x-hydroxy alcanoic acid): x = e.g. 3; H =hydroxy: A= alcanoic acid e.g A= alkanoic acid B=butyric acid, Hx=hexanoic acid, O=octanoic acid, V = valerci acid.	
Py-GC/(MS)	Pyrolysis-gaschromatography/(mass spectrometry)	
QSAR	Quantitative structure activity relationship	
RHEED	Reflection high-energy electron diffraction	
RNA	Ribonucleic acid	
rpm	Rotations per minute	$[1 \cdot min^{-1}]$
RS	Reference substance	

Abbreviation	Description	Unit symbol
RT-PCR	Reverse transcriptase polymerase chain reaction	
SAN	Styrene-acrylonitrile (styrene copolymer)	
SBR	Styrene-butadiene rubbers	
SCAS	Semi continuous activated sludge (test)	
SDS	Sodium-dodecyl-sulfate	
SDS-PAGE	Sodium dodecyl sufate polyacrylamide gel electrophoresis	
SEM	Scanning electron microscopy	
Sky-Green®	Aliphatic polyester made of adipic acid and succinic acid, butanediol and ethylene glycol	
SOP	Standard operating procedure	
TA	Thermal analysis	
TAE	Tris-acetate-EDTA Buffer	
TC	Total carbon	
TEG	Tetraehtylene glycol	
TEM	Transmisson electron microscopy	
TEMED	N,N,N`,N´-Tetramethylendiamin	
Th	Theoretical (biogas, CO_2, O_2)	
$ThCO_2$	Theoretical CO_2 evolution	
ThOD	Theoretical oxygen demand	
TLC	Thin layer chromatography	
TOC	Total organic carbon	
TOF	Time-of-flight	
TRIS	2-amino-2hydroxymethyl-1,3-propanediol	
TS	Test substance	
UV	Ultra Violet	
UV-VIS	Ultraviolet-visible spectroscopy	
WWTP	Waste water treatment plant	

Table of Contents

1 Introduction and aim of the present study

The biodegradation of polymers plays an important role because polymers are used in high amounts in our daily life. The life cycle of polymers from production to waste disposal or recycling is often complex and a huge part of the total amount of produced polymers is unaccounted for after the materials application. In order to understand the big picture on polymers one has to remember that polymers may occur in many different forms such as solid materials or even (water) soluble compounds used in huge amounts in the pharmaceutical, personal care products, dyes and lacquers, glues, construction and oil & gas field industry (e.g. Kinetic hydrate inhibitors (KHI)).

Up to know only little is known especially on aquatic environmental biodegradation of polymers but many scientific studies have investigated soil or compost biodegradation. To better understand mechanical biodegradation process of synthetic polymers in the environment and also provide suitable test methods for future research, this project aims to investigate the general aerobic biodegradation of selected synthetic polymers in marine and freshwater environments. The main focus is

- to summarize known information on biodegradation of polymers in the aquatic environment with specific focus on the marine environment from the literature
- to identify if standard biodegradation tests may be used for the evaluation of polymer biodegradation and to identify potential shortfalls
- to compare the biodegradation potential and biodegradation pathways in marine and freshwater environment
- to check whether molecular or structural properties of the polymers may have influence on the biodegradation in different environments
- to confirm the possible pathways of biodegradation and identify similarities and differences
- to partially investigate the microorganism community link the composition of microbial communities to the biodegradation potential
- to check whether it may be possible to accelerate marine biodegradation tests and what options are available if standard test cannot be applied to investigate marine biodegradation

Along with these topics, investigation of parameters of biodegradation tests such as physico-chemical parameters and also biological parameters such as biomass were investigated. Generally, tests for ready biodegradability on one hand and highly complex simulation tests (environmental fate) on the other are the most frequently used approach for measuring biodegradability of chemical substances. In those tests the influence of biomass could be important. It was investigated if a change in the biomass concentration could significantly accelerate biodegradation or the test results in marine and freshwater biodegradation tests. In this study the effect of the biomass concentration on the biodegradation was examined in online CO_2 evolution tests. Two different test substances were investigated with three different biomass concentrations using activated sludge from municipal wastewater treatment as inoculum suspensions.

On one hand it would also be beneficial on a scientific base to have a full profile on microorganism communities, parallel substance specific analysis and data from such biodegradation experiments starting at the beginning and lasting until final biodegradation is observed. To do this in two or three different kinds of aquatic environment and with at least three to five polymers (which would still be just not enough) would require much effort, time and money. Therefore this was not intended in this work and focus was set to a water soluble type (PEG) and two water insoluble polymers (Ecoflex and Ecovio). The focus of this research mainly compares different tests and strategies and the outcome of these tests and the data presented from molecular analysis is just one small part. In the strategy of biodegradation tests it is important to have a box of valuable tests with as much validity as possible. It is intended that the results provided by this work and the second dissertation on analytical data [1] will deliver a sound basis on which other polymers can be investigated and maybe better, faster and cheaper tests will be developed In industrial or contract trials for registration and evaluation purpose details such as the composition of microorganism communities will not be analyzed and considered if the methods for determination are too complex or expensive even though these details might be scientifically significant and necessarily to consider. This gap between very important details from scientific research and industrial applicability of a test system is still huge. But today it is imperative to bridge this gap and deliver resilient data with easy applicable tests. As it is, it seems still not possible to estimate biodegradation on the basis of fast test and transfer the knowledge to other environmental compartments or even other polymers.

The information generated by this approach may provide insight on the gap between ready biodegradation tests and simulation studies e.g. by giving better predictive value for the real environment while the test is still easily carried out. Since biodegradation research requires sophisticated analytical equipment and knowledge, all analyses that are necessary for the investigations and experiments discussed in this document are described in detail in a second thesis [1]. Today, some mechanisms for biodegradation of polymers are known [2] but systematic studies comparing different environmental compartments e.g. marine environment, freshwater environment, are missing. In this study, some pathways and mechanisms of biodegradation were investigated using sum parameters and substance specific analytical techniques.

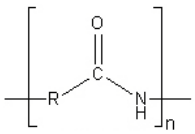
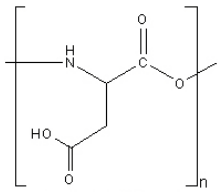
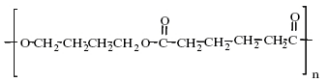
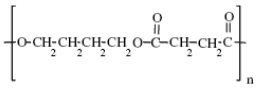
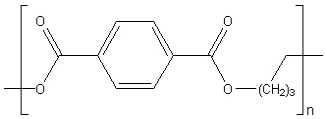
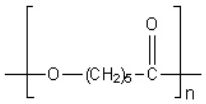
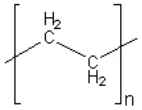
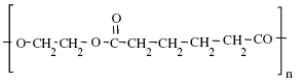
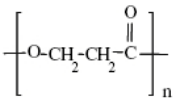
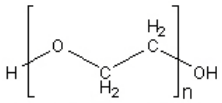
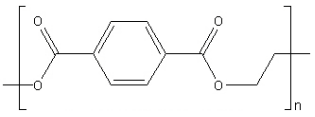
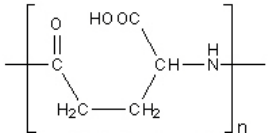
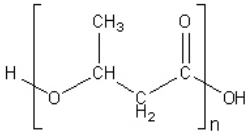
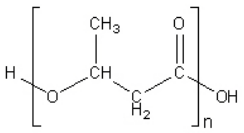
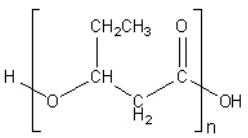
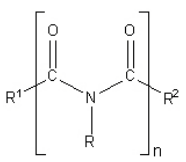
Certain polymers were selected based on their properties. Poly(ethylene glycol) (PEG) was used as water soluble polymer with at least some biodegradation potential in marine and freshwater systems. Poly(vinyl pyrrolidone) (PVP) was selected as water soluble polymer because it has been demonstrated to have low biodegradation potential [3]. Two water insoluble thermoplastic polymers, Ecoflex and Ecovio, both polyesters based on aliphatic-aromatic constituents were taken because they are biodegradable in soil and compost and comparisons to aqueous environments could help to understand the different biodegradation pathways in both environmental compartments.

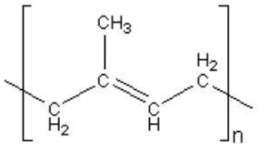
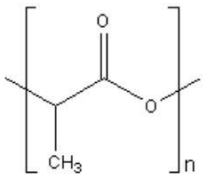
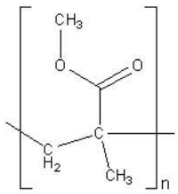
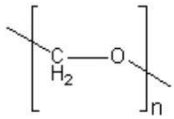
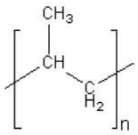
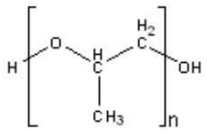
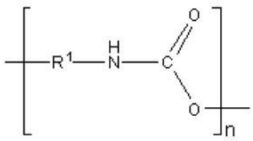
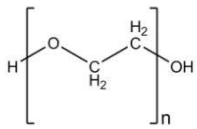
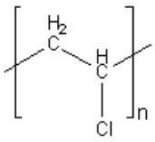
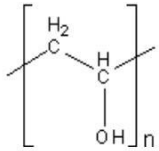
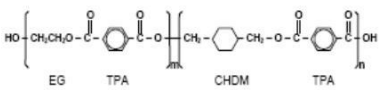
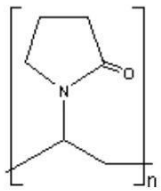
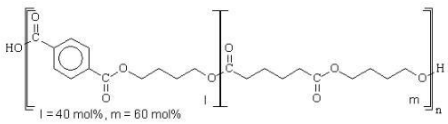
The results of standardized biodegradation tests in freshwater were compared to marine biodegradation. Up to now, tests on marine biodegradability are limited to only a few studies [4-15] maybe because of the low biodegradation potential of the medium. Marine biodegradation has been observed mainly for aliphatic and aromatic hydrocarbons [16], polyaromatic hydrocarbons (PAHs) [17;18], surfactants [19] and phthalate esters [20] and mostly “small molecules” from pharmaceutical, household, or crop protection/agricultural applications.

1.1 Polymers and their effects in the environment

Today, polymers surround us almost everywhere in our lives. In discussing this huge class of chemical compounds one has to carefully organize all the different structures to maintain a critical perspective on properties and effects observed in experiments. It is especially imperative to select what is beneficial for a specific application. The main polymers discussed further in this work are given in Table 1 to provide a selection of some important polymers. Only a selection of those is investigated more closely in this thesis. The selection was based on a) solubility, b) biodegradation potential and c) current situation and published data on different tests.

Table 1 - Names and structures of the main polymers and their groups described in this work

Name	Abbr.	Structure	Name	Abbr.	Structure
Poly(amide)	PA		Poly(aspartic acid)	PASP	
Poly(butylenes adipate)	PBA		Poly(butylenes Succinate)	PBS	
Poly(butylene terephthalate)	PBT		Poly(ε-caprolactone)	PCL	
Poly(ethylene)	PE		Poly(ethylene adipate)	PEA	
Poly(propio lactone)	PPL		Poly(ethylene oxide)	PEO	
Poly(ethylene terephthalate)	PET		Poly(glutamic acid)	PGA	
Poly(hydroxy alkanoate)	PHA		Poly(hydroxy butyrate)	PHB	
Poly(hydroxy valerate)	PHV		Poly(imide)	PI	

Name	Abbr.	Structure	Name	Abbr.	Structure
Poly(isoprene)	PIS		Poly(lactic acid)	PLA	
Poly(methyl metacrylate)	PMMA		Poly(oxy methylene)	POM	
Poly(propylene)	PP		Poly(propylene glycol)	PPG	
Poly(urethane)	PUR		Poly(ethylene glycol)	PEG	
Poly(vinyl chloride)	PVC		Poly(vinyl alcohol)	PVA	
SkyGreen®			Poly(vinyl pyrrolidone)	PVP	
Aliphatic aromatic copolyester					

Polymers can be classified into many groups based on their different properties. From the material point of view and especially with regard to biodegradability and environmental topics it is beneficial to distinguish between water soluble and water insoluble polymers. The first ones are generally not intended to end up in the environment because they should normally be reused or recycled in order not regain the resources. Recycling may either be energetic, physical or chemical recycling.

Water soluble polymers may be intended for use in aquatic compartments such absorbents in waste water treatment or oil & gas field chemicals or they may enter the environment unintentionally because they are incorporated in personal care products, lacquers and many other products.

Today, biodegradation is more an interesting feature for water soluble polymers because of their use in other products but for plastic materials that are often designed to be very recalcitrant. Nevertheless another part of invisible polymers in the environment are water insoluble materials of micro or nano particle size or pellets that derive from deterioration of larger parts and that are found in huge amounts in the environment an generating still unknown effects. In the following paragraphs it is discussed where problems may originate from and the effects that can occur and what is known today. The example provide, shows how much connected the world is and that it is imperative to think before acting.

1.1.1 Polymers as "nutrients" for (marine) mammals

Considering the fact that everything that has entered the aqueous environment can surface anywhere in the world, especially water insoluble polymeric materials have been reported to serve as food for animals. The direct effects such as starvation have been often reported especially in daily press and media but also by scientists [21]. The indirect effects such as accumulation of particles or other chemicals adsorbed to polymer particle surface though, have not yet been investigated much. In a global world these might be even more problematic in the future. Especially these effects may not only be prompted by water insoluble materials. A brief description will be given in chapter 1.1.2.

1.1.2 Polymers as carriers for persistent, bio-accumulating and toxic chemicals

It has been investigated that polymeric materials may work as transporter system for persistent organic pollutants such as PCB's PAH's Pesticides and DDT [22], PCB's [23;24] and DDE and nonyl phenols [24] as well as phenantrene [25]. Analytical methods were developed to extract analytes from resin or thermoplastic polymers and it was found that these substances tend to adsorb to the surface in high concentration, which enables them to "travel" into every possible environmental compartment in the oceanic system on the planet. In summary, these substances may end up in marine creatures and the food chain.

For phenantrene it was shown that tendency to adsorb to polymers is far greater than to sediment particles. Also variations were more than an order of magnitude greater between adsorption to PE, PP and PVC from seawater. Desorption was observed to be faster from sediment particles than from polymers and varied again more than an order of magnitude between polymers. It was also estimated using equilibrium partitioning methods that adding as little as 1µg of contaminated PE per gram of sediment inhabited by lugworms (*Arenicola marina*) the effect would result in a significant increase in phenantrene accumulation over time [25].

1.2 The importance of the marine environment

Biodegradation is a major topic today in risk assessment strategies [26]. Mostly, the focus is set on freshwater systems because in generally the direct entry of chemical substances is through these systems. One could ask now why the marine system is so important and especially why the degradation (DEG) of polymers should be investigated in marine systems.

First, the marine system is the largest aqueous system on the planet. The marine ecosystem accounts for over 90% of the biosphere, the oceans cover 71% of the Earth's surface and contain 1.410 ^{21}L of water (97% of the total water on Earth) [27]. The second reason is that investigating biodegradation in the marine ecosystem is still not very common and only little is known. Today, neither marine biodegradation nor polymers are covered by the REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemicals) [26] but it is important to investigate differences between marine and standard freshwater tests and also to try to improve tests for this special application as a preventive measure and in order to support R&D activities on the development of new products. It should also be possible to transfer the generated knowledge to other marine biodegradation tests in chemical industry. Polymer biodegradation in the marine environment is also required for some special applications (e.g. under OSPAR contract) and therefore a necessary tool [28].

1.2.1 Size matters

It was long thought that marine systems offer low cell density and biodegradation occurs very slow because of this fact. On the contrary it has been often reported [27] that using lately developed tools, the number of marine prokaryotes detected is in the range of 10^5-10^7 mL^{-1} [27]. The question then is, whether biodegradation in marine and freshwater are so different and why. Most microbes found in marine ecosystems are exceptionally small, which is also the reason why they eluded us until very recently new tools were available.

Small cell size has great significance when it comes to physical processes that effect life. At this scale, the rate of molecular diffusion becomes most important for the transport of substances into and out of a cell, meaning, small cells feeding by absorption (osmotrophy) may take up nutrients more efficiently than larger cells. In Table 2 the size for some microorganisms is given.

Table 2 - Size range of some representative marine prokaryotes (Where one value is given as size, this is the diameter of spherical cells)

Organism	Characteristics	Size [µm]	Vol. [µm3]
Thermodiscus sp.	Disk-shaped. Hyperthermophilic *Archaea*.	0.08 x 0.2	0.003
'Pelagibacter' (SAR11)	Crescent-shaped. *Bacteria* ubiquitous in ocean plankton	0.1 x 0.9	0.01
Prochlorococcus sp.	Cooci. Dominant photosynthetic ocean *Bacteria*	0.6	0.1
Vibrio sp.	Curved rods. *Bacteria* common in coastal environments and associated with animal tissue	1 x 2	2.
Staphylothermus marinus	Cocci. Hyperthermophilic *Archaea*.	15	1800
Thiploca auracae	Filamentous. Sulfur *Bacteria*	30 x 40	40'000
Beggiatoa sp.	Filamentous. Sulfur *Bacteria*	50 x 160	1'000'000
Epulopiscium fishelsoni	Rods. Bacteria symbiotic in fish gut.	80 x 600	3'000'000
Thiomargarita namibiensis	Cocci. Sulfur *Bacteria*	750	200'000'000

As the cell size increases, the volume (V) increases more rapidly than the surface area (SA) which shows that the critical factor affecting nutrient uptake is the SA/V ratio. Prokaryotic cells with large SA/V ratios are more efficient in nutrient uptake.

The explanation can be supported by studies using low nutrient media and increasing the nutrient concentration which did not lead to increase in sell size. But also if nutrients are severely limited as in most marine habitats, selection will favour small cells because of their efficiency [27].

Cells use various strategies to increase their SA/V ratio. Especially spherical sells are the least efficient shapes regarding diffusion of nutrients and therefore many marine microorganisms are long and filamentous shaped. It is interesting that even though larger organisms are present in marine water, the smaller ones are the most abundant ones (Table 3). That such small cells play a vital role in marine life has only been found out lately [27].

Unfortunately it is not clearly known whether small cell size is due to starvation or genotypically determined and it is also unknown if cells size determines or influences the rate of biodegradation or whether biodegradation occurs or not.

Table 3 - Classification of plankton by size (additional information for bacteria: some filamentous Cyanobacteria and sulfur-oxidizing bacteria occur in larger size classes)

	Size category	Size range [µm]	Microbial groups	
Size ↓	Femtoplankton	0.01 - 0.2	Viruses	Abundance ↑
	Picoplankton	0.2 - 2	*Bacteria*, *Archaea*, some flagellates	
	Nanoplankton	2 – 20	Flagellates, diatoms, dinoflagellates	
	Microplankton	20 – 200	Ciliates, diatoms, dinoflagellates, other algae	

1.2.2 Chemical and physical properties of the marine ecosystem

Seawater is generally slightly alkaline (pH 7.5 to 8.4). It consists of over 80 solid elements, gases, and dissolved organic substances of which the concentration varies considerably according to the location and physical factors. Generally these parameters are summarized in the degree of salinity (in ‰). The open ocean has normally a salinity of 34-37‰. Due to rainfall and evaporation salinity changes a bit. The major ionic compounds of seawater are sodium (Na^+, 55% w/v), chloride (Cl^-, 31% w/v), sulfate (SO_4^{2-}, 8% w/v), magnesium (Mg^{2+}, 4% w/v), calcium (Ca^{2+}, 1% w/v) and potassium (K^+, 1% w/v). Together, these ions constitute to over 99% of the weight of salts. The minor ions are hydrogen carbonate (HCO_3^-), bromide (Br^-), borate ($B_4O_7^{2-}$), silicate (SiO_4^-) and iron (Fe^{3+}). These make less than 1% of the salts in seawater and the trace elements remaining contribute to even less than 0.01% [27] (p 9-10).

It is important, to recognize the especially low concentration of inorganic carbon even though the oceans are the biggest reservoir for CO_2. Carbon dioxide reacts with water to carbonic acid which dissociates rapidly to bicarbonate and carbonate as given in Equation 1.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

Equation 1 - Dissociation of carbonic acid and carbon species distribution

This reaction tends to stay in equilibrium buffering the pH of seawater within a narrow range but with a huge capacity. At the normal pH of seawater almost all carbon in the water phase is found as bicarbonate. The correlation between carbon species and pH is shown in (Figure 1).

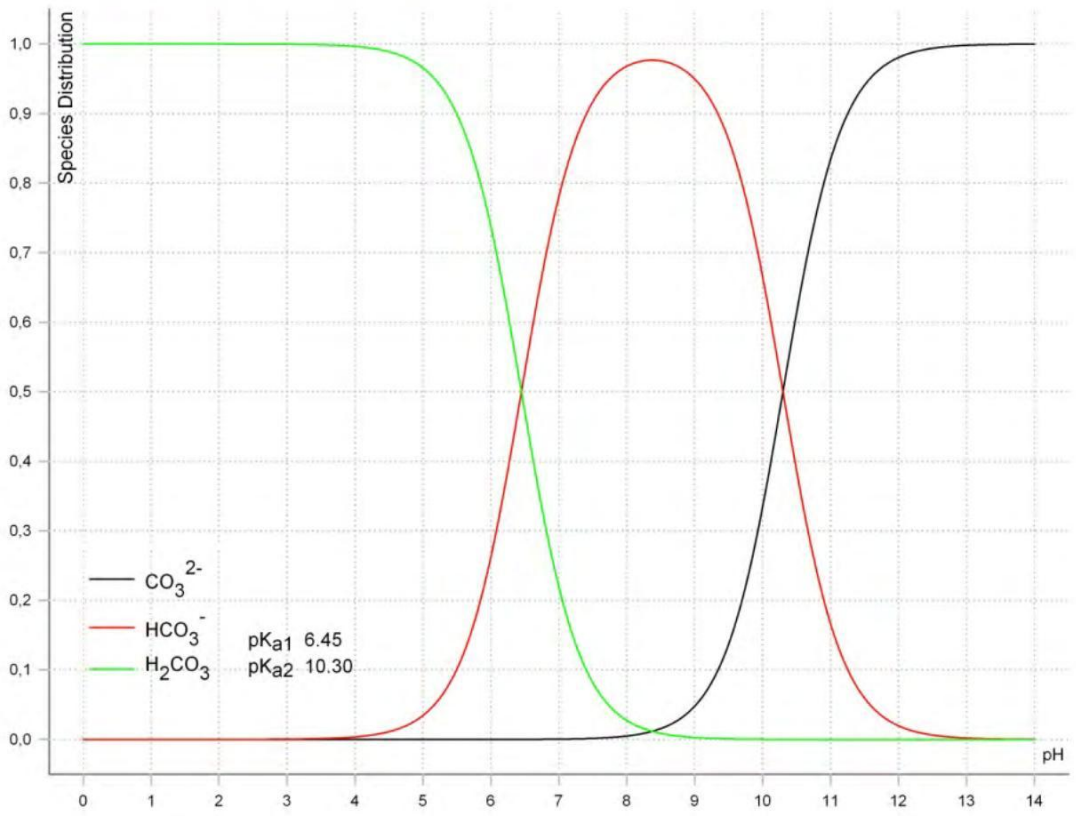


Figure 1 - Carbon species distribution in aqueous media at 20°C and changing pH

2 Current situation on biodegradable polymers in the environment

2.1 An introduction to biodegradation

The biodegradation of polymers is important within the frame of sustainable development. The different stages of biodegradation (biodeterioration, biofragmentation and assimilation) have to be researched and validated. Especially assimilation has been neglected and the tools have been inadequate to certify real biodegradability and integration of the material in biogeochemical life cycles [29].

Biodegradation is an important parameter in the assessment for many different substances concerning environmental fate [30]. The concepts have been applied and improved [31] since the early 70s and conventional tests and assessments were done during the last decades for different groups of chemical substances such as surfactants [32-37], pesticides [38-43], pharmaceuticals [44-48] and industrial chemicals [20;49-53].

Biodegradability is a key feature of chemical (and natural) substances [54;55]. It is imperative for some but also not useful for other compounds. Concerning polymers, one has been focusing mainly on soil or compost biodegradation. Many aspects were investigated such as the influence of structure, conditions, microorganisms etc. [56;57] but generally there is almost no systematic information on the fate in aqueous environments especially the oceans.

Marine biodegradation can be an important parameter for certain applications of especially water soluble polymers that are used in high amounts in every day products such as PVP or PEG or KHI's for oil- & gas field applications. Nowadays marine biodegradability is also required by regulations for some markets and for environmental risk assessments especially in oil- & gas field.

2.2 Biodegradable polymers

In polymer research biodegradation is useful to obtain plastics for certain applications were biodegradation enhances the value of an application. This is mostly applicable to soil or compost biodegradation of packaging materials [58] or mulching films [59]. Biodegradable polymers were described in the literature through recent years [60-62]. Also manufacturing of "Bioplastics" was investigated using natural and/or petrochemical/fossil resources [63;64].

Generally, there are two different types of biodegradable polymers. First, the ones that are rapidly converted to carbon dioxide and water in an appropriate environment (e.g. cellulose, starch and aliphatic polyesters) and the class of oxo-biodegradable polymers (natural rubber and lignocelluloses). Also polyolefins are representatives of the latter category. Non-stabilized polyolefins oxidize rapidly in the environment (PE 20% weight loss in 5 months; PP 80% weight loss in 5 months). Only the formulation and additional compounds such as antioxidants give them their stability [65]. It is often assumed that bioplastics are by definition environmentally friendly or sustainable. As lifecycle analyses (LCA) or ecoefficiency analyses show, that may not always be the case. If an assessment of environmental (and ecological) facts for each stage of a products lifecycle regarding

natural resources, consumed energy (from generally fossil fuels) and produced waste is done, relevant comparative information will be obtained [65]. It has also been shown by *WITT ET AL.*, that it is not the resource that determines biodegradability but rather the chemical structure [66]. In producing biodegradable plastics, it is most important to minimize energy consumption and environmental pollution during production [65].

2.3 Polymers from renewable resources

In the process of designing new products, also renewable resources may play role for circumventing difficulties with common polymers such as unknown environmental fate and recalcitrance [67]. But in order to design products specifically for any new application or use and to obtain the best eco-efficiency rating it is absolutely necessary to consider the overall performance and the complete lifecycle of a product to establish whether natural or petrochemical resources offer the best and most environmentally friendly source of energy and material [64]. The actual situation on polymers from renewable resources in terms of interest, present degree of advancement and prospective development was given by *GANDINI* et al. [68].

At this point, most investigations on processing polymers from renewable resources are focused on poly(hydroxy alkanoates) [69;70], starch or cellulose based polymers [71-73], lactide or caprolactam polymers [74-78] and block co-polymers with ethylene glycol [79]. Also chemo-biotechnological process to convert polyolefins into biodegradable thermoplastics has been described [80].

It is important to recognize that “sustainable” and “renewable” are not the same. Materials that are made from renewable materials are not necessarily more sustainable or better highly eco-efficient than others. This knowledge is important to think in every direction when developing new materials as it becomes more imperative to have materials with complete life-cycles and many different applications as possible [81]. In the ongoing discussion about carbon footprint, global warming and fading resources we need to provide a sound basis of reliable aspects and not dwell on marketing strategies only. This may also mean that products made from polymers from different origin may provide better properties although they will be partly or completely produced from fossil resources. It will also lead to very specialized but highly improved products that require a complete network of researchers, producers, marketing strategists, consumers and recycling units to enable a complete life cycle for developed products.

This will also require everyone from producer to consumer to think of the effects, the intended application of a product and take responsibility in one’s actions.

The directions of polymer research must be defined very carefully because environmental friendly and biodegradable are two terms that are not necessarily the same. There are several questions to be asked such as [67]:

- What is a biodegradable polymer?
- What is a polymer expected to do in the environment?
- What tests are needed to determine rate of biodegradation and acceptability of polymers in the environment?

- Does biodegradation deliver a useful solution for the application and the polymer?
- Is the complete lifecycle eco-efficient?

2.4 General aspects of degradation and terminology

During recent decades uprising concerns about plastic wastes found in different environmental compartments, discussions in- and outside the scientific community some terms regarding degradation were used in many different contexts. This led to some confusion that has to be cleared. In general there are two terms, biodegradation and biodeterioration. Biodegradation implies that the material needs to be degraded by microorganisms, such as bacteria and fungi under aerobic or anaerobic (methanogenic or sulfidogenic) conditions. Biodeterioration should be used for abiotic degradation of substances through weathering, UV-light, water (hydrolysis) and parameters such as pH, temperature and concentration of minerals and nutrients. The ASTM definitions, for all the collectively but erroneously used descriptions for biodegradation are given as follows [82]:

- A degradable polymer is designed to undergo a significant change in its chemical structure und specific environmental conditions resulting in the loss of properties.
- A biodegradable polymer is one in which degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae.
- A hydrolytically degradable polymer is one that degrades by hydrolysis.
- An oxidative degradable polymer is one that degrades by oxidation.
- A photo degradable polymer is one that degrades by the influence of natural day-light.

As indicated (Figure 2) all degradation pathways will lead to fragmentation in the first place when occurring in the environment. However they are strongly differentiated in the fact, that only biodegradation of the original polymer may result in complete mineralization.

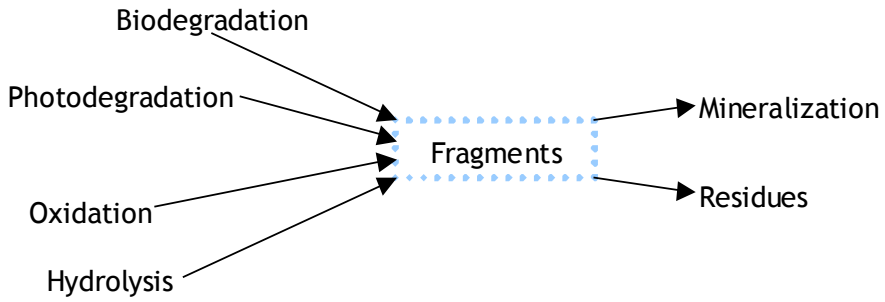


Figure 2 - Degradation and deterioration of substances

For environmental acceptance of certain polymers partial biodegradation is mandatory. Biodegradation is not restricted by time. It may be very slow or very rapid and dependent on many different factors.

Because of a huge increase in polymer wastes during recent years, biodegradability as a special and possibly controllable property becomes more and more imperative, thus vital in polymer research. But ecological con-

siderations on the use and production of biosynthetic and synthetic polymers are necessary and important [83]. Degradation in general may occur in different steps, depending on ambient conditions and on the chemical and physical structure of the observed compound or composite. Figure 3 shows possible pathways on polymer degradation [84].

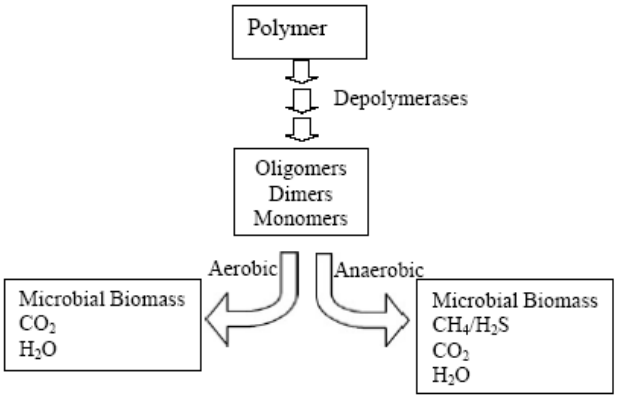


Figure 3 - Degradation of polymers under various conditions [84]

Polymer degradation under various, but not only marine conditions, is reliant on generally the chemical structure of a polymer or polymer composite. Not the origin, the resources a polymer was manufactured of determine biodegradability but the chemical structure [64;66]. Also structure-activity-relationship (SAR) plays an essential role in biodegradable polymer development [85]. Knowing this and second that physical properties avail biodegradability furthermore; certain guidelines have been developed for conveying research and development into the right directions. The given directions for researching environmentally biodegradable polymers have been presented a few years ago [67].

- Naturally occurring polymers often biodegrade (note: the natural polymers e.g. from sequoia wood are very recalcitrant)
- Chemically modified natural polymers may biodegrade depending on the extent of modification
- Synthetic addition polymers with carbon-chain backbones do not biodegrade at molecular weights greater than ~500g·mol^{1}
- Synthetic addition polymers with heteroatoms in their backbones may biodegrade
- Synthetic step-growth or condensation polymers are generally biodegradable to a greater or lesser extent depending on:
    - Chain coupling (ester>ether>amide>urethane)
    - Molecular weight (lower degrades faster than higher)
    - Morphology (amorphous degrades faster than crystalline)
    - Hydrophilicity versus hydrophobicity (hydrophilic polymers are faster degraded than hydrophobic ones)

In general two distinct mechanisms depending on the nature of the polymer and the environment determine the processes. First, abiotic or biotic hydrolysis followed by bio assimilation (hydro-biodegradation), which can

be accelerated by photo oxidation. And second, peroxidation followed by bio assimilation of low molar mass products (oxo-biodegradation), which happens particularly to carbon-chain polymers. Abiotic peroxidation can be controlled accurately by the use of appropriate antioxidants, which lead to higher persistency as well as photo stabilizers or additives.

Physical characteristics like molecular orientation, crystallinity, cross-linking, chemical groups or side chains determine the accessibility to degrading-enzyme systems. A review by *SCOTT* [86] provides an overview for 'green polymers' and their management in regard to waste recycling and biodegradation as well as application in all-day use.

Degradation is an irreversible process in chemical structure of a polymer involving changes in properties. Polymers can be degraded by five different mechanisms [14]:

- Photo degradation by natural (day) light
- Oxidation by chemical additives
- Thermal degradation by heat
- Mechanical degradation by mechanical effects
- Biodegradation by microorganisms

Biodegradation can be subdivided into complete/total biodegradation (mineralization) and partial (primary) degradation. Primary degradation leads to stable (catabolites) and/or temporary (metabolites) degradation products [87-89].

Biodegradation is catalyzed by microorganisms leading to ultimate formation of carbon dioxide, water and new biomass. In summary the chemical process can be described in Equation 2 and Equation 3 [89;90]:

$$C_{(Polymer)} + O_2 \longrightarrow CO_2 + H_2O + C_{(residue)} + salts$$

Equation 2 - Aerobic biodegradation of polymers

$$C_{(Polymer)} \longrightarrow CO_2 + CH_4 + H_2O + C_{(residue)} + C_{(biomass)} + salts$$

Equation 3 - Anaerobic biodegradation of polymers

Complete/total biodegradation (also: mineralization) can be observed when no $C_{residue}$ remains.

A recently developed concept by Swift has introduced environmentally acceptable biodegradable polymers [82]. Consequently, the definition must include the degree of biodegradation as well as the impact of polymer by-products on the environment.

Biodegradation can occur in two different environmental types (aerobic and anaerobic) with two subdivisions each (aquatic and solid) [89;90]. The microorganisms of different environments show also different potential in biodegradation of organic compounds [91].

Test systems used for biodegradation tests of anthropogenic substances have a number of crucially important parameters affecting the degradation processes. One of which is the immense variety of degradation pathways in very different environmental situations. Therefore many test organisms would be required, reflecting the different aquatic and terrestrial compartments, if one would like to achieve real and detailed knowledge on the actual situation in a native environment [92].

Three key elements are indispensable for biodegradation of a certain material to occur: first, the basis for any biodegradation is the existence of microorganisms with an appropriate metabolic pathway to synthesize enzymes specific for the target substance to initiate depolymerization and mineralization of monomers and oligomers. Second, environmental factors such as temperature, moisture, salts, oxygen and pH rendering the biodegradation processes possible, and third, the substrates (sample) structure influences the degradation process. Therefore, chemical bonds, degree and type of branching, degree of polymerization (DP), degree of hydrophobicity, stereochemistry, molecular weight distribution (MWD), crystallinity and morphological aspects [90]. During the first phase, cleavage of side chains or backbone will lead to an increasing contact interface between microorganisms and polymers. The decomposition into smaller fragments normally occurs outside microorganisms cells due to the size of the polymer macromolecules. Involved enzymes cleave ether randomly internal linkages of the polymer chain (endo-enzymes) or sequential at the end/terminal monomer units in the main chain (exo-enzymes). The second step, mineralization, normally takes place after sufficiently small oligomeric fragments are transported into the cells, where, after bio assimilation, mineralization leads to the final products CO_2, CH_4, H_2O, N_2, H_2, salts, minerals and new biomass (BM) [90].

Plastics with outstanding, user-friendly properties and very good thermoplastic processability but at the same time full biodegradability have drawn attention through recent years. Especially in agriculture and packaging these products, namely aromatic-aliphatic co-polyesters, have shown good compliance regarding biodegradation [60;66;93-97].

After the development of methods corresponding as realistic as possible to environmental conditions, the task is to investigate mechanisms of microbial degradation and dependency of chemical composition and physical structure of the test substances [98].

The fact that laboratory conditions are very different and often not comparable to their environmental counterparts must be taken into account when limit values for degradation tests are being established. Higher test substance concentration and lower inoculum concentration as well as time limits in degradation tests play a major role for the fact that results obtained in lab tests are generally lower than those measured in the field.

2.5 Biotic degradation

One reason why substances may accumulate in the environment is due to the fact that the evolution cannot keep pace with the rapid introduction of newly developed compounds into the environment. Also chemical structure determines highly whether a substance is biodegradable or not. There are possibilities to promote biodegradation such as I) changes in chemical molecular structure and II) the development of microorganisms

that are capable of using a certain carbon source as substrate or III) making substances more conductive to microbial populations attack.

Many factors contribute to recalcitrance. Microorganisms may lack the necessary genetic information which they may acquire by plasmid transfer or *de novo* enzyme synthesis. Also the compounds may be too large to be assimilated into the cell or no membrane transport system may exist. Compounds can be insoluble and microorganisms may lack the proper nutrients. Recalcitrant compounds can be oxidized when a readily biodegradable organic carbon (OC) source is available but in the absence the recalcitrant compound is not degraded (e.g. alkane or lignin degradation). Polymers are known to show mostly complex biodegradation behavior. The initial rate of its degradation often follows a "Freundlich" or modified "Langmuir" isotherm rather than "Michaelis-Menten" kinetics [99].

A significant problem with degradation studies is that these studies are in most cases time-consuming long-time studies. Acceleration might eventually be possible to a certain degree when changing parameters like temperature, salinity, pH, UV-light, special enzymes/organisms or pre-adaptation of organisms (SCAS Test) but would on the other hand lack environmental relevance.

HOWARD gives an overview of the biodegradation with fungi and bacteria of certain PURs, which were thought not to be degradable for a long time [100]. A huge part of biodegradation research is taken by experiments of degradation of PHA and its derivates. These experiments show degradation by fungi as well as bacteria in many studies [8;101]. *MABROUK & SABRY* published a study on degradation of PHA derivates by marine organisms (*Streptomyces sp.* SNG9) [102] as well as *LEATHERS ET AL.* who studied the degradation of PHA co-polymers by a marine bacterium (*Pseudoalteromonas sp.* NRRL B-30083*) [103].

The degradation of polyesters such as PHA and its bio- or chemosynthetic related polyesters with a focus on microbiology, biochemistry and molecular biology of PHA degradation has been presented by *JENDROSSEK* [104]. Especially intracellular and extracellular degradation, the search for and characterization of microorganisms, the influence of physico-chemical parameters and mechanisms of depolymerase reactions are discussed in detail.

It has been shown, that biotic degradation of polymeric structures is a function of a) the chemical structure of a polymer, b) the presence of degrading microbial populations and c) the environmental conditions encouraging microbial growth. A review on polymer microbial degradation and its research advances shows mechanisms and microorganisms involved and grouped as biopolymers, chemically modified and natural polymers and recalcitrant polymers [84].

Polymer materials may be used as potential source of carbon and energy for heterotrophic microorganisms including bacteria and fungi. Additives used in polymer manufacturing may serve as good nutrients for ambient degrading organisms [105].

Degradation under aerobic condition results in microbial biomass, CO_2 and H_2O as final products either from degradation pathways of additives or the polymeric material itself or both, while degradation under methano-

genic conditions results in CH_4, CO_2 and H_20 and under sulfidogenic conditions in H_2S, CO_2 and H_2O as final products [105].

In most cases of biodegradation, the first step is an enzymatically-catalyzed hydrolysis of ester, amide or urethane bonds. This primary depolymerization is a surface erosion process still unknown. It leads at least to water soluble intermediates, which can be assimilated by microorganisms and are further metabolized. However, a second mechanism can be described as biodegradation, which is not catalyzed by enzymes. Abiotic hydrolysis can also lead to intermediates, which will be taken up by microorganisms later and can be metabolized [94;106].

Microbial assimilation of polymers is limited by the cell membrane structure of degrading organisms. Enzymes of the latter such as lipase, polymerase etc. may help in decreasing size and molecular weight of macromolecules to dimmers, monomers and oligomers before uptake by a much wider range of microorganisms. [105].

It was observed that some unknown “Priming effect” can occur if an easily degradable compound is added and more than the predicted amount of CO_2 is evolved. The effect decreased when the amount of substance added additionally increases. It has also been reported that the addition of such substances to the biodegradation test can significantly increase the number of microorganisms. Addition of 5 wt% glucose resulted in 10-100 times more microorganisms but the increase did not influence the final biodegradability of the tested polymers [107]. A similar study investigated influences of sludge inocula in ISO 14851 Tests. Microorganisms were taken for municipal and industrial wastewater treatment plants (WWTP) and the results show that municipal sludge degraded starch based polymers and PCL to similar degrees. In some cases starch based materials were degraded more easily. Degradation in industrial sludge was in both cases better but it was observed that degradation of cellulose (used as reference) was lower in industrial sludge than in municipal sludge. Acclimatization of microorganisms to the substance did not provide expected results of an increased biodegradation degree [108].

In summary, it is still unknown under which circumstances biomass and biodiversity may or may not have any effect on biodegradability of a substance, and if biodegradation can be described as a function of biodiversity and/or biomass. The influence of both parameters is also very complicated to assess

2.6 Abiotic degradation

Concern for the environment as well as special applications have led to the development of degradable polymers. Synthetic hetero-chain-polymers are often susceptible to hydrolytic cleavage that can be followed by microbial bioassimilation (e.g. polyesters) while carbon-chain polymers undergo degradation by peroxidation and formation of low molecular carboxylic acids further used as carbon source by bacteria and fungi. This process can be controlled by controlling the peroxidation step. It is possible to control photolysis, photo-biodegradation, hydro-biodegradation, antioxidant-controlled photo-biodegradation and thermo biodegradation through chemical structure and by additives. The change in mechanical properties is due to chemical modification and leads to smaller molecular mass products that are susceptible to microorganisms. Products with

these special properties are very important in medicine, agricultural technology, water- and fertilizer conservation, controlled release, forestry, and packaging [109].

Abiotic degradation can be very easily determined, though the experiments are sometimes long-time studies as well. Hydrolysis and photo degradation are easy to determine via weight loss for example. Other physical parameters such as elongation, breaking loads, moisture absorption, shear characterization and change in diameter are standard test methods in polymer research and thus easily applicable. Additionally, tracer studies (with isotope labeling), and development of accelerated test methods (change in parameters such as salinity, temperature, pressure, pH, UV light) would provide new challenging areas of research.

2.6.1 Hydrolytic degradation

It is generally known that polymers with ester bonds may be degraded by hydrolysis because hydrolytic processes under natural conditions can cleave ester bonds [105].

The hydrolytic degradation along with changing mechanical properties of hydrogels from microbial poly(aspartic acid) was investigated by *KUNIOKA & CHOI* [110]. Microbial poly(γ-glutamic acid) and poly(ε-lysine) (M_w 100'000 - 1'000'000 g·mol^{-1}) were found not degraded near room temperature but were hydro biodegradable at 60°C

PLA/PEO/PLA triblock copolymers with short PLA blocks were synthesized and hydrolysis was investigated using DMSO/D_2O medium in the presence of TFA. Data shows that PLA/PLA intra-chain and PEO/PLA connecting ester bonds were cleaved at comparable rates in the selected homogenous medium [111].

Monofilaments of PBT-PTMO along with PET, poly(hexamethyleneadipamide) and PP and poly(1,4-butylene-terephthalate-co-tetramethylen-oxalate) co-polymer were studied in saline and distilled water at different temperatures below and above glass transition temperature (T_g). Below T_g there was no change in mechanical properties observed in either distilled or salt water besides for PBT-PTMO were a decrease with increasing ageing time was detected. After around 300 days at 25°C total strength loss was observed in both media, respectively. The poor hydrolytic stability of PBT-PTMO can be attributed to higher moisture regain. The salinity did not have any significant effect on strength loss of the materials. Hydrolytic degradation of PBT-PTMO was confirmed by increasing acid carbonyl and hydroxyl groups and increasing consumption of ester groups [112].

DAVIES ET AL. studied the influence of water and accelerated ageing on glass epoxy composites [113]. Acceleration was simulated by increased temperature and physical properties were measured. Un-reinforced matrix resins were immersed in medium as well. Results have shown that sea water was absorbed less rapidly than distilled water. Weight gain below 1% by absorbing media had no influence to the shear strength of the material. Higher weight gains reduced the shear strength up to 25%.

E-glass/vinylester composites were studied, to evaluate their performance in aqueous media by *KARBHARI & ZHANG*. Comparisons are made between deionised water and potassium based pH-buffered solutions and determined via measuring physical parameters. It was observed that the coefficients of apparent diffusion and

levels of moisture gain are the highest for the deionised water immersed samples at 60°C and these results in the highest levels of tensile strength and modulus degradation [114].

A comparison between marine and freshwater exposure as weathering experiments of photo degradable polyethylenes show that under marine conditions, degradation is even slower than under freshwater conditions. That statement applies to enhanced degradable polymers as well as common polymers as control samples [115;116]. Also, physical parameters are determined in a study of 42-year immersion of seawater on natural rubber. No effect of marine organisms (microorganisms, invertebrates etc.) on rubber was observed and the water absorbed was less than 5% in this long-time experiment at a depth of 24.3m (80ft) [4]. Glass-fiber reinforced polymer (GRP) laminates used for marine constructions were investigated concerning seawater immersion ageing. Water uptake behavior has been behavior for polyester, phenolic and vinylester glass-fiber reinforced polymers and neat resin castings as well as losses in mechanical properties. Phenolic glass-fiber reinforced polymers showed anomalous water uptake behavior; flexural strength fell about ~20% for polyesters and vinylesters and 25% for phenolic glass-fiber reinforced polymers. Interlaminar shear strength fell for all glass-fiber reinforced polymers about 12-21% [7].

Additional information on sea water immersion on carbon-fibre composites (glass/PE, carbon/PE, glass/vinyl ester, carbon/vinyl ester) was published by *KOOTSOOKOS & MOURITZ*. The samples were immersed in seawater at 30°C for over two years. Significant moisture absorption and chemical degradation of the resin matrix especially at the fibber/matrix region was observed. The flexural modus and strength decreased while the mode I interlaminar fracture toughness was steady [12].

The development study for potentially degradable materials for marine applications compares polypropylene-starch (PP-ST) blends [9] and polyethylene-polyethylene oxide (PE-PEO) blends [117] in stabilized and non-stabilized forms (containing certain additives). The study suggests certain oxidizing agents to help degradation processes within a timeframe of approx. 6-9 months for degradation of PE-PEO and PP-ST blends. Thus with special agents the timeframe could be controllable. Determination of PE and PE-ST composite degradation in marine environments and strawline of a marsh was simulated and physical parameters, such as weight loss, tensile properties, starch loss and carbonyl content were analyzed. Low degradation was observed in marine water for both control PE and PE-ST while deterioration could be observed in strawline of a marsh [118].

Another study comprises information about disintegration rates of low-density polyethylene (LD-PE), PS, 2% ethylene carbon monoxide (ECO) copolymer, 10% ECO copolymer, PE with vinyl ketone graft (PE-graft), and PE-ST blends in aqueous media. Comparisons were made to UV light no water control environments. Eco polymers disintegrated more rapidly than other films evaluated. The aqueous environment significantly delayed if not inhibited degradation [119]. Another study investigated hydrolysis of biodegradable polymers and bio-composites under 50°C and 90% relative humidity. With increasing time (0-30d) the mechanical properties significantly decreased because of easy hydrolysation of ester bonds. If hydrolysation inhibitor is present in the polymer, tensile strength and material properties increase compared to polymers without inhibitors [120].

2.6.2 Photo degradation and UV-Oxidation

Studies on photo degradation or photo-degraded polymers are presented on various polymers. Acceleration is generally possible with varying light intensity but the photodegraded polymers are just smaller particles which are still intact able to act as stressors in the environment. In general physical parameters are measured like oxygen and water uptake [121;122]. This is also one of the few studies available for the marine environment.

In order to investigate biological degradation or oxidation of polymers after photo degradation experimental methods were developed using degraded PS, PE and PP [122] and also for a photodegraded PS-vinyl-ketone copolymer [121]. The method of making these polymers degradable through UV light is assumed to make materials more susceptible to microbial attack and therefore even poly olefins biodegradable. The methods were tested with soil microbial communities and sewage sludge and carried out in respirometers. The procedure may easily adapted to aquatic systems as well. It was shown that generally the photodegraded polymers are better biodegradable than the undegraded ones. But still bio degradation takes very long especially for PS and PS-vinyl-ketone copolymer. Methods were enhanced using tracers to follow the biodegradation of PS polymers since the method was at first not precise enough to detect biodegradation with such slow process [121].

Ultraviolet (UV) oxidation is a destruction process that oxidizes organic contaminants in water. It works by the adding oxidizing agents such as ozone (O_3) or hydrogen peroxide (H_2O_2) to the contaminated water. The contaminated solution is passed through a chamber where it is exposed to intense UV radiation. UV radiation is provided by UV light bulbs and oxidation of target contaminants is caused by direct reaction with the oxidizers, and through the action of UV light in combination with ozone and/or hydrogen peroxide. A major success factor is how well UV light is transmitted to dissolved contaminants. High turbidity of the water or absorption of UV light in the desired wavelength may cause interference. The water should be relatively free of heavy metal ions and insoluble oil or grease to minimize the potential for fouling of the lights. This system does not destroy some volatile organics such as trichloro-ethane (TCE). Instead, the contaminants may be vaporized and would need to be treated in an off-gas system. Energy requirements are very high, which is the largest drawback to this technology. The UV/oxidation technology is a commercially available as a water treatment technology that has been used for more than 10 years. A majority of these applications are for groundwater contaminated with petroleum products or with a variety of industrial solvent-related organics such as trichloro ethane, dichloro ethane, and vinyl chloride.

UV treatment is used to destroy volatile organic carbon (VOCs) and explosive compounds such as TNT in aqueous systems. Typically, easily oxidized organic compounds, such as those with double bonds (e.g., trichloro ethane, perchloro ethane, and vinyl chloride), as well as simple aromatic compounds (e.g., toluene, benzene, xylene, and phenol) are rapidly destroyed in UV/oxidation processes.

Results of a study comprising butyl acrylat (BA) degradation in different aqueous media, such as riverine, distilled, artificial and natural sea water, show that degradation simulated with a 125W medium pressure mercury lamp, led to the formation of a polymeric product composed mostly of aliphatic chains. After hydrolysis of BA to butanol and acrylic acid, followed by decarboxylation of acrylic acid and finally recombination of formed

radicals a new polymer was formed mostly of aliphatic chains with only some ether-like bonds. It seems that marine organic substances and dissolved inorganic ions inhibit photo degradation compared to samples in riverine water. Photo catalyzed reactions in TiO_2 and ZnO suspensions lead to rapid disappearance of BA [123].

Representative samples of commercial photo degradable PE's were examined and an ethylene-carbon monoxide (E/CO) copolymer was photodegraded most rapidly but to biodegrade most slowly. An antioxidant iron dithiocrabamate photo degradable PE and a starch filled iron catalyzed PE showed a higher production in carboxylic acids during photo oxidation than E/CO and therefore resulting in rapid microbial growth. It was observed that microbial exo-enzymes are able to recognize relatively high molar mass carboxylic acids and remove them from the polymer surface in a way that water cannot remove the acids by leaching. Hence, the oxidation products of oxidized PE's do not present a threat to the environment. Abiotic iron-catalyzed photo- or thermo oxidation is the rate-limiting step in the process of bio assimilation. Abiotic degradation must therefore precede biotic degradation. The molecular weight is not the limiting factor. Products with a molecular weight of 40'000 $g \cdot mol^{-1}$ were degraded readily. Much more important is the nature of the photochemical or thermo chemical modification [124].

The thermal degradation of poly(EO-PO-EO) triblock copolymers was studied using size exclusion chromatography/MALDI-TOF-MS, size exclusion chromatography/NMR and size exclusion chromatography/GC-MS. The degradation was studied in air and it was observed to start after around 21 days in the PPO block forming volatile formate and starting the breakdown [125].

2.6.3 Fenton reaction

H.J.H Fenton discovered in 1894 that several metals have special oxygen transfer properties which improve the use of hydrogen peroxide. Some metals have a strong catalytic power to generate highly reactive hydroxyl radicals (·OH). From that day on, the iron catalyzed hydrogen peroxide oxidation process has been called Fenton's reaction. The reaction is used for example by wastewater treatment plants etc. to treat a large variety of water pollutants such as phenols, formaldehyde, BTEX, pesticides, rubber chemicals and many more. The Fenton's Reaction is currently one of the most powerful oxidizing reactions available [126]. Applications of the Fenton's reaction are:

- WW treatment, contaminated soils and sludge
- Organic pollutant destruction
- Toxicity reduction
- Biodegradability improvement
- BOD/COD removal
- Odor and color removal
- Destruction of resin in radioactive contaminated sludge

The peroxide is broken down into a hydroxide ion and a hydroxyl free radical. The hydroxyl free radical is the primary oxidizing species and can be used to oxidize and break apart organic molecules. One primary advantage of the Fenton's Reaction is that it does not produce further organic compounds or inorganic solids such as permanganate and dichromate, since there is no carbon in the peroxide. This makes the Fenton's Reaction more appealing than a biological process, if the goal is removal of organic compounds. However, there are organic species that show resistance to oxidation by the Fenton's Reaction. Small chlorinated alkanes, n-paraffins, and short-chain carboxylic acids, compounds that are typical oxidation products of larger molecules seem to resist further fragmentation by the Fenton's Reaction.

$$Fe^{2+} + H_2O_2 \Longrightarrow Fe^{3+} + \dot{O}H + OH^-$$

Equation 4 - Fenton's Reaction - generation of radical species I

$$Fe^{3+} + H_2O_2 \Longrightarrow Fe^{2+} + \dot{O}OH + H^+$$

Equation 5 - Fenton's Reaction - generation of radical species II

The mechanism of reaction with respect to hydrogen peroxide as described above (Equation 4 and Equation 5) is very complex and may change with conditions of the reaction. Generally, though, the reaction follows a mechanism similar to the one listed below, (Equation 6 and Equation 7). It is still in debate whether the reactive species is a hydroxyl or ferryl radical:

$$RH + \dot{O}H \Longrightarrow \dot{R} + H_2O$$

Equation 6 - Fenton's Reaction - generation of radical species III

$$\dot{R} + Fe^{3+} \Longrightarrow R^+ + Fe^{2+}$$

Equation 7 - Fenton's Reaction - generation of radical species IV

If the reaction is carried to completion, then ultimately the organic molecules break down into CO_2 and water, which are the normal end products of a combustion reaction. Also similar to a regular combustion reaction, organic destruction by the Fenton's Reagent is highly exothermic. Unlike combustion, Fenton's Reaction is associated with foaming, often very heavy and thick in the early parts of the reaction, especially for large compounds with high amounts of carbon.

$$\cdot OH + C_6H_6 \Longrightarrow (OH)C_6H_6$$

Equation 8 - Fenton's Reaction (addition)

$$OH + CH_3OH \Longrightarrow CH_2OH + H_2O$$

Equation 9 - Fenton's Reaction (hydrogen abstraction)

$$\cdot OH + [Fe(CN)_6]_4^- \Longrightarrow [Fe(CN)_6]_3^- + OH^-$$

Equation 10 - Fenton's Reaction (electron transfer)

$$\cdot OH + \cdot OH \Longrightarrow H_2O_2$$

Equation 11 - Fenton's Reaction (radical interaction)

It is required to adjust the pH to 1-5: if the pH is too high the iron will precipitate as $Fe(OH)_3$ and will reduce H_2O_2 to oxygen [127;128]. Nevertheless, it was shown that significant photo-Fenton reaction may also take

place in weakly acidic aqueous environmental compartments containing fulvic acids. Fenton reaction in natural waters may have been underestimated since it may take over a few hours until reaction starts [129].

Oxidative biodegradability of PEU and PCU was investigated in an accelerated *In Vitro* system using 20% H_2O_2 and 0.1M Cobalt chloride solution. It could be observed that changes were consistent with those seen in long term *In Vivo* studies. Overall PCU was less degraded and the degraded surface layer of the films was thinner than observed in PEU. Also the *In Vitro* system could be inhibited adding an antioxidant to the PU film. The accelerated method was found suitable to simulate real environmental oxidation within shorter time [130].

Cellulose and hemicelluloses substrates were degraded using a chelator-mediated Fenton reaction. The Catechol chelator-mediated reaction clearly degraded hemicelluloses substrates significantly faster and more effective while hydroxyamate chelator-mediated reactions showed inhibitory effects. For cellulosic substrates no accelerated degradation process was observed for both chelator-mediated reactions. But it was observed that chelator-mediated Fenton reaction of cellulose proceeded depolymerization [131].

Recently the photo-fenton degradation was reported for water soluble polymers such as PEG, PVP and PAM in WW treatment [132]. It was demonstrated that it is technically feasible to photo degrade the polymers and use the treatment for industrial water cleaning. It was observed that Fe(II) had a negative effect on the treatment though.

2.6.4 Ultrasound degradation

The degradation of polymers using ultrasound was investigated for PEG after it was found that no studies were reported on well defined link-functionalized polymers. It has been demonstrated that cleavage of a polymer chain occurs preferentially at weak bonds incorporated in the chain and that mechanically induced cleavage can be localized almost exclusively to a single to a single weak site [133].

2.7 Effects of biomass on biodegradation

Knowing the ecological behaviour and impact of chemical substances is a main responsibility of the manufacturing chemical industry. Substances may pass accidentally or being applied intentional to the environment and a critical risk assessment is required to ensure that they do not constitute any unknown threat to the environment. This process is based on a highly complex system to provide all relevant data and to ensure chemical safety. The REACH legislation [134-136] regulates and guides the step-by-step approach in order to establish valuable and reliable data on the behaviour and ecological impact of chemicals, active substances and others.

In this context knowledge on the biodegradability is one of the most important aspects to assess environmental behaviour because a biodegradable substance is expected to cause less ecological problems [137]. For regulation of chemicals in the European Union generally two types of biodegradation studies are used today. Short-term laboratory tests such as the OECD 301 studies [138] and long-term simulation tests such as OECD 307 and 308 [138].

For the majority of chemicals short-term biodegradation tests in the aerobic aquatic environment have been conducted [139-141]. The biodegradation of surfactants was investigated in more detail because personal care products have an increased potential to enter the environment [19;32;33;36;42;142-146]. Generally, the methods for measuring biodegradability can be divided into two principal groups: direct measurement of parent compound concentrations and indirect measurement of parent compound bioconversion, such as carbon dioxide production, decrease in dissolved organic carbon (DOC), cumulative oxygen consumption (biochemical oxygen demand (BOD)), and decrease in chemical oxygen demand (COD). The most important and most frequently used tests are those for ready biodegradability [138]. These are the most stringent tests, offering only limited opportunities for biodegradation and acclimatization of the inoculum.

Long-term biodegradation simulation tests were used more regularly in crop protection and veterinary medicine (active ingredients) biodegradation assessments since these products are often intentionally released into the environment [147-149]. These tests are quite complex and difficult and require experience, knowledge and time and are also expensive. On the other hand they have a fairly higher predictive value for the real environment in contrast to standard biodegradation tests. These tests might in future become also much more relevant for other areas than the evaluation of crop protection or pharmaceutical products.

For the evaluation of chemicals it would be desirable to develop a fast standardized test method with a higher predictability for the situation in the environment than the tests on ready biodegradability, which reflect mainly the situation in the waste water treatment plant. This standard method should be less time and cost consuming than the available simulation tests. As a first step for such a development we investigated the effect of varying biomass concentration in standard short-term tests.

Such an approach has not been reported very often [150]. In the environment, the biomass concentration varies dependent on the geographic position, the medium, the season and other influences. This leads to the question how the biomass concentration influences the biodegradation and if the relationship is linear or characterized by threshold levels.

2.8 Biofilms

Biofilms (BFs) are accepted to play an important role in biodegradation and their activity has been investigated [151]. In nature the existence of biofilms is believed to be the bulk of bacterial biomass as an adherent community. The construction of biofilms seems to follow many pathways and a cell-to-cell communication known as quorum sensing may play a key role in the development [152].

The deterioration of polymer surfaces is an interfacial process controlled by the conditions prevailing directly at the surface. Microorganisms present, attach themselves to and colonize the surface in the form of biofilms. Some features are displayed in biofilms that cannot be found in microorganisms in suspended forms. In biofilms, the cells are embedded in a polymeric matrix of their own origin, mainly consisting of polysaccharides and proteins. They also contain populations of bacteria, fungi, protozoa and in some cases, if the conditions allow, they host even higher microorganisms such as nematodes and larvae. Because of its unique and complex

form of microbial life, the cells incorporated in biofilms can tolerate much higher concentration of biocides than in suspension. Also biofilms can be very heterogenic environments. Because of the oxygen consumption of microorganisms, anaerobic/anoxic zones can form within a biofilm forming habitats for anaerobe microorganisms which could not proliferate in the aerobic conditions in the water phase [153].

The requirements for biofilm formation are quite simple: surface, humidity, nutrients and microorganisms. As microorganisms are ubiquitous most of them live in immobilized form. Biofilms represent one of the oldest forms of community based life on this planet. From an ecological point of view, biofilms offer an important advantage as a form of life to the cells. First, the possibility of forming stable micro consortia, second, the facilitated exchange of genetic material, third, the accumulation of nutrients from the bulk water phase, fourth, the protection against toxic substances and fifth, protection against desiccation are just a few examples [153].

A biofilm is composed mainly of water (80-95%), extracellular polymer substances contributing of about 85-98% of the organic matter, than the microorganisms, entrapped organic and inorganic particles (e.g. humic substances, debris, clay and silica minerals, gypsum, etc.), substances sorbed to extracellular polymer substances, cells or particles and substances dissolved in the interstitial water [153]. Biofilms can result in different mechanisms of polymer deterioration as shown in Figure 4.

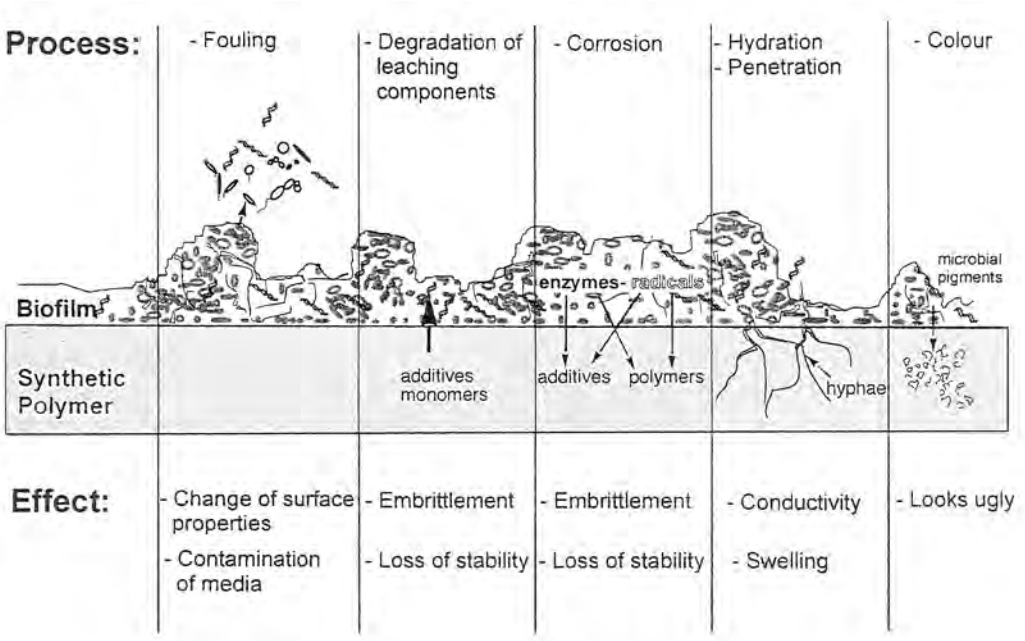


Figure 4 - Processes and effects of biofilms on polymer surfaces

The first one is known as “fouling”. This is referred to as the change of surface properties and contamination of the surrounding media by biofilm formation on polymer surfaces. The second mechanism is the “DEG of leaching components” such as additives and monomers. In this case microorganisms do not necessarily have to penetrate the polymer surface. Components may leach out, providing a food source. This form of degradation results in embrittlement and loss of stability. The third mechanism can be described as “corrosion”. Again it results in embrittlement and loss of stability of the polymer. It is an interfacial process depending strongly on the parameters of the microenvironment. In contrast, the microorganisms penetrated the surface and make

use of their enzymes to release additives and polymers/monomers for use as nutrients. The fourth mechanism is “hydrolysis and penetration”. Because biofilms consist of over 80% water they provide an ideal electrolyte that increases surface conductivity and also swelling. The fifth mechanism, however not resulting in degradation, but rather in a change of looks is that some microorganisms produce pigments. Some are lipophilic and tend to diffuse into the polymers giving a strange coloration [153].

2.9 Degradation of polymers in compost and soil

2.9.1 Biodegradability and compostability in the context of the European packaging regulation

The European Packaging Directive 94/62/EC aims to harmonize national measures concerning the management of packaging and packaging wastes in order to prevent or reduce any impact on the environment. First priority is given to reduce packaging waste production, then to re-use, recycling and recovery and the reduction of the final disposal of such a waste [154]. The European Packaging Directive 94/62/EC (December 31, 1994) specifies composting as a form of recycling for packaging wastes. CEN TC261 SC4 (European Normalization Committee) was given mandate to regulate and develop norms and criteria in testing materials for compostability. Regarding the acceptance for composting and organic recovery, basically three criteria are required; first biodegradation of the specific material, second disintegration and third, no influence on the quality of the compost [88].

A standard composting test based on the determination of biodegradability under dry, aerobic conditions via measurement of evolved CO_2 is described by many methods (ISO 14855 [155], CEN TC 261/SC5/WG2, ASTM D5338-92 [156], OECD 304 [138], [88]). Relevant organic constituents with more than 1% of the dry weight have to be investigated for biodegradation. In the appropriate laboratory scale test not only bacteria, but also fungi, moulds and actinomycetes are available as active microorganisms [154]. The test was run in a ring-test and standardized and described [157-159]. To be accepted in composting plants, biodegradability of plastics alone is not sufficient. Also disintegration, as the physical falling apart into fine, visually indistinguishable fragments at the end of a typical composting cycle is an important factor. It is measured in a pilot- or full-scale composting test with sieving the compost at the end of exposure and precise sorting analysis [88].

2.9.2 Composting tests & degradation in soil

Until the 1980s composting was not used very much as a solid waste treatment (4% for Europe and less in the USA). The poor quality of the compost with high heavy metal content and other visual contaminants was the reason. In the late 1980s the idea of introducing source-separated waste collection led to high quality compost mainly in German speaking and Benelux-countries. In the Netherlands composting was required by law, from 1994 onwards. The composting techniques have had a rather fast evolution since then. The first composting units were only run as open units with little control over the process. Nowadays, mostly in-vessel composting with control over moisture, pH, aeration, retention time, temperature and other factors is done [88].

In compost and soil environments the most important factor concerning biodegradability is aerobic biodegradation by bacteria and fungi. For standardized composting tests, the inoculum is generally finished compost from composting plant [87]. But it has also been demonstrated, that the degradation environment plays an important role. Therefore several degradable polymers were tested in lab-scale composting tests, exposure in thermal-hydrolytic environment using water at 60°C and exposure in thermal-oxidative dry oven environment at 60°C. It was found that next to chemical and biological transformation, physical effects such as restructuring or reorganization of the macromolecules may happen at typical compost environmental temperatures. It was observed that PE-based films behaved all similar but different to all other polymers tested. PE-based polymers should degrade in thermal-oxidative environment but when applied in lab-scale compost tests with sufficient oxygen available and at high temperature no biodegradation was observed within the test duration [160].

For determining whether mineralization has occurred, the summary parameters dissolved organic carbon, biological oxygen demand or CO_2 evolution is measured. In dissolved organic carbon measurement, the initial concentration is compared with the final concentration. In the case of biological oxygen demand or CO_2 evolution the values acquired throughout the test are accumulated and compared with the respective theoretical data calculated from the chemical formula. Dissolved organic carbon measurement is not applicable if water insoluble polymers are to be tested; only biological oxygen demand and CO_2 evolution can be measured. For investigation of primary degradation, additional analytical tests such as gel permeation chromatography (GPC) and/or MALDI-TOF are necessary as plausibility check [87].

In the ISO 14855 (also European Standard Draft WI 261 085), a terrestrial test system, both inoculum and test conditions provide good conditions for polymer degradation. The purpose of the test is to demonstrate complete biodegradation and differentiate the latter from abiotic deterioration. In this test it is mandatory that the substance must be degraded to the maximum degree achievable (duration of 180 d), which cannot be compared to conventional treatment of biowaste by composting procedures, for the European Standard On Requirements (European Standard Draft WI 261 236 1998) clearly states that degradation of test materials may be completed when using compost in the soil. The test period of 180 days opposes the mean residence time of the test materials in bio waste treatment plants (BCTP) of approx. 42 days (6 weeks). If the test duration is divided by the residence time, one obtains a value between 1 and 4.3. If the maximum test period (180 d) was applied, than it is about 4 times longer than the mean residence time in conventional bio waste treatment. In comparison, aquatic batch tests acquired in 28 d compared to conventional WWTP dwell times of 12 h for biological sewage treatment, give a quotient of 56. The test is 56 times longer than conventional treatment because of the necessity to give microorganisms a sufficient time to adapt [87]. This phenomenon can then be seen in the final graph describing the degradation process. The lag-phase is usually very clear and indicates the adaptation time. The prolonging of the composting test is due to the fact that the ripened compost used might have a lesser potential to degrade the test compounds than the fresh compost formed in conventional treatment. Also the load factor of about 6 (6:1 w/w, compost: test material) in the test system requires lengthening of the test duration. In conventional biowaste treatment plants the load is certainly substantially smaller (higher load factor). In Aquatic tests the load factor can be found between 1.25 and 2.

Tests of many different polymers (PE, PVC & PP and others), were tested with different methods in soil and traditional methods were compared to new methods [161-163]. The Polymers were tested with and without photo exposure (using ASTM D1436-75) prior to degradation tests. Methods used for biodegradation determination were the bio meter technique, measuring the conversion of the plastic material to CO_2, residual weight change measuring after solvent extraction or tedious and less effective manual retrieval, and traditional tensile strength measurement to connect values to previous literature. Also GC/MS and GPC experiments were carried out for supplemental information such as plasticizer degradation and molecular weight distribution. The soil for incubation was composed of 50% sand, 21% silt and 29% clay in terms of texture. Organic matter content was 5% and the pH was between 5.5 and 6.5 [161]. The moisture content was adjusted to 50%, which is considered ideal for aerobic biodegradation. The pH was adjusted to 7.5, which is considered the near optimum for hydrocarbon degradation, with $CaCO_3$ five days prior to the experiment [164].

The cumulative CO_2 evolution background from the soil only was typically determined at 4µmol during the 160 day test period. The PE samples evolved between 500 and 860µmol CO_2 with a lack in correlation between photo exposure and CO_2 evolution. The PE sample with 7.7% starch evolved around 2000µmol CO_2 and the PVC sample around 2500µmol CO_2. It is possible that the CO_2 evolved during the 160 days might originate from the additives, colorants or other substances, rather than the polymer resin [161].

GPC measurements show that photo exposure had in general little effect on M_w but some effect on M_n. Only one PE sample showed a decrease in M_w as well as M_n to 58 and 48% and additional degradation in soil to 37 and 29% of the start value. The study shows only a limited correlation of used techniques. CO_2 evolution does not necessarily correlate with molecular weight decrease and tensile strength or elongation measurements. This complicates the prediction of biodegradation of especially complex polymers. Especially residual weight determination seems to be the most unsuited method for determining degradation especially for starch containing polymers [161].

Different copolyesters with aromatic constituents were synthesized and characterized and afterwards used in biodegradation tests with compost eluate and also in soil burial tests. The polymers were synthesized with dimethyl terephthalate and different diols (1,2-ethanediol, 1,3-propanediol, 1,4-butanediol) in certain ratios (Table 4) [95].

Table 4 - Educt composition for the synthesis of model oligoesters

Oligomer	Dimethyl terephthalate [mol·g^{-1}]	Diol [mol·g^{-1}]
OliET	0.161/31.266	1,2-Ethanediol, 0 322/19.996
OliPT1	0.131/25.440	1,3-Propanediol, 0.157/11.948
OliPT2	0.131/25.440	1,3-Propanediol, 0.197/14.992
OliPT3	0.131/25.440	1,3-Propanediol, 0.1262/19.938
OliPT	0.131/25.440	1,3-Propanediol, 0.262/19.938
OliBT	0.111/21.556	1,4-Butanediol, 0.222/20.002

The results from the performed Sturm-test with a compost eluate as inoculum source show that the used all ester-type oligomers and polymers synthesized from aliphatic diols and/or aromatic dicarboxylic acids are biodegradable. The rate of biodegradation is dependent on the chain length of the aliphatic constituents and the degree of polymerization as can be seen in Table 5 [95].

Table 5 - Carbon Balance of oligomer degradation in Sturm-test and size exclusion chromatography results

			Carbon [% of theoretical value]			
Sample	SEC [% degradation]	CO_2 [%, excluding COD]	biomass	Soluble components	Residual polyester	Σ
OliET	61.3	54.6	3.8	2.8	39.0	100.2
OliPT1	13.2	10.2	<0.1	0	87.1	97.3
OliPT2	19.1	16.3	<0.1	2.5	78.8	97.4
OliPT3	26.3	23.2	<0.1	4.0	74.9	102.2
OliBT	61.3	53.1	2.5	3.4	38.8	97.8
PHBV	--	54.5	46 6	3.8	0	104.9

The results clearly show that monomers as well as dimers were unambiguously degraded and mineralized by microorganisms and not just dissolved into the test medium. Of basic importance is the finding that biodegradation decreases drastically with increasing chain length of the aromatic ester oligomers. In this regard, bioavailability is dependent only on solubility. Oligomer solubility is dependent mainly on the number of RU's and to a lesser extent to the chemical structure which explains why no significant differences were found in the solubility of oligomers with different diol components. From the results of this it seems possible to be able to estimate the fraction of intermediate residues in copolyesters containing terephthalic acid. Block polyesters, having been shown to be degradable by microorganisms, will exhibit a high amount of slowly degradable residues, because generally the length of the distinct blocks exceeds two repeating units [95].

The microbial susceptibility of high molar mass copolyesters of terephthalic acid and different co-monomers has been investigated in parallel dependence on their composition. It has been shown that the optimal copolymer composition of terephthalic acid with aliphatic co-monomers with regard to biodegradation and melting point is in the range of ~40 to 50mol% of terephthalic acid. These copolymers have melting points above 90°C when the fraction of terephthalic acid is higher than 40mol% of the acid content. Even polymers with melting points above 140°C show significant weight loss in composting tests within 12 weeks. At higher temperatures biological attack is advantageously accompanied and supported by chemical hydrolysis enhancing biodegradation of the copolyesters. Degradation at room temperature is caused only by biological attack. Significant hydrolysis was not observed under sterile test conditions or even in soil burial tests [96].

Soil biodegradation was tested with tensile test pieces of P(3HB) and a copolymer P(HB-co-HV) (90%3HB, 10% 3HV). The test pieces were incubated at constant temperatures of 15, 28 or 40°C for up to 200 days. Hydrolytic degradation was compared at a range from 4 to 55°C in sterile buffer solution for 98 days. The degradation was

determined observing weight loss/surface erosion, molecular weight distribution and mechanical strength. In sterile buffer, no weight loss was determined, while in soil incubated tests degradation rates from 0.03 to 0.64% weight loss per day was observed depending on polymer, temperature and soil. The copolymer was degraded more easily and higher temperatures resulted in higher degradation rates. Molecular weights in soil and sterile buffer decreased at the same rate at 40°C incubation temperature but remained almost unaffected at lower temperature. This indicates that molecular weight decrease is due to simple hydrolysis but not to biodegradation by microorganisms. In general, degradation resulted in loss of mechanical properties. 295 microorganism strains capable of degrading P(3HB) and P(HB-co-HV) were isolated and identified. most were Gram-negative bacteria (*Acidovorax* and *Variovorax* sp.), some *Bacillus* strains, *Streptomyces* strains and moulds mainly belonging to *Aspergillus* sp. and *Penicillium* sp [165].

P(HB-co-HV) films were investigated in soil biodegradation tests in order to study the contribution of fungal microorganisms to the process of degradation. The various grooves, traces and cavities observed on the dented surface demonstrated that the degradation was a concerted effect of microorganisms colonizing the surface including fungi, bacteria and A*ctinomycetes*. During exposure, a succession of microbial consortia with a distinctive increase in fungal population was observed in the soil around the polymer. At the end, fungal strains were the dominant form of microorganisms in the population. The comparison of degradation ability of microbial strains showed that fungi contributed highest to the process [166].

Often, the biodegradation of substances depends on the conditions applied to the inoculum during sampling, storage and preparation. It was described that polymer biodegradation with compost stored for different periods and at different temperature gives independent biodegradation results for PCL and PBS polymers but same results for cellulose. It was also shown that different Microorganisms are present depending on the storage temperature. It is therefore suggested, that easily biodegradable reference substances may not always be suitable as basis for validity criteria but more precise standards on the preparation of the inoculum could guarantee more reliable results [167].

2.10 Degradation of plastics in marine environments

Pseudoalteromonas sp. NRRL B-30083 was isolated as predominant PHBV degrading bacterium from a tropical marine environment. PHBV samples were prepared in culture. The activity of the bacterium in the presence of PHBV was very low although the strain produced distinct zones of clearing on solid medium [103].

A degrading marine microorganism, *Streptomyces* sp. SNG9, was used in the investigation of PHB and PHBV degradation. The microorganism grew efficiently in a simple mineral liquid medium with PHB as the sole source of carbon. The cells excreted depolymerized and degraded the polymer to complete clarity within 4 days. Natural PHB and PHBV degradation was observed by SEM. Additional carbon sources easily accessible, inhibits degradation of the polymer. SEM showed that degradation could be detected at the surface of the polymers used. The generally smother surface of un-inoculated samples becomes irregular with lots of erosion pits during the experiment [102].

Two deep-sea isolates *Aspergillus ustus* (fungus) and *Rhodosporidium sphaerocarpum* (yeast) and for comparison two marine surface yeasts (*Candida guilliermondii* & *Debaryomyces hansenii*) and one terrestrial isolate of *Aspergillus ustus* were investigated. Growth, physiological parameters and PHB degradation were followed after incubation in high-pressure autoclaves in artificial sea water at 27°C and pressures of 0.1, 5, 10, 20, 30, 45 or 50 and 100 MPa (~ 10000m water depth) for 21 days (yeasts) and 28 days (filamentous fungi). Independently of the origin of the isolates, growth decreased with higher pressure with a limit between 30 and 50 MPa. Metabolic activity started to decline from 20 MPa, ceasing at growth limit pressures. Under atmospheric pressure PHB degradation was observed for all strains in soil. In liquid media the rate was lower and decreasing further with rising hydrostatic pressure. Beyond 30 MPa no PHB degradation could be observed [8].

The biodegradability of P(HB-co-HV) blended with starch in the presence or absence of PEO as enhancer was investigated in marine coastal waters southwest of Puerto Rico for a time of about one year. Two sample points were within a mangrove stand, two others off-shore; one of these on a shallow shoulder of a reef, and the other at a location in deeper water. Considerable flux in microorganism population was detected throughout the year and in general, the population densities at the deep-water station was one order of magnitude less than at the other three. Starch-degraders were 10-50 fold more prevalent than P(HB-co-HV) degraders. The formulations contained 30 to 50% starch and the degradation according to weight loss was as expected shortest for pure starch (~2% weight loss per day) and longest for pure P(HB-co-HV) (0.1% weight loss per day). PEO incorporation slightly retarded the biodegradation process. Consistent with the relatively low microbial population at the deep-water station, biodegradation exhibited an initial lag period but the degradation rates later on where comparable to the ones obtained from other stations. Presumably, significant biodegradation occurred only after colonization of the plastic, which is dependent on the resident microbial population and it can be inferred, that the extended degradation lag-periods would occur even more in open ocean waters where microorganisms are sparsely populated [168].

A marine bacterium (strain NK-1, JCM10458) was isolated from the Pacific Ocean deep-sea floor (1165m) in Japan. It was classified to belong to the *Marinobacter* sp. and as a motile, Gram-negative, aerobic, rod-shaped bacterium. It was then cultivated in NaCl containing media with P(3HB) as the sole source of carbon. The excreted extracellular enzyme, P(3HB)-depolymerase, was purified and analyzed. The enzyme showed stability below 37°C at pH 7.5-10.0 and a molar mass of 70kDa. The N-terminal amino acid sequence showed similarities to N-terminal and internal sequences of *Pseudomonas stutzeri* depolymerase. Enzymatic P(3HB) degradation yielded products such as monomer, dimer and trimer molecules of 3-HB. The isolated depolymerase was also capable of hydrolyzing P(3-HP) and P(4-HB) [169].

2.11 General degradation of different polymers

The relatively newly researched group of biodegradable polymers as previously shown in

Table 1 includes many different substances ranging from starch-, sugar cane- [170], lactic acid-, PHA-, lignin-, aliphatic- or aromatic- based polymers. Mainly polyesters play a major role with respect to industrial relevance. In the 1970s production and processing of PHB as a biodegradable plastic material was developed. The group of

PHA-polyesters is produced and intracellular accumulated by various microorganisms. *BIOPOL*® was the first commercially available biodegradable polyester. Probably because of its high price the production was stopped in the 1990s after the production rights were sold from *IMPERIAL CHEMICAL INC. (ICI)* to *ZENECA* and later *MONSANTO* [94;106]. Beside the natural PHA polyesters, a number of synthetic aliphatic polyesters have been developed and shown to be also enzymatically degradable [96]. The most important one currently used is poly(ε-caprolactone) (PCL), predominantly used in starch blends [73]. Its problem is only the low melting temperature of about 60°C which excludes it for many applications [106].

On the opposite of many aliphatic types of polyester, aromatic polyesters such as PET or PBT provide excellent material properties and are used very widely because of the low prices and high applicability. Until today these polymers are considered resistant against microbial attack [106]. The task of combining both, excellent material properties with superb biodegradability, and combinations of aliphatic and aromatic polyesters were investigated closely. The combination of 1,4-butanediol, adipic acid and terephthalic acid turned out to be the most promising one, especially with regard to the price [96].

2.12 Common plastics

2.12.1 Polyolefins and Oxo-biodegradable polymers

Polyolefins are widely used and it was reported that they accumulate in the environment at a rate of 25 million tons per year [163]. The initial step in microbial oxidation of n-alkanes is known to be hydroxylation, resulting in corresponding primary or secondary alcohols, which can further be oxidized to aldehydes or ketones followed by the acid. Carboxylated n-alkanes are considered to be degradable following an analogues procedure observed in fatty acids, the β-oxidation. This results in depolymerized substances by two C-atoms in turn. Neither any evidence of cleavage at the centre of a carbon chain nor cleavage of long carbon-chain acids has been reported so far [171]. The degradation of commercially available PE-wax used as the sole source of carbon for soil microorganisms shows that mostly small molecules were assimilated and metabolized faster than larger ones in the process. It might be possible that microorganisms absorbed molecules up to approx. 100 $g \cdot mol^{-1}$, which results in the removal of the molecules from the culture supernatant. Based on this theory, connecting weight loss to β-oxidation and uptake of smaller molecules into microorganisms cells, a mathematical model has been proposed [171]. The microorganisms were grown on PE-wax with molecular mass from 100-10'000 $g \cdot mol^{-1}$ for three weeks. Then, the Polymers were removed and submitted to GPC analysis. The weight loss was around 31%. The M_w and M_n values were observed to drift to a higher value suggesting that low molecular weight fractions were consumed faster [171].

As PE is classified as hydrocarbon, it can be subjected to the following metabolic pathways (Equation 12, Equation 13 and Equation 14):

$$RCH_3 \Rightarrow RCH_2OH \Rightarrow RCHO \Rightarrow RCOOH$$

Equation 12 - Metabolic pathway for hydrocarbons (terminal oxidation)

$$H_3CRCH_3 \Rightarrow\Rightarrow\Rightarrow CH_3RCOOH \Rightarrow HOH_2CRCOOH \Rightarrow OHCRCOOH \Rightarrow HOOCRCOOH$$

Equation 13 - Metabolic pathway for hydrocarbons (di-terminal oxidation)

$$RCH_2CH_2CH_3 \Rightarrow RCH_2CH(OH)CH_3 \Rightarrow RCH_2C(O)CH_3 \Rightarrow RCH_2OC(O)CH_3 \Rightarrow RCH_2OH + CH_3COOH$$

Equation 14 - Metabolic pathway for hydrocarbons (sub-terminal oxidation)

A Molecule submitted to one of these reactions becomes carboxylated sooner or later and can be subject to a β-oxidation (Figure 5). A series of weight losses due to terminal separation of two carbon-atoms at each step should follow. Short n-alkanoic acids may be absorbed into cells [171].

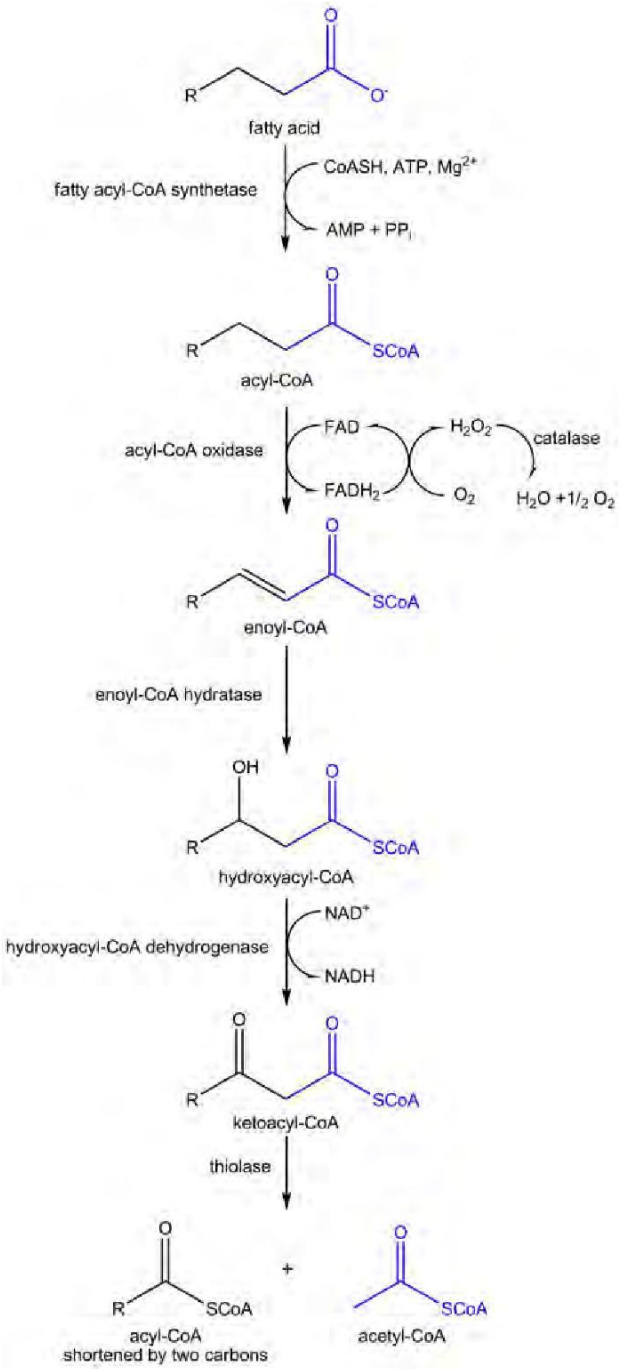


Figure 5 - Fatty acid oxidation

An investigation of ethylene-propylene copolymers, LDPE and isotactic-PP films (100µm) after UV irradiation in an accelerated weathering chamber (60°C, 4x400W medium-pressure mercury vapor lamps, radiation >290nm) was carried [172]. They describe degradation tests in compost and culture while measuring viscosity and the photo oxidation via FT-IR. Degradation increased with increasing irradiation time (0, 50, 100h). After 6 months of exposure to the medium, LDPE was the slowest sample to be degraded (22%). Viscosity showed a decrease during the exposure as well as with increasing irradiation time. FTIR spectra show an increase in hydroxyl as well as carbonyl groups with rising irradiation time and SEM photographs show also more surface deformation in irradiated samples [172].

The ability of microorganisms to attack PE or modified PE films was investigated for Streptomyces sp. And Actinomycetes sp. Showing that extracellular enzymes were detected that seem able to attack PE polymers [163;171;173]. LDPE modified with starch was tested for biodegradation in soil microcosms for 6 months. It was shown, that inoculation of soil with *P. chrysosporium* enhanced the degradation and biomass increased much more than in non-inoculated soil [163]. For the incubation of 10-day-heat-treatet starch-PE degradable plastic films, incubated for 3 weeks at 37°C, extracellular culture concentrates of *Streptomyces viridosporus* T7A, *Streptomyces badius* 252 and *Streptomyces setonii* 75Vi2 were prepared. Degradation by active enzymes was observed through changes in FT-IR spectra, mechanical properties and PE-MWD. The study confirms the change in the polymer structure measured via GPC and FT-IR as well as material properties, after degradation by the active enzymes [173].

Three poly ethylene samples (HDPE +3% additive, LLDPE +7% additive and HDPE +3% additive) were tested in different biodegradation processes. Hydrolytic exposure for up to 60 says showed only up to 3% weight loss for the first polymer. The other results are negligible. The oxidative exposure resulted in an 8% weight loss for LLDPE and 1.8% and 1.5% for HDPE samples respectively. A composting exposure [174] did not show any weight loss for all three samples but a loss in tensile strength was detected of about 18-20%. A microbial degradation test showed no results for all three samples [175].

The biodegradability of poly(ethylene wax) was studied comparing degraders of two different classes, bacteria and fungi. GPC patterns were obtained before and after a consortium of KH-12 and a fungus, *Aspergillus* sp. AK-3 were incubated in culture media containing PE-wax as their sole carbon source for three weeks. The total consumption rates and the β-oxidation rate for both degraders were determined numerically and the degradability was compared. The qualitative features as degraders are considered quite similar as far as the mechanism of biodegradation is concerned. Regardless of the class, PE biodegradation is based on two factors: the gradual weight loss of large molecules due to β-oxidation and the direct consumption or absorption of small molecules by cells. However it is demonstrated that there is a significant difference between degradation rates of the two consortia. Particularly, the β-oxidation rate for KH-12 is more than 36 times as large as that for *Aspergillus* sp. AK-3. Moreover the degradation by the consortium persists even for the molecular weight of nearly 5000 $g \cdot mol^{-1}$ while the degradation for the fungus disappears when the molecular weight reaches approx. 1600 $g \cdot mol^{-1}$. The limit in size for direct consumption by cells was estimated as approx. 1500 $g \cdot mol^{-1}$. Different PE-wax kinds were used for experiments but it seems incorrect that different molecular weight will affect biode-

gradability. Only lower molecular weights will lead to better biodegradation but experimental results suggested rather different biodegradation rates for different classes of microorganisms. Differences in biodegradability might be due to permeability differences in cell membrane structures because β-oxidation is associated with cell membranes. And also Gram-negative bacteria are thought to have more affinity to PE-wax than for example cell walls of fungi. This is an example for mathematical simulation of exogenous biodegradation processes where a molecule is truncated at its terminal end by a certain chain section [171]. A mathematical model for endogenous cleavage (poly(vinyl alcohol)) was performed, showing that both types of degradation can be analyzed and characterized using experimental data and mathematical modelling [176].

Biodegradation of polyethylene using oxo-additives has been discussed in controversy. Most investigations focus on degradation/deterioration of the polymers. Methods used for analysis are mostly not suitable to determine biodegradation by microorganisms. It is often discussed that biofilm forms on the surface of oxo-degraded PE films and from that and the observed shifts in molecular weight or physical strength it is concluded that biodegradation occurs. Biofilm formation does not necessary mean that the material is biodegraded. In the first instance it only proves that the surface features biofilm formation, it does not have to be biodegradable itself. Most existing microorganisms live in biofilms and often the biofilm does not degrade the material on which it is hosted. It could be possible that the oxidation leads to a more brittle and fissured surface that in comparison to standard PE films offers better properties for the formation of biofilms. Oxo-biodegradable PE [81;86] are polyolefin polymers that have predetermined breaking points inserted by carbonyl groups into the polymer backbone. This feature enhances deterioration or degradation (abiotic) of the polymer chain under irradiation or hydrolytic breakdown or such. This leads only to fragments of the original polymer rather than real biodegradation. Up to now, no scientific evidence has been published that these fragments later biodegrade by mineralization of microorganisms and lead to the production of carbon dioxide, water and biomass. All tests that were published up to now focus on methods that are only suitable to detect fragmentation (molecular weight loss, physical properties etc.) but not biodegradation by microorganisms of the "short" polyolefin fragments. Nevertheless these test results on abiotic degradation introduced oxo-PE's into the field of degradable polymers [177]. It is important to remember though, that these substances only break down into small particles but do not completely mineralize in the environment some parts only may be degraded. The oxidative degradation under accelerated test conditions is easily performed but lack environmental relevance. The only information that was published on oxo-PE (bio-) degradation in aqueous media is that these so called "oxo-biodegradables" of thermally degraded full carbon backbone polyolefins were observed in standard closed bottle tests for 100 days in river water. Mineralization was observed in terms of CO_2 evolution within 100 days of incubation in river water [15;177].

Often tests are carried out to rather specific norms or guidelines (British ON S 2200 ON S 2300, ASTM etc.). Often these guidelines describe the experimental design but have no accurate pass/fail criteria. Test results are not comparable to others without such. On the other hand, it is beneficial that these guidelines can better distinguish between different environmental compartments (e.g. there are many ASTM test designs for different situations and environments and only few European/OECD guidelines for composting, biodegradation in soil and WWTPs).

The standard guideline ASTM D6954-04 is often referred to in the context of biodegradation results of oxo-degradable polymers. The guideline recommends a tiered approach to test materials exposed to a combination of oxidation and biodegradation. Tier 1 focuses on physico-chemical parameters during oxidation and degradation of the material. Tier 2 measures biodegradation and tier 3 assesses the ecological impact.

- Tier 1 requires the material to be tested at temperatures, oxygen content and humidity comparable to the environment on which the statement shall focus (meaning: do not test at 90°C and 100% humidity and intense UV light when degradation shall be comparable to a river water environment). Lab results shall not be transferred to real environment but rather confirmed by tests.
- Tier 2 measures biodegradation in bio meter flasks. So far no results for oxo-degradable products have been published by the producers or any scientific party.
- Tier 3 uses the residues from Tier 2 and submits them to an eco-toxicity test. So far, some data seems to be available following the requirements from EN 13432 and OECD 208.

Overall: mass balance, comparisons between different polymers, tests in real environment and especially the biodegradation data are lacking. Only little information is available on real biodegradation by microorganisms of oxo-degradable products.

The reports and statements available so far from *RAPRAA* (UK), *OWA* (Belgium) and *SYMPHONY* plastics etc. describe only fragmentation and never biodegradation. All reports that supposedly describe biodegradation are not available or provided by OPA or any company. Only the eco-toxicity of the additives has been tested to comply with EN 13432 and ASTM 6954-04 (these guidelines require that no harmful residues are left) (OWS Report MST-4/1-d2wb & d2wc, Eco-Sigma Report Sept. 2008).

Biodegradation was reported [178] using UV-degraded PE. The authors observed that generally the polymer breaks down by irradiation to form some smaller fragments. They observed biodegradation with *Pseudomonas* sp. Strains that seemed to be able to use the smaller oxidized fragments of the original material but only to some extent. Microorganisms were able to form bio films on the surface (which does not proof biodegradation itself without further data). The molecular weight distribution shows a shift to higher molecular weight during the process, indicating some small parts have been possibly degraded further. It has been shown that if the oxidized polyethylene has a molecular weight less than 5000 $g \cdot mol^{-1}$, a significant fraction of it will be in the range of 100-2000 $g \cdot mol^{-1}$ and this fraction can be rapidly biodegraded.

These results are significant as they reveal that biodegradation is mainly because of the consumption of pro-oxidant aided oxidation products. The shift toward high molecular weight during biodegradation also suggests that pro-oxidant has ceased its action during the abiotic oxidation stage and is not helping the biodegradation. However, the high-molecular weight region in the distribution exhibits little changes indicating that *P. aeruginosa* is just able to utilize the end chain products and unable to perturb the whole of the polymer volume.

The second important study focusing on abiotic and biotic degradation [179] shows as well the positive results of the abiotic fragmentation as described by others. It is stated, that biofilm formation is most likely stimulated because of the presence of fragments of small sizes when compared to standard PE films where no biofilm

formation is observed. Biodegradation experiments where performed with naturally aged samples but the mineralization rate was low (12.4% at 58°C and 5.4% at 25°C in compost after 3 months). This confirms that almost no biodegradation occurs and that probably only smallest fragments below 1000 $g \cdot mol^{-1}$ might be biodegraded as was shown by [178].

The authors of another study [178] observed that generally the polymer breaks down by irradiation to form some smaller fragments. They observed biodegradation with Pseudomonas sp. Strains that seemed to be able to use the smaller oxidized fragments of the original material but only to some extent. Microorganisms were able to form bio films on the surface (which does not proof biodegradation itself without further data). The molecular weight distribution shows a shift to higher molecular weight during the process, indicating some small parts have been possibly degraded further. It has been shown that if the oxidized polyethylene has a molecular weight less than 5000 $g \cdot mol^{-1}$, a significant fraction of it will be in the range of 100-2000 $g \cdot mol^{-1}$and this fraction can be rapidly biodegraded. These results are significant as they reveal that biodegradation is mainly because of the consumption of pro-oxidant aided oxidation products. The shift toward high molecular weight during biodegradation also suggests that pro-oxidant has ceased its action during the abiotic oxidation stage and is not helping the biodegradation. However, the high-molecular weight region in the distribution exhibits little changes indicating that *P. aeruginosa* seems just able to utilize the end chain products and unable to perturb the whole of the polymer volume.

The test system used in this study may be problematic for long term tests and especially to observe recalcitrant substances. Also one cannot be sure, what organisms survive for the duration of the test and also the oxygen content may drop significantly. Also, almost no biodegradation was observed with the films (below 10%). Only the acetone extractable fractions showed some CO_2 evolution which also may be caused from remaining acetone. It would be very important to verify and distinguish mineralization and deterioration of oxo-polymers in different environments. Until no evidence on real biodegradation is available, oxo-degradables should be used carefully because the fragments may accumulate in the environment. So far, from a scientific point of view it seems if PE is degraded abiotic to fragments <1000 $g \cdot mol^{-1}$and carbonyl index ($CO_{(i)}$) is high, possibly real biodegradation by microorganisms may occur to these fragments. More proof is required on what happens to larger fragments! CO_2 and biomass investigation is also only scarcely available and do not confirm biodegradation. UV or hydro-biodegradation has been proven many times and it would be important to know if fragmentation can be directed specifically to gain fragments as small as possible. The statements from the Oxo-Biodegradable Plastics Association (OPA) are mostly just statements at this time but little proof has been seen on real biodegradation of the fragments. That is definitely a downside for their argumentation. The OPA statement from August 14 in response to EBP statement from July 22, 2009 shows a few important aspects:

- EN 13432 delivers insights in composting of plastics but not biodegradation in the environment (which from the experience described in this work takes ultimately longer).
- Composting and biodegradation in the environment are two different parameters and require different approaches.

If it can be proven that oxo-PEs really biodegrade and form CO_2, biomass and water in the end and if that happens with the complete polymer and not only some part of small fragments within a reasonable time-frame depending on the environmental compartment, this would be very helpful. In terms of recycling, the statement may be a benefit. If biodegradation can be fully proven for oxo-degradables and recycling is also possible this would be beneficial.

Recently biodegradation of polyesters improved when a thermophilic hydrolase (activity optimum at 65°C) was investigated [180-182]. *Thermobifida fusca* was isolated and expressed in *E. coli* and the enzyme exhibits 65% sequence similarity with to a lipase from *Streptomycetes albus* and combines characteristics of a lipase and an esterase. It was shown to be even able to degrade PET from beverage bottles and can open the door to enzymatic degradation of synthetic polymers that have prior been not degradable at all [183].

2.12.2 Phenol formaldehyde resins and poly(methyl metacrylate)

The biodegradation of three synthetic ^{14}C-labeled polymers (PS, PMMA and phenol formaldehyde) was investigated using 17 fungal species, five groups of soil invertebrates and a variety of mixed microbial communities from sludge, soils, manures, garbage and decaying plastics. Fungi were not able to degrade any of the plastics. Degradation rates found were <1% within 35 days. Soil invertebrates were not able to degrade any polymer at all as well as mixed microbial communities. In comparison to lignins in soil for example, which show degradation rates of approx. 2-15% within 1 month these polymers are extremely recalcitrant [184].

2.12.3 Biodegradable, water soluble polymers

Widely used commodity and specialty water soluble polymers for industrial, and biomedical applications and consumer products have a fundamental concern with degradation in a living organism, may it be *in vivo* or in the environment, with a desire of no adverse responses of an organism. Future water soluble materials are being designed to be completely biodegradable. Today, remediation of water soluble polymers generally implies adsorption on sewage sludge and ultimate removal for land application, land filling or incineration. Hydrogels, such as super-absorbents are often land filled or incinerated. The difficulty in recovering water soluble materials when compared to common plastics leads to an urgent goal in developing better materials in regard to biodegradability. Neither recycling nor any other option used for common plastics can be taken as one for water soluble polymers [89]. Phenolic resins (phenol formaldehyde polymers, production of >2.2 million metric tons in the US) were thought to be not biodegradable. *GUSSE ET AL.* studied the biodegradation with three independent lines of evidence linked to the ability of white-rot fungi, *Phanerochaete chrysosporium*, to biodegrade the polymers. The chromatic transformation of the growth medium indicated initial biodegradation three days after inoculation. A formed degradation product, ^{13}C-labeled phenol, was detected with GC-MS and SEM revealed physical evidence of degradation. White-rot fungi have evolved to produce a very powerful and somewhat non-specific bank of enzymes called liginases degrading lignin. Since the molecular structure of lignin is similar with the one of phenolic resins and the enzymatic arsenal of white-rot fungi is adept to lignin degradation it was assumed that these microorganisms could degrade phenolic resins as well [185] as it is reported that the white-rot fungus is also able to degrade PE [186].

2.12.4 Poly(ethylene glycol) and poly(propylene glycol)

PEG, a synthetic polyether with a molecular weight of 20'000 $g \cdot mol^{-1}$ has been degraded under anaerobic conditions in enrichment cultures inoculated with mud of limnic and marine origins. Three strains of rod-shaped, gram-negative, non-spore forming anaerobic bacteria were isolated in mineral medium with PEG as the sole source of carbon and energy. All strains degraded PEG monomers, dimmers, oligomers and polymers up to a molecular weight of 20'000 $g \cdot mol^{-1}$ completely by fermentation to nearly equal amounts of acetate and EtOH. The monomer, ethylene glycol, was not degraded. It inhibited the strains probably due to blocking of the cellular uptake system, since none of the strains excreted extracellular depolymerase enzymes [187].

Enrichment cultures of methanogenic microorganisms capable of degrading ethylene glycols and poly(ethylene glycols) were obtained from sewage sludge. Degradation products were identified as EtOH, acetate, methane and ethylene glycol monomers (in the case of PEG). The cultures metabolized those glycols best, being close to the molecular weight of the substrate on which they were enriched. The study showed about 82% degradation for PEG 20000 and 83% PEG 1000 within 12 days and 100% PEG 400 within 4 days in anaerobic culture medium. The rates cannot be easily compared to aerobic data since those are mainly based on biological oxygen demand, dissolved organic carbon or CO_2 determination, while this study is based on product recovery [188].

FRINGS and co-workers investigated different enzymes obtained from PEG-grown, strictly anaerobically fermenting cells. A diol dehydratase and a PEG degrading enzyme, PEG acetaldehyde lyase, were detected. Both enzymes are oxygen sensitive and dependant on a reductant. The diol dehydratase was inhibited by glycerol up to 95% while the PEG-degrading enzyme was only slightly affected [189].

Two anaerobic bacteria, *Desulfovibrio desulfuricans* DG2 and *Bacteroides sp.*, were isolated from methanogenic enrichment cultures from a municipal sludge digester. The first metabolized oligomers form ethylene glycol to tetra ethylene glycol. The second one metabolized diethylene glycol and polymers of PEG up to 20'000 $g \cdot mol^{-1}$. Both microorganisms produced acetaldehyde as metabolite and acetate, EtOH and hydrogen as catabolites. Cell extracts of both microorganisms were able to degrade glycols and PEGs up to 400 $g \cdot mol^{-1}$. The extracts of *Bacteroides sp.* strain PG1 could not dehydrogenate long PEG (≥1000 $g \cdot mol^{-1}$) but the bacterium grew with PEGs up to 20'000 $g \cdot mol^{-1}$ suggesting that it possesses a mechanism for PEG depolymerization not present in cell extracts. On the other hand, extracts of *Desulfovibrio desulfuricans* DG2 dehydrogenated long PEG molecules, but whole cells could not grow on the same PEGs as a substrate indicating no conversion of PEG to a product absorbable by the bacterium. Unexpectedly, no microorganism' could degrade PPG while propanediol served as substrate for both. The depolymerization systems in both strains seems limited to ether-linked ethoxy units [190]. Another anaerobic bacterium, *Pelobacter venetianus* from marine anoxic sediments was investigated. Two porins, pore-forming proteins, were characterized and identified to play an important role in fermenting high molecular weight PEGs (20'000 $g \cdot mol^{-1}$). Fermentation proceeds inside the cytoplasm through a vitamin B_{12}-dependent hydroxy group shift from terminal to sub terminal C atom which results in an unstable half-acetal breaking down to acetaldehyde residues. With this investigation it was also shown that PEGs are able to cross outer and inner membrane intact before depolymerization [191].

The metabolism of PEG was investigated with a synergistic mixed culture of *Flavobacterium* and *Pseudomonas* sp., both individually unable to utilize PEGs. A PEG dehydrogenase was found in extracts with high substrate specificity to PEGs from diethylene glycol to PEG 20'000 $g \cdot mol^{-1}$. Metabolic products formed from tetraehtylene glycol degradation were isolated and analyzed with GC/MS, identifying TEG-monocarboxylic acid plus small amounts of TEG-dicarboxylic acid, diethylene glycol and ethylene glycol. Interpretation of the data led to the following pathway for degradation of PEG (Equation 15) [192].

$$HO(CH_2CH_2O)_nCH_2CH_2OH \Rightarrow HO(CH_2CH_2O)_nCH_2CHO \Rightarrow HO(CH_2CH_2O)_nCH_2COOH \Rightarrow HO(CH_2CH_2O)_{n-1}CH_2CH_2OH \Rightarrow\Rightarrow\Rightarrow$$

Equation 15 - Pathway for poly(ethylene glycol) degradation

PEGs biodegradation with a molecular weight up to 13'000 to 14'000 $g \cdot mol^{-1}$ were investigated with a river water isolate (strain JA1001, *Pseudomonas stutzeri*). Cultures metabolized about 2g polymer per liter in less than 24h and $10 g \cdot L^{-1}$ in fewer than 72h. The limit in size of the polymer of about 13500 $g mol^{-1}$ sustaining any growth of the isolates and the presence of a PEG-oxidative activity in the periplasmic space suggests, that PEGs cross the outer membrane and are subsequently metabolized in the periplasm. The oxidation was found to be catalyzed by an enzyme, PEG-dehydrogenase, which is a single poly peptide. The characterization of the enzyme revealed glyoxylic acid as the product of PEG-oxidation and cleavage. Glyoxylate supports growth by entering cells and introducing its carbon atoms in the general metabolism through dicarboxylic acid cycle [193].

GU & PAN isolated PEG 3400 degrading bacteria from tap water and wetland sediments. Only one *Sphingomonas* strain was obtained in enrichment cultures from each inoculum, but 9 degraders (out of 15 isolated bacteria) were isolated on agar plates. Three of those were Gram-negative bacteria belonging to the genus of *Pseudomonas* and *Sphingomonas*. The other six isolates, Gram-positive bacteria, belonged to *Rhodococcus*, *Williamsia*, *Mycobycterium* and *Bacillus* genii [194]. It was also shown in a previous study, that it seems slow growing bacteria contribute more to the degradation of polymeric materials than the fast growing ones over an extended period of time [195]. It is obvious that the diversity in PEG degrading microorganisms in native environments is significantly greater than first assumed. A phylogenetic tree was constructed based on a distance matrix analysis of 16S rDNA sequences from the study and related bacterial strains in *GENBANK*. It should be noted, that *Rhodococcus*, *Williamsia*, *Mycobycterium* belong to the *Actinomycetes*, as well as the only known Gram-positive PEG degrader, *Pseudonocardia* sp. Strain K1. A further investigation of biochemical pathways of *Actinomycetes* in comparison with those demonstrated for *Sphingomonas* and *Pseudomonas* species [194].

Biodegradation in river water dissolved organic carbon-Die-away tests of PEG (PEG 300) an PPG (PPG 425) was investigated by *ZGOLA-GRZESKOWIAK ET AL.* PEGs were isolated from the water matrix with a solid-phase extraction using a graphitized carbon black sorbent and the analytes were derivatized with phenyl isocyanate and determined with HPLC-UV detection. PPGs were extracted with a liquid-liquid extraction from the water matrix and chloroform as solvent. Derivatization was performed with naphthyl isocyanate and determined with HPLC-fluorescence detection [196]. All degradation rates of both PEG and PPG reached 99% during the test (within 14 days). Two pathways of biodegradation could be observed. One is the fragmentation of PEGs into shorter homologues with random chain scission. The second pathway, which is more widely accepted, is the terminal oxidation of the hydroxyl group, formation of an aldehyde and than carboxylic acid and sequential shortening

of the backbone by a single ethylene unit. A lack of analytical methods for the determination of ethylene glycol, di- and tri-ethylene glycol hinders the solution of this problem [196]. On the biodegradation of PPGs not much is published yet. It has been found out that several bacterial strains are able to degrade the substances; however, most PEG-degrading microorganisms do not grow on PPGs. The biodegradation pathway could be different [196]. For PEG biodegradation a clear change in molecular weight distribution can be observed. It seems that with the decrease of higher molecular weight chains the amount of shorter chains rises at first. If PPGs are compared to that, a difference can be clearly stated. PPGs are degraded as fast as PEGs but no change in molecular weight pattern can be observed. This means that PEGs are degraded into smaller homologues first; PPGs show no change in molecular weight pattern [196].

A PEG degrading, ether cleaving enzyme in cell-free extracts of a strictly anaerobic fermenting bacterium which can utilize PEGs from 100 to over 20'000 $g \cdot mol^{-1}$ was investigated by *SCHRAMM* and *SCHINK* [197]. The isolate was assigned to the genus of *Acetobacterium* on a basis of morphological, physiological and biochemical properties. Along with the ether cleaving enzyme activity a slightly lower diol dehydrogenase activity was found. An analogous mechanism was postulated: since acetaldehyde was found to be the first product of PEG degradation, the polymer must be attacked by hydroxyl shift reaction similar to the ediol dehydratase reaction, forming a hemiacetal of acetaldehyde disintegrating non-enzymatically to free aldehyde (see Equation 16).

$$HO\text{-}CH_2\text{-}CH_2\text{-}O\text{-}R \rightarrow [H_3C\text{-}CH(OH)\text{-}O\text{-}R] \rightarrow H_3C\text{-}CH\text{-}C(H)=O + HO\text{-}R$$

Equation 16 - Metabolization of PEG by hydroxyl shift reaction

The fact that tetra ethylene glycol dimethyl ether which does not contain a free hydroxyl group, cannot be degraded by this enzyme and that the ether cleaving enzyme is specifically inhibited by glycerol and various corrinoids at mircomolar concentration corroborate this idea. There is no doubt that such corrinoide-dependent hydroxyl shift reaction can only occur within the cytoplasm. The observations indicate that PEGs have to enter the cell before degradation can occur and the question remains how synthetic polymers with average molecular weight up to 1000 $g \cdot mol^{-1}$ and in some cases up to 20'000 $g \cdot mol^{-1}$ can cross the cytoplasmic membrane [197]. Also aerobic degradation has been shown to occur within the bacterial cells [192].

Different bacterial strains known to be able to attack poly(oxyethylene)-type nonionic surfactants and were isolated by enrichment culture technique from SW of the Arno River. Alkyl- and alkyl phenol polyethoxylates as well as poly(ethylene glycols) were degraded and assimilated by bacterial strains in axenic cultures. Matching each bacterial isolate with several types of nonionic surfactants, the routes of degradation were identified. In accordance with previous studies, the first attack led to the cleavage of the poly(oxyethylenes) from the nonionic surfactant. It was then found that systems being able to degrade PEG segments of nonionic surfactants are unable to degrade free PEGs, whereas PEG degraders are not able to utilize PEG chains from nonionic surfactants. Biodegradation is strongly affected by both, the presence of adapted degrading microorganisms and the surfactant molecular structure. In particular, the presence of a terminal free hydroxyl group in the hydrophilic part of the nonionic surfactant or in PEGs resulted as fundamental prerequisite for biodegradability [198].

SUZUKI ET AL. observed the Ozone treatment upon the biodegradability of water soluble polymers. PEG (M_W 8'000 g·mol^{-1}), PVA (M_W 28'000 g·mol^{-1}), PVP (M_W 27'000 g·mol^{-1}) and poly(acryl amide) (M_W 280'000 g·mol^{-1}) were investigated in river-bed mud/sediment as inoculum. Ozonation increased biodegradability of the polymers except poly(acryl amide) but the samples were degraded only slowly. Without Ozonation, biodegradability was almost not observed [199]. PEG degradation in model WW treatments with PEG of 10'000 g·mol^{-1} and wet-air oxidation process were compared with biological oxidation processes of PEG (200-35'000 g·mol^{-1}). A degrease of biodegradation with rising molecular weight was observed and when wet air oxidation was applied as pre-treatment, the biological oxidation time was reduced by an order of magnitude at least resulting in higher degradation rates (TOC removal) as well [200].

2.12.5 Polyurethanes

Polyurethanes are known to be susceptible to microbial attack, especially fungi. The degrading enzymes have been investigated and one has been purified and characterized. A surface binding and a catalytic domain have been reported, in analogy also known already for PHA degrading depolymerase. No significant homology could be detected between both amino acid sequences, besides in the site-specific surface-binding region. A review about microbial degradation of PUR, polyester PUR, polyether PUR, biodegradation of the urethane bond and the characteristics of their degrading enzymes is given by *NAKAJIMA-KAMBE* [201]. Biodegradation of PUR by these microorganisms is, however incomplete and their growth is not supported by PUR alone. Additional carbon sources are required as are nutrients. PUR biodegradation is thought to mostly be caused by esterase.

Soil fungal communities are involved in PU biodegradation and PU films were observed in sandy loam soils at different organic carbon content and different pH (5.5 and 6.7). After 5 months of burial the fungal communities were compared between surface and native soil. DGGE showed that communities on PU surface was less diverse than in soil and only few species found on the surface were detectable in the soil. Also the soil type influences the microorganism communities depending on the pH and organic carbon. The interesting point is that PU is highly susceptible to soil biodegradation and independently degraded almost completely in both soil types but by different communities [202].

2.12.6 Polyamides

Nylon-66 (PA-66) was found to be degradable significantly by lignin degrading white-rot-fungus. The characteristics of the nylon degrading enzyme was found to be identical to those of manganese Peroxidase but the reaction mechanism for nylon degradation was suggested to differ from manganese peroxidase. Nylon-6 fibers are also degraded by the enzyme. The erosion observed suggests that nylon is degraded to soluble oligomers [186]. Similar results were observed by screening 58 fungi strains where most were isolated from a factory producing nylon-6. No degradation was observed, but white rot fungus (*Bjerkandera adusta*) from culture collection was able to degrade the polymer [203].

2.12.7 Polyimides

Another class of polymers, the polyimides, have been investigated and discussed concerning their degradation probabilities by fungi (*Aspergillus versicolor*, *Cladosporium cladosporioides* and *Chaetomium sp.*) [204].

The microbial degradation of polyimide coatings has been monitored utilizing EIS. Samples were exposed to a mixed fungal culture originally isolated from degraded polyimides. Identified species include *Aspergillus versicolor*; *Cladosporium cladosporioides* and a *Chaetomium* sp. Active growth of fungi on polyimides give distinctive EIS spectra through time. This indicates a loss in polymer integrity in comparison to un-inoculated samples. Obtained data was supported with SEM, showing extensive colonization of polymer surfaces by fungi. Polymer samples were inoculated for 122 days at ambient temperature in 0.2M NaCl solutions. At the beginning no differences in impedance was detected between sterile and inoculated samples. After the test was finished a decrease of impedance could be detected with rising frequency [204].

2.12.8 Poly(isoprene)

The degradation of poly(cis-1,4-isoprene) and vulcanized natural rubber by *Streptomyces coelicolor* and *Pseudomonas citronellolis* was investigated. Investigations were compared to a non-degrading strain, *Streptomyces lividans* 1326, as control. Molecular weight distribution analysis of the polymer molecules before and after the experiment showed time-dependent increased low-molecular weight polymer molecules for the degrading strains but not for *S. lividans* (control). Three degradation products were isolated and identified belonging to the group of methyl-branched di-ketones. A possible pathway for oxidation has been proposed (Figure 6).

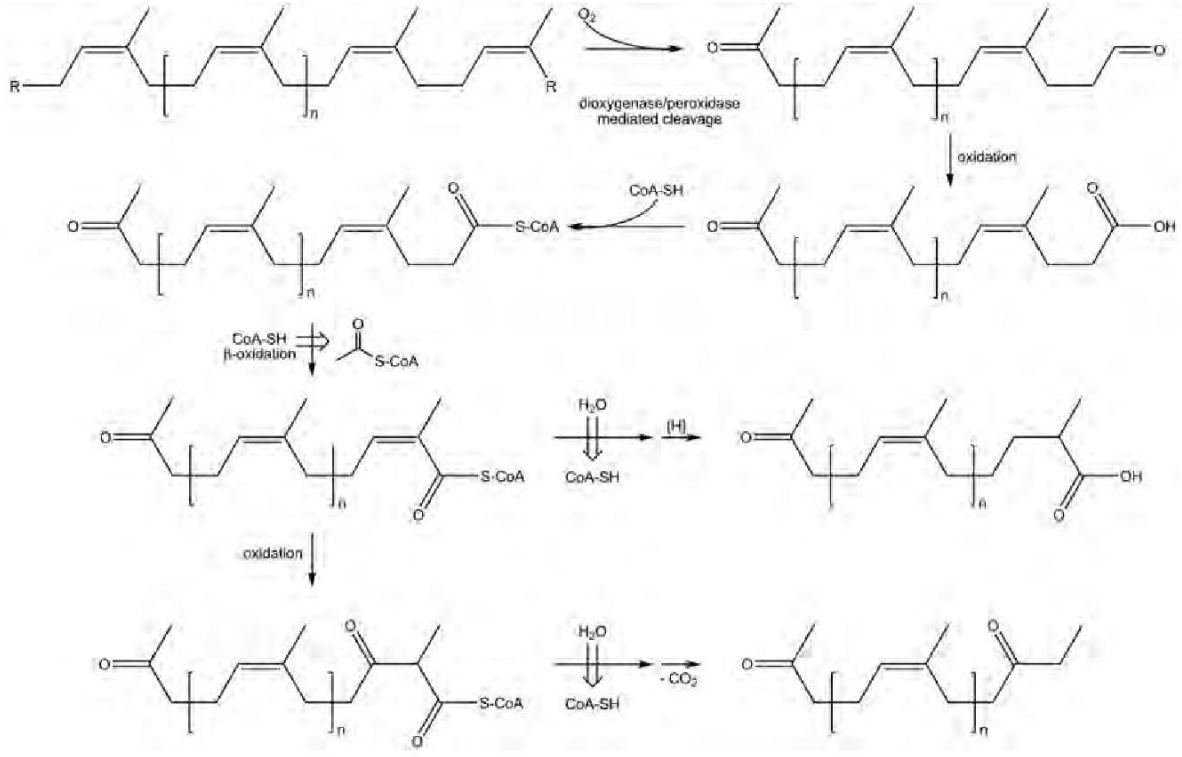


Figure 6 - Hypothetical biochemical route for degradation of poly(cis-1,4-isoprene) to compounds 2 to 4 by S. coelicolor

Based on the fact that a significant and time-dependent decrease in molecular weight to low-molecular weight fractions had been observed, it could be concluded that the mechanism was mainly endo type cleavage. On the contrary, partially occurring exo type breakdowns cannot be ruled out. In the case of only exo type cleavage the molecular weight would remain very high until all polymer molecules were degraded since the molecule would be attacked from one end to the other. This phenomenon has not been observed yet for any rubber-degrading bacterial strain. Analysis showed also a sharp cut-off for molecules with less than 5000 $g \cdot mol^{-1}$. The smaller products might have been absorbed and used preferentially by microorganisms [205].

2.13 Specialty polymers

2.13.1 Acrylic polymers (Superabsorbents)

White-Rot Fungus, *Phanerochaete chrysosporium*, was able to degrade two synthetic super absorbent cross linked acrylic polymers. The amount of converted CO_2 measured, increased with an inclining amount of polymer added to the cultures. When all culture fluid was absorbed and a gelatinous matrix had formed, the fungus still grew and mineralization was still observed. Neither the polymers nor the degradation products were toxic to the fungus. All of the polymers incubated in the liquid fungal culture were depolymerized into water soluble products within 15-18 days while the mineralization rate was very low. Depolymerization was only observed in nitrogen limited cultures where the lignin degradation system is secreted. It is remarkable though that the water soluble products of the depolymerization were mineralized in both, nutrient limited and sufficient cultures and the rate of mineralization of those products was more than two times greater in nutrient sufficient cultures. It could also be shown that most of the carbon of the polymer was recovered in the fungal mycelia mat and therefore been converted to fungal metabolites [206].

2.13.2 Poly(vinyl pyrrolidone)

The first study on aerobic PVP degradation in FBBR studies was monitored using MALDI-TOF-MS. No oxidation of end groups or shift in repeating units was observed even after 30 days. A decrease in molecular mass could be drawn to adsorption onto sludge particles [3] The samples were fractionated by GPC to improve the detection of high mass oligomers which are suppressed by low molecular oligomers due to the polydispersity of the polymer parent compound. No oxidation of end groups or shift in repeating units was observed even after 30 days (Figure 7).

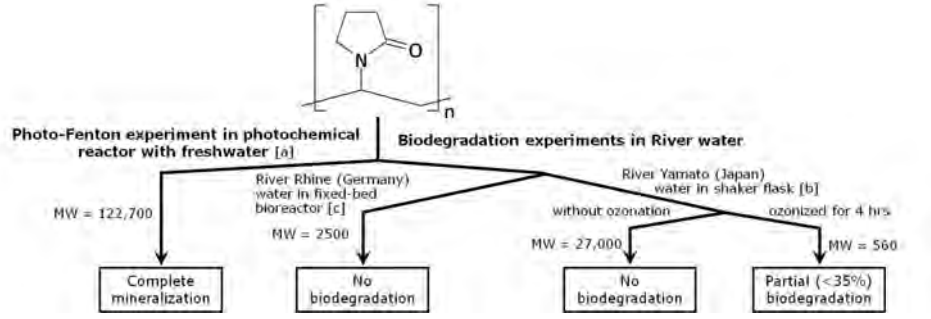


Figure 7 - Biodegradation pathways for poly(vinyl pyrrolidone) (a [132], b [199], c [3])

A decrease in molecular mass could be drawn to adsorption onto sludge particles [3]. The authors assume that this poor biodegradability results from the end group of the investigated PVP sample and a higher degradation may be obtained with a different end group. Not much has been reported on PVP biodegradation [207] which is important because it is known that huge amounts are used today in manifold applications such as personal care products, lacquers and dyes and for remediation of pollutants in water treatment. PVP may enter the environment directly or through waste water treatment, because PVP is not biodegraded nor completely eliminated in wastewater treatment and also may enter directly through one of its applications [3;208].

2.14 Bioplastics

In the group of biodegradable polymers, polyesters [73;209-211] play the main and most important part. Many are already available on the market [212] and their degradation has been studied extensively especially in soil or compost [183]. Until a few years ago, these polyesters have only been of aliphatic nature without any exceptions. Well known are the bacterial polyesters PHB (and also *BIOPOL*®) or the synthetic materials PCL and PLA as well as *BIONOLLE*®. Problems such as high prize, worse physical properties or lower eco-efficiency than conventional polymers pose as problems in marketing and usage of those biopolymers [93]. In the 1990s a new and somewhat more empirical approach has been made to improve the use and processing properties of aliphatic polymers while maintaining their biodegradability [96]. This led to combinations of biodegradable aliphatic polymers with aromatic polyesters, having better properties in both regards in the resulting polymers. The degradation mechanisms and the effects of environment, different microorganisms and enzymes all influence the extent of biodegradation. It was shown that certain enzymes may degrade specific bonds (as shown as in Figure 8) such as α-ester bonds (Protease, Proteinase K), β-ester bonds (PHB-depolymerase) and γ~ω-ester bonds (Lipase) [213].

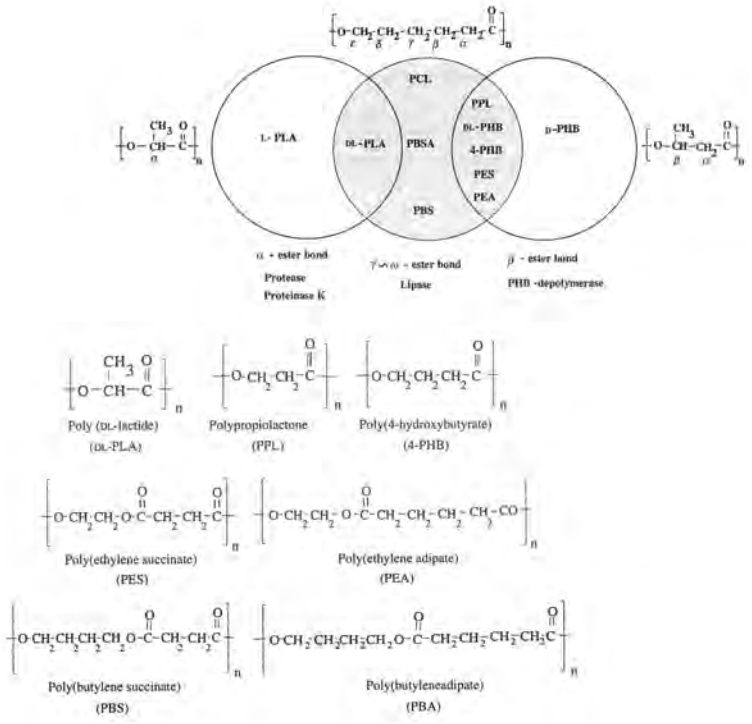


Figure 8 - Different enzymes involved in the degradation of specific ester bonds (adapted from [213])

2.14.1 Poly(hydroxy alkanoate), BIOPOL®

PHA was first discovered and isolated and characterized by *LEMOIGNE* at the *PASTEUR INSTITUTE, PARIS* in 1926 [62]. An overview of PHA bio polyesters physiological and engineering aspects has shown that PHA is suited for negative ecological impact products because of their good biodegradation properties [70;214]. PHA's are biosynthesized polymers [215;216] with similar properties to conventional plastics but the ability of being completely biodegradable to CO_2 and H_2O. Over 90 different HA monomers have been identified of being constituents in bio polymeric PHA. The industrial production of microbiological PHA's is described in detail by *HANKERMEYER ET AL.* [209;210]. The physical parameters of PHA polymers, molecular structures, molecular weight distribution, chemical structure and composition, thermal properties crystallinity and morphology and mechanical properties and performance in comparison to other polymers has been investigated closely since the discovery of PHA's [62;217] as well as different techniques for detecting and analyzing PHA's [209]. PHB for example is also produced in nature in a wide variety of bacteria as carbon storage. Its usefulness for some applications [217] is limited by its brittleness, deficiency in thermal stability and difficult processability. However, the addition of polyhydroxyvalerate or even other polymers overcome these problems [186;218]. PHB polymers are very important today because they have good material properties as well as biodegradability [219].

Since microorganisms view PHB and PHBV as an energy source, biodegradation is dependent on microbial active environments, surface area and structure, disposal environment, moisture level, and other nutrient materials [209;218]. Also stereochemistry plays an important role in biodegradation processes of PHA's when synthesized by microorganisms PHA's are 100% stereo specific with all carbon atoms in the R(-) configuration. Man-made PHA's are racemic mixtures of R and S enantiomers. The S(+) PHA's interfere with biodegradation because microorganisms do not recognize the S(+) constitution. A 100% P(S)-HA cannot be degraded microbiologically at all. Because of the difficulties arising from separation of racemic mixtures of synthetic polymers, microbial production is the best option for industrial mass production [210]. An experiment of PHBV and PE-Starch blends in an freshwater environment (Lake Lugano, Switzerland) showed that steady degradation rates of 10-20mg·d^{-1} of PHBV degraded even under relatively extreme conditions (at low temperature, no sunlight exposure and seasonal variations in oxygen content). Conversely, PE-Starch blends showed no degradation after one year [210].

For industrial production microbial synthesis is the best way to obtain PHB's since it ensures the right stereochemistry to enable biodegradation processes. microorganisms synthesize and store PHB under nutrient-limited conditions and degrade and metabolize PHB when conditions are reversed completely [209;210]. Traditional methods used gravimetric and respirometric tests on determining degradation behaviour of PHB [105]. PHV and P(HB-co-12%-HHx) was compared with *ECOFLEX*® in terms of biodegradation in activated sludge [220]. Samples (films) were periodically removed, washed and dried before analysis. Additionally, films were put in special lipase solution in phosphate buffer saline (PBS) to monitor enzymatic degradation. average molecular weight analysis was performed with an HPLC system with a chloroform mobile phase [220].

Both microbial polymer films (PHB and P(HB-co-12%-HHx) degraded faster than *ECOFLEX*®. After 18 days 40% P(HB-co-12%-HHX) and 20% PHB was degraded, while *ECOFLEX*® lost only 5% of its residual weight. Also the average molecular weight was decreased in the first two cases above average. SEM showed also changes in surface morphology before and after the study. *ECOFLEX*® was not affected very much, but its surface was rather smooth and without many holes in the first place. This led to the conclusion that surface morphology may in some way correlate with biodegradation behaviour [220]. After testing P(HB-co-5%-HHx), PHB and P(HB-co-20%-HHx) it was found that HHx content changes resulted variations in polymer crystallinity [221]. It has also been discovered that biodegradation is dependent on the sample crystallinity. Therefore, surface morphology and crystallinity play an important role in biodegradation behaviour. Surface morphology has an influence on the contact between water, enzymes, microorganisms and the polymer. Crystallinity determines the intrusion rate of enzymes, water and microorganisms into the polymeric structures [220;222].

PHB and PHBV samples were degraded in natural environments like soil, water, and compost and sewage sludge, incubated under lab conditions. The degradation rate was determined 45% weight loss in sewage sludge for 200 days of incubation at most. Isolated cultures were used for further experiments. The optimum concentration was determined at 0.3% (w/v) PHB. Supplementation of soluble carbon sources reduced the degradation rates [223]. degradation of PHBV occurs most rapidly in anaerobic sewage sludge, then in estuarine sediment, aerobic sewage, soil and slowest in sea water [218]. Also tests with different soil types and PHB polymers were reported and have shown that biodegradation was best in activated sludge soil but at different temperatures for the different polymers. This shows that defined structures are degraded best by specific microorganisms which may have different temperature optima. Generally biodegradation rate was PHB>Sky-Green®>MaterBi® [224].

Anaerobic degradation of certain plastic materials has not been investigated very closely and therefore only few reliable studies are available. Three different systems for testing anaerobic degradation were compared and PHB and PHBV were used as test compounds. The best system proved to be a test system where methane production was determined with a gas meter. The test compounds degraded very fast in anaerobic tests (99% within 30 days) [225]. In another comparison wet mesophilic test methods (ASTM D5210-91, Iso 11734 & DIN 38414 T8) were compared to dry mesophilic methods (ASTM D5511-94) and it was observed, that suitability of the methods is not equal since different results were produced. This may be due to the differences mainly in nutrient media and buffer systems used. Three different linear polymers (PHB, PHBV and PCL) were investigated in regard to anaerobic degradation. Two sources of inoculum were used for degradation experiments: methane sludge and sewage sludge. Tests were incubated at 35 and 37°C for usually 42 days. Biogas production was measured and converted into %ThBiogas and then plotted against incubation time. Degrading strains were isolated and used for new tests. Samples were incubated for 9 weeks at 35°C (6 weeks, 37°C for PCL) and degradation was determined by weight loss. Results show degradation rates of 100% after 10 days in methane sludge for PHB and 60% (PHBV) and 30% (PCL) after 42 days. Independently of the test system used and compared, a general direction is visible. PCL was the most resistant polymer to anaerobic degradation. PHB showed the fastest degradation under anaerobic condition. It might be probably the degradation product of PHBV, 3-hydroxyvaleric acid, posing as problem in the growth of microorganisms [226].

Another study of anaerobic degradation of PHB, PHBV, PCL, PCL-Starch and BTA polymers, investigating weight loss and biogas formation as well as starch content in Polyester-starch blends via GPC, showed fastest degradation of PHB followed by PHBV, PCL and BTA polymers. Interestingly, synthetic polyesters besides PCL degrade far slower under anaerobic conditions than under aerobic conditions as demonstrated in the literature [227]. *SCHIRMER ET AL.* demonstrated the degradation of P(3HO) samples by *Pseudomonas fluorescence* GK13 bacteria. 25 gram-negative bacteria and one gram-positive bacterium capable of growing on the samples and utilizing it as the sole source of carbon and energy were isolated from soils, lake water and activated sludge. Only the gram-positive strain was able to hydrolyze P(3HO) and P(3HB). Most isolates degraded only P(3HO) and MCL (MCL) hydroxy acid (HA) co polymers. One strain, *Pseudomonas fluorescens* GK13, was isolated and depolymerase was collected and purified. It hydrolyzed P(3HO), copolymers of MCL HA and para-nitrophenyl esters of fatty acids. The dimeric ester of 3-HO was determined to be the main product of enzymatic hydrolysis [101].

A detailed review study by *JENDROSSEK* presents different studies focusing on extra- and intracellular degradation of PHA polyesters by microorganisms. Also the identification, isolation, characterization, biochemical properties, molecular biology and functional analysis, mechanisms and regulation of depolymerise enzymes as well as mobilization and accumulation and hydrolysis of PHA *In Vitro* by bacteria are discussed [104]. The degradation of PHB occurs via a pathway that does not involve CoA esters. As shown by *E.A. DAWES*, a depolymerase initiates hydrolysis to yield free D(-)-3-hydroxybutyrate which is further oxidized at acetoacetate by an NAD-specific dehydrogenase. This enzyme is completely inhibited by NADH, pyruvate and 2-oxoglutarate; NADPH has no effect. The acetoacetate is further converted to acetoacetyl-CoA by an acetoacetate/succinate CoA transferase. This means that PHB metabolism is a cyclic process with acetoacetyl-CoA serving as precursor for PHB synthesis and also as a product of its degradation (Figure 9) [228].

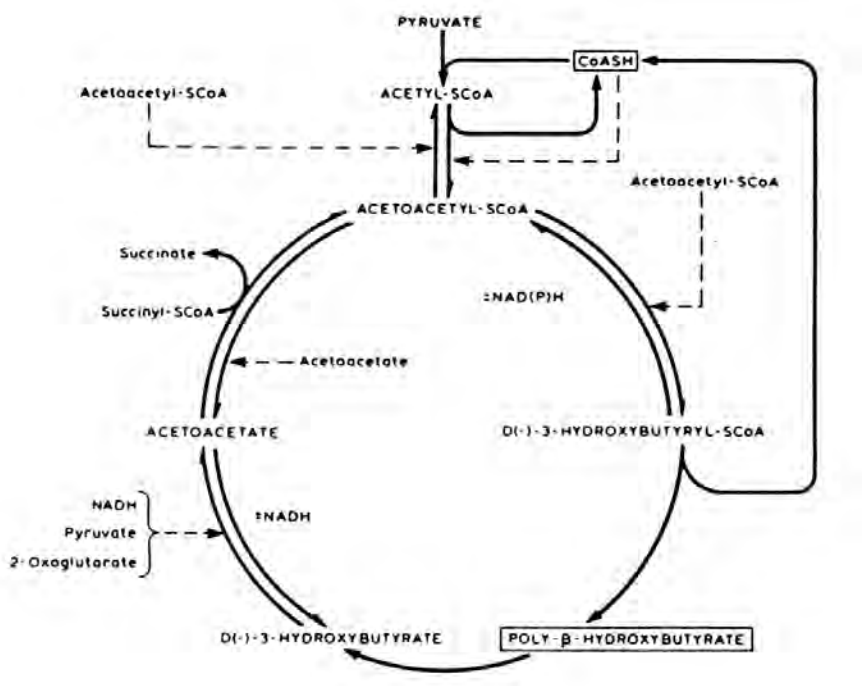


Figure 9 - Poly(hydroxy butyrate) metabolism cycle

MATAVULJ and *MOLITORIS* developed a screening procedure for testing fungal degradation of *BIOPOL*® polymers with different test media. 143 pure fungal strains were screened using different culture media spiked with *BIOPOL*®. All fungal strains were able to grow on all test media. One medium, a mineral Peptone/Yeast extract culture medium (mPY), showed best results in *BIOPOL*® degradation. Being poor in easily available organic carbon sources it generally supported slow, and in some cases only weak colonial growth with often late expression of the degradation ability (sometimes not before 5 weeks after incubation). However, the degradation activity was best on mPY medium and in some cases activity was only found on this medium it is recommended for PHA degradation screening. The incubation time should be at least 6 weeks [229].

The relatively slow degradation of *BIOPOL*® in different soils, sludge from a lake or freshwater, as inoculum but very quick degradation in aerobic sewage sludge prompted an investigation of different parameters in biodegradation processes. Therefore the dependence of *BIOPOL*® degradation on inoculum size, pH and agitation of the culture was analyzed. Inoculum size showed to have no effect on the degradation rate within the range of applied initial concentration. The change in pH influenced the degradation rate to a high degree. Over a period of about 12 weeks the loss in polymer weight increased to 100% at pH 7.5 and pH 8.0. Also the turbidity of the culture fluid increased, indicating cell growth. Below pH 6.0 and above pH 9.0 the degradation rate dropped below 10% of the initial polymer concentration after 12 weeks. Also almost no cell growth could be observed. These results are in agreement with the pH of municipal sewage sludge which is in the slight alkaline range (pH 7-8). The agitation of the culture fluid had relatively little influence n biodegradation of *BIOPOL*®. A significant effect could only be measured under alkaline conditions at pH 9.0 and above. The degradation rates in shaken cultures were significantly higher than those in non-agitated or daily shaken flasks [230].

Three polymer samples (PHB, PHB/10% HV and PHB/20% HV) were tested for biodegradation in 5 different soil types, 2 composts, 2 freshwater types and seawater at different temperatures (15°C, 28°C & 40°C and incubation times. The highest rates of degradation in soil were obtained at 40°C. 74-93% of the initial weight of the homo polymer and 53-90% of the 10% HV copolymer remained after 200 days. In the university freshwater pond only 4-7% weight loss could be determined after half a year while in the canal the same amount could be detected after 2 months already. In seawater about 61-71% of the initial weight remained after half a year. In general the degradation rates increase with rising amount of 3HV content in the polymer. 325 microorganisms degrading PHB in vivo was isolated from the soils including 154 bacteria, 77 streptomycetes and 94 moulds. At 15°c the micorflora consisted mainly of Gram-negative bacteria and streptomycetes. At 28°C also Gram-positives and moulds were frequently isolated and at 40°C moulds and streptomycetes predominated. In freshwater, bacteria were the predominant cultures (52 isolates). Only 9 streptomycetes and 3 moulds were isolated [13;231].

Eight aliphatic polyesters (P(3HB), P(3HB-*co*-14% 3HV), P(3HB-*co*-10% 4HB), poly(ε-caprolactone), poly(ethylene succinate), poly(ethylene adipate), PBS, poly(butylene adipate)) were investigated in biodegradation tests in different environmental natural waters at 25°C for 28 days in modified MITI-Tests. Weight-loss as well as biological oxygen demand was used to establish biodegradation curves. Table 6 shows results of the tests (Table 6) [11].

Table 6 - Biodegradation of poly(hydroxy butyrate) & poly(caprolactone) in fresh- and seawater

	Sample	Freshwater (river)		Freshwater (lake)		Seawater (bay)		Seawater (ocean	
		weight loss biodegradation [%]	BOD biodegradation [%]	weight loss biodegradation [%]	BOD biodegradation [%]	weight loss biodegradation [%]	BOD biodegradation [%]	weight loss biodegradation [%]	BOD biodegradation [%]
1	P(3HB)	100 ± 0	75 ± 16	93 ± 7	52 ± 7	41 ± 16	27 ± 10	23 ± 13	14 ± 10
2	P(3HB-*co*-14% 3HV)	100 ± 0	79 ± 2	100 ± 0	71 ± 1	100 ± 0	84 ± 2	100 ± 0	78 ± 5
3	P(3HB-*co*-10% 4HB)	100 ± 0	90 ± 1	74 ± 26	55 ± 17	70 ± 30	51 ± 27	59 ± 15	43 ± 14
4	poly(ε-caprolactone)	100 ± 0	75 ± 8	100 ± 0	77 ± 1	100 ± 0	79 ± 2	67 ± 21	56 ± 9
5	poly(ethylene succinate)	100 ± 0	83 ± 2	100 ± 0	77 ± 2	2 ± 1	1 ± 1	5 ± 2	3 ± 2
6	poly(ethylene adipate)	100 ± 0	70 ± 3	95 ± 5	68 ± 8	100 ± 0	65 ± 13	57 ± 14	46 ± 13
7	PBS	2 ± 1	3 ± 1	22 ± 14	12 ± 8	2 ± 2	1 ± 1	2 ± 3	2 ± 0
8	poly(butylene adipate)	24 ± 7	20 ± 4	80 ± 13	50 ± 10	34 ± 2	20 ± 2	11 ± 10	10 ± 5

15 different polyester samples of P(3HB-co3HV) and P(3HB-co-4HB) were prepared and biodegradation was investigated with a bacterium isolated from laboratory media and identified as *Pseudomonas piketii*. It was found that the bacterium also grew on 3HB, glucose, fructose, citrate and succinate but only 3HB was able to induce the PHA depolymerase enzyme apart from 3HB. The enzyme was purified and its molecular weight was determined as about 40 kDa. The optimum activity was observed at pH 5.5 and 40°C. H-NMR analysis revealed that the main degradation product of the P3HB polymers was 3-hydroxybutyric acid [232].

The biodegradability of P(HB-co-HV) blended with starch as well as mechanical properties were determined under aerobic and anaerobic conditions via weight loss. The starch content ranged from 0 to 50% (w/w) in the polymer tested. With increasing starch content biodegradation increases as well. A mixed microbial culture degraded pure PHB in over 20 days but the 50% starch P(HB-co-HV) polymer was already gone in less than 8 days. The tensile strength declined from 18MPa to 8MPa while Young's modulus increased from 1.525 MPa to 2.489 MPa, but the overall mechanical properties remained in a useful range. Also aerobic degradation was faster than anaerobic processes [233].

In a study on P(HB-co-HV) with different portions of 3-HV reaching from 17 to 60 mol% it could be shown that with increasing 3-HV content the water contact angle and hence hydrophobicity increases. It is widely accepted that when increasing the side chain lengths of the constituents the surface hydrophobicity inclines as well. Along with the higher 3-HV content it can be also observed by FTIR-ATR (attenuated total reflectance) and

calculation of the crystallinity index (CI) and amorphous index (AI), that AI values increase and CI values decrease along as the polymer becomes more and more amorphous. These findings suggest that the degradation rate is more dependent on crystallinity than on hydrophilicity [234] seeming more important than the dependence on molecular weight of PHB and P(HB-co-HV) degradation [235]. Similar findings were reported earlier for PHB degradation already [236;237] and other structures [222]. It has been shown that a correlation of the degree of crystallinity, morphology, glass temperature, mechanical properties and biodegradability exists for PHA blends. PHB homopolymers are very brittle but when mixed with other biodegradable polymers mechanical properties can be changed and relevant materials can be manufactured that can have similar properties as PE or PP or others [238].

2.14.2 Polythioesters

Polythioesters are new class of biopolymers, which can be basically synthesized with the PHA biosynthesis system. A study by *KIM ET AL.* [239] approached the issue of biodegradation of poly(3-mercaptopropionat) (poly(3MP)) trying to isolate microorganisms being able to degrade the polymer. About 74 different environmental samples were screened but neither bacteria nor fungi were found hydrolyzing poly(3MP). Also soil, compost and activated sludge were applied to search for microorganisms probably non-cultivable and considering also microbial communities but again, even after an exposure of more than half a year, no poly(3MP) degrading organisms were found [239].

2.14.3 Polyesters with synthetic aromatic and aliphatic components

Since this group of polyesters has shown biodegradable properties, the group has been investigated closely through recent years [240]. Especially the degradation in soil and compost was the main feature of interest. This is mostly due to the market situation for these materials since they have major applications in packaging and agricultural industry. It was shown that there are already huge differences in biodegradation for soil and compost compartments as shown in Figure 10 [96;212] for different aliphatic-aromatic polyesters.

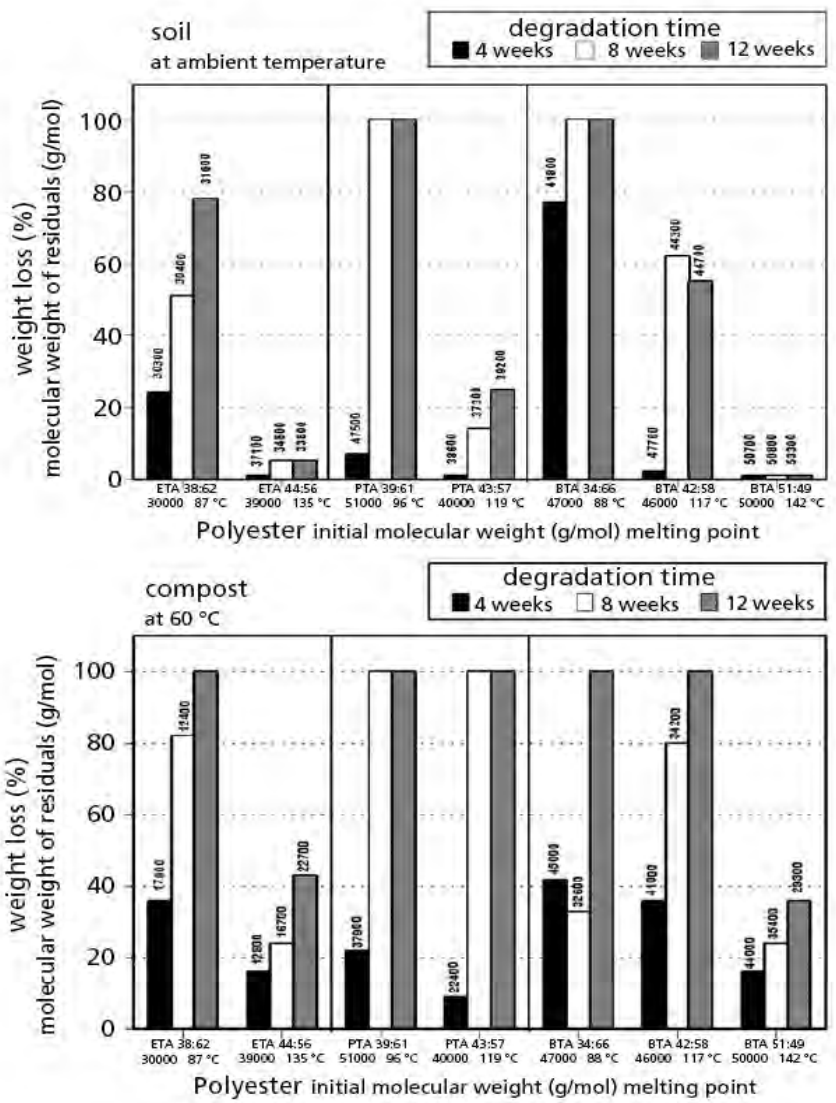


Figure 10 - Weight losses of aliphatic-aromatic copolyester films (100µm) in soil and mature compost; components: E = > 1,2-ethanediol. P = > 1,3-propanediol, B: 1,4-butanediol, A: adipic acid, T: terephthalic acid; numbers reflect the ratio of aromatic/aliphatic acid component in mol%, (e.g., ETA38:62 copolyester from 1,2-ethanediol, adipic acid and terephthalic acid with 38 mol% terephthalic acid in the acid component)

It is not surprising considering the ecological differences of environmental compartments, that biodegradation in aqueous media are much slower because of the different components and microorganisms [93;220;240-242]. The architecture and biodegradation of different BTA Polymers, *Bayer Tir 1874*®, PHB and *Bionolle*® were investigated in regards to biodegradation by microorganisms in compost and soil.

A commercially available lipase, *Rhizomucor miehei* has been chosen for the degradation in several test assays. Also, a screening for polyester-degrading microorganisms has turned up, that especially *Actinomyces sp.* are surprisingly effective in degrading aliphatic/aromatic copolyesters with up to 60 mol% of the aromatic component [93].

The biodegradation of the aliphatic-aromatic polyester *BIOMAX*® was investigated in a lab-scale bioreactor at 58°C. The reactor was inoculated with microorganisms obtained from compost and supplemented with powdered test substance as well as an additional energy source. After an acclimation period, the microorganisms were capable of degrading the major components of *BIOMAX*® and degradation was monitored by laser diffrac-

tion. The particle size distribution shifted to smaller sizes until the diameters were indistinguishable from bacteria. microorganisms types were investigated using 16S rRNA gene sequencing and the bacteria belonged to about 35 different groups with the majority being new species [243].

The aliphatic-aromatic copolyester Ecoflex® has been reported to easily biodegrade under compost environmental conditions [240;241]. The polymer was developed especially for applications using compost as the route of disposal. Today these types of polymers offer very good combinations of biodegradation and material properties and can be used for manifold applications. The tests were done under thermophilic conditions to reproduce municipal or industrial composting facilities. The biodegradation was also described under moderate environmental soil conditions with 29 strains of enzyme-producing soil bacteria, fungi and yeasts. A screening procedure was developed and the results show that after 21 days of exposure the polymer could be degraded especially by some of the microorganisms. Since the duration of these tests is rather short and moderate environmental conditions pose more limited ability for biodegrading the polymer, Ecoflex® was observed to be partially degraded within the timeframe. To study real environmental compartments the tests would require lasting longer. The microorganisms preferentially degraded the bonds between aliphatic components and the biodegradation, as expected, is faster for oligomers than for polymer chains. Degradation intermediates were detected and identified by GC-MS as monomers of the co-polyester. GPC results suggest that exo-enzyme type degradation occurs where microbes preferentially hydrolyze the ester bonds at the termini of polymeric chains [244]. Using polymers based on aliphatic-aromatic constituents together with other polymers such as PLA or PCL might lead to special co-polymers that offer increased biodegradability and good material properties depending on the requirements of the application both for the consumer and the environment.

In contrast, biodegradation of Ecoflex in soil has been already investigated as well as in compost. Especially the thermophilic environment promotes biodegradation. The study's performed in soil take much more time, but Ecoflex is mineralized as well only it takes at least over 150-200 days.

The group of biodegradable polyesters also contains multiblock poly(ether-ester)s based on PBS as "hard" and poly(ethylene oxide) as "soft" and hydrophilic components. When the content of PEO ($M_w \equiv 1000\ g \cdot mol^{-1}$) is varied between 10-50 w% material, structural, physical and biodegradation properties change. Biodegradation was observed in phosphate buffer and a lipase from *Candida rugosa*. Weight loss of the samples was in the range of 2-10 w% and significant molecular changes were confirmed by GPC to be up to 40% of initial values leading to the conclusion that degradation occurs through bulk degradation in addition to surface erosion of the PBS-PEO polymers [245].

The biodegradation of polymers containing lactic acid was observed to increase with the rising amount of lactic acid when copolymers of lactic acid, terephthalic acid, and ethylene glycol were synthesized and biodegraded by different fungal species (*Aspergillus* sp., *Mucor* sp., *Alternaria* sp. and *Rhizopus* sp.) [246].

2.14.4 Mater-bi®, Eastar bio®, PLA and PCL

The biodegradation of PLA, PCL and others (such as PBS and PBSA) has been investigated mostly in compost or soil environments [167;170;247].

PCL is synthetic polyester that can easily be biodegraded by microorganisms. PCL-degraders are widespread in the environment. PCL is degraded mainly by lipase and esterase [186]. PLA, also a biodegradable plastic is uptaken by animals and humans. Its application in medicine is widely studied and extensively developed. The general mechanism of degradation is thought to be non-enzymatic hydrolysis. The crystallinity seems to play an important role in that process as well. Several enzymes can degrade the polymer as well such as proteinase K, pronase and bromelain. Only few degrading microorganisms have been characterized yet. They are also not thought of being very widespread in the environment [186].

Four polymers (*MATER-BI®*, *EASTAR BIO®*, *NATUREWORKS PLA®*, PCL) were investigated in solid and liquid [248] and soil media [224]. Cellulose and PE were used as positive and negative control. 20µm films and PCL powder were tested. Quantitative tests were carried out following ISO 14851 and ISO 14853 in liquid phase. Qualitative tests were carried out in solid phase (aerobic) and liquid phase (anaerobic) as described in ISO 14851, ISO 14853, ASTM G 21-90 and ASTM G 22-76. Quantitative assessment shows that *MATER-BI* (42%) was the most degraded substance followed by PCL (40%), *EASTMAN BIO* (15%), and PLA (4%) after 28 days. Qualitative information obtained shows that microorganisms largely colonized especially Eastman bio and PCL in solid phase experiments. In liquid phase experiments no changes could be observed after incubation. But with pre and post incubation characterizations the differences could be seen [248]. *MATER-BI®* biodegradation was also observed for aerobic and anaerobic conditions using organic fractions of municipal solid wastes and anaerobic WWTP sewage sludge. Within 72 days of composting in aerobic sludge the polymer was degraded to 27%. Anaerobic degradation was faster and also the same in terms of biodegradation when compared to cellulose reference within of 32 days [162]. These results indicate that *MATER-BI®* biodegradation is better in anaerobic than aerobic environment.

PLA biodegradation has been investigated mainly in compost and was found easily degradable to 80-100% within 7 weeks similar to Avicell (Cellulose) [170]. When biodegradation was compared between real and simulated composting conditions a more detailed understanding developed. Cumulative measurement respirometric system (CMR) and gravimetric measurement respirometric systems (GMR) were compared and showed similar trends for simulated composting. The results were around 75-85% after 58 days of exposure (DOE). In real compost environment, biodegradation was correlated to molecular weight distribution shifts and breakdown. M_W of 4100 g·mol^{-1} was reached after 30 days of exposure. Results match well with theory and biodegradation mechanisms but still with some variability [249].

Hydrolytic degradation of PLA/PEO/PLA triblock copolymers was investigated after polymerization of PLA in the Presence of PEG 2000. The early stages of ester-bond cleavage occurred randomly along the PLA blocks. With advancing degradation, a swollen hydro gel layer composed of PLA/PEO/PLA copolymers with short PLA blocks, expanded from the surface. Once the polymer was placed in aqueous medium it absorbed large amounts of

water. Short PLA blocks derived from degradation of parent long blocks as confirmed with NMR. The hydro gel layer remained at the surface via hydrophobic interactions of micro domains. The degradation and material properties of these polymers should depend on initial degree of polymerization, crystallinity, LA/EO ratio and processing [250].

UV-Irradiation effects on enzymatic degradation of PLA were investigated by *TSUJI ET AL*. Therefore, amorphous and crystalline PLLA (PLLA-A and PLLA-C) films were investigated under UV-irradiation for 10 and 60h. The Proteinase K-catalyzed enzymatic degradation was observed. Molecular weight of both PLA samples can be altered when UV-irradiation time is changed. Weight loss of UV-irradiated PLLA films was similar or higher when compared to non-irradiated samples. UV-irradiation is expected to cause chain cleavage and the formation of C=C double bonds. It seems that this effect accelerates the decrease molecular weight supports faster enzymatic degradation [251].

Biotic and abiotic degradation of PLLA oligomers of molecular weight 260-2880 $g \cdot mol^{-1}$ has been studied in an aquatic aerobic headspace biodegradation test for six months. Water soluble dispensable PLLA's (MW 260-550 $g \cdot mol^{-1}$) were biodegraded at temperatures of 25°C and also 58°C. The larger, crystalline and hydrophobic oligomers (MW 550-2880 $g \cdot mol^{-1}$) could only be biodegraded at 58°C. The average molecular weights decreased both during abiotic and biotic degradation. The surface and inner structures of biotic degradation PLLA was more porous than those of abiotic experiments. These experiments show that abiotic hydrolysis is not the only explanation, though essential, for PLLA degradation. Also enzymatic cleavage seems to play its role [252].

Different poly(L-lactide-*block*-ε-caprolactone-*block*-L-lactide) polymers were synthesized to investigate biodegradation and hydrolysis effects at pH 7.4 and 37°C. The rate of hydrolysis depends on a sensitive combination of morphology and composition. The initial chain cleavage (day 0-7) was suppressed most by those systems with the highest ε-caprolactone (CL) crystallinity. In addition, microorganisms secreting PCL depolymerase (cutinase) show the ability to degrade systems with longer caprolactone sequence lengths. It appears that initial caprolactone crystallinity and overall composition controls the hydrolytic degradation since the PLLA phase is more susceptible to random chain scission. It has been shown that wild-type *Fusarium solani* and *Fusarium moniliforme* degraded those copolyesters with longer caprolactone sequence, while cutinase-negative strain *Fusarium solani* (mutant strain without cutinase) does not [253].

Different lactic acid based polyesters were investigated under controlled composting conditions. Therefore, poly(lactic acids), poly(ester-urethanes) and poly(ester-amides) were synthesized and the effects of different structure units were observed. Ecotoxicological impact of the compost was evaluated. All polymers degraded over 90% within 6 months. Toxicity was detected in poly(ester-urethanes) where chain linking of lactic acid had been carried out with 1,6-hexamethylene diisocyanate. The other polymers, chain-linked with 1,4-butanediisocyanate, showed no toxic effects [254].

A method for rapidly testing if polymers are biodegradable using oxygen consumption as the observed parameter and two consortia of fungi, one containing five and one three different fungi strains. Minor differences in the consortium result in major differences in the ability of the consortium to utilize the polymer as carbon

source. The exposure isolates biodegradation and therefore is excellent for reference material development for biodegradable polymers. It is also possible to pre-treat samples to initiate other degradation mechanisms, such as photo degradation, hydrolysis or physical deterioration. The test has the potential do indicate if a polymer is or is not biodegradable, but a ranking with regard to biodegradability requires longer times of exposure than this procedure allows. The method is quite sensitive, detecting small changes in organism's metabolism. Results are at hand within mere days. A well defined consortium is needed, since small changes in composition of the consortium results in markedly different results [255]. This test can be applied only if a sufficient understanding of microorganisms can be relied on since a change in consortium determines excessively the outcome of the results. Therefore, the environment were the test is set up is crucial as are the used strains.

In addition PCL, PE and PCL/PE blends were investigated using a 5 microorganism consortium. Polymers were exposed for 16 weeks in an NSM containing 0.8 mgL^{-1} potato dextrose. After exposure the samples were cleaned and analyzed for weight loss, changes in molecular weight (GPC), molecular changes (FT-IR) and tensile strength. While tensile strength began to decrease in PCL samples after 1 week of exposure, the molecular weight distribution showed no changes. FT-IR indicated a loss of amorphous PCL from the surface of the samples. PE samples were observed to be very recalcitrant and the consortium was not able to degrade the samples at all [256]. Systematic investigations on biodegradation of packaging materials made from PVA and PCL are described in detail by *HEINZ HASCHKE* et al. [257-259].

Block copolymers (PCL-PEG and PCL-PEG-PCL) were synthesized using a PEG with 4600 g·mol^{-1}, and enzymatic degradation was observed in a pH 7.0 phosphate buffer solution with *Pseudomonas* lipase. PEG introduction increases Hydrophilicity in the molecule and also makes the molecule more amorphous, the crystalline parts only being the PLA-blocks. The degradation of the homopolymer and also of the block-copolymers was observed to be nearly the same. The assumption was made that this feature is due to the increased hydrophilicity [260], but considering the study described for PHB degradation [234] it might also be possible that it is due to crystallinity.

2.14.5 Cellulose and cellulose-based polymers, lignocelluloses, lignin

Recent studies of cellulose, lignocelluloses and lignin, all being major parts of plant biomass and inevitable to carbon lifecycles, show that each polymer is degraded by a variety of microorganisms which produce a set of different enzymes that work synergically [261]. Most of the cellulytic microorganisms belong to eubacteria and fungi but some anaerobic protozoa and slime molds are able to degrade cellulose. The interactions lead to complete mineralization under aerobic and anaerobic conditions. Hemicellulose is degraded to monomer sugars and acetic acid. The biodegradation needs additional accessory enzymes such as xylan esterase, ferulic and *p*-coumaric esterase acting together to efficiently hydrolyze wood xylans and mannans. The biodegradation of lignin is somewhat more complex since its high molecular weight and the complexity of its structure as well as the insolubility delay biodegradation. Extracellular, oxidative and unspecific enzymes, releasing highly unstable products being subject to many further oxidation steps, catalyze the initial steps of lignin depolymerization.

This process has been referred to as “enzymatic combustion”. “White-rot”-fungi species are the most efficient microorganisms known up to date, degrading lignin [262].

Lignocelluloses degradation was studied using ^{14}C-labelled substrate in marine and freshwater tests and it was observed that ^{14}C was found partially in different sugars and also in mineralization product CO_2. The total lignin degradation was below 30% after over 600h [263]. Thermophilic anaerobic biodegradation of ^{14}C-labeled Lignin, ^{14}C-labeled Cellulose and ^{14}C-labeled Lignocellulose were studied for 60-days at 55°C. It was observed that degradation enhances at these elevated temperatures and degradation rates were 10- 15-fold higher than in previous studies at 25°C [264]. Later, 68 *Basidiomycetes* species were screened for enzymes involved in lignin degradation. Laccase activity was found in 50% of the fungi, 40% expressed aryl-alcohol oxidase and 29% showed availability of Mn-dependent polymerase. Laccase activity was highest obtained in the cultures and the over two enzymes were active significantly lower [265].

2.14.6 Starch and starch-based polymers

The biodegradability of starch and inulin and respectively oxidized forms of dialdehyde derivates was studied in biological oxygen demand and modified Sturm-tests. A higher degree of oxidation of dialdehyde starch and dialdehyde inulin results in a lower rate of oxygen consumption and mineralization over the incubation period (between 100% for starch and 25% for dialdehyde starch-100% oxidized; 60 day-Sturm-test and between 82% for inulin and 20% for dialdehyde inulin-100% oxidized; 60 day-Sturm-test). It is also demonstrated that the oxidized dialdehyde inulin derivates degrade far less than the equivalent starch counterparts do. The decrease in degradation can be attributed to changes in the polymer structure due to intra- and intermolecular acetal formation. Apparently, the oxidized starch and inulin derivates adopt different conformations, resulting in different susceptibility to microbial attack [266]. Anaerobic degradation has also been studied, but not many experiments have been performed up to now [267]. Few data on starch base plastics by white rot fungus *P. chrysosporium* was reported [268].

2.14.7 Polyamides

OPPERMANN ET AL. Reported the degradation of bio-polyamides (poly(γ-glutamic acid)) by certain microorganisms and in different media reaching from freshwater & soil to sewage sludge. Samples were analyzed with viscosimetric means determining a decrease in viscosity over the time of incubation. The stability against proteolytic attack was investigated using different protease enzymes. The ability of a non-adapted microbial community to degrade the test substance was tested in different media was investigated in enrichment cultures. Twelve isolates were able to use poly(γ-glutamic acid) as carbon source. To verify degradation pathways, different polymers were used as carbon source. One isolate was investigated closer. During the first 20h of inoculation only a small change in poly(γ-glutamic acid) concentration could be detected, but a significant decrease in average molecular weight was observed. During the next 70h both, average molecular weight and concentration dropped significantly. This observation of a two-phase degradation corresponds with an introductory fragmentation and a subsequent degradation of the oligomers. The amount of free glutamic acid increased

which could be determined with HPLC via the reaction with o-phthalaldehyde used in pre-column modification for amino acid analysis [269].

2.14.8 Poly(aspartic acid)

The biodegradable, water soluble, synthetic polypeptide has gained attention for being used as dispersant, detergent builder and in biomedical applications because of its biodegradation and environmentally friendly properties. Already, some studies have reported the structure dependent degradation behavior in activated sludge. Also a study regarding PAA degradation in river water and isolation of a degrading species has been reported. Analysis of PAA was done with GPC and it can be determined that the isolated *Sphingomonas* sp. strain degraded only low molecular weight PAA components but the cell extract could hydrolyze PAA polymers up to 150'000 g·mol^{-1} [270].

2.14.9 Poly(vinyl alcohol)

PVA is sort of a polymer with some special features regarding its structure and characteristics. It's a vinyl polymer with a main chain linked by only C-C bonds equal to those found in PE, PP, PS and also in specialty polymers such as poly acrylic acid or poly(acryl amide). Among all vinyl polymers manufactured, PVA is the only one known to be biodegradable by microorganisms [186]. PVA is water soluble but also has thermoplastic features. It can be molded in various shapes such as containers and films. This feature is used to make water soluble, biodegradable carriers for fertilizers, pesticides or herbicides and such. PVA degrading microorganisms are not ubiquitous in the environment and almost all reported strains able to degrade PVA belong to the *Pseudomonas* genus. Several enzyme systems have been reported to degrade PVA. The pathway was proposed to degrade PVA first by the action of a dehydrogenase, to yield poly(vinyl ketone), which was subsequently cleaved by a hydrolase enzyme to yield products with either methyl ketone or carboxylate termini (Figure 11). Several enzyme systems have been reported to degrade PVA. The carbon chain bond is always cleaved first of either a dehydrogenase or an oxidase and than it is followed by hydrolase or Aldolase reactions [186].

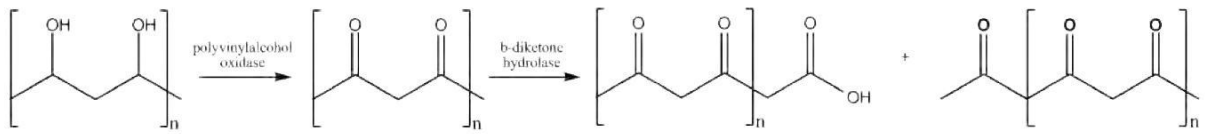


Figure 11 - Biodegradation of poly(vinyl alcohol) by Pseudomonas sp.

Anaerobic degradation has also been studied, but not many experiments have been performed up to now [267]. Mixed polymer films based on PVA, protein hydrolysate and glycerol were investigated in aqueous environment using unadapted current mixed culture from WWTP's. PVA was degraded in pure form only after 10 days of lag-phase. It was observed that when mixed polymers were used, the protein component and glycerol were degraded first and PVA was degraded in the second stage. 1st order kinetics described the process and when adapted organisms were used, lag-phase shortened and degradation occurred in one single step with a 1.5 fold increased breakdown rate. The number in PVA degraders during the process was observed to be 100

fold increased when substrate was present. The addition of protein hydrolysate and glycerol enhanced biodegradation more than assumed from proportional regression of individual components [271].

2.15 Analytical methods for polymer determination

In environmental analysis many methods are established to day. They range from simple physical or wet-chemical [272] tests to immunological tests and bioanalytical [273;274] arrays and further to sophisticated methods [275] with complicated setups and procedures. Mostly three steps (sampling, separation or enrichment and detection) are important and may be adapted to the task that is performed. Most methods described are used for "small molecules". Polymers are somewhat complex because of their often high molecular weight and it is more complicated to analyze these compounds in environmental matrices.

Analytical methods used for evaluating biodegradation of polymers are based on the chemistry of biodegradation either in aerobic or anaerobic tests. The level of biodegradation may be assessed by accurately establishing changes in concentration of the polymer (GPC, MALDI-TOF), the oxygen uptake (OxiTOP®), evolution of CO_2 (conductivity, TIC/DIC), removal of carbon (DOC/TC) or the incorporation of the polymer into biomass (radio labeling techniques) [89].

Substance specific analytical tools are powerful but often complicated. As a basis separations combined with mass spectrometry provide a huge variety for evaluation of many different problems [276].

2.15.1 Comparison of degradation techniques and analytical methods

A wide range of analytical methods has been generally used for the analysis of different polymers. An overview of several methods was presented [277;278], including sections about gas chromatography (GC), gel permeation chromatography (GPC), high performance liquid and thin layer chromatography (HPLC and TLC), atomic absorption and plasma emission spectroscopy (AAS and ICP), IR spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), surface analysis with scanning electron microscopy (SEM), reflection electron energy loss spectroscopy and reflection high-energy electron diffraction (RHEED), X-ray photoelectron spectroscopy (XPS) or electron spectroscopy for chemical analysis (ESCA) and further techniques. Also ultraviolet-visible spectroscopy (UV-VIS), X-ray diffraction (XRD) and thermal analysis (TA) techniques were used and described. Mass spectrometric methods for polymer analysis on synthetic are described as well [279-282] as is sample preparation and matrix/analyte effects [283]. Molar mass profiling and degree of polymerization/polydispersity determination can also be performed using solution capillary electrophoresis of DNA-polymer conjugates [284] for uncharged water soluble polymers that can be uniquely conjugated to DNA. As shown, Table 7 provides an overview of selected methods of biodegradation research with sophisticated analytical techniques.

Table 7 - Methods for biodegradation research linked to analytical techniques

Method	Polymer form (physiology, morphology)	Inoculum and degradation criteria monitored	Comments	Selected references
Gravimetry	Film or physical intact forms	Wide range of inocula from soil, water, sewage or pure species from culture collections	Robust method, good for isolation of degrading microorganisms. High reproducibility. Disintegration cannot be differentiated from biodegradation	[105;246;249;285]
Respirometry	Film, powder, liquid and virtually all forms and shapes	O_2 consumed or CO_2 produced under aerobic conditions or CH4 produced under methanogenic conditions	most adaptable to many materials. Specialized instrumentation may be required. When fermentation is the major mechanism of degradation, the method gives underestimated results	[105;138;140;163;249;286]
Surface hydrolysis	Films or sheets, pieces and others	Generally aerobic conditions, pure enzymes are used. Hydrogen ions released are monitored as incubation progress	Prior information about degradation of sample by microorganisms and particular enzymes is needed for a target specific test.	[105;201;222]
EIS	Films or coatings resistant to water	Test polymers should adhere on conductive materials. Electrochemical conductance is recorded	Sample must be initial water impermeable for signal transduction. degradation can proceed quickly and as it is registered no further degradation process can be distinguished	[105;204]
Radio labeling	All kinds of materials	Marine, soil, sewage, compost sediment etc.	Samples need to be 14C labeled	[263;287;288]
GPC/SEC	Virtually most polymers soluble in different solvents such as PEG, PVP, Ecoflex, Ecovio	Freshwater, Saltwater, CO_2-balance, DOC	Problems with environmental samples because extraction may be required	[289-291]
GC, GC/MS	Ecoflex and others, PHB, Xanthan, polysaccharide, Avicel®. Requirement: small molecules. MWD low!	Soil leachate, CO_2-balance; compost	Molecular weight can be limiting factor for this type of analysis!	[125;241;292-295]
HPLC, LC/MS	PEG, PVP, Requirement: small molecules. MWD low	Freshwater, Saltwater, CO_2-balance, DOC	Molecular weight can be limiting factor for this type of analysis	[289]
MALDI-TOF	PEG, PVP, Ecoflex, Ecovio molecules with higher molecular weight, synthetic polymers	Freshwater, Saltwater, CO_2-balance, DOC	Parameters optimized, important for polymer analysis	[125;289;290;294-298]
AFM	Particles adhered or dispersed to a substrate		Surface analytical procedure	[79;235;299]

Method	Polymer form (physiology, morphology)	Inoculum and degradation criteria monitored	Comments	Selected references
TEM	Thin and vacuum resistant, electron transparent samples	SW, sea water, activated sludge	Surface analytical procedure	[10;299;300]
NMR	Solid powder or liquid samples			[125;250;290]
SEM	Gold sputtered solid samples	Bacterial degradation, surface area	Surface analytical procedure	[222;246;250;299]
FT-IR	PE-Wax, PHB, Xanthan, polysaccharide, Avicel®, solid or liquid samples	--	fingerprinting technique	[163;292;301]
XRD	well-ground sample powders			[250;299]
DSC				[248;250]

2.15.2 Pyrolysis gas-chromatography/mass-spectrometry

Pyrolysis Ionization Mass Spectrometry can be used as rapid characterization technique for polymer analysis [302]. (Py-GC/MS) is known to be a relatively easy to use technique for direct analysis of polymers of small quantities (in mg range) of air-dried sediment samples or in extracts of polymers after Soxhlet extraction with dichloromethane (DCM) and precipitation in hexane. During analysis under inert atmosphere, copolymers often decompose to monomers and other information-rich fragments. These fragments are identified with MS after separation with GC. A problem though is the exact allocation of identified fragments to the specific polymer if one used a mixture of different polymers from a native sample.

Matrix effects due to inorganic phases, interference by organic matter and the lack of intense specific markers hamper accurate quantitative analysis of synthetic polymers by Py-GC/MS. Nonetheless, the technique is very rapid and requires a small size of untreated sample, as demonstrated in the analysis of suspended particulate matter (SPM), and therefore, is ideally suited for the rapid determination of polymer pollution. For more reliable identification and especially quantification, data from direct Py-GC/MS can be complemented by analysis of the purified plastic material as reference standard. However, isolation of polymers from sediments requires larger sample quantities and time-consuming steps (Soxhlet extraction), which may affect the overall yield [303;304].

Biodegradation of plastics in aqueous environment using soil leachate was analyzed using GC by determining CO_2 in the gas phase in dependence on ratio of liquid and gas volumes and pH of the liquid phase. Results were checked using standard TOC/TIC measurement and the method was confirmed to be useful because of simplicity, robust, and variable in test conditions [292;293].

2.15.3 Gel-permeation-chromatography

Gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC) is used to analyze polymers according to their molecular weight distribution. The one problem with this method is that it can hardly be applied to aqueous solution. Therefore a separation of the target analytes from its matrix (SPM, water etc.) would be needed [305]. *SE ET AL.* provide an overview about using GPC equipped with a low-angle laser light-scattering (GPC-LALLS) detector for the analysis of different polymers and block copolymers [291].

A promising approach seems to be the combination of GPC with Py-GC/MS. It has proven to be a powerful tool in compositional analysis of polymers, thus desirable because it allows one to monitor the composition of structurally similar monomers as a function of molecular weight [295]. Via a solvent evaporative interface, the eluent of the GPC experiment was deposited on common aluminum foil, cut at selected GPC retention times (RT) corresponding to certain molecular weights, and along with the polymer residue analyzed via Py-GC/MS.

2.15.4 Elektrospray ionization mobility analysis

Charge reduced ESI mobility analysis for high resolution distribution determination of water soluble polymers were proven an effective tool, since some weaknesses of MALDI and other techniques were overcome [306]. The technique was then applied to water insoluble polymers such as PMMA and PS [307].

2.15.5 Sophisticated liquid chromatography mass spectrometry techniques

Soft ionization techniques such as electro spray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) have provided research personnel with the possibility to evaluate polymer samples with several different mass analyzers. These methods have been used and optimized [298] for repeat unit and end group determination as well as for analyzing relative concentration and mass determination. In general the determination of average mass values with high accuracy is based on the polymers analyzed having a relatively narrow polydispersity index (PDI) of about 1.2 [296]. To overcome the mass discrimination effects, occurring with more broadly dispersed polymers, such as high molecular weight oligomers within the molecular weight distribution, incorporating GPC prior to MS is presented as possible strategy. With GPC-MS the complexity of broad molecular weight distribution is minimized by independent evaluation of near mono-dispersed chromatographic fractions [296]. Sample preparation [308] and different techniques [309] and limitations [310] were investigated.

Fractions can be collected either manually in a series of different vials, which is rather time consuming and tedious since every sample needs to be prepared prior to MS analysis, or in a more automated approach. On-line couplings of GPC with ESI are commonly used since solution infusion characteristics allow these techniques to be easily combined. GPC couplings with MALDI are reported [311], however to date most GPC-MALDI analyses are based on deposition of GPC eluent on a solvent evaporative device, such as a MALDI plate. This is not a true on-line hyphenation when compared to GPC-ESI/MS but rather a two-step combination of the methods. It is to be considered in this context as automated or semi-automated procedure [295;296].

The analysis of polymers using sophisticated MALDI techniques are reported for PVP [3], PEG [311-315], POE and POP [297], PBA and PBAS [316], PP and PS [312;313], PBA and PMMA [302;312], aromatic polyamides [317]. Time-of-flight secondary ion mass spectrometry is also a highly useful tool for depth profiling of polymers and polymer blends. It was reported for various studies on PLA [318] and could also be used to investigate microbial attack to polymeric surfaces.

2.15.6 Nuclear magnetic resonance

The characterization of PLA/PEO/PLA triblock copolymers and the investigation of its hydrolysis process were determined with ^{1}H and ^{13}C NMR, size exclusion chromatography, X-ray diffraction and differential scanning calorimetry [250].

2.15.7 Microscopy

In order to study morphological alterations of certain polymer structures during or after biotic as well as abiotic degradation processes one may use scanning electron microscopy (SEM). This method can specifically be used to study bacterial hydrolysis for example. Sheets of different plastics are exposed to environmental conditions for a certain time span, than analyzed and compared to original, untreated samples. *MOLITORIS ET AL.* describe a method for using SEM in a study of polyhydroxyalkanoate (PHA) degradation [222] to observe specific PHA degrading bacteria. SEM can be used probably also to get an understanding what happens to certain polymers under special condition such as being in saline environments regarding physical properties. Merging that information, when obtained for different polymer structures, with known guidelines in polymer research and development, combined with a mass spectrometric method of analysis might prove a valuable asset in developing new polymer structures. The visualization of morphologies and enzymatic degradation of PLLA by proteinase-K, extracted from *Tritirachium album*, has been shown using SEM/TEM, AFM, X-ray diffraction, and HPLC. Enzymatic degradation of single crystals progressed from the edges rather than the chain-folding surfaces. molecular weight of PLLA chains in the crystals and the thickness of mono lamellar parts remained unchanged [299].

2.15.8 Further methods

There is a broad variety of further methods used for analyses of polymers and polymer composites. Depending on the intended result or the desired information these methods might be useful in a certain way. For the purpose of determining polymer degradation in marine environments these methods are only of minor interest. *ANDERSON* describes in a few review publications methods like ^{13}C- and ^{19}F-NMR two dimensional studies, Fourier-transform IR spectroscopy (FT-IR) studies of coating durability and weathering phenomena, X-ray diffraction for elemental composition, crystallinity and a few others [277;278;319-323].

2.16 Empirical and mathematical methods of degradation modeling

Modeling of bioprocesses such as biodegradation or the prediction of the environmental concentration (PEC) plays an important role in evaluation of results as well as predictions or estimations of an impact in environmental assessment. Not many mechanistic models exist for polymer biodegradation up to now [324]. Generally these models were applied to “small molecules” (active substances and others) from pharmaceuticals, plant science products, personal care products, etc.

Some recently developed mathematical or empirical methods were applied to predict the results of biodegradation experiments [325-327]. One of the empirical models named CATABOL is based on expert knowledge that is used to predict ecotoxicological and toxicological behavior of chemical structures in specific test methods. Such models are evaluated in regard to assess biodegradation in standardized tests using QSAR methodologies [328-330] but also special models for predicted environmental concentration (PEC) assessment [331] or applied to specific processes such as Fenton oxidation [332] or particle size distribution are used [333].

2.17 Microbiological methods

Microbiological methods have been applied for many years to investigate communities from environmental samples and many comparisons have been published [334]. Also optical methods such as different microscopy techniques were investigated [334;335].

There are different ways to culture and analyze suspensions of microorganisms from the environment but especially marine native samples are problematic because many organisms are uncultivable in the laboratory. In the beginning, cultures were established on agar-plates [336] but recent investigations using molecular phylogeny have shown that a huge abundance of previously unknown microorganisms can be found and that cultivation is not as easy for some organisms as thought to be. New methods are based mainly, dilution culture [337] and some strains have been investigated closely [338-341]. Also gel micro droplets as encapsulation of cells has opened a new path to untapped resources of uncultured communities [342]. Genetic evidence suggests that diverse uncultivated microbial taxa dominate most natural ecosystems. The technique of isolating cells by dilution into sterile medium takes advantage of the fact that substrate concentration and cell numbers in natural waters are typically three orders of magnitude less than in laboratory media [343]. This technique has also been described and used for high-throughput culturing in micro titer plates (MTP). It was observed that in dilution culture the cultured species were 14 to 1400 fold higher than in common methods [344].

2.18 Molecular fingerprinting techniques and microbial diversity

Molecular methods are a valuable tool for detection, identification and characterization of microorganisms found in environmental samples, foods and other complex ecosystems [345]. Today culture independent molecular methods are required for understanding total micro biota since culture-based methods do not cover the entire microbial diversity in complex matrices because of selectivity.

Biodiversity [346;347] has become a huge issue during recent years and there are several approaches available to assess the diversity in communities [348-350]. Diversity in marine habitats has been studied extensively through recent years [340;351-353] and data on biodegradation in marine environmental compartments is still sparse [17;142;354-359]. Many reports on chemical and molecular approaches have been reported [360;361] and techniques such as fluorescent in-situ hybridization (FISH) [362;363], flow cytometry [364-369], PCR or RT-PCR [370;371], DGGE/TGGE [372-374], DNA or functional gene microarrays (FGA) [375-381], and terminal restriction fragment length polymorphism [382] are widely used to determine microbial communities and establishing phylogenetic trees [353;383-385].

BROSIUS et al. [386] described the complete nucleotide sequence of the 16S ribosomal DNA (rDNA) from *Escherichia coli* which led to further development of designed primers for detection of single bacterial species, as well as universal primers intended for population analyses. The 16S rDNA has several benefits making it a suitable identifier. 16S rDNA occurs in all bacteria consists variable and fixed regions and can therefore be used for species differentiation. Genes that encode the 16S rDNA can identify an organism's taxonomic group relationships between organisms [387]. The benefit of using 16S rDNA is that it can be rapidly sequenced. Online electronic databases with large amounts of available sequences (EMBL database of the European Bioinformatics Institute: http://www.ebi.ac.uk/embl/ and the BLAST database of the National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/BLAST/, allow comparison of determined sequences [388]. The identification of unknown bacterial populations and species by 16S rDNA analysis is shown schematically in Figure 12.

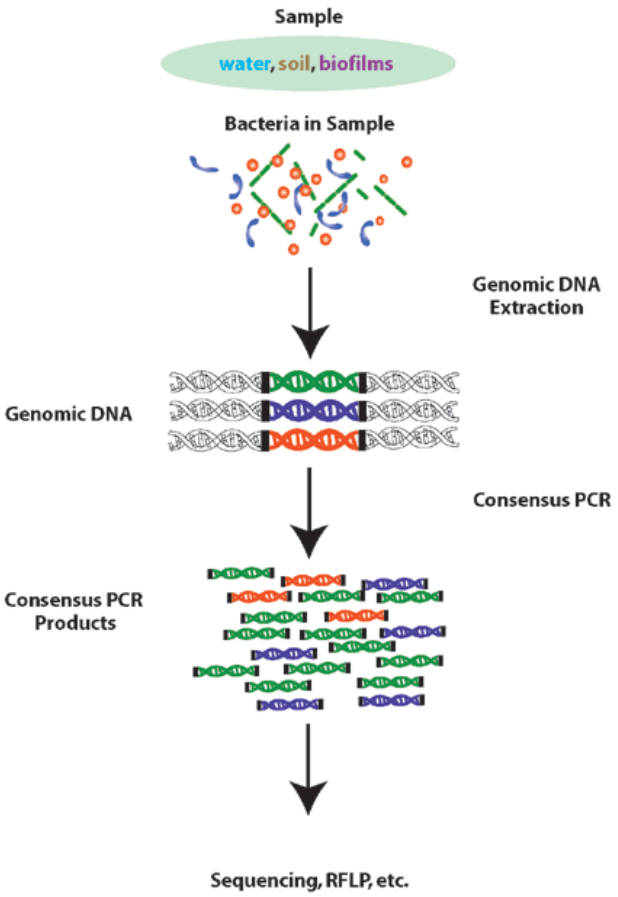


Figure 12 - Procedure for processing of genomic DNA

2.18.1 DNA Extraction

Many protocols exist today for extracting environmental DNA and processing of samples [389;390]. Optimization of the extraction is important for quantitative investigations or when low volumes (2-300 mL samples) are available. Comparisons of results when using different sample volumes are mostly complicated [391].

2.18.2 DNA-Amplification using PCR

Originally theoretically developed by *KARY MULLIS* in the early 1980s the procedure of DNA amplification was first described briefly investigating the mutation that causes sickle cell anaemia [392;393]. Details of the method were discussed more and more over the next few years [392;394]. PCR revolutionized molecular genetics by allowing rapid duplication and analysis of DNA and was also rewarded with the Nobel Prize in 1993.

PCR is used to amplify a specific region of DNA in order to produce a large number of nearly identical copies. The method uses a heat stable DNA replication enzyme called a DNA polymerase, the four desoxynucleotide building blocks of DNA and two small single-stranded DNA segments called primers, which flank the "target" region of DNA to be amplified and are complementary to each strand (meaning the matching strand to which its bases pair).

There are 3 basic steps in PCR carried out at different temperatures (for optimal conditions):

- DNA denaturation (meaning to separate the double-stranded DNA into single strands).
- Primer binding or hybridization to each of the single strands of DNA at either the beginning or the end of the target sequence, depending upon the single-strand of DNA. Hybridization combines complementary, single-stranded DNA into a single molecule. This process is called annealing.
- DNA polymerase elongation. The enzyme attaches to the primer-single-stranded DNA duplex and synthesizes the complementary strand of DNA, using the existing single-strand as a template.

Newly synthesized DNA strands can serve as additional template for complementary strand synthesis. PCR rapidly amplifies DNA because both strands are copied resulting in exponential increase in the number of copies. Assuming there is only a single copy of the target gene before cycling starts:

- Cycle Single-strand Copy Number
- Cycle 1 4 copies (22)
- Cycle 2 8 copies (23)
- Cycle 3 16 copies (24)
- … …
- Cycle 35 68.7 billion copies (236)

After 35 cycles of PCR, theoretically over 68 billion copies are made. PCR starts with many copies of the target gene in reality so the result is typically higher. Each cycle only takes a few minutes. Factoring in the time to change temperatures, the entire process can be done in several hours.

Many primers have been designed to amplify variable regions of the rDNA. It was shown that gene sequence specific primers give better amplification results and lesser artefacts than universal primers [395]. The review of *ERCOLINI* [396] summarizes primers targeting the different variable regions of the 16S rDNA. The 16S rDNA primers can be universal, targeting theoretically all bacteria from a sample, or they can be species-specific and detect specific bacterial groups such as LAB (LAB). The heterogeneity of 16S rDNA, resulting in multiple copies of the sequence, is one of the disadvantages when using it as a target region for amplification [397]. The average number of 16S rRNA genes per genome has been reported to be 4.1 for the domain Bacteria [398]. *SCHMALENBERGER* et al. [399] found that the heterogeneity varied between the different variable regions on 16S rDNA. For the regions V2-V3 an average of 2.2 bands per organism was found. For the V4-V5 region, 1.7 bands were observed and 2.3 bands were detected in the V6-V8 region. Other target regions or genes have been suggested to overcome the disadvantages of 16S rDNA. The RNA polymerase subunit gene (rpoB) seems present in only one copy [400;401]. In contrast, the use of rpoB presents a disadvantage since the database of the sequence is less documented than of 16S rDNA. Another approach for studying diversity uses group-specific primers or amplification of bacterial functional genes. Functional genes are especially suitable when investigating structure-function relationships [402].

2.18.3 Denaturing Gradient Gel Electrophoresis

A large number of molecular methods have been developed for examination of microorganisms in complex samples. Denaturing gradient gel electrophoresis (DGGE) is a widely used molecular fingerprinting method [403-405] that separates polymerase chain reaction (PCR) generated DNA products. It was shown that DGGE may not be suitable for detection of the most abundant organisms but more for numerical important organisms in environmental samples [406]. The PCR of environmental DNA generates templates of differing DNA sequence that represent many of the dominant microorganisms. PCR products from a given reaction are of similar size (in terms of bp) and conventional separation by agarose gel electrophoresis gives only one single DNA band however non-descriptive. DGGE removes this limitation by separating PCR products based on sequence differences that result in differential denaturing characteristics e.g. melting based on the GC content of the DNA fragments in a gradient of DNA denaturants and an electronic field. The “melting domains”, defined as stretches of base-pairs with identical melting temperature are sequence-specific [407]. The DGGE Procedure is schematically shown in Figure 13.

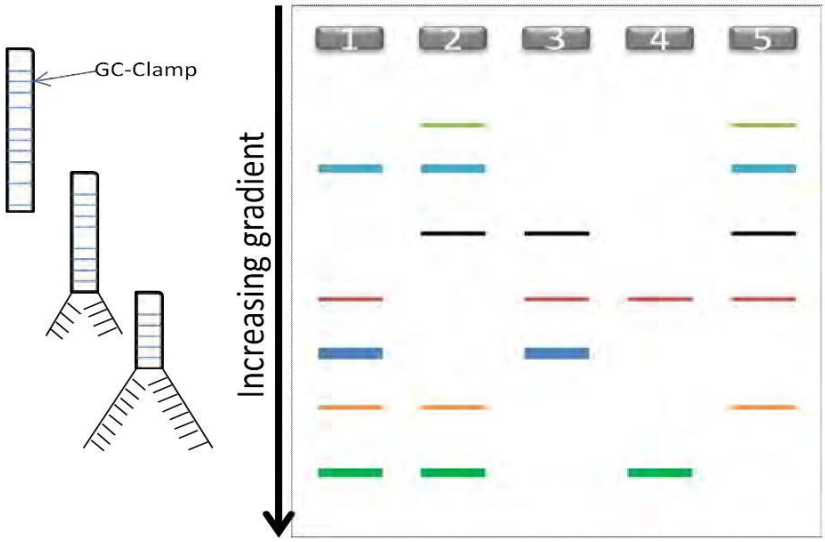


Figure 13 - The principle of denaturing gradient gel electrophoresis (DGGE). Double stranded DNA fragments from PCR are separated (PAA-gel with denaturing gradient). Increasing gradient of denaturants causes DNA to melt and separate while moving through the gel. The GC-clamp attached to the 5'-end of the PCR fragment prevents complete denaturation

With the broad range of available PCR primers DGGE can be used to investigate broad phylogenies or target organisms such as pathogens or xenobiotics degraders.

For initial fingerprinting analysis, the DGGE gel can be used directly. The bacterial profiles from the gel are also useful when analyzing multiple samples over time, and to reveal profile differences. Time studies can also be achieved when samples taken at different time points are compared on the same gel. To identify the origin of DNA in gel bands of special interest, the bands can be recovered from the gel and sequenced. By sequencing the band, the bacteria present in the sample can be determined, based on the DNA sequence information (Figure 14).

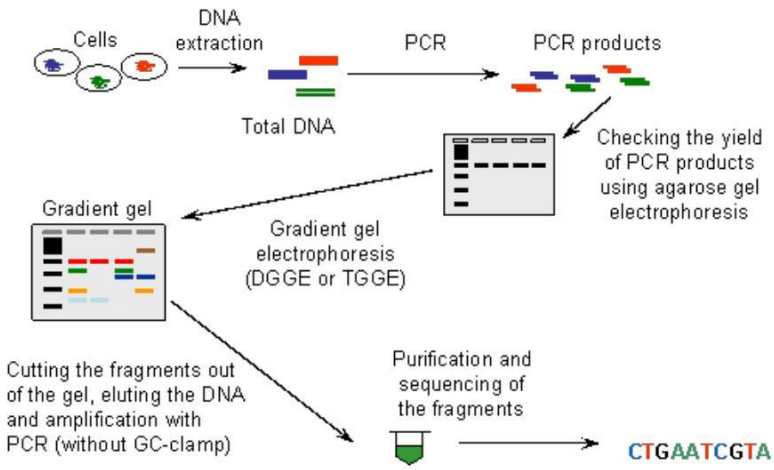


Figure 14 - From sampling to bacterial detection and identification. DNA extractions, amplification and separation on a denaturing gradient gel before bands of interest is sequenced

The DGGE approach represents a rapid and reproducible method of studying population dynamics and to determine cultivable and uncultivable microorganisms.

2.18.4 Limitations of molecular methods

Generally, sampling and sample handling are known to produce biases. An obvious source of variability for molecular methods is the extraction of bacterial DNA especially from a complex matrices or mixtures of cultured bacteria. Environmental samples include proteins, humic acids, fulvic acids, enzymes and polysaccharides, making it a difficult sample for all types of analyses. Interference, inhibition or enhancements of the following analyses are often unknown.

PCR reaction itself provides many pitfalls. The main issue associated with the analysis of complex samples is the presence of substances in the DNA mixture. These substances can inhibit or at least affect PCR amplification. To minimize this effect, well established and verified PCR conditions and procedures were used in the present study. Also, the band intensity may reflect the relative amount of particular bacteria or a bacterium for which the PCR amplification is favored. Despite these and other limitations, DGGE is still considered as one of the few techniques allowing a fast and reproducible microbial analysis of microorganism community.

2.18.5 Statistical support and database development

Today, it is very important that the information obtained with the described methods is available to the scientific community. Databases are available and growing extensively today [408-410]. It has also been established to support information using modeling techniques and combine all the information obtained and to evaluate the data statistically [411].

2.18.6 Bacterial detection limits

One of the major problems and concerns for any quantitative bacteriological analysis is the detection limit. The sensitivity of PCR-DGGE is based on the PCR reaction and its ability to amplify bacterial DNA. To get the best possible results, the product must be as pure and as concentrated as possible. Theoretically, one cell in a 10µl sample added to a PCR reaction of 100µl total volume, corresponding to 100 $cfu \cdot ml^{-1}$, can be amplified by PCR. Generally, the sensitivity in complex samples is reduced due to a wide range of inhibitory substances.

The fact that PCR does not distinguish between alive and dead cells is both an advantage and disadvantage. PCR amplification is dependent on intact nucleic acid, rather than viable or non-viable cells. Positive PCR amplification and the presence of a PCR product do not imply that the target organisms were viable. PCR can detect viable but non-cultivable (VBNC) and dead cells. This is a benefit in marine systems, since many microorganisms seem uncultivable. In consequence PCR amplification may result in false positive results. Despite the possibility of false positives, the predominant population will represent the cultivable bacteria during storage and the bacterial profile of DGGE should be represented by bacterial DNA from the dominant, viable species rather than the dead cells.

2.18.7 Polyester cleaving enzymes

Different BTA copolyesters have been investigated in an aerobic compost medium and especially *Actinomyces* species and fungi could be determined as highly capable of cleaving the ester bonds. With these Isolates degradation screening experiments can be accelerated considerably. Similar experiments with BTA polymers as well as PHA samples were done under anaerobic conditions. About approx. 100 isolates could be obtained and identified as mostly Clostridia [226]. Interestingly, the substrate spectrum of those microorganisms is rather narrow. Those degrading natural polyesters cannot affect synthetic ones and vice versa. For PHA degradation specific depolymerase enzymes are responsible which cannot degrade aliphatic polyesters, while less specific lipase and hydrolase enzymes are responsible in degradation of the latter.

The isolation of a polyester-cleaving enzyme from a thermophilic *Acetomyces*, *Thermomospora fusca* (DSM 43793), which proved to be a rather efficient microbial isolate in cleaving polyester-bonds is described by *KLEEBERG* et al. [182]. The cleaving enzyme is produced only after induction with insoluble BTA co-polyester. It has a molecular weight of 25.5 kDa and a pI-value of 6.3. The enzyme showed a 10 times higher hydrolysis activity compared to *Pseudomonas sp*. lipase and it was capable of cleaving aliphatic-aromatic polyesters as well as pure aliphatic polyesters and polyester amides. The enzyme showed no activity regarding PHB, which can be interpreted, that it seems to be a lipase. Further investigations show that BTA polymers were fully degraded to monomers after a short time. The cleaving organism did not further utilize the monomers.

A polyester-degrading extracellular hydrolase from thermophilic actinomycete *Thermomospora fusca* was produced and investigated. The excretion of the enzyme could be achieved with an optimized medium and only in the presence of a polyester from 1,4-butanediol, terephthalic acid and adipic acid with around 40-50 mol% terephthalic acid [180;412].

3 Materials and methods

3.1 Chemicals and laboratory material

Utilized chemicals (salts, solvents, acids etc.) and material as well as molecular Markers, PCR equipment, Enzymes and Kits were bought from Merck/VWR-International® Darmstadt, Germany or Sigma Aldrich, Germany in analytical grade. Consumables and required materials are given in Table 8 and technical equipment is stated in Table 9.

Table 8 - Consumables used in this work

Consumable supplies	
Glass pasteur pipettes, open jet, length 150 mm, 230 mm (VWR®, Darmstadt, Germany)	Glass fiber filter GF6, ∅ = 55 mm, 0.45 µm (Schleicher & Schüll®, Dassel, Germany)
Agar plates, sterile, VWR International, Germany	Syringe filter: Spartan 13/0.45 RC, 0.45 µm, brown rim L (Schleicher & Schüll®, Dassel, Germany)
Eppendorf Tips, grey, yellow, blue, Eppendorf AG Hamburg, Germany	Single-use fine dosage syringes Omnifix®-F 1mL (B.Braun®, Melsungen, Germany)
Eppendorf PCR vessels, 1.5 & 2.0 mL, Eppendorf AG Hamburg, Germany	Single-use sterile syringes 10mL, 20mL 60mL (B.Braun®, Melsungen, Germany)
Digralski-spatula, VWR International, Germany	Cannulae: Stercican®, ∅=0.80, 40 mm (B.Braun®, Melsungen, Germany)
Piston pipettes 1, 2, 5, 10, 20 mL amber grad. (Hirschmann®, Eberstadt, Germany)	Sterile filtration units MF75, 75mm 0.45µm pore size, Nalgene, Rochester, NY 14625. U.S.A.
Serological pipettes, 5mL, 10mL, Falcon, VWR International, Germany	MTPs 96-well, Eppendorf
Falcon Tubes 10mL, 50mL	Beakers and other glass vessels

Table 9 - Technical equipment used in this work

Technical equipment	
Carbon Analyzer TOC 3000 & TOC 4000, Shimadzu, Duisburg, Germany	Dispenser, Eppendorf AG, Hamburg, Germany
UV-Vis Spectrophotometer, Perkin-Elmer Lambda 2, Perkin Elmer, Rodgau, Germany	Eppendorf Research Pipette, various sizes Eppendorf AG, Hamburg, Germany
Ultrasonic bath Transonic T460, Elma, Singen, Germany	Eppendorf Multipette and Tips, Eppendorf AG, Hamburg, Germany
Temperature bath, Julabo VC, Seelbach/Germany	Precision balances: Scout® Pro SP202, range: 1mg–200g, e=0.01g; Scout® Pro SP6000, range: 1g–6000g, e=1.0g (Ohaus®, Pine Brook, NJ, USA)
Magnetic stirrer: IKA Combimag REO & RCT, 0-1250 min-1 (Janke & Künkel, Staufen i. Breisgau, Germany)	Microbiological bench, Köttermann 8511, Köttermann, Uetze/Hänigsen
Tube shaker/Vortexer: REAX 2000, 200-2400 min-1 (Heidolph®, Kelkheim, Germany)	Membrane pump MZ2C, Vacubrand, Wertheim, Germany

Technical equipment	
Conductivity-meter WTW Cond 340 and Cond 340i, WTW Weilheim, Germany	Centrifuge Hettich Rotana Type 3500, Hettich, Tuttlingen, Germany
Drying oven Type B12, Thermo Electron/Heraeus, Hanau Germany	Centrifuge Hettich Universal 16, Hettich, Tuttlingen, Germany
Drying oven T5042K, Heraeus, Hanau Germany	Centrifuge Hermle Z300K, Hermle, Wehingen, Germany
Analytical balance: Type A 200 S, range: 0–200 mg, e=0.1mg (Sartorius®, Göttingen, Germany)	MilliQ Plus water purifying system, Millipore, Schwalbach, Germany
Precision Balance Mettler PC4400, Mettler-Toledo, Gießen, Germany	Schott pH-meter Type CG842, Schott, Mainz, Germany
Precision Balance Mettler PM4000, Mettler-Toledo, Gießen, Germany	Precision Balance Mettler PM460, with IR-drying module Mettler LP16, Mettler-Toledo, Gießen, Germany
Precision Balance Mettler Toledo AT200, Mettler-Toledo, Gießen, Germany	Precision Balance Mettler Toledo AT20, Mettler-Toledo, Gießen, Germany
Precision Balance BP2100S, Sartorius, Germany	BioRad UV Detection system
BioRad DCode™ Universal Mutation Detection System	BioRad Gradient mixing system Model 385
Agarose Gel Chamber (Self-made by BAH Helgoland)	Eppendorf PCR Thermo cycler Master cycler, Master cycler gradient
Eppendorf Thermo mixer 5436 Comfort	Eppendorf Centrifuge 5417R

3.2 Evaluated polymers (Test substances)

The polymers studied in this work are given in Table 10. They were carefully selected to deliver specific properties for biodegradation tests. It was intended to compare specific tests with water soluble and water insoluble polymers and where no biodegradation occurs with polymers where biodegradability has already been demonstrated partly or completely in aqueous or solid environment. Also it was important that analytical methods could be established to determine these polymers in the desired aqueous marine and wastewater media as given in the work of *BERNHARD* [1]. To select the polymers for this work the literature was reviewed and summarized in this work. As a result the following polymers were selected because of properties, absence and/or presence of data probability of success for this project in regard to the aim of the study.

PVP samples were used as a polymer mostly known not to be biodegradable. It was evaluated using marine medium to check whether under long-term conditions no biodegradation would also be observed and also to confirm that the tests could be extended using the same medium for quite some time for testing biodegradability. Ecoflex and its similar polymers were selected because of their ability to biodegrade in compost and soil environments. I had to be shown that under marine and freshwater conditions biodegradation would occur. Also it should be investigated that biodegradation is different when the environmental compartments are switched and if there is some sort of possibility to use knowledge about biodegradation in one compartment to estimate biodegradation in another one. PEG samples were selected because PEG is available in a broad range of molecular weight, it is water soluble and it is known to biodegrade in at least WWTPs. Using PEG it was a main aspect of this work to compare different test systems and media used for biodegradation research.

Table 10 - Polymer Samples used for biodegradation studies

Product Name	Sample #	Type	composition
Cellulose (β-1-4 Glucose) (Starch: α-1-4 glucose)		condensation polymer of glucose	cellulose subunits
Ecoflex® F BX 7011	P01	aliphatic aromatic co-polyester (BTA polymer)	22.2 mol% terephthalic acid; 27.8 mol% adipic acid; 50 mol% buntanediol
Ecoflex® 200-300µm	P01a	aliphatic aromatic co-polyester (BTA polymer)	22.2 mol% terephthalic acid; 27.8 mol% adipic acid; 50 mol% buntanediol
Ecoflex® <100µm	P01b	aliphatic aromatic co-polyester (BTA polymer)	22.2 mol% terephthalic acid; 27.8 mol% adipic acid; 50 mol% buntanediol
Ecovio® 2099 L BX 8145, Ecoflex Blend (+ PLA 4041D + Ecoflex Batch SL1 + Ecoflex Batch FBA	P04	Blend	53% Ecoflex F BX 7011 45% NatureWorks PLA 4041D 1% Ecoflex Batch SL1 + 1% Ecoflex Batch FBA
Ecovio® 2129-2, L BX 8180, Ecoflex +PLA 4041D + Citrofol + Ecoflex Batch FBA	P05	Blend	19% Ecoflex F BX 7011 70% NatureWorks PLA 4041D 10% Citrofol B1 + 1% Ecoflex Batch FBA
Pluriol E 200	P06, P06b	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 1000	P07	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 2000	P08	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 4000	P09	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 6000	P10	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 8005	P11	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 9000	P12	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 20000	P17	polyalkyleneglycol, polyether	ethylene glycol
Pluriol E 35000	P18	polyalkyleneglycol, polyether	ethylene glycol
Kollidon 12 PF (PVP)	P33	poly vinyl pyrrolidone	vinyl pyrrolidone
Kollidon 17 PF (PVP)	P34	poly vinyl pyrrolidone	vinyl pyrrolidone
Kollidon 25 (PVP)	P35	poly vinyl pyrrolidone	vinyl pyrrolidone
Kollidon 30 (PVP)	P36	poly vinyl pyrrolidone	vinyl pyrrolidone
Kollidon 90F (PVP)	P37	poly vinyl pyrrolidone	vinyl pyrrolidone

Additionally for the tests three substances, namely aniline, sodium benzoate and cellulose (Avicell) were used as reference substances to confirm the activity of the test media as described by the guidelines (OECD and ISO etc.). Specific physicochemical parameters of the selected polymers are provided in Table 11.

Table 11 - Physicochemical data on the polymer samples

Probe #	Mn	Mp	Dp	Mw [g·mol^{-1}]	density [g·mL^{-1}]	soluble in	TC [mg·g^{-1}]
Cellulose				50000-500000	1.52-1.59 other source: 0.6	DMS + paraformaldehyde mixture	429
P01	32,852	53,815	1.92	63,049	1.25-1.27	hexafluoroisopropanoic acid (HFIP), THF, CHCl3	630
P01a	12,429	40,136	7.04	87,184	1.25-1.27	hexafluoroisopropanoic acid (HFIP), THF, CHCl3	630
P01b	11,994	41,356	7.17	84,729	1.25-1.27	hexafluoroisopropanoic acid (HFIP), THF, CHCl3	630
P04	49,339	78,880	2.12	104,490 (Natureworks 4041D)	1.24-1.26	hexafluoroisopropanoic acid (HFIP), THF, CHCl3	567
P05	53,408	79,354	2.42	128,980 (Natureworks 4041D)	~1.3	hexafluoroisopropanoic acid (HFIP), THF, CHCl3	549
P06, P06b	120	275	2.10	251	1.09-1.12	Water, many organic solvents	540
P07	680	967	1.37	930	1.09-1.12	Water, many organic solvents	540
P08	1424	2114	1.41	2009	1.09-1.12	Water, many organic solvents	540
P09	3967	4401	1.14	4519	1.09-1.12	Water, many organic solvents	540
P10	5444	7550	1.36	7426	1.09-1.12	Water, many organic solvents	540
P11	8051	10599	1.28	10310	1.09-1.12	Water, many organic solvents	540
P12	10909	14846	1.34	14629	1.09-1.12	Water, many organic solvents	540
P17	15548	25807	1.71	26647	1.09-1.12	Water, many organic solvents	540
P18	25075	45161	2.30	57759	1.09-1.12	Water, many organic solvents	540
P33				2000-3000		Water	648
P34				7000-11000		Water	648
P35				28000-34000		Water	648
P36				44000-54000		Water	648
P37				1000000-1500000		Water	648

3.2.1 Project and sample codes

In order to accurately label all test related material, results and data a systematic structure was used. The following paragraphs give explanations on how the labeling system is build and what information it contains.

3.2.1.1 Project codes for degradation tests

All projects and samples were labeled following a unique and standardized procedure. All projects and samples can be identified with the codes described below. The sample and project codes contain a number of information, e.g. the code 29G P7 #0.1 MA is composed of the parts 29G (test method), P7 (sample ID), #0.1 (serial number) and MA (characterizing medium and inoculum).

Table 12 - Labeling schematics for biodegradation tests (first part): 29G P7 #0.1 MA

first part: test method, name of experiment (e.g. 29G)		second part: sample ID (e.g. P7)		third part: serial number (e.g #0.1)	fourth part: inoculum (e.g. MA)	
21G	DOC die away test, OECD 301A, ISO 7827	sample ID (see also sample list)		project number/serial	medium/inoculum	
22G	CO_2 evolution test, modified "Sturm-Test" OECD 301B, ISO 9439	P1	Ecoflex® granules	#0.1	MA	marine inoculum, synthetic marine water
23G	closed bottle test, BOD, OECD 301D, ISO 10707, BASF SOP-DEG 7.1.23	P1a	Ecoflex® 200-300µm 04/0595-1	#0.2	MAn	marine inoculum, natural marine water
29G	combi test (measurement of DOC & CO_2 evolution) ISO 9439:1999, OECD 301A & B; BASF SOP-DEG 7.1 29	P1b	Ecoflex® <100µm 04/0595-2	#0.3	KA	WWTP inoculum, activated sludge
32G	marine CO_2 evolution test ISO 16221:2001, ISO 9439:1999, OECD 306; BASF SOP-DEG 7.1.32	P4	2099 EcoVio® L BX 8145 granules	(…)	KAef	WWTP inoculum, WWTP effluent
33G	Marine Closed Bottle Test, BOD Measurement. OECD 306, ISO 16221:2001	P5	2129-2 EcoVio® L BX 8180 granules	#1.0	SW	SW, river Rhine
36G	conductivity test, modified-Sturm-Test, ISO 9439:1999, OECD 301B; BASF SOP-DEG 7.1.36	(…)	(…)	(…)	GW	GW
MB	Microbiology Experiment	P37	Kollidon 90F (PVP)	(…)		
MoB	Molecular Biology Experiment	CFU	CFUs	#0.1		

3.2.1.2 Sample labeling for all test assays

The samples are individually labelled following always the same procedure. First, the project code is used, followed by clear identification of each test assay used as given in the following Table (Table 13).

Table 13 - Identification of test assay (second part): 29G P7 #0.1 MA/BC1

first part: 29G P7 #0.1 MA		second part: /BC1	
project ID (see above for detailed information)		sample ID	
/PSn	TSn	/BC1	BC 1
LU	Luisenpark (marine inoculum)	/BC2	BC 2
B1	Tank 1,2,3,4, mix	/RS	RS
20	Incubation Temperature [°C]	/IH	IH
Pur	Pure inoculum	/PS1	TS 1
dil	Inoculum pre washed and diluted. 1L inoculum with 2L synthetic marine water	/PS2	TS 2
1:200	Dilution before plating with synthetic marine water	/PS3	TS 3
#a	Sample or aliquot #a, #b, #c…		

3.2.1.3 Labeling of samples and spectra

Spectra recorded for each sample are labeled following the next scheme (Table 14):

Table 14 - Identification of spectra and data (third part): 29G P7 #0.1 MA/BC1 MS d01 #0.1

first part: 29G P7 #0.1 MA/BC1	second part: MS		third part: d1	fourth part: #0.1
project & sample identifier	Type/method of analysis		day of sampling	ascending serial number for each spectra or file taken
	MS	MALDI TOF mass spectrometry	d00	#0.1
	GPC	gel permeation chromatography	d01	#0.2
	PCR	PCR	d02	#0.3
	MB	Microbiology Experiment	d03	(…)
	MoB	Molecular Biology Experiment	d60	#2.1

3.3 Media, buffers and solutions

3.3.1 Biodegradation test media and solutions

The tests used to determine aerobic biodegradation are based on the measurement of evolved carbon dioxide and/or dissolved organic carbon (DOC) removal as described in OECD guidelines. Tests were performed in CO_2-evolution tests (modified Sturm-tests, OECD 301B)), dissolved organic carbon Die-Away tests (OECD 301A) or a combination of both or in online CO_2-evolution test. All tests are based on the determination of the ultimate biodegradability of organic compounds by aerobic microorganisms, using a static aqueous test system and the evolution of CO_2 or the removal of dissolved organic carbon as analytical parameter. An amount of 1.50-liter test mixture was prepared in 2-liter vessels containing an inorganic medium and the polymer samples, respectively, as the sole source of carbon at a concentration of 20 $mg \cdot L^{-1}$ of organic carbon for PEG and PVP samples and 100 $mg \cdot L^{-1}$ of organic carbon for the aliphatic aromatic polyester. The vessels were aerated with CO_2-free (50 $ml \cdot min^{-1}$) and incubated at 20 ± 2°C. Test Media for biodegradation tests were generally prepared in two different ways. The media for tests using WWTP activated sludge as inoculum based on OECD 301 were always freshly prepared following Table 15.

Table 15 - Mineral media for OECD 301 based tests

	Ingredient 1	Ingredient 2	Ingredient 3	Ingredient 4	Solvent/total volume
Solution 1	KH_2PO_4	K_2HPO_4	$Na_2HPO_4 \cdot 2H_2O$	NH_4Cl	H_2O
	8.50 g	21.75 g	33.40 g	0.50 g	ad 1000 mL
Solution 2	$CaCl_2$	$CaCl_2.2H_2O$	---	---	H_2O
	27.50 g	36.40 g			ad 1000 mL
Solution 3	$MgSO_4 \cdot 7H_2O$	---	---	---	H_2O
	22.50 g				ad 1000 mL
Solution 4	$FeCl_3 \cdot 6H_2O$	---	---	---	H_2O
	0.25 g				ad 1000 mL

The test assay was then prepared using 10mL Solution 1 and 1mL of solutions 2 to 4 each in 1000mL demineralized water. For marine biodegradation tests the procedure was generally to use synthetic marine medium as described by ISO 16221 and Inoculum as described below. In some cases fresh sea water was used instead. Table 16 and Table 17 give an overview of how the synthetic marine medium was prepared for the test assays.

Table 16 - Mineral media for OECD 306 or ISO 16221 based tests (I)

	Ingredient 1	Ingredient 2	Ingredient 3	Ingredient 4	Ingredient 5	Ingredient 6	Solvent/total volume
Solution A1	NaCl 478.0 g	Na_2SO_4 80.0 g	KCL 14.0 g	$NaHCO_3$ 0.4 g	KBr 2.0 g	NaF 0.06 g	H_2O ad 10 L
Solution A2	$MgCl_2 \cdot 6H_2O$ 406.6 g	---	---	---	---	---	H_2O ad 2 L
Solution A3	$CaCl_2 \cdot 2H_2O$ 73.5 g	---	---	---	---	---	H_2O ad 500 mL
Solution A4	$SrCl_2 \cdot 6H_2O$ 6.65 g	---	---	---	---	---	H_2O ad 250 mL
Solution A5	K_2HPO_4 34.0 g	---	---	---	---	---	H_2O ad 250 mL
Solution A6	NH_4Cl 6.69	---	---	---	---	---	H_2O ad 250 mL
Solution B	Yeast-Extract 15 mg	---	---	---	---	---	H_2O ad 100 mL
Solution C1	$MnSO_4 \cdot H_2O$ 30.23 mg	$ZnSO_4 \cdot 7H_2O$ 42.8 mg	$(NH_4)_6MO_7O_{24}x4H_2O$ 36.85		---	---	H_2O ad 500 mL
Solution C2	$FeCl_3 \cdot 6H_2O$ 44.5 mg	EDTA 55.5 mg	---	---	---	---	H_2O ad 5010 mL

Using solutions A1 to A6 the required amount of synthetic mineral medium is prepared as described below. The solutions A1 to A6 can be stored for up to 6 months at room temperature in the dark and used again. Solutions B and C1 and C2 should always be prepared freshly. The synthetic marine medium is prepared using solutions A1 to A6, solution B and solution C1 and C2 as indicated in Table 17 to result in the amount of 20L synthetic marine medium with a salinity of approx. 35‰.

Table 17 - Mineral media for OECD 306 or ISO 16221 based tests (II)

Solution A		Solution B	Solution C	
Solution A1 10 L	Solution A2 1066 mL	Solution B 20 mL	Solution C1 10 mL	Solution C2 10 mL
Solution A3 206 mL	Solution A4 18 mL	---	---	---
Solution A5 25 mL	Solution A6 20 mL	---	---	---
H_2O Make up to 20 L				

Activated sludge for the comparison of biodegradability was collected from an aeration tank of the WWTP Mannheim, Germany, fed with municipal and industrial sewage. The inoculum was preconditioned (aged) for 1-2 days to reduce the endogenous CO_2 production rate and sieved using a 0.8 mm pore size mesh. It was washed once with tap water and adjusted to a concentration of $5g \cdot L^{-1}$ of dry matter. The different media used in all tests were characterized. For tests using freshwater and microorganisms from WWTP aeration tanks the parameters are comparable to OECD and ISO guidelines [138;155]. The used concentration of microorganisms was 30 $mg \cdot L^{-1}$ calculated to the dry mass of the inoculum suspension. Marine synthetic medium was prepared following ISO 16221 (see Table 16 and Table 17, paragraph 3.3.1). The microorganisms (MOs) were obtained from filter units of a salt water aquarium at Luisenpark Mannheim, Germany or from the upper 10-30cm of the North Sea at the western shores of Sylt Westerland, Germany. The inoculum suspension was sieved using a mesh with 70µm pore size and was normally aerated for about 7 days with CO_2-free air to reduce the carbon content. Prior to the experiments the inoculum suspension was mixed with marine medium to obtain different dilutions of microorganisms. Parameters from the marine medium are comparable to OECD 306 [413] and ISO 16221. Media used in biodegradation studies were characterized and the data evaluated. Marine colony forming units were investigated using marine agar plates (see below). This is only used as control method since most marine microorganisms are not detected because of their inability to grow on agar plates.

3.3.2 Media used in microbiological analyses

The plates were prepared using marine agar (Difco Marine Agar 2216, Becton Dickinson, Sparks MD, USA), 55.1 g·L-1, dissolved and sterilized at 121°C for 15 min, onto sterile Petri dishes. Plates were poured using approx 5 mL agar per plate under a sterile environment and left at least overnight before the investigation. The plates were stored at 4°C in a storage room in sealed plastic bags to prevent water loss prior to incubation. The inoculum suspension was diluted 1:100 and 1:1000 with synthetic seawater and 50µL were plated on the agar plates. Cultivable cells were counted after normally 7 days of incubation at room temperature (20 ± 2°C).

3.3.3 Media used in molecular fingerprinting analyses

Molecular analysis of 16S rDNA was used in special biodegradation tests with synthetic polymers to evaluate the influence of different media on the biodegradation. Since differences between parallel test batches may occur in biodegradation tests that cannot be explained easily, the medium in certain test batches was selectively analyzed to provide an insight on the microbial community. The media used are given in Table 18, Table 19 and Table 20.

Table 18 - Solutions and buffers for DNA extraction

Name	containing	amount	Name	containing	amount
STE-buffer (pH 8)	EDTA	1 mM	SDS-Tris-EDTA (pH 8)	EDTA	20 mM
	Tris	50 mM		Tris	50 mM
	Saccharose	6.7 % (w/v)		SDS	20 % (w/v)

Name	containing	amount	Name	containing	amount
EDTA-Tris (pH 8)	EDTA	250 mM	TE-buffer (pH 8)	EDTA	1 mM
	Tris	50 mM		Tris	10 mM

Table 19 - Solutions, buffers and primers used for polymerase chain reaction and agarose gel electrophoresis

Name	containing	amount	Name	containing	amount
10x TBE-buffer (pH 8)	Boronic acid	900 mM	PCR-Stop-Mix (pH 7.9 with acetic acid)	Bromocresol-purple	0.25 %
	Tris-base	900 mM		Glycerol (97%)	50 %
	EDTA	20 mM		Tris-base	0.05 M

Primer	Tm [°C]	length	Mol weight [$g \cdot mol^{-1}$]	GC-content [%]	Sequence
P3(GC-clamp)	103.6	57	17254.3	91.2	5‘-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CCC CCC TAC GGG AGG CAG CAG-3‘
907rw	-	20	6053.9	35.0	5‘-CCG TCA ATT CMT TTR AGT TT-3‘
341f	51.2	17	5246.4	70.6	5- CCT ACG GGA GGC AGC AG-3‘

Table 20 - Solutions and buffers for denaturing gradient gel electrophoresis

Name	containing	amount		Name	containing	amount
50x TAE buffer (1L)	Tris	2 M (242g)		SDS-Tris-EDTA (pH 8)	EDTA	20 mM
	Acetic acid (100%)	57 mL			Tris	50 mM
	EDTA (0.5M)	100 mL			SDS	20 % (w/v)
Denaturing Solution		0 %	15 %	55 %	80 %	
	38% Acryl amide/Bis-AA	79	79	79	79	mL
	50x TAE	5	5	5	5	mL
	Formamide (deionized)	0	30	110	160	mL
	Urea (dis. in Formamide and H_2O)	0	31.5	115.5	168	g
		Made up to total volume: 500 mL				
Elution-buffer	NH_4Ac	0.5 M				
	EDTA	1 mM				
	SDS	0.1 % (w/v)				

3.4 Different methods for testing degradation

3.4.1 General introduction

Standardized methods introduced by the American Normative Reference (ASTM), Japanese Industrial Standards (JIS) and European Normative reference (ECN) for polymer testing are described by *CALMON-DECRIAUD* [14] for solid as well as liquid media. *GARTHE* et al. published a general overview, describing especially *ASTM* (ASTM) methods standard test methods [414].

The current situation offers only limited flexibility of methodologies for testing biodegradation of polymers because methods are developed either based on material types or the environments of application. For example, testing of microbial degradation offers in general only a small variety of fungal and bacterial species or a small number of environments applied for testing. Finding the same small variety of environmental conditions is hardly possible [105]. For there are many different metabolic pathways in natural environments we can fin also many different biodegradation processes that reflect again in a variety of different types of test methods such as static, semi-continuous and continuous test systems, anaerobic and aerobic, limnic and marine tests [87].

Different tests for determination of the biodegradability of water insoluble polymers are described in the literature (e.g. [415]). The headspace test, based on CO_2 measurement which can be performed as either closed or aerated culture test, depending on the size of the sample ant its oxygen demand was closely investigated. The amount of microbial carbon was determined by carbon/protein ratio along with CO_2 distribution between gas and liquid phase during the exposure. The amount of biomass was found to be too small to affect the results in biodegradation. It was also shown that higher amounts of test substance (300-600 $mg \cdot L^{-1}$) can be used when testing water insoluble polymers [416]. The combined CO_2/DOC test [141] can be adapted and used when testing soluble polymers such as PVP, PEG or PPG etc. the method can also be adapted using marine media. Making test results comparable internationally, the tests should be carried out best following internationally recognized (OECD) or standardized (ISO or CEN) methods [87]. The following tables describe some methods found in literature.

3.4.2 Overview of known areas of expertise

As a first approach, environmental or technical areas were biodegradation can be observed were checked for information available. The main interest was focused on four different systems namely, Sewage sludge, composting, freshwater and marine environment.

Table 21 gives an overview of what information and in which area of expertise may be available and it uncovers areas where not much interest was focused on up to now. The table shows basically the available test methods today and where rules for biodegradation where discovered or where model systems are available on chemicals in general and on polymers specifically.

Table 21 - Overview of the current test methods and scientific knowledge on biodegradation in the environment

	Sewage sludge	Composting	Freshwater environment	Marine environment
Available tests	Std tests: ASTM, OECD 301,302, etc DIN, ISO	D5338-92; OECD 304 etc. ISO 17556, 16929 :2002; EN 13432	Karrenbrock et al. Vom Wasser, 1999, 92, 361-371	OECD 306, ASTM D6691
Biodegradation rules for chemicals	QSAR model: CATABOL TD [328] Many studies exist in that area but not for polymer structures	Limited knowledge on chemicals	Many studies exist in that area	Only few information are known to exist
Biodegradation rules for polymers	Soluble polymers are partly investigated. Some biodegradable ones also.	The focus on polymer degradation is set to composting environments. Fungi and the elevated temperature are the reason for better degradation and deterioration of polymers	Only few information are known to exist	Only few information are known to exist

3.4.3 Comparison of standardized test methods

The following section gives a short comparison on different standardized methods for biodegradation research based on OECD, ISO and ASTM methods.

3.4.3.1 ASTM test methods

The ASTM [156] offers a set of designed methods applicable to certain environmental conditions for evaluating biodegradation of plastic materials [105].These methods require either specifically designed and characterized environments or certain strains of established cultures of microorganisms. Table 22 gives an overview of ASTM-test methods.

Table 22 - ASTM standard test methods

ASTM code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
D5209-92	Aerobic degradation of plastic materials in municipal sewage sludge	Indigenous microorganisms in sewage sludge	CO_2 evolved	OECD 301
D5210-92	Anaerobic degradation of plastic materials in municipal sewage sludge	Indigenous microorganisms in sewage sludge	CO_2 and CH4 evolved	OECD 311
D5247-92	Aerobic biodegradability of degradable plastics by specific microorganisms	Streptomyces badius ATCC39117 Streptomyces setonii ATCC39115 Streptomyces viridosporus ATCC39115 or other organisms agreed upon.	Weight loss, tensile strength, elongation and MWD	---
D5271-02	Aerobic biodegradation of plastic materials in activated sludge and WW	Municipal sewage treatment plant sludge	Oxygen consumption	OECD 303

ASTM code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
D5338-92	Aerobic biodegradation in composting conditions	2-4 months old compost	Cumulative CO_2 production, DMR, CMR, GMR	
G21-90	Resistance to fungi	Aspergillus niger ATCC9642 Aureobasidium pullulans ATCC15233 Chaetomium globosum ATCC6205 Gliocladium virens ATCC9645 Penicillum pinophilum ATCC11797	Visual evaluation	
G22-76	Resistance to bacteria	Pseudomonas aeruginosa ATCC13388	Visual evaluation	
D6691-01	Determination of aerobic biodegradation of plastics in marine environment	Inoculum consists of a minimum of nine test organisms. Marine solution is prepared in the lab following a standard procedure. Inoculum is verified by standard identification test	CO_2 evolved	OECD 306, ISO 16221
D6692-01	Determination of biodegradation of radiolabled polymeric plastics in seawater	Most ASTM methods use natural sea water as inoculum. No standardized method is available. See also D7081-05	CO_2 evolved, DPM counts (LSC), specific radioactivity	
D6340-98	Determination of aerobic biodegradation of radiolabled plastic materials in aqueous or compost environments	Natural mixed culture or compost matrix	CO_2 evolved, DPM counts (LSC), specific radioactivity	
D6003-96	Determination of weight loss from plastic materials exposed to simulated municipal solid-waste aerobic compost environment	Simulated compost, commercial compost seed	Weight loss	
D7081-05	Standard specification for Non-floating biodegradable plastics in the marine environment	--	--	--

3.4.3.2 OECD screening Tests

The OECD Guidelines [138] (Table 23) divide tests into three categories. The first consists tests for ready biodegradation. The second summarizes tests for inherent biodegradation and third comprises complex simulation tests which are closest to native environmental compartments but rather expensive and complicated demanding a lot of expertise. Most important for practical uses, are the tests determining ready biodegradability of substances. These are the most stringent ones; offering only limited opportunities for biodegradation and acclimatization of the inoculum [140]. Nevertheless, simulation tests are becoming more and more important today, since very complex risk assessment strategies require a broad knowledge on products and chemicals.

The ready biodegradability tests are based on the removal of organic compounds measured as dissolved organic carbon, catabolic CO_2 and/or biological oxygen demand. Biological oxygen demand has the benefit of being a direct biological parameter of aerobic biodegradation compared to dissolved organic carbon removal, which indicates only the strict elimination of carbon from an organic source. The latter parameter therefore allows

only indirect conclusions to be made. Respirometric tests allow also poorly water soluble compounds to be tested and also tests in automated systems, thus playing a role in modern biodegradability testing [140].

Table 23 - OECD standard test methods

	OECD Code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
TESTS ON READY BIODEGRADABILITY	301A	Static aerobic aquatic test using standard conditions. (DOC-Die-Away-Test)	Standardized defined inorganic test medium and mixed microorganisms. Aerated and stirred test through 28 days and 3-4 samplings a week. Non volatile and not significantly adsorbable test compounds. Water solubility at 10-40 $mg \cdot L^{-1}$ DOC	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	ISO 7827 EU 92/69/EWG C.4A
	301B	CO_2-Evolution Test. Static aerobic aquatic test using standard conditions. Measurement of biogenically evolved CO_2 and comparison to $ThCO_2$	Standardized defined inorganic test medium and mixed microorganisms. Aerated and stirred test through 28 days and 3-4 samplings a week. For Polymers a modification with higher buffer capacity, higher test temperatures and longer test duration can be used.	CO_2-evolution	ISO 9439 EU 92/69/EWG C.4C
	301C	MITI-I. Designed for use in Japan. With special inoculum preparation and obligatory specific analysis.			Only for Japanese region EU 92/69/EWG C.4F
	301D	Closed bottle test. Static aerobic aquatic test system using standard conditions.	Oxygen supply from test water. Oxygen measurement with electrode. Low inoculum concentration. Comparison of BOD with ThBOD or COD. DOC removal determination possible.	BOD measurement. In completely filled bottles.	ISO 10707 EU 92/69/EWG C.4E
	301E	Modified test. Static aerobic aquatic test using standard conditions with low bacteria concentration (e.g. river water)	Standardized defined inorganic test medium and mixed microorganisms. Aerated and stirred test through 28 days and 3-4 samplings a week	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	ISO 7827 EU 92/69/EWG C.4B
	301F	Respirometric test. Static aerobic aquatic test using standard conditions. Comparison of BOD to ThBOD or COD	Test compounds which are water soluble or insoluble at the test concentration Of 100 $mg \cdot L^{-1}$ substance or ThOD.	BOD measurement. In closed respirometers. Add. Information for water soluble compounds by DOC removal	ISO 9408 EU 92/69/EWG C.4D
TESTS ON INHERENT BIODEGRADABILITY	302A	Semi continuous activated sludge test (SCAS). Semi static aerobic aquatic test system using organic test compounds and easily biodegradable organic medium.	Daily fill and draw of test vessel. Water soluble non volatile not significantly adsorbable organic compounds at concentration between 20 and 50 $mg \cdot L^{-1}$ DOC	DOC measurement before and after replacement to determine ultimate biodegradation within a test time of 26 weeks	ISO 9887 EU 88/302/EWG C

	OECD Code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
	302B	Static test (Zahn-Wellens-test). Static aerobic aquatic test using standard conditions.	Higher concentration of TS and activated sludge (up to 1µg·L^{-1} dry substance). May be difficult to distinguish between abiotic elimination by adsorption and biodegradation. For water soluble non volatile organic compounds between 50 and 400 mg·L-1 DOC	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	ISO 9888 EU 88/302/EWG C
	302C	MITI-II same as MITI-I (OECD 301C) but with different test and inoculum concentration to improve biodegradability			Only for Japanese Government
SIMULATION TEST	303	Activated sludge simulation test. Continuously operated aerobic aquatic test system using organic test compounds and easily biodegradable organic medium.	Water soluble or satisfactorily dispersible non volatile organic compounds at concentration of normally 10-20 mg·L^{-1} DOC	DOC or COD measurement in influent and effluent of test and blank. Test time of 12 weeks.	ISO 11733 EU 88/302/EWG C
OTHER TESTS	304	Biodegradability in soil. Static test using soil as medium and inoculum in a closed system. Radio labeled TSs	Incubation time up to 64 days.	Evolved $^{14}CO2$ determination by alkali adsorption and liquid scintillation counting.	
	306	Marine biodegradation. Static aerobic test using sea water. Shake flask (60 days) or closed bottle test with 28 day test duration.	Test compounds at concentration from 2-40 mg·L^{-1} DOC	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	
	307	Aerobic and Anaerobic Transformation in Soil Systems	High Environmental relevance. Soil system from the environment	Substance specific analysis, radio labeled analytes	
	308	Aerobic and Anaerobic Transformation in Aquatic Sediment Systems	High Environmental relevance. Sediment/water system from the environment	Substance specific analysis, radio labeled analytes	
	309	Aerobic mineralization in SW - Simulation Biodegradation Test	High Environmental relevance. SW system from the environment	Substance specific analysis, radio labeled analytes	

A study based on the results of a large number of chemical substances, compares the different biodegradation tests (OECD 301, 303A, 302B) and the different measurement techniques to provide an assessment in terms of passing or failing threshold criteria. The comparison revealed high consistency for CO_2, biological oxygen demand and dissolved organic carbon measurements. The compared data pairs formed a solid basis for a reliable prediction of the carbon-based removal of chemical substances in WWTP's [139].

3.4.3.3 ISO screening tests

With its increased use, the disposal of plastic wastes has become a major environmental issue. More and more biodegradable plastics emerge as one of many available options to solve these issues. Since the working group on biodegradability of plastics was created in 1993, rapid advances have been made in this area. The approach and the development of standardized methods and definitions are described by *SAWADA* [417]. The following table (Table 24) shows an overview of ISO standards [155] for biodegradation tests.

Table 24 - ISO standard test methods

	ISO code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
TESTS ON READY BIODEGRADABILITY	7827	Static aerobic aquatic test using standard conditions. (DOC-Die-Away-Test)	Standardized defined inorganic test medium and mixed microorganisms. Aerated and stirred test through 28 days and 3-4 samplings a week. Non volatile and not significantly adsorbable test compounds. Water solubility at 10-40 $mg \cdot L^{-1}$ DOC	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	OECD 301 A
	9408	Respirometric test. Static aerobic aquatic test using standard conditions. Comparison of BOD to ThBOD or COD	Test compounds which are water soluble or insoluble at the test concentration Of 100 $mg \cdot L^{-1}$ substance or ThOD.	BOD measurement. In closed respirometer. Add. Information for water soluble compounds by DOC removal	OECD 301 F
	9439	CO_2-Evolution Test. Static aerobic aquatic test using standard conditions. Measurement of biogenically evolved CO_2 and comparison to $ThCO_2$	Standardized defined inorganic test medium and mixed microorganisms. Aerated and stirred test through 28 days and 3-4 samplings a week. For Polymers a modification with higher buffer capacity, higher test temperatures and longer test duration can be used.	CO_2-evolution	OECD 301 B
	10707	Closed bottle test. Static aerobic aquatic test system using standard conditions.	Oxygen supply from test water. Oxygen measurement with electrode. Low inoculum concentration. Comparison of BOD with ThBOD or COD. DOC removal determination possible.	BOD measurement. In completely filled bottles.	OECD 301 D
TESTS ON INHERENT BIODEGRADABILITY	9887	Semi continuous activated sludge test (SCAS). Semi static aerobic aquatic test system using organic test compounds and easily biodegradable organic medium.	Daily fill and draw of test vessel. Water soluble non volatile not significantly adsorbable organic compounds at concentration between 20 and 50 $mg \cdot L^{-1}$ DOC	DOC measurement before and after replacement to determine ultimate biodegradation within a test time of 26 weeks	OECD 302 A
	9888	Static test (Zahn-Wellens-test). Static aerobic aquatic test using standard conditions.	Higher concentration of TS and activated sludge (up to $1 \mu g \cdot L^{-1}$ dry substance). May be difficult to distinguish between abiotic elimination by adsorption and biodegradation. For water soluble non volatile organic compounds between 50 and 400 mg·L-1 DOC	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$.	OECD 302 B

	ISO code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
SIMULATION TEST	11733	Activated sludge simulation test. Continuously operated aerobic aquatic test system using organic test compounds and easily biodegradable organic medium.	Water soluble or satisfactorily dispersible non volatile organic compounds at concentration of normally 10-20 $mg \cdot L^{-1}$ DOC	DOC or COD measurement in influent and effluent of test and blank. Test time of 12 weeks.	OECD 303 A
OTHER TESTS	10634	Guidance for poorly water soluble test compounds	Description of several techniques for preparation of poorly water soluble organic compounds and introduction to the test vessel with aqueous media.	n/a	
	10708	Two-phase-closed-bottle-test. Static aerobic aquatic test using standard conditions	Closed test system with sufficient oxygen in headspace. Oxygen measurement with electrode. Comparison of BOD with ThBOD or COD. DOC removal determination possible.	BOD measurement	
	11734	Anaerobic biodegradability. Static aquatic test using anaerobic digested sludge medium and inoculum.	Test duration 60 days and test concentration at 20-100 $mg \cdot L^{-1}$ organic carbon	Measurement of biogas (CO_2 & CH_4) pressure or volume and IC	OECD 311
	14592	Low concentration water test. Static (shake flask method) or dynamic (river simulation) aerobic SW system.	Primary biodegradability and degradation kinetics of TS at realistic environmental conditions. Substances known principally degradable and water soluble at test concentration Non volatile and suitable analytical method is necessary.	Specific analyses or radiolabled compounds.	Part one is similar to OECD 309, Part Two describes a flow rover model
	14593	CO_2-Headspace test. Static aerobic aquatic test using standard conditions. Gas-tight closed bottles with sufficient oxygen	Measurement of biogenically evolved CO_2 after acidification in the gas phase or alkalization in form of DIC and comparison to $ThCO_2$	CO_2-evolution. Volatile substances can be analyzed.	OECD 310
	14851	Test modification of MITI-I & II. Used for polymer testing	Higher buffer capacity, test temperature and longer test duration. Carbon balance is possible.	Oxygen consumption (BOD)	ISO 9408
	14852	CO_2-Evolution Test. Static aerobic aquatic test using standard conditions.	Especially for Polymers: a modification with higher buffer capacity, higher test temperatures and longer test duration can be used. Measurement of biogenically evolved CO_2 and comparison to $ThCO_2$	CO_2-evolution	ISO 9439
	14855	Aerobic composting test. Static aerobic test.	Determination of ultimate biodegradability of test material in an optimized simulation of an intensive aerobic composting process at about 60°C. Duration 45 days. Optional disintegration and weight loss determination at the end of the test.	Continuous CO_2 measurement, , DMR, CMR, GMR	

	ISO code	Purpose	Involved microorganisms and key features	Parameters monitored	Similar to
	15462	Guidance for selection of biodegradation tests.	Overview of current biodegradation methods with a short description of their principles, scopes and recommendations	n/a	
	16221	Water quality - Guidance for determination of biodegradability in the marine environment	Natural or artificial seawater	TIC, DOC, CO_2, BOD	OECD 306

The ISO 14851 and ISO 14852 methods are specially designed for aerobic biodegradability determination of polymeric and plastic materials and their additives in aquatic batch tests. The sample is exposed to an inoculum under laboratory conditions in aqueous solution. In these cases the inoculum is either activated sludge or a compost suspension [418].

3.4.3.4 Tests used for this study and applied modifications

The methods used to evaluate biodegradation of the selected polymers in this study are mainly described in OECD guidelines [138] and were modified for special requirements as given inTable 25.

Table 25 - Modified methods applied to study polymer biodegradation based on standard test methods

	Purpose	Involved microorganisms and key features	Parameters monitored
OECD 301A	Static aerobic aquatic test using standard conditions. (DOC-Die-Away-Test)	With standard activated sludge inoculum and also with marine medium (synthetic medium with microorganisms from sea water aquarium)	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$
OECD 301B	CO_2-Evolution Test. Static aerobic aquatic test using standard conditions. Measurement of biogenically evolved CO_2 and comparison to $ThCO_2$	With standard activated sludge inoculum and also with marine medium (synthetic medium with microorganisms from sea water aquarium)	CO_2-evolution
OECD 301D	Closed bottle test. Static aerobic aquatic test system using standard conditions.	With marine medium (synthetic medium with microorganisms from sea water aquarium) and also with natural sea water	BOD measurement. In completely filled bottles.
OECD 306	Marine biodegradation. Static aerobic test using sea water. Shake flask (60 days) or closed bottle test with 28 day test duration.	With marine medium (synthetic medium with microorganisms from sea water aquarium) and also with natural sea water	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$
Combi test	A combination of 301A and 301B	All different kinds of media	DOC removal. Comparison of $DOC_{(start)}$ to $DOC_{(end)}$ and CO_2-evolution

Especially because longer biodegradation periods were expected and the tests were required to fit biodegradation as well as sophisticated analytical determinations the test systems had to be adapted a bit. Special valves

were necessary to withdraw samples using syringes or pipettes without opening the system, to add medium or inoculum solution and to spike new test substances.

Generally the CO_2-Evolution or "Sturm"-test (OECD 301B) was used for insoluble polymers and dissolved organic carbon-die away tests were used for water soluble polymers. The test used was determined by the water solubility of the test substances. CO_2 evolution can only be measured using a specific test design, while dissolved organic carbon, which is the measured parameter in the carbon-die-away tests, can only be determined when a water soluble substance is tested. There are a few combined or enhanced methods [141] available that are used to measure either two parameters in one test or that utilize easier measurement techniques. For testing of water soluble compounds in marine or WWTP/freshwater media in this study, the combined dissolved organic carbon/CO_2-test was used. To compare data, some other methods were also used for some polymers. What method was applied and which data was acquired will be indicated later in each section along with each of the results described.

One problem with these test methods is that they are designed in general to monitor degradation of more easily degradable polymers. Those macromolecules with slow degradation rates and a high resistance to environmental influences cannot be evaluated using these methods. New methods for highly sensitive detection of polymer degradation for resistant polymers such as PE, PET or PP are needed. Microbial test systems, which simulate relevant environmental conditions, do not allow a proper workout of microbial degradation. Therefore it is necessary to gain an insight on the first steps of degradation. The initiating organisms must be determined in a simplified system. For this task, substance specific analytical methods are required. The existing methods are often not easily applied because of the difficulties with high molecular weight, the weight distribution of polymers and the often complicated environmental media. In this study, analytical methods were applied to investigate the biodegradation of polymers in different ways [1;289].

3.4.4 Aquatic and terrestrial tests

The Problem with aquatic batch tests compared with terrestrial tests is the considerably lower degradation potential, because especially fungi and *Actinomyces* species do not have optimum growth conditions in water, for these organisms are especially important in polymer degradation. Though a big advantage is the lower requirement of resources and the possibility of getting proper carbon balances when analyzing aquatic tests, which characterizes the extent of biodegradation better than only CO_2 evolution alone [87;97;419].

Numerous tests are utilized for simulating biodegradation processes under lab conditions based on static, semi-continuous or continuous principles, operated under aerobic or anaerobic conditions, with limnic or marine test media. In all tests the following factors are important in influencing the biodegradation process [92].

- Concentration of the test substance should be high enough for the analytical methods chosen but sufficiently low in case of toxicity or when real environmental concentration are to be simulated.
- Physico-chemical properties such as volatility or water solubility determine the bioavailability and abiotic elimination in water systems.

- Composition and concentration of nutrients in the test medium, especially nitrogen and phosphorus, as well as sufficient buffer capacity.
- Presence or absence of other degradable substances in the same medium may affect the process.
- Conditions and properties of the test system such as volume, shape, open or closed bottles, temperature, pH, mode of mixing or shaking and oxygen/air supply.
- Test duration and life cycle time of microorganisms.

Biodegradation tests should be standardized to provide comparable and reproducible data as far as possible, though especially different approaches are still required for the variety of environmental areas and chemical structures. One criterion that may only be standardized up to a certain degree is the inoculum. Most tests require mixed cultures and tests with pure cultures are mostly used for metabolic pathway analyses. The inoculum is usually described by its origin and any pre-treatment procedures [87;92]. Some methods use controlled environmental conditions that are more easily standardized but they generally lack environmental realism and relevance on the other hand.

The choice of analytical parameters also affects the results obtained. Generally dissolved organic carbon (DOC) and biological oxygen demand (BOD) are the methods of choice. The advantage of these sum parameters is that the methods are quite simple in application and handling. When dissolved organic carbon measurement is performed it is not always possible to distinguish biological degradation processes from abiotic elimination such as adsorption to the inoculum or stripping into the headspace. biological oxygen demand on the contrary gives a clear answer on biodegradation processes with the disadvantage of not taking transformation of test substance into biomass into account [92].

3.4.4.1 The importance of the inoculum

Literally thousands of biodegradation studies have been performed during the last decades in industry and academia but still, there is no standard condition for each system that will give the same results with high reproducibility. The effect of the biomass, the type of inoculum, the consortium of microorganisms, their ability to transform a certain carbon substrate, their surface/volume ratio, the enzymatic kit available and many other factors such as nutrients, pH, Oxygen concentration etc. determine possible biodegradation.

3.4.4.2 Ready biodegradability

The term “ready biodegradable” is used as criterion for substances, which achieve a defined degree of degradation (70% DOC removal or 60% biological oxygen demand or CO_2 of the theoretical value) in the course of 10 days in an appropriate test (e.g. OECD 302 A-C). Their disappearance in the environment is assumed to be in the medium or long-term range [87].

3.4.4.3 Limit values for biodegradation

If the 90% limit value for complete biodegradation of a test substance by the end of the test, based on biological oxygen demand and CO_2 evolution (DIN or European Standard Requirements), is achieved, one can assume that the substance is completely biodegradable and no degradation products are formed in any significant amount. If relevant regulations and directives require e.g. a 70% decrease in dissolved organic carbon, it is assumed, that biodegradation will be complete in natural environments and the initial substance will no longer be present in significant amounts. Demanding higher rates would not be appropriate because the lack of accuracy of analytical methods used in these tests [87].

Biological oxygen demand and CO_2 evolution measurements have a serious disadvantage. A part of the test substance carbon is not oxidized but rather used by microorganisms to synthesize new biomass; hence it will not be taken into account of the analysis. For evaluation this portion must be estimated and kept in mind when calculating limit values. More precise values can be obtained when a carbon balance is performed as described in ISO methods ISO 14851 (1998) and ISO 14852 (1998) [155]. The 60% limit value was established practically for complete biodegradation and is used preliminary in all test methods based on biological oxygen demand and CO_2 evolution measurements. It is also the limit for determining ready biodegradability according to OECD criteria [87].

3.4.4.4 Comparison of respirometric methods based on OECD 301

In 1981 the OECD published the first set of tests, for the evaluation of potential biodegradation of chemicals. Later, in 1993 these methods were updated to a new version [138]. The international organization of standardization (ISO) published also a number of biodegradation standards that are similar to those of OECD in some extend [155]. An Overview can also be found in ISO 15462 and [92;155].

3.4.5 New enzymatic test methods

The problem with traditional test methods is mostly that they are quite time consuming. Some tests can take as long as one year to deliver reproducible and useful results. Enzymatic or laboratory test on the opposite may lead to results within a few hours, days or weeks, using defined and controlled test systems [60]. These tests are not ideal in regard to natural environmental conditions but their transferability to those has been demonstrated for some polyester [420].

Biodegradation of polymers can be seen as a surface process. Since the surface correlates reciprocal with its degree of fragmentation one could obtain a large surface by using nanoparticles. A new test system was developed based on the use of nanoparticles with enzymatic degradation tests that one could use to obtain results for samples not easily biodegradable. The degradation of polyester nanoparticles was carried out using lipase enzymes (subgroup of esterase). Acidic groups generated by the enzymes during cleavage of the ester bonds were determined via titration. The degradation rates were calculated from the slope of the NaOH-solution consumption. Results in comparison to the degradation of polyester films showing linear correlation between

degradation and surface size show that the degradation of nanoparticles is disproportionately high. For particles with a size of 100nm the rate was for times higher than expected considering the surface enlargement. This is due to the fact that the nanoparticles are completely amorphous. Crystalline structures are degraded slower than amorphous structures [421;422].

DEG experiments of slowly degradable aromatic homo polyesters with enhanced enzymatic tests show that these ester bonds can also be cleaved in principal. Comparing a film of 1,5-pentandiol and terephthalic acid (PPeT) with nanoparticles it could be determined that no degradation was observed for the film but the nanoparticles were degraded within hours. It can also be assumed that this phenomenon has a direct link to morphology. The next test investigates aliphatic aromatic copolyesters. The degradation graph of nanoparticles shows a fast start with high degradation potential and a following moderate part where degradation is only very low. It can be seen, that we have clearly two systems in this polyester: one that is degraded very easy (more amorphous) and a second, which is more inert to cleaving enzymes (crystalline). Also the size of the nanoparticles plays an important role in this effect [421;422].

3.5 Effects of biomass concentration on biodegradation

This part of the study describes to what extend effects of the biomass concentration on the biodegradation in a laboratory test system (Online CO_2-Evolution Test [423]) have been observed [424]. For this purpose, samples of microorganism-suspensions from a wastewater treatment plant (WWTP) were analyzed first with different methods in regard to their biomass concentration. The methods were statistically evaluated and the best method was then used to determine and adjust biomass concentrations in tests. Afterwards laboratory tests with 2 different substances (di-ethylene glycol (DEG) and poly(vinyl alcohol) (PVA)) and several biomass concentrations have been performed using activated sludge as inoculum. The test was conducted with three different biomass concentrations (low, (3 $mg \cdot L^{-1}$), standard (30 $mg \cdot L^{-1}$) and a high (300 $mg \cdot L^{-1}$)) of dry matter.

Activated sludge was collected from an aeration tank of the WWTP Mannheim fed with municipal and industrial sewage. It has a capacity of about 725'000 population equivalents with an amount of water of 103'300 $m^3 \cdot day^{-1}$ during dry weather [425]. The inoculum was preconditioned to reduce the endogenous CO_2 production rate. This was done by sieving the sludge by a 0.8-mm pore size mesh to remove coarse particles, washing it once with tap water, bringing it to a concentration of 5 $g \cdot L^{-1}$ of dry matter and finally aerating the sludge suspension for 1 day. The concentration of activated sludge in the tests was adjusted to 3, 30 and 300 $mg \cdot L^{-1}$ at the start of exposure by spiking the test medium with an appropriate volume of the sludge stock suspension.

3.5.1 Determination of Biomass

In order to determine and manage the biomass concentration in the tests, different methods were investigated first for their relevance and applicability [426;427] .

The first method was based on the determination of dry mass using centrifugation. Four replicates with 50 mL of activated sludge suspension each from the same WWTP were centrifuged at 5000 xg and 4°C for 15 min to separate biomass and aqueous phase. After decanting the supernatant the pellet was washed twice with approx. 50mL MilliQ-water and centrifuged again in-between each washing step. The pellet was re-suspended with MilliQ-water and transferred quantitatively to weighing bottles. Then, the biomass was dried at 105 °C over night. The weight difference of the weighing bottles was recorded with 0.1 mg accuracy and the dry mass was calculated by subtracting the previously recorded weight of the empty bottles.

The second method applied for biomass determination was based on filtration. 50 mL from each of the 4 replicates were separated from the aqueous phase using vacuum filtration using Machery-Nagel or Schleicher & Schüll blue ribbon filter paper (110 cm diameter and 2 µm pore size). Before and after the filtration, the filters were dried and weighted on an analytical balance with infrared drying unit attached (to 0.1 mg accuracy.) The weight of the dried filter was subtracted in the end from the total weight to determine the biomass concentration.

The third method applied was the determination of protein concentration using BIURET-assay [428-430]. The assay is based on the reaction of carbamoyl-urea with copper sulphate in alkaline aqueous media. A red-violet complex is formed between Cu^{2+} and two Biuret molecules which can be determined using UV-Vis spectrophotometry at a wavelength of 550nm. The reaction is typical for compounds with at least two CO-NH-groups as found in peptides or proteins. A specified amount of sludge suspension was centrifuged to separate cell material from the aqueous phase and after decanting the supernatant carefully 2 mL of MilliQ water was added. After res-suspending the pellet, 1 mL sodium hydroxide solution (3M) was added and the tubes were sealed and incubated for 5 min at 90-100°C and afterwards cooled town to room temperature on ice/water. After addition of 1 mL of 2.5% (w/v) $CuSO_4 \cdot 5\ H_2O$ solution the reaction tubes were sealed, shaken and incubated for 30 min at room temperature. Afterwards the tubes were centrifuged at 4500 rpm and 20°C for 15 min to precipitate the copper(II)hydroxide and other insoluble cell material. The supernatant was decanted and the extinction was measured at 555nm against a blank control. The corresponding protein content was calibrated using Bovine Serum Albumin (Sigma Aldrich). The determination of protein content allows an estimation of the biomass concentration whereas 1 g protein corresponds to 2 g of biomass [426].

The fourth method applied in this study was based on the determination of living bacteria known as colony forming units (CFU). About 4-5$g \cdot L^{-1}$ biomass corresponds to 8-9$\cdot 10^{13}$ cells [431;432]. Plating technique was applied using standard casein/soy-peptone agar plates (Merck, Darmstadt, Germany) and 100 µl of the diluted sludge suspension were pipetted on the plates and spread out with a Digralski spatula. As diluent a peptone-salt solution (1 g peptone and 8.5 g NaCl made up to 1 L with MilliQ-water and sterilized) was used. As the cell density was expected to be in the range of 7-9$\cdot 10^{13}$ cells per mL sludge suspension a dilution factor of 10^8 was used to dilute the medium resulting in about 30-300 colony forming units on the agar plates. After plating of the diluted cell suspension, the agar plates were incubated for 3 days at 30°C in temperature controlled incubation chambers. After counting the colony forming units, the biomass concentration was calculated [433]

3.5.2 The applied online CO_2 evolution test system

The principle of the used online CO_2 evolution test OECD 301B [138] and ISO 9439 [155] was modified as described previously [423]. It is used for determination of the ultimate biodegradability of organic compounds by aerobic microorganisms, using a static aqueous test system and the evolution of CO_2 as analytical parameter. The total volume of 1.5L test mixture was prepared in 2-liter vessels containing an inorganic medium and the organic compound as the sole source of carbon at a concentration of 20 mg of organic carbon (OC) liter^{-1}. Activated sludge was used as a mixed inoculum. The vessels were aerated with 1 to 2 bubbles of CO_2-free air per s (50 ml min^{-1}) and incubated at 20 ± 2°C for at least 28 days in our study but depending on the rate of biodegradation. Agitation was done by stirring with a magnetic stirrer (length 40 mm) at about 800 rpm. The exhaust gas from the vessels was passed through a glass chamber (volume 150 ml) that contained 50 ml of a 0.25 M aqueous KOH solution. Here, CO_2 was trapped and continuously measured by changes in conductivity, using a conductivity electrode. The absorption solution was continuously stirred with a magnetic bar (length, 10 mm) at 400 to 500 rpm. After conversion of the measured conductivity (mS·cm^{-1}) to produced CO_2 (mg·L^{-1}) and subtracting the blank values, biodegradation was calculated and expressed as a percent of the theoretically possible evolved CO_2 ($ThCO_2$). The carbon balance based on dissolved organic carbon (DOC) measurement was calculated to obtain additional information on the removal of carbon. The determination of CO_2 evolution alone may not be sufficient to characterize and quantify their biodegradability. During biodegradation new biomass formed by the microorganisms and some of the organic carbon of the test substance used is transformed to biomass but not biochemically oxidized. Therefore analytical parameters such as CO_2 evolution will often not reach 100% of the respective theoretical values even in the case of complete biodegradation of a test material and an insufficient degradation could falsely be assumed from the test results.

Along with the test substances (PVA and degradation) and control substance assays (aniline), blank values were prepared only using inoculum suspension and no additional carbon source. Evolved CO_2 in these vessels was due to endogenous oxidation activity of the microorganisms of the inoculum and took place without any addition of a biodegradable carbon source. In biodegradation tests, it was essential to determine this endogenous activity and to correct the CO_2 measured from the test assays with the test substance by subtracting the blank values.

3.6 Carbon balances

In a complete investigation with PHB as model polymer carbon balances were established as a tool to investigate biodegradation of polymers. In controlled 4L scale tests it was shown, that the degree of degradation could not be determined with satisfactory accuracy from CO_2 release (evolution) alone. Instead, the biodegradation process was described establishing carbon balances for the degradation with *Acidovorax facilis* and a mixed culture consortium from compost. The quantitative determination of biomass and residual polymer were the main problems. The amount of biomass derived from protein measurement depends strongly on the assumption of the protein content of the biomass. And when using selective oxidation of the biomass with hypochlorite as alternative, problems arose from insoluble metabolites. The results confirm both analytical and

theoretical approaches ending up at values very close to 100% within an acceptable standard deviation [97]. In regard to OECD criteria readily biodegradable substances must show at least 60% CO_2 evolution. Thus a lot of polymers such as PHB would not meet the criterion. In measurements using sum parameter such as dissolved inorganic carbon, dissolved organic carbon it is not possible to distinguish between microbial products or non-degradable intermediates, thus carbon balances can be used to more accurately determine biodegradation.

3.6.1 Carbon balance for modified Sturm-tests

For the determination of the carbon balance in "Sturm"-tests (insoluble test substance, carbon dioxide evolution is measured) the following assumption is made: biomass concentration at begin of exposure in test substance assay equals biomass concentration at begin of exposure in blank control assay. And for insoluble test substances: dissolved organic carbon at begin of exposure in test substance assay equals = dissolved organic carbon at begin of exposure in blank control assay.

Table 26 - Abbreviations used in carbon balance equations for "Sturm"-Test carbon balance

Abbreviation		Abbreviation	
TS	Test substance	BC	Blank control
TS_C	carbon fraction from test substance	BM_C	Carbon fraction from biomass
CO_2	Carbon originating from carbon dioxide	BM	Biomass
DOC	Dissolved organic carbon	DIC	Dissolved inorganic carbon

Begin of exposure: blue (start) End of exposure: green (end)

The general assumption for biodegradation tests is given in Equation 17 for the test substance and in Equation 18 for the blank control.

$$TS_{C(start)} + BM_{C(start)} + DOC_{(start)} = TS_{C(end)} + CO_{2(TS, DOC, BM)} + BM_{C(end)} + DOC_{(end)}$$

Equation 17 - Carbon balance for the test substance (I)

$$BM_{C(start)} + DOC_{(start)} = CO_{2(TS, DOC, BM)} + BM_{C(end)} + DOC_{(end)}$$

Equation 18 - Carbon balance for the blank control (I)

In modified Sturm-Tests biodegradation is evaluated using dissolved inorganic carbon as parameter. For test substance, calculation must be written as indicated by Equation 19.

$$CO_{2(TS, DOC, BM)(End)} = TS_{C(start)} + BM_{C(start)} + DOC_{(start)} - TS_{C(end)} - BM_{C(end)} - DOC_{(end)}$$

Equation 19 - Carbon balance for test substance (II)

And for blank control calculation:

$$CO_{2(DOC, BM)(End)} = BM_{C(start)} + DOC_{(start)} - BM_{C(end)} - DOC_{(end)}$$

Equation 20 - Carbon balance for blank control (II)

Subtracting Equation 19 and Equation 20 results in test substance - BC as follows:

$$CO_{2(TS-BC)(end)} = TS_{C(start)} + \Delta BM_{C(TS-BC)(start)} + \Delta DOC_{(TS-BC)(start)} - TS_{C(end)} - \Delta BM_{C(TS-BC)(end)} - \Delta DOC_{(TS_{BS})(end)}$$

Equation 21 - Carbon balance for modified Sturm-Tests (I)

Taking into account the assumptions stated above, that $\Delta BM_{C(TS-BC)(start)} = 0$ and $\Delta DOC_{(TS-BC)(start)} = 0$, it can be written:

$$\Delta CO_{2(TS-BC)(end)} = (TS_{C(start)} - TS_{C(end)}) - \Delta BM_{C(TS-BC)(end)} - \Delta DOC_{TS-BC(end)}$$

Equation 22 - Carbon balance for modified Sturm-Tests (II)

$$\Delta CO_{2(TS-BC)(start)} = \Delta TS_{C(start-end)} - \Delta BM_{C(TS-BC)} - DOC_{(TS-BC)(end)}$$

Equation 23 - Carbon balance for modified Sturm-Tests (III)

$$\Delta TS_{(start-end)} = \Delta CO_{2(TS-BC)(end)} - \Delta BM_{C(TS-BC)(end)} - DOC_{(TS-BC)(end)}$$

Equation 24 - Carbon balance for modified Sturm-Tests (IV)

3.7 Carbon Balance for polymers

Table 27 - Abbreviations used in carbon balance equations for polymer carbon balance (I)

Abbreviation		Abbreviation	
C_{GA}	total carbon content in test assay	C_{CO2}	carbon content from summarized carbon dioxide
C_{Bio}	carbon content from biomass	C_{DOC}	carbon from dissolved organic carbon
C_{DIC}	carbon from dissolved inorganic carbon	C_{POL}	carbon from insoluble organic carbon of the polymer

3.7.1 Determination of biodegradation - general balance

The main task is to determine the degree of biodegradation of test substance with respect to the remaining substance within a test system. Assumption: carbon content at begin of exposure = Carbon content at the end of exposure.

$$C_{BIO(start)} + C_{DIC(start)} + C_{POL(start)} + C_{2(start)} + C_{DOC(start)} = C_{BIO(end)} + C_{DIC(end)} + C_{POL(end)} + C_{2(end)} + C_{DOC(end)}$$

Equation 25 - Carbon balance for polymers (I)

The sum of all carbon contents at the start of the experiment, C_{GA} is composed of:

$$C_{GA} = C_{BIO(start)} + C_{DIC(start)} + C_{POL(start)} + C_{DOC(start)}$$

Equation 26 - Carbon balance for polymers (II)

The carbon content of the remaining polymer may be determined from the equation. A direct determination using analytical techniques depends on the techniques currently available since the remaining polymer needs to be separated from the biomass first.

$$C_{BIO(start)} + C_{DIC(start)} + C_{POL(start)} + C_{2(start)} + C_{DOC(start)} = C_{BIO(end)} + C_{DIC(end)} + C_{POL(end)} + C_{2(end)} + C_{DOC(end)}$$

Equation 27 - Carbon balance for polymers (III)

$$C_{GA(start)} = C_{CO_2(start)} + C_{BIO(start)} + C_{DIC(start)} + C_{POL(start)} + C_{DOC(start)}$$

Equation 28 - Carbon balance for polymers (IV)

$$C_{POL(start)} = C_{GA(start)} - C_{CO_2(start)} - C_{BIO(start)} - C_{DIC(start)} - C_{DOC(start)}$$

Equation 29 - Carbon balance for polymers (V)

From the balance given in Equation 28 with the following data, the degree of biodegradation of a polymer can be calculated as follows:

$$D_{completePOL}[\%] = \frac{\left(\Delta C_{CO_2} + \Delta C_{BIOMASS} + \Delta C_{DIC}\right) \cdot 100}{C_{GA}}$$

Equation 30 - Carbon balance for polymers (VI)

Table 28 - Abbreviations used in carbon balance equations for polymer carbon balance (II)

Abbreviation		Abbreviation	
C_{GA}	carbon content at the begin of exposure	C_{DOC}	carbon content of dissolved organic carbon at the end of exposure
C_{CO2}	carbon content of the summarized CO_2 produced at the end of exposure	C_{DIC}	carbon content of dissolved inorganic carbon at the end of exposure
C_{BIO}	carbon content of the biomass in the test system	C_{POL}	carbon content of insoluble organic carbon from the remaining polymer

Knowing this, the following equation (Equation 31) gives the corresponding values for the remaining polymer:

$$D_{completePOL}[\%] = 100\% = \frac{\left(\Delta C_{CO2} + \Delta C_{BIOMASS} + \Delta C_{DOC} + C_{POLremaining}\right) \cdot 100}{C_{GA}}$$

Equation 31 - Carbon balance for polymers (VII)

Table 29 - Abbreviations used in carbon balance equations for polymer carbon balance (II)

Abbreviation		Abbreviation	
$C_{POL(remain)}$	carbon content of the remaining, un-dis. polymer [mg·L^{-1}]	ΔC_{BM}	difference in carbon content of the BM (end - start) [mg·L^{-1}]
C_{GA}	summarized carbon content at begin of exposure [mg·L^{-1}]	ΔC_{DOC}	difference in organic carbon content (end - start) in [mg·L^{-1}]
ΔC_{CO2}	difference between CO_2 (end of exposure - begin of exposure) [mg·L^{-1}]		

Special tasks as to inquire the increase in biomass or the degree of degradation of CO_2 for insoluble polymers can be made with the following presumptions:

- C_{POL} corresponds to the insoluble organic carbon content of the polymer.
- C_{DOC} dissolved organic carbon content of the test substance assay equals the dissolved organic carbon content of the blank control at the start of the experiment (valid for insoluble polymers)

- C_{DOC} of the blank control at the end of exposure is negligible, $C_{DOC}BC_{end}$ = zero. If a test is stopped before complete biodegradation has occurred, the blank controls need to be determined and included in the calculation.

3.7.2 Isolated focus on the biodegradation balances

The following considerations focus only on the test substance assay (TS).

3.7.2.1 Carbon dioxide balance

The production of CO_2 over the complete test duration is consisting of all single values added from each measurement of dissolved inorganic carbon in the absorption solution of the modified "*Sturm*-Test", whereas C_{CO2}= C_{CO2POL}+C_{CO2BC} and C_{CO2POL}= C_{CO2}- C_{CO2BC} and C_{O2POL}= C_{O2}- C_{O2BC}. The CO_2-biodegradation degree of the polymer is given in Equation 32 and Equation 33.

$$D_{t_{CO2}} = \frac{\left(CO_{2POLEnd} - CO_{2POLStart}\right) - \left(CO_{2BCEnd} - CO_{2BCStart}\right)}{ThCO_{2POL}} \cdot 100$$

Equation 32 - Carbon dioxide balance for polymers (I)

$$D_{t_{CO2}} = \frac{\left(\Delta CO_{2POL} - \Delta CO_{2BC}\right)}{ThCO_{2POL}} \cdot 100$$

Equation 33 - Carbon dioxide balance for polymers (II)

Table 30 - Abbreviations used in carbon balance equations for carbon dioxide balance

Abbreviation		Abbreviation	
$DtCO_2$	CO_2-DEG degree of the polymer [%]	CO_{2BCEnd}	CO_2-content of the BC (BC) the end of exposure [mg·L^{-1}]
$CO_{2POLEnd}$	total CO_2 production at the end of exposure in the TS assay [mg·L^{-1}]	$\Delta CO_{2BCstart}$	difference of the CO_2-content of the BC [mg·L^{-1}]
$CO_{2POLStart}$	total CO_2 production at the begin of exposure in the TS assay [mg·L^{-1}]	ΔCO_{2BCEnd}	difference of the CO_2-content of the BC [mg·L^{-1}]
$CO_{2BCStart}$	CO_2-content of the BC (BC) the begin of exposure [mg·L^{-1}]	$ThCO_{2POL}$	theoretically possible amount of CO_2-production if the polymer is degraded completely [mg·L^{-1}]

With the assumption that the CO_2 content at the begin of exposure equals zero (0 mg·L^{-1}) and the CO_2 values calculated and summarized from the dissolved inorganic carbon-measurements which are already corrected with the blank control values, Equation 34 can be written:

$$D_{t_{CO2}} = \frac{\left(CO_{2POLEnd}\right)}{ThCO_{2POL}} \cdot 100$$

Equation 34 - Carbon dioxide balance for polymers (III)

3.7.2.2 Increase of biomass

The biomass in a test system can be calculated either by determining the protein content or the dry mass. Generally it can be assumed that the biomass consist of about 50% protein and about 50% carbon. Hence, it can be stated that c(protein) = (c)biomass - c(carbon content), which is valid for complete as well as incomplete biodegradation processes. Also, the dry substance (dry mass) = 2·biomass - c(carbon content), which is valid for complete biodegradation only. In general it can be stated that C_{BIO}= $C_{BIO(start)}$+$C_{BIO(increase)}$ or C_{BIO}(increase)= C_{BIO}-C_{BIO}(start). If caused by the test substance, Equation 35 and Equation 36 can be used with the assumption that the biomass in the test substance assay at the start of exposure equals the biomass concentration in the blank control.

$$C_{BIO(increase)POL} = C_{BIO,POL(end)} - (C_{BIO,BC(end)} - C_{BIO,BC(start)})$$

Equation 35 - Increase of biomass (I)

$$C_{BIO(increase)POL} = C_{BIO,POL(end)} - C_{BIO(increase)BC}$$

Equation 36 - Increase of biomass (II)

3.7.3 Calculating the carbon balance for polymers in modified "Sturm"-tests

The most difficult task is the analytical determination of the polymers and to distinguish between biomass and the polymer. Depending on the substance and/or analytical technique applied this has not been solved up to now for most cases. It is therefore also questionable if it is useful to include a DOC balance in the calculation or if it would make more sense to use summarized parameters as TOC or others for example.

3.7.4 Dissolved organic carbon balance

For calculating the degree of biodegradation based on dissolved organic carbon, the following assumptions are made (Equation 37 and Equation 38).

$$DOC_{start} = DOC_{POL,start} + DOC_{BIO,start}$$

Equation 37 - Dissolved organic carbon balance (I)

$$DOC_{end} = DOC_{POL,end} + DOC_{BIO,end}$$

Equation 38 - Dissolved organic carbon balance (II)

Generally the same concentration of inoculum is used in each vessel of a test setup and therefore it can be assumed that $DOC_{BIO,start}$ = $DOC_{BC,start}$ and that this assumption results in the fact that DOC_{start} =$DOC_{POL,start}$+ $DOC_{BC,start}$. For the term DOC_{end}=$DOC_{POL,end}$+$DOC_{BIO,end}$ the DOC degradation degree is given in Equation 39. Where Dt_{DOC} = DOC elimination degree of the polymer [%], DOC_{start} = total DOC content in the test substance assay at begin of exposure [mg·L^{-1}], $DOC_{BC,start}$ = DOC content in the blank control at begin of exposure [mg·L^{-1}], DOC_{end} = total DOC content in the test substance assay at the end of exposure [mg·L^{-1}] and $DOC_{BC,end}$ = DOC content in the blank control at the end of exposure [mg·L^{-1}].

$$D_{t_{DOC}} = \left[1 - \frac{DOC_{end} - DOC_{BC,end}}{DOC_{start} - DOC_{B,start}}\right] \cdot 100$$

Equation 39 - Dissolved organic carbon degradation degree (I)

Because it is not possible to distinguish between $DOC_{POL,end}$ and $DOC_{BIO(increase)end}$ with the DOC determination in the test substance assays, they must be used as "one" value together in the calculation of the biodegradation degree (DOC_{tot}) (Equation 40 and Equation 41).

$$D_{t_{DOC}} = \left[1 - \frac{DOC_{POL,end} + DOC_{BIO(increase)} - DOC_{BC,end}}{DOC_{POL,start} + DOC_{BIO,start} - DOC_{BC,start}}\right] \cdot 100$$

Equation 40 - Dissolved organic carbon degradation degree (II)

$$D_{t_{DOC}} = \left[1 - \frac{DOC_{tot} - DOC_{BC,end}}{DOC_{POL,start}}\right] \cdot 100$$

Equation 41 - Dissolved organic carbon degradation degree (III)

If the assumption is made, that the dissolved organic carbon of the blank control at the end of exposure with an idealized characteristics of the biodegradation process, is negligible, meaning $DOC_{BC,end}$=zero, one can calculate the dissolved organic carbon removal (biodegradation degree) as given in Equation 42.

$$D_{t_{DOC}} = \left[1 - \frac{DOC_{tot}}{DOC_{POL,start}}\right] \cdot 100$$

Equation 42 - Dissolved organic carbon degradation degree (IV)

3.8 Carbon measurement

The measurement of the analytical parameters (CO_2 evolved, dissolved organic carbon) was done as required by the extent of degradation. Carbon analysis was performed using a Shimadzu TOC V-CSN and TOC-5000A analyzer with auto sampler measuring CO_2 with near IR spectroscopy after combustion of the sample (for total carbon) or evolved CO_2 after acidification of the samples (for inorganic carbon). The analysis was confirmed using standards which have been statistically evaluated over the complete measurement period (see 4.4, p. 119).

3.9 Molecular biology

Samples (Table 31) were taken from actual biodegradation tests on Sunday 24, August 2008. 400mL of each sample was taken, centrifuged at 10000xg at 5°C for 20 minutes. The supernatant was carefully siphoned off using a sterile and cut off tip of a polyethylene Pasteur pipette connected to a vacuum pump. The precipitate was re-suspended with 10mL sterile synthetic sea-water. The samples were stored at 4-6°C until aliquots were removed, centrifuged and re-suspended in STE-Buffer for DNA extraction on Tuesday 26, August 2008.

Table 31 - Samples submitted to DNA analysis, amount and sample ID

Sample code	Amount of sample		Sequential (sample) identifier for DNA template tubes
	From biodegradation test [mL]	Volume after pre concentration step [mL]	
32G P01 #0.1 MA/BC1 PCR d1270 #0.1	400	10	1
32G P01 #0.1 MA/BC2 PCR d1270 #0.1	400	10	
32G P01 #0.1 MA/BC3 PCR d1270 #0.1	400	10	2
32G P01 #0.1 MA/BC4 PCR d1270 #0.1	400	10	
32G P01 #0.1 MA/RS1 PCR d1270 #0.1	400	10	3
32G P01 #0.1 MA/RS2 PCR d1270 #0.1	400	10	4
32G P01 #0.1 MA/PS1 PCR d1270 #0.1	400	10	5
32G P01 #0.1 MA/PS2 PCR d1270 #0.1	400	10	6
32G P01 #0.1 MA/PS3 PCR d1270 #0.1	400	10	7
32G P01 #0.1 MA/PS4 PCR d1270 #0.1	400	10	8
32G P01 #0.1 MA/PS5 PCR d1270 #0.1	400	10	9
32G P01 #0.1 MA/PS6 PCR d1270 #0.1	400	10	10
32G P01b #0.2 MA/BC1 PCR d535 #0.1	400	10	11
32G P01b #0.2 MA/BC2 PCR d535 #0.1	400	10	
32G P01b #0.2 MA/RS1 PCR d535 #0.1	400	10	12
32G P01b #0.2 MA/PS1 PCR d535 #0.1	400	10	13
32G P01b #0.2 MA/PS2 PCR d535 #0.1	400	10	14
32G P04 #0.1 MA/PS1 PCR d535 #0.1	400	10	15
32G P04 #0.1 MA/PS2 PCR d535 #0.1	400	10	16
32G P04 #0.1 MA/PS3 PCR d535 #0.1	400	10	17
32G P05 #0.1 MA/PS1 PCR d535 #0.1	400	10	18
32G P05 #0.1 MA/PS2 PCR d535 #0.1	400	10	19
32G P05 #0.1 MA/PS3 PCR d535 #0.1	400	10	20
21G P08 #0.1 MA/BC1 PCR d206 #0.1	400	10	21
21G P08 #0.1 MA/BC2 PCR d206 #0.1	400	10	22
21G P08 #0.1 MA/RS1 PCR d206 #0.1	400	10	23
21G P08 #0.1 MA/PS1 PCR d206 #0.1	400	10	24
21G P08 #0.1 MA/PS2 PCR d206 #0.1	400	10	25
21G P10 #0.1 MA/PS1 PCR d206 #0.1	400	10	26

Sample code	Amount of sample		Sequential (sample) identifier for DNA template tubes
	From biodegradation test [mL]	Volume after pre concentration step [mL]	
21G P10 #0.1 MA/PS2 PCR d206 #0.1	400	10	27
21G P12 #0.1 MA/PS1 PCR d206 #0.1	400	10	28
21G P12 #0.1 MA/PS2 PCR d206 #0.1	400	10	29
Luisenpark 2007-06-04	400	10	30
Luisenpark 2007-06-18	400	10	31
Luisenpark 2008-07-03	400	10	32
Luisenpark 2008-08-01	400	10	33
Luisenpark 2008-08-14	400	10	34
Sylt Westerland 2006-10-27	400	10	35
Sylt Westerland 2006-12-12	400	10	36

3.9.1 DNA Isolation

The DNA from aquatic samples taken from biodegradation experiments was isolated using phenol-chloroform extraction [390]. 1.5mL of each re-suspended samples were transferred into an Eppendorf-Tube (1.5mL). The samples were centrifuged for 5 min at 12'000 rpm. The supernatant was discarded and the procedure was repeated again if the pellet was still very small. The amount extracted from each sample taken can be seen in Table 32.

Table 32 - Extracted Volume and required amount of Tris-EDTA-buffer for extraction

Sequential (sample) identifier for DNA template tubes	Extracted sample volume [mL]	Volume of TE-Buffer added to DNA-pellet [µL]	Dilution (end volume [µL])	Total volume [µL] and dilution ratio
1	4.5	30		30
2	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
3	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
4	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
5	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
6	3.0	30		30
7	1.5	30		30
8	6.0	30		30
9	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
10	1.5	30	1:1 (60)	60 (1:1 H_2O /TE (v/v))

Sequential (sample) identifier for DNA template tubes	Extracted sample volume [mL]	Volume of TE-Buffer added to DNA-pellet [µL]	Dilution (end volume [µL])	Total volume [µL] and dilution ratio
11	1.5	30		30
12	6.0	30		30
13	1.5	60		60
14	1.5	60		60
15	1.5	60		60
16	1.5	60		60
17	1.5	60		60
18	6.0	30		150 (3:2 TE/H_2O (v/v))
19	1.5	60		60
20	1.5	30		30
21	1.5	30	1:5 (150)	150 (3:2 TE/ H_2O (v/v))
22	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
23	1.5	30		30
24	9.0	30		60 (1:1 H_2O/TE (v/v))
25	1.5	30		30
26	9.0	30		30
27	9.0	30		30
28	1.5	30	1:5 (150)	150 (3:2 TE/H_2O (v/v))
29	3.0	30	1:5 (150)	150 (3:2 TE/H_2O (v/v))
30	1.5	60	1:1 (60)	60 (1:1 H_2O/TE (v/v))
31	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
32	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
33	1.5	30	1:1 (60)	60 (1:1 H_2O/TE (v/v))
34	1.5	30	1:5 (150)	150 (3:2 TE/H_2O (v/v))
35	9.0	30		30
36	1.5	30		30

The remaining pellet was then re-suspended in 380µL STE-Buffer (see p. 83 paragraph 3.3.3 Media used in molecular fingerprinting analyses) and 100µL Lysozyme (10mg·mL^{-1}) were added. The suspensions were mixed by vortexing for a few seconds and the tubes were incubated for 15min at 37°C. Then, 50µL EDTA-Tris, 30µL SDS-Tris-EDTA and 10µL RNase (10mg·mL^{-1}) were added. The samples were mixed by carefully shaking the tubes and then incubated for 10-30min at 37°C and afterwards for 30min at 50°C. After incubation, 70µL of

NaCl-solution (5M) were added and the samples were carefully mixed. About 700µL of Phenol-Chloroform (1:1) were added to the samples. After mixing the tubes were centrifuged for 10min at 13'000rpm at 4°C. The supernatant phase (water phase containing the DNA templates!) was carefully transferred to a new Eppendorf-tube and the DNA was precipitated wit 600µL cold iso-propanol for 2h at -20°C. After about two hours the samples were centrifuged for 20min at 13'000rpm and 4°C. The supernatant was discarder (decanted) and the DNA-template (pellet) was washed with ice-cold iso-propanol/water solution (70:30 (v/v)) and centrifuged again for 20min at 13'000rpm and 4°C. This must be done very quickly without intermission because the DNA may dissolve partially in the water solution. After decanting the washing agent the DNA-templates were left to dry for about 10-30min and afterwards the almost dry pellets were suspended in 30µL TE-buffer. 2µL of this DNA-template were used to check the extraction on a 0.8% (w/v) agarose-gel. The DNA-templates were stored at -20°C for further experiments.

3.9.2 Agarose gel electrophoresis

The required amount of Agarose (Table 33) was weighed in and made up with 0.5x TBE-buffer. The Gel was heated using a microwave (750 W) for approx 1-2 minutes depending on the amount of solution prepared (approx 100mL for 1 gel with 28 sample spots) and when completely dissolved and clear the solution was poured into the mount, respectively. After cooling for 30min the Agarose gels were loaded with samples and placed in the electrophoresis chamber. The samples were prepared by mixing of the respective amount of DNA-templates (Table 33) with PCR-Stop-Mix (5x) solution to result in a total sample volume of 25µL in micro plates. These samples were then transferred into the slots on the agarose gel. Electrophoresis was performed at conditions of 80-100V, for 50 to 60min.

Table 33 - Agarose concentration and amounts of sample loaded on the gel for electrophoresis

	Agarose concentration [% (w/v)]	Amount of DNA-template used [µL]
Genomic DNA (complete DNA)	0.8	2
PCR-product	1.2-1.5	5
Restriction-templates	2-4	20

Agarose gels were afterwards dyed using ethidium bromide solution for approx. 5-7min and placed in distilled water for up to 30min. Pictures were taken using a BioRad UV-scanner system.

3.9.3 Polymerase chain reaction

To select the most suitable method for PCR Analysis different enzyme-kits were tested with selected samples from the biodegradation tests. The selected PCR Kits are described in Table 34, primers and media in Table 19 (p. 84).

Table 34 - Kits tested for polymerase chain reaction results on selected samples

	Manufacturer	Name of polymerase kit	taq-polymerase Lot-number	PCR Buffer Lot-number	Enhancer Lot-number
1	Quiagen	Taq DNA Polymerase 5 u·µL^{-1}	111870307	11870833 10x $MgCl_2$	11871150 and ATG002.4
2	Eppendorf	Taq DNA Polymerase 5 u·µL^{-1}	T101183K	T101177K 15mM Mg^{2+}	T101747M 5x Taq MasterPCR
3	Mo Bi Tec	MoBiTaq(K) 5 u·µL^{-1}	H9701.1	#0206 10x	-
4	PerkinElmer/ Applied Biosystems	AmpliTaq DNA polymerase 2 u·µL^{-1}	D00329 07/31/02	D05395 11/30/03 10x PCR Buffer 15mM $MgCl_2$	BSA G0049 7/31/98 25mM $MgCl_2$
5	Boehringer Mannheim	Taq DNA Polymerase 5 u·µL^{-1}	14562165-28/31/Aug98	83808722-28 10xPCR MasterMix	

Following each of the protocols for the selected PCR-Kits the preparation scheme given in was calculated to obtain PCR templates with a total volume of 100µL. For each taq-Polymerase a positive control (*Roseobacter* sp.), a negative control and two samples from the extracted DNA (sample: 2 and 7, Table 32) were used. This means that for each taq-Polymerase 4 Eppendorf-tubes were required. The amounts given (Table 35) were multiplied by four (or any required number for multiple PCR tubes) and added into the first tube (without DNA template). Then, 99µL were transferred into each following tube and DNA-templates were added in the tubes for samples and positive control, respectively.

Table 35 - Preparation scheme for polymerase chain reaction template preparation with different polymerases

Method Number	taq-Polymerase	Abbreviation for sample	H_2O [µL]	DNA Template [µL]	Polymerase Buffer [µL]	Enhancer[µL]	dNTP's [µL]	Primer P3 [µL]	Primer 907r [µL]	taq- polymerase [µL]	Total amount [µL]
1	PerkinElmer	PE	80	1	10	2 (BSA)	4	2	2	1 (2*u*)	100
2	Quiagen	QQ	60	1	10	20 (Q)	4	2	2	0.5 (5*u*)	100
3	Quiagen	Q	80	1	10	-	4	2	2	0.5 (5*u*)	100
4	Boehringer	B	80	1	10	-	4	2	2	0.5 (5*u*)	100
5	MoBiTaq(K)	Mobi	80	1	10	-	4	2	2	0.5 (5*u*)	100
6	Eppendorf	EP	60	1	10	20	4	2	2	0.5 (5*u*)	100
7	PerkinElmer	PE0BSA	80	1	10	-	4	2	2	1 (2*u*)	100
8	PerkinElmer	PEdBSA	76	1	10	4 (BSA)	4	2	2	1 (2*u*)	100
9	PerkinElmer	PE+Mg^{2+}	72	1	10	2(BSA) + 6 $MgCl_2$ (15µM)	4	2	2	1 (2*u*)	100

After these different PCR Kits were tested, protocol #1 (Table 35) was selected because of its high reproducibility and best results with the samples. It was further used for all other PCR experiments. Each DNA-template from each water sample was used to prepare 100µL PCR-template as described using the method selected above. A positive and negative control was always prepared as well to check the PCR method for reproducibility and errors. The PCR method generally used in this study is given in Table 36.

Table 36 - Method parameters for polymerase chain reaction experiments

Step	Temperature [°C]	Time interval [hh:mm:ss]
Eppendorf MasterCycler Gradient PCR temperature-time-program		
Lid temperature	105	Complete run
1	94	00:05:00
2	94	00:01:00
3	65	00:01:00
	-0.5 00:00:00 R = 3° C·s^{-1} G = 0°C	
4	72	00:01:00
5	GOTO 2 Repeat 20x	
6	94	00:01:00
7	55	00:01:00
8	72	00:01:00
9	GOTO 6 Repeat 12x	
10	72	00:10:00
11	72	00:01:00
	-1.0 00:00:00 R = 3° C·s^{-1} G = 0°C	
12	GOTO 11 Repeat 22x	
13	Hold 5.0°C	
14	End (total runtime approx: 02:58:00)	

All PCR-products were checked on Agarose-gels for a fragment of about 520bp (Figure 15) against a 1 kb DNALadder (US Patent No. 4,403,036, from *GIBCOBRL*). These PCR products were further used for DGGE analysis.

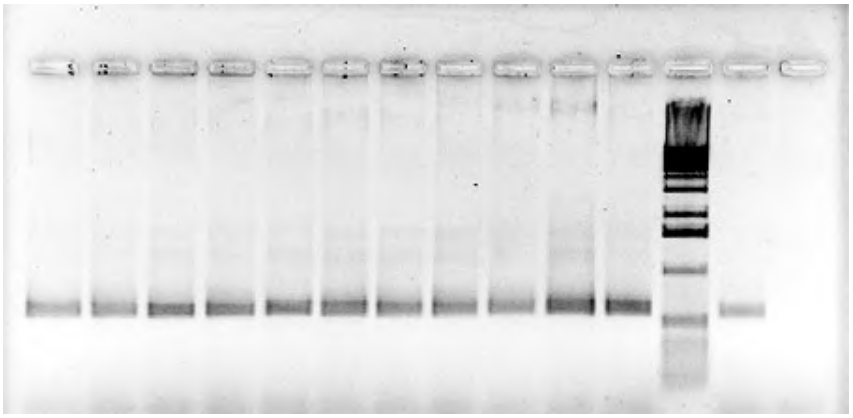


Figure 15 - Example of agarose gel after polymerase chain reaction products were checked against a 1 kb DNALadder

3.9.4 Denaturing gradient gel electrophoresis

DGGE was performed using a BioRad DCode™ Universal Mutation Detection System. The DGGE formamide/urea/polyacrylamide gel was cast using a gradient mixing system. 14mL of each solution (15% and 70% formamide/urea) were prepared using a 0% and 70% stock solution. Required solutions are presented in

Table 20 on p. 84. Therefore the 15% solution was prepared by mixing 3.2mL of the 70% solution with 10.8mL of the 0% solution. In each vessel 11µL TEMED (N,N,N',N'-Tetramethylethan-1,2-diamine) and 78µl ammonium persulfate (APS) as polymerization initiator were added, the Tubes were shaken briefly and the solution was poured into the gradient mixer and the separating gel was cast. The stacking gel was prepared later using only 0% (formamide/urea) acryl amide solution. 40µL of the PCR templates prepared from the samples were mixed with 15µL undiluted PCR-stop-mix (Table 19 p. 84) in a clean and sterile micro plate. The samples were transferred onto the gel and DGGE was performed at 100V for 15h at 60°C in 0.5x TAE buffer. The DGGE polyacrylamide gels were dyed using SYBR Gold solution for approx. 20 min. Pictures were taken using a BioRad UV-scanner system (Figure 16) and DGGE bands were separately cut out of the gel numbered and stored each until further use at -24°C in labelled Eppendorf tubes.

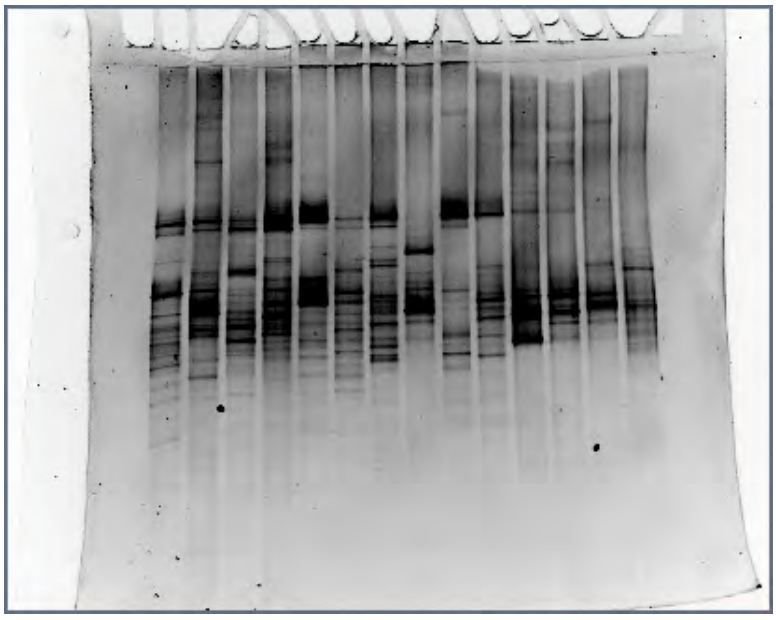


Figure 16 - Example of a denaturing gradient gel electrophoresis gel prior to cutting out bands

3.9.5 Elution of cut DGGE bands

The bands were cut systematically as indicated in Figure 17. Therefore each band was labeled numerically after the sample sequentially in alphabetical order (a, b, c, …, n) in the direction of the electrophoresis of the gel.

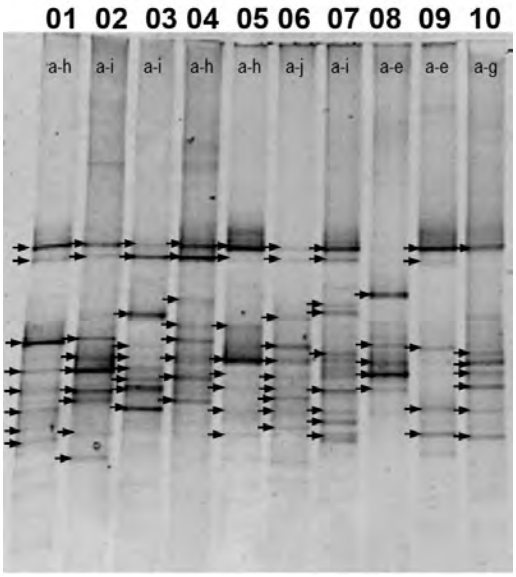


Figure 17 - Example of alphanumerical labeling of DNA bands for sequencing

The cut bands were re-suspended in 150µL elution-buffer (Table 20, p. 84) for 5h at 40°C on a shaker. Afterwards the samples were centrifuged at 12'000 rpm for 1 min at 4°C. The supernatant was transferred to a new 1.5mL Eppendorf-tube and the pellet was re-suspended again using 50µL elution-buffer. After vortexing, the samples were centrifuged again at 12'000 rpm (15'300 xg) for 1 min at 4°C. The supernatant was added to the first volume and the pellets (gel-bands) were discarded later after confirmation of the results. The supernatant was precipitated using 400µL ice-cold EtOH (100%, p.a.) over-night. After precipitation, the samples were centrifuged at 4°C for 20 min at a minimum of 12'000 rpm. The supernatant was discarded and the pellets were left to dry for a few minutes until the EtOH was evaporated. The remaining DNA pellet was resuspended using 10µL PCR-clean water. For PCR 1µL of the DNA Template was used following method 1 (Table 35, p. 109) to obtain 100µL templates for sequencing. The validity of the templates was checked with agarose-gel electrophoresis (Figure 18 and Figure 19). For this last step primer P3 was replaced by primer 341f (without GC-clamp, see Table 19).

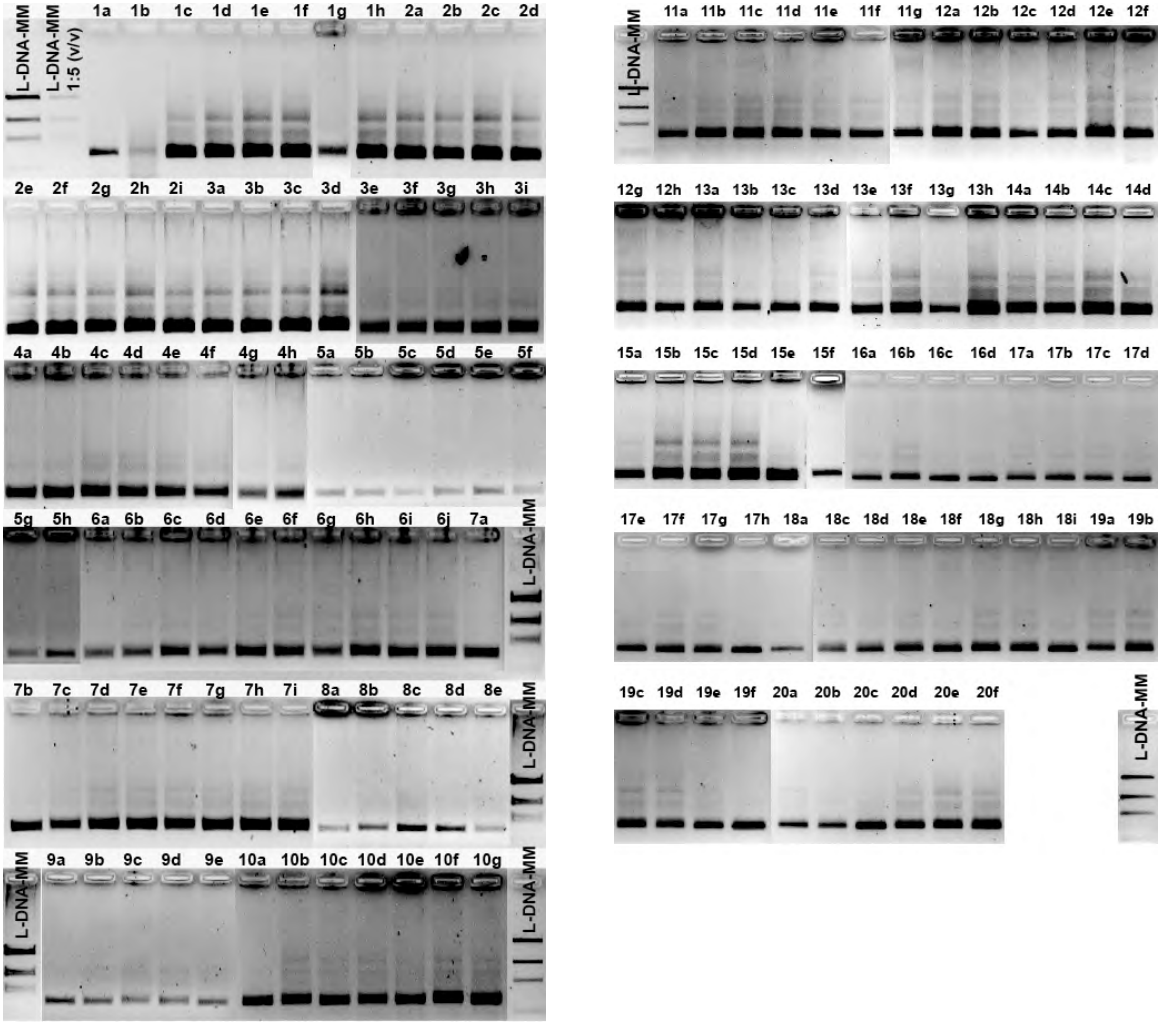


Figure 18 - Control of DNA templates after PCR w. primers 341f and 907rw for sequencing (samples 1 to 20)

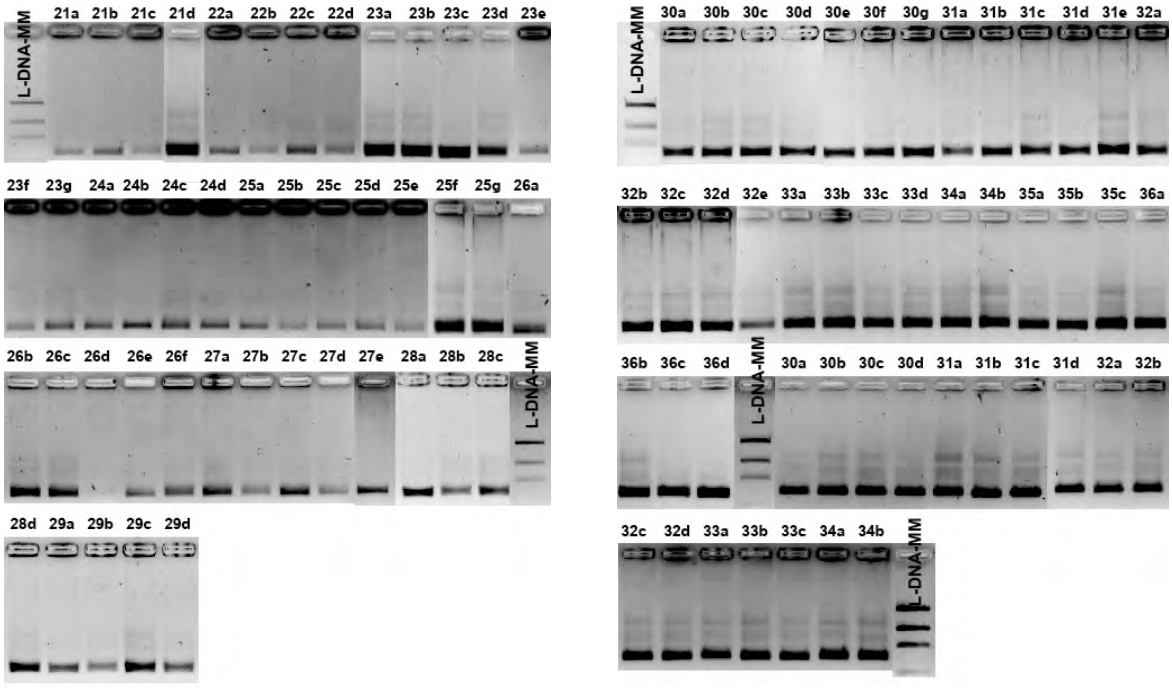


Figure 19 - Control of DNA templates after PCR w. primers 341f and 907rw for sequencing (samples 21 to 34)

3.9.6 DNA Sequencing

DNA Sequencing was performed by the Bioanalytical Department (GVC/N) of BASF SE, Ludwigshafen and the resulting sequences were cross checked for approx. best 10 matches against nBLAST (National Center for Biotechnology Information, National Library of Medicine and National Institute of Health) (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) database automatically without alignment. The data was evaluated manually step by step and unclear results were aligned using Vector NTI Software and again crosschecked against nBLAST database.

4 Results

4.1 Properties of biodegradation test media

The test media for biodegradation tests with activated sludge were prepared following OECD guidelines and confirmed by measurement of the important parameters such as carbon content and/or dry mass as required by the guideline. All criteria were met for each experiment. No further investigation was performed on this medium, since much data has been collected already and many aspects have been reported previously. Parameters on media used for marine biodegradation tests were analyzed more carefully since not much has been reported yet and also the guideline does not require narrow criteria but only documentation of the data.

As can be seen in Figure 20 the collected data from the inoculum suspensions for marine biodegradation tests show that some parameters such as sampling temperature, salinity, total organic carbon and pH show only narrow variation but other parameters such as inorganic carbon, colony forming units and of course total carbon vary significantly. The data on carbon concentrations can be compared with each other. Variation in total and inorganic carbon can be explained by the huge capacity of marine water to store CO_2. Experiments have shown that it is useful to age medium for marine tests for at least 7 days prior to the test using CO_2 free air. The inorganic carbon content will drop and later, measurements will be more accurate. The data presented for temperature, salinity, pH, dry mass can only be seen as independent sets of data. Colony forming units (assigned to the second (right) y-axis) must also be seen as an independent parameter in this figure.

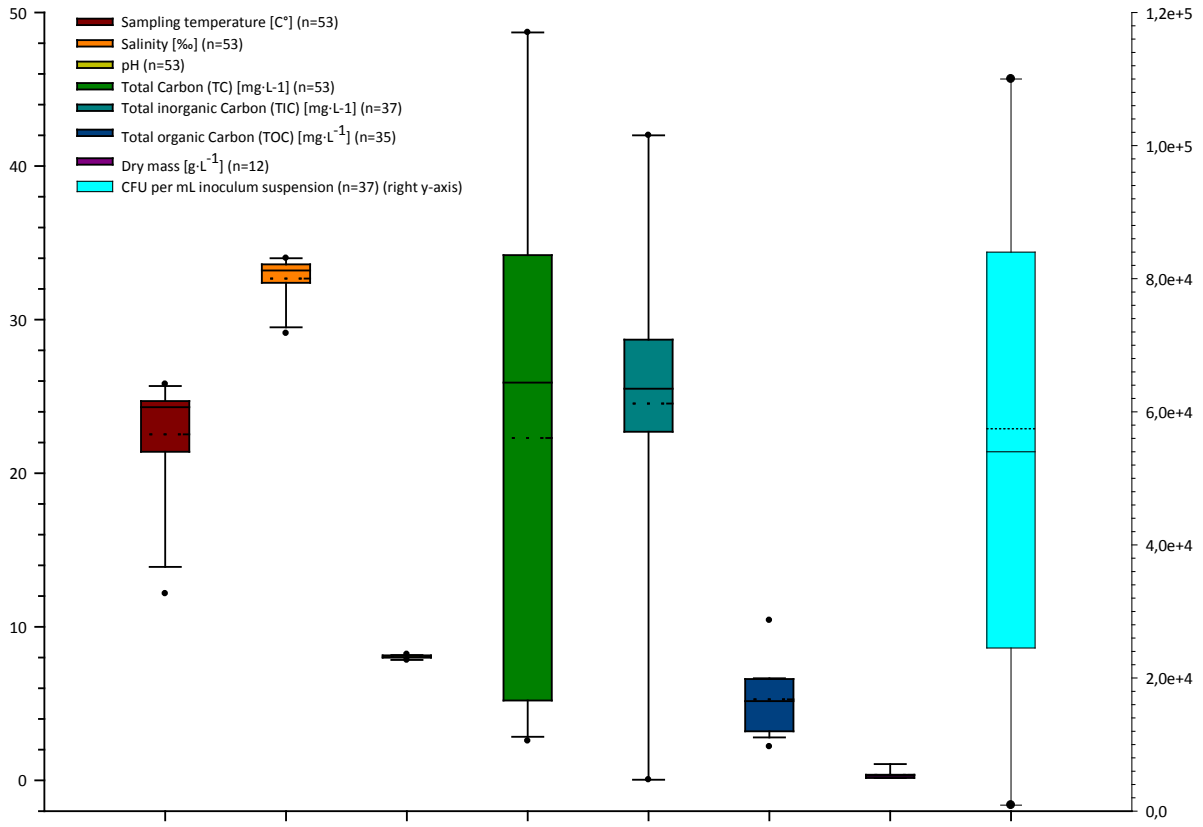


Figure 20 - Parameters of marine test medium for biodegradation tests prior to test phase

4.2 Microbiological analysis of marine media

In order to confirm that some microorganisms are present in the medium used for marine biodegradation tests some microbiological investigations were performed (3.3.2). Generally one has to remember, that marine microorganisms are mostly famous for not being cultivable in the laboratory on agar plates or nutrient media. Still, counting and estimating average colony forming units was used to confirm on short notice that media used were comparable and for providing additional data. Generally the medium/inoculum samples were plated on Difco Marine Agar plates undiluted and 1:10, 1:100 and 1:000. As a start, incubation temperature was investigated. Therefore samples were stored at different temperatures (6°C, 20°C and 30°C) during incubation time. It could be observed that 20°C (RT) served best for the development of the cultures during the incubation time (Table 37).

Table 37 - Effect of different incubation temperatures on colony forming unit counts

	Inoculum 20°C	Inoculum 30°C	Inoculum 6°C
Mean CFU/mL	$8.89 \cdot 10^4$	$8.08 \cdot 10^4$	$1.58 \cdot 10^3$
Std. Deviation.	$2.1 \cdot 10^4$	$7.1 \cdot 10^4$	$5.6 \cdot 10^2$
Std. Deviation from the mean	$1.2 \cdot 10^4$	$3.6 \cdot 10^4$	$2.8 \cdot 10^2$
Std. Deviation from the mean [%]	13	44	18

The physico-chemical parameters and statistical evaluation of colony forming units' counts are given in Figure 20. It can be seen, that cell counts vary quite a bit but over 50% of all values are in the range of 10^4. This allows estimating how much inoculum suspension is used to spike the synthetic marine medium. Generally the results have shown that a ratio of 1:10 (v/v, inoculum/synthetic medium) offers best results in biodegradation tests performed in this study. A typical example for marine inoculum suspension after 7 day of incubation at 20°C on agar plates is shown in Figure 21.

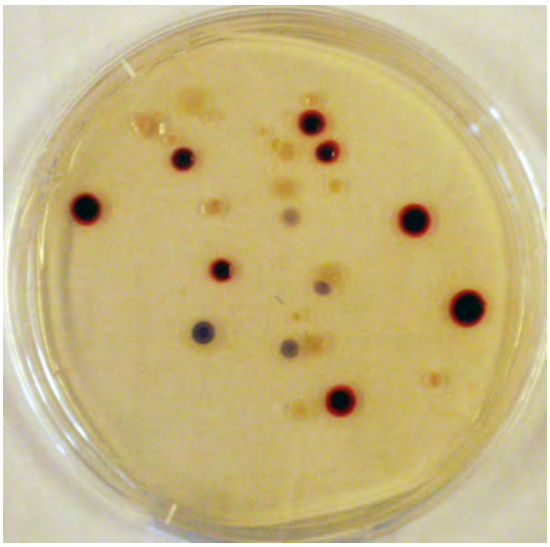


Figure 21 - Agar plate with marine medium after 7 days of incubation at 20°C (1:100 dilutions)

CFU counts were recorded each day for up to 7 days during aging of the medium for biodegradation tests. Experiments with aged medium showed that no change could be observed when data was compared to non-aged/fresh inoculum suspension.

Another experiment confirms the estimated correlation between growths of biomass/increase in colony forming units during degradation of an easily degradable substance. PEG was used to investigate the effect and to demonstrate that colony forming units can be used as a parameter to measure roughly the amount of cells in marine medium although only about 1% of all marine microorganisms might be cultivable in the laboratory. The experiment was done to a) verify correlation between colony forming units count and biodegradation and b) to precondition marine microorganisms to PEG and to study if afterwards degradation would be much easier, specifically if degradation of PEGs with higher molecular weight distribution would occur more easily. The last part will be discussed later in chapter 4.8. As can be seen in Figure 22 while TOC decreases because of biodegradation of the PEG, colony forming units counts increases from 10^5 to almost 10^7.

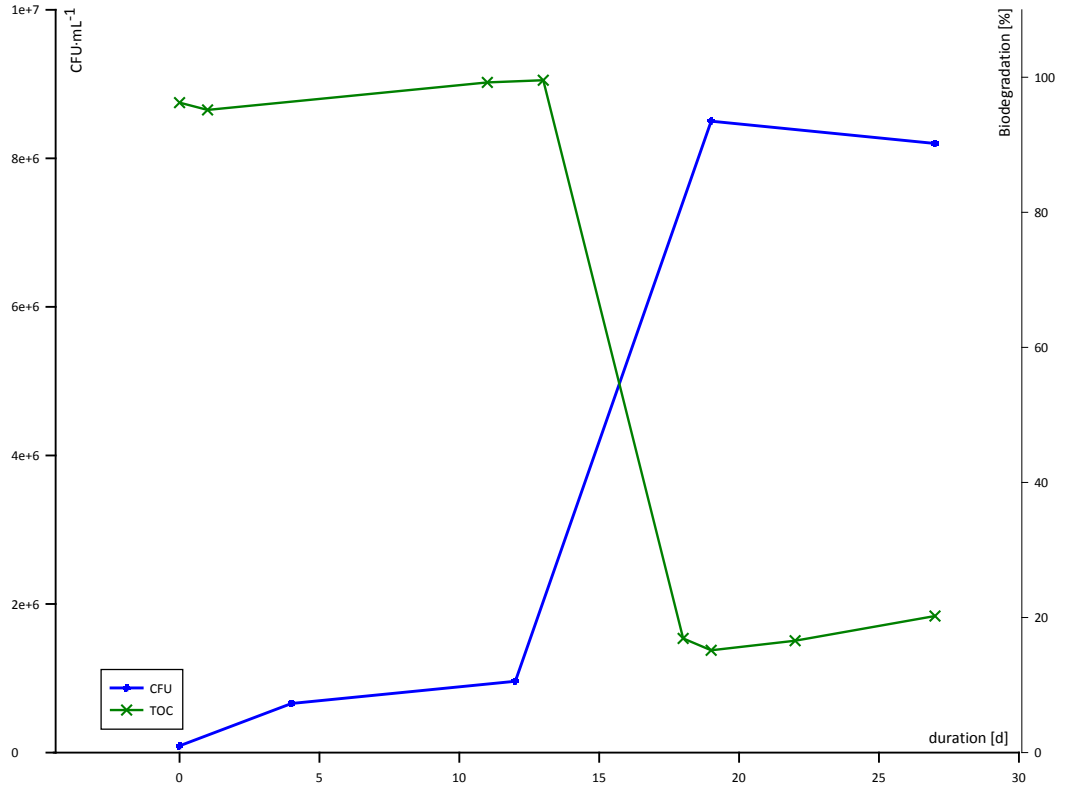


Figure 22 - Correlation between number of colony forming units and degradation in biodegradation tests (PEG 2'000)

4.3 Molecular Biology Experiments with medium samples

A few samples of marine inoculum suspension taken from Luisenpark Mannheim, Germany and from Sylt Westerland were investigated on molecular level as reference samples to biodegradation tests. The samples are given in Table 31 and Table 32 (Samples 30-36). The samples were processed as described above and DNA was sequenced. Figure 23 shows DGGE results for five samples taken from sea water aquarium at Luisenpark

and used for biodegradation tests. It is interesting that only few bands were observed and only few hits where achieved from those samples.

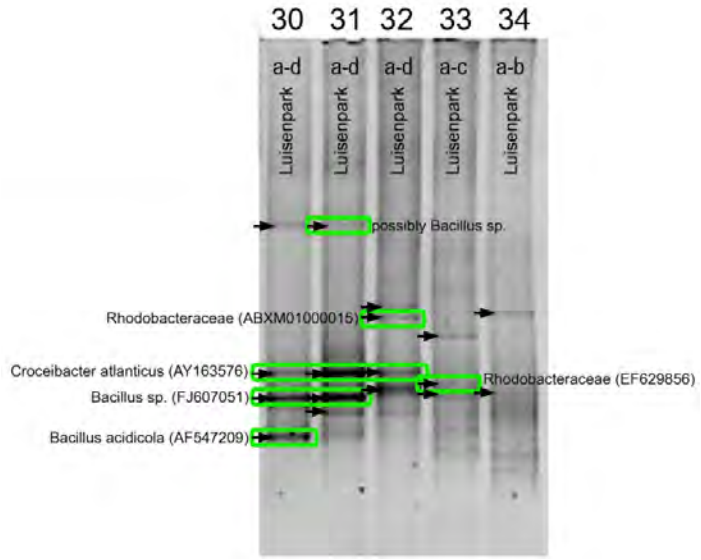


Figure 23 - Denaturing gradient gel electrophoresis from Samples 30 to 34 with selected/identified bands (bands marked with arrows were submitted to sequencing and those with green frames were identified with forward and reverse sequence in nBLAST)

The same samples and also 3 samples from Sylt Westerland were analyzed twice (Figure 24) to make sure that results are reproducible. The results show again, that only few organisms could be detected. Along, some bands were not accurately matched with existing data because only one of the two sequences matched results or alignment was impossible because of limited data from sequencing.

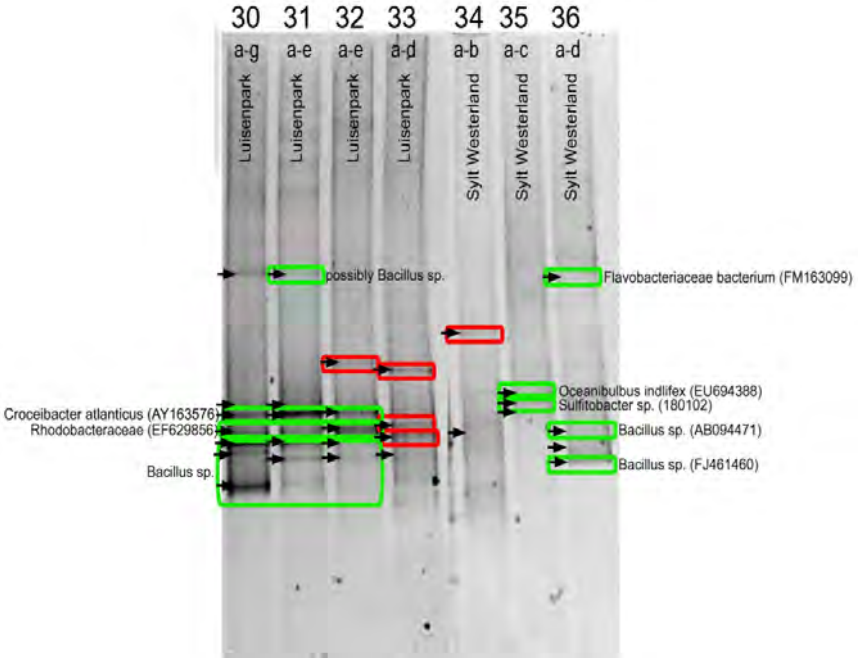


Figure 24 - Denaturing gradient gel electrophoresis from Samples 30 to 36 with selected/identified bands (bands marked with arrows were submitted to sequencing and those with green frames were identified with forward and reverse sequence in nBLAST. Bands that were only identified with low accuracy or where only one sequence (forward or reverse) matches database sequences are framed with red boxes)

When the data is compared with results obtained from degradation experiments it is interesting, that the test assays differ much from the analyzed media and there is also high variability between the test assays. Only a few organisms were found in the medium samples and none of these organisms were detected in the test assays at the end of the biodegradation experiments, but many other organisms were found.

During this research no method was found to accelerate marine biodegradation tests in any way. Higher concentrations of microorganisms and/or changes in the concentration of the test substance did not result in the desired acceleration of the tests. The assumption can be made, that influences and interactions between different types of bacteria, fungi, viruses and others may also complicate the processes. If degraders would be found, one would need to confirm degradation by isolating the organism and by the use of the cultures in another experiment. If no degradation is observed there, or if a steady state is reached, this does not necessarily mean that degradation is not possible. Also effects within the food chain or other population dynamics can cause such results.

4.4 Basic carbon analysis statistics

To ensure correct carbon measurement (TC/TOC/TIC), the carbon analyzers used where cross checked and calibrated on a random basis under GLP and ISO17025 certification. Before and after each analysis, standards where run to confirm correct setup and working of the instrumentation. The data shown in Figure 25 gives a good overview of the reliability and confirms the accuracy of the measurement. The data was collected during the complete time of measurement of any biodegradation test.

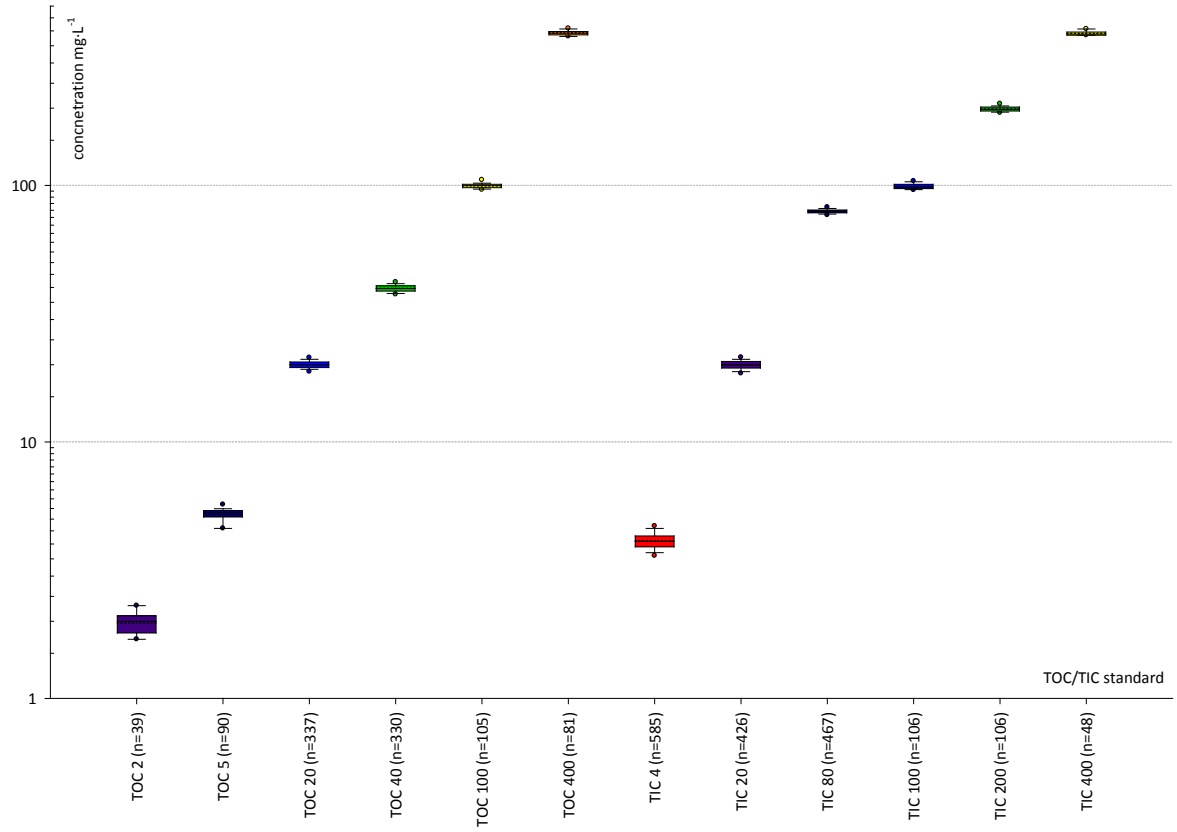


Figure 25 - Total carbon/total organic carbon/total inorganic carbon measurement and calibration statistics

4.5 Evaluation of blank control assays from biodegradation tests

To evaluate the results of biodegradation tests, it is imperative to estimate the influence of different factors to the outcome of results. Therefore certain parameters were monitored and documented. To confirm each biodegradation test, blank controls and reference substances are submitted under the same conditions. All determined/measured blank controls where cross checked to ensure the lowest possible variation between biodegradation tests. The data was obtained from basically the same test designs in each of the tests. Measurements were made during different tests for each parameter throughout the duration of the test to show variation between many tests and also throughout the tests. It was distinguished between medium (marine and WWTP) and measurement parameter/endpoint (DOC or CO_2). Additionally, the NaOH solution blank control (DIC) prior to each measurement interval when trapping flasks were switched and measured was statistically evaluated. Figure 26 shows that generally dissolved organic carbon values of blank control assays are quite low and very steady in both test media. Also NaOH solution shows only low variability. Since the data for NaOH solution showed no significant difference between marine and WWTP tests, the data is given in this figure in one box together for both media. It is interesting, that CO_2 evolution from the blank control assays shows much higher variation although carbon dioxide free air was used to aerate the test assays. This explains why carbon dioxide evolution tests may give higher variation in the final results as well because blank control data is subtracted in the calculation of the biodegradation degree of a tested substance. It is also interesting, that marine biodegradation tests show higher variation in carbon dioxide blank control levels than in WWTP tests when comparing 50% of the data (given by the box). However, the distribution of the data seems different. When 90/10 and 95/5 percentile is compared, WWTP tests show higher variation than marine tests.

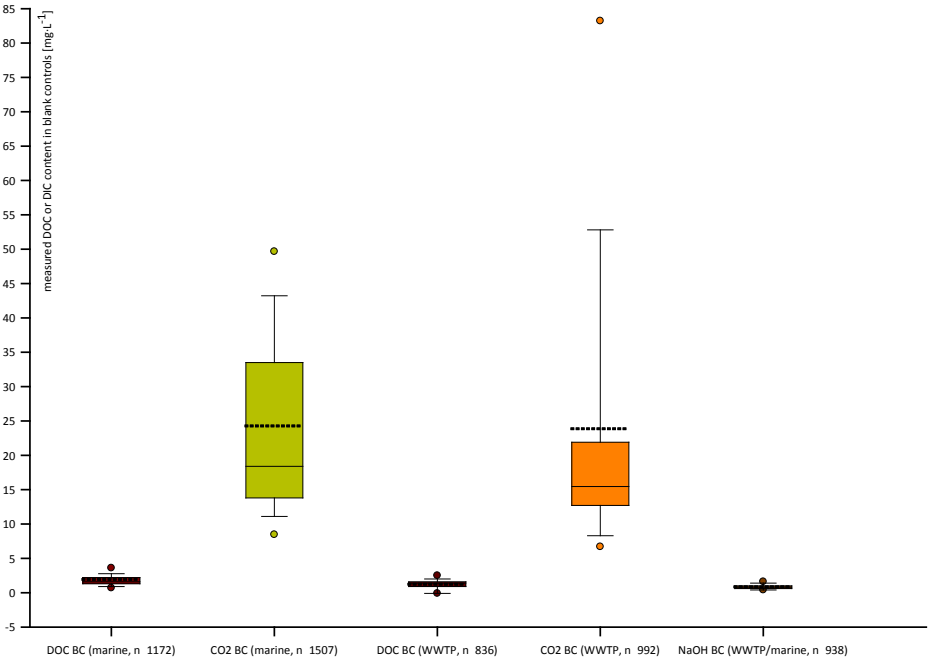


Figure 26 - Statistical evaluation of measured blank control data from marine and WWTP biodegradation tests (data based on tests of a maximum duration between 60 days of exposure for activated sludge tests and up to 180 days for marine tests. Data of blank controls show variation characteristics that seem independent from experimental duration when single values are observed, which could be due to variations in a) airflow or b) quality of the carbon dioxide free air and c) variations caused additionally by the medium itself

4.6 Reference substance statistics

In standard biodegradation tests aniline is mainly used as reference substance to proof that the medium has basic biodegradation potential. A typical biodegradation curve for standard dissolved organic carbon-Die-Away tests is given in Figure 27.

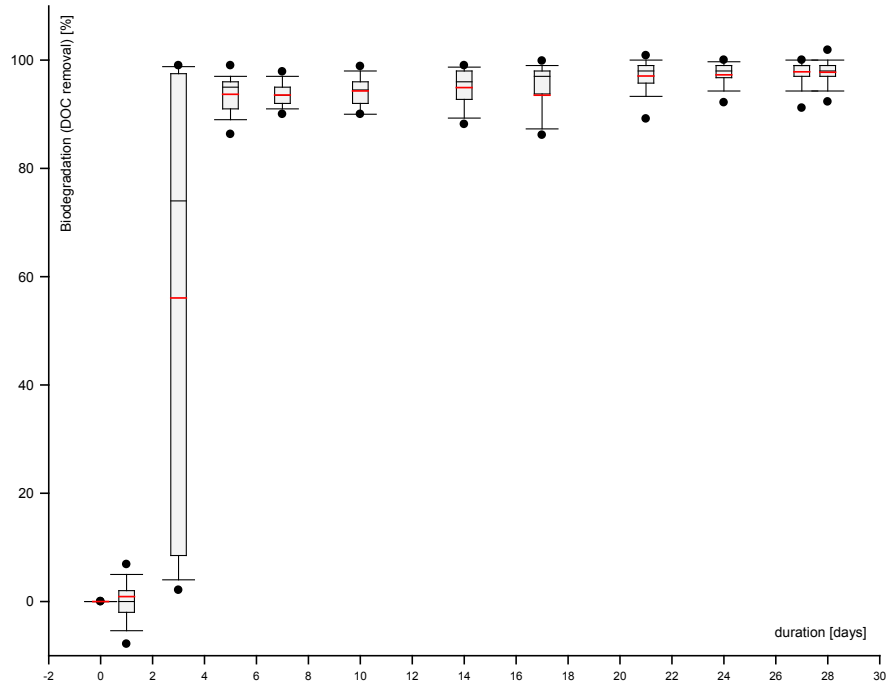


Figure 27 - Biodegradation of aniline in standardized OECD 301 A test (DOC Die-Away), n=42

The same biodegradation test but with measurement of carbon dioxide evolution (OECD 301B) gives the following results for reference substance (aniline) biodegradation (Figure 28).

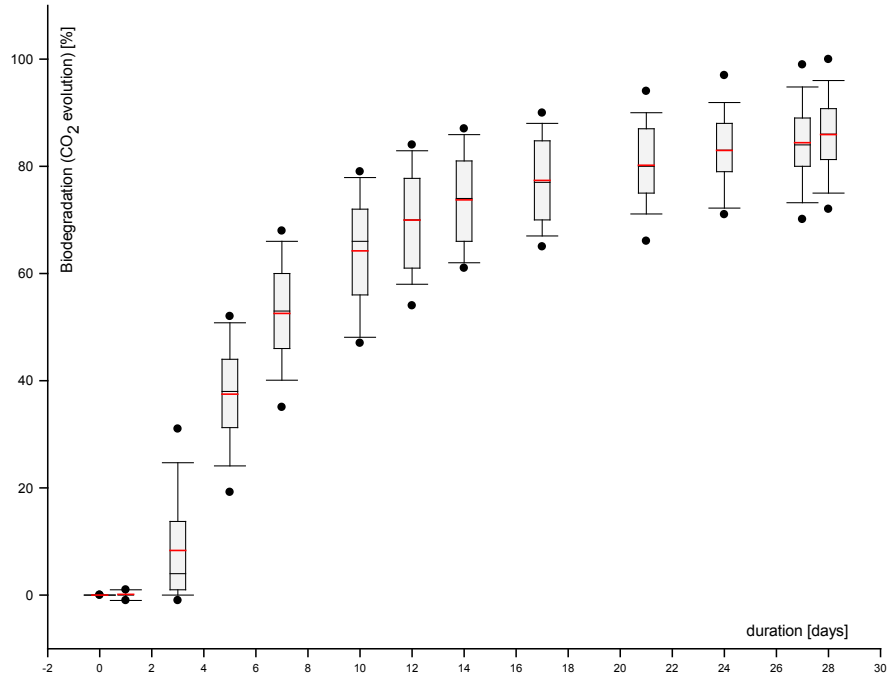


Figure 28 - Biodegradation of aniline in standardized OECD 301 B test (CO_2 evolution), n=100

If both figures are compared (see overlay in Figure 29Figure 30), it is obvious that the evolved carbon dioxide can be measured only with a short delay of about a few days. This is due to the test setup and to the biodegradation process. Also dissolved organic carbon based values are mainly higher at the end because formed biomass or bound residues are not detected anymore.

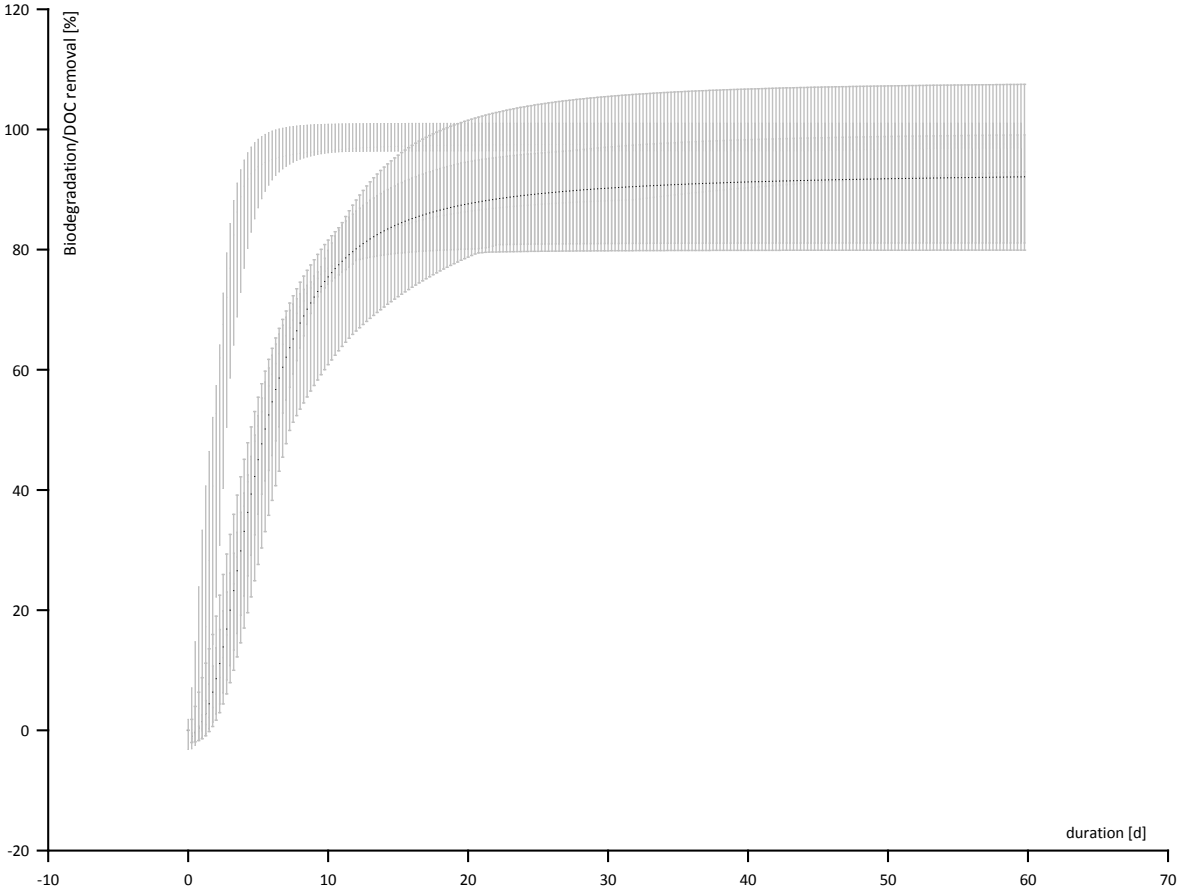


Figure 29 - Dissolved organic carbon removal and CO_2 evolution curve of aniline biodegradation in WWTP biodegradation tests (overlay)

Therefore CO_2 evolution is the more reliable parameter for biodegradation but it is also the more complicated one to determine and is much more prone to interference as can be seen in the following paragraphs. In marine biodegradation tests aniline can be used as well but mostly Na-benzoate is taken instead. To confirm differences between both substances within the same test medium, especially in marine tests, a biodegradation test in marine medium (OECD 306) was performed with the two substances over 28 days as well as a standard WWTP-test. The results of the marine test are shown in Figure 30.

It is interesting, that in marine tests, aniline degradation takes longer than Na-benzoate biodegradation, while in activated sludge tests biodegradation of both substances occurs within the same timeframe. The biodegradation kinetic for benzoate in marine tests is almost comparable to kinetic of aniline in WWTP standard tests. If Na-benzoate and aniline are submitted to a biodegradation test using WWTP activated sludge as inoculum (OECD 301), aniline and Na-benzoate show biodegradation rates that differ much less than in marine tests. The rate and biodegradation degree in WWTP test is similar for both test substances. No differences can be de-

tected with the used measurement techniques. Biodegradation of those substances in WWTP tests occurs very rapid in WWTP activated sludge which is why no difference can be observed.

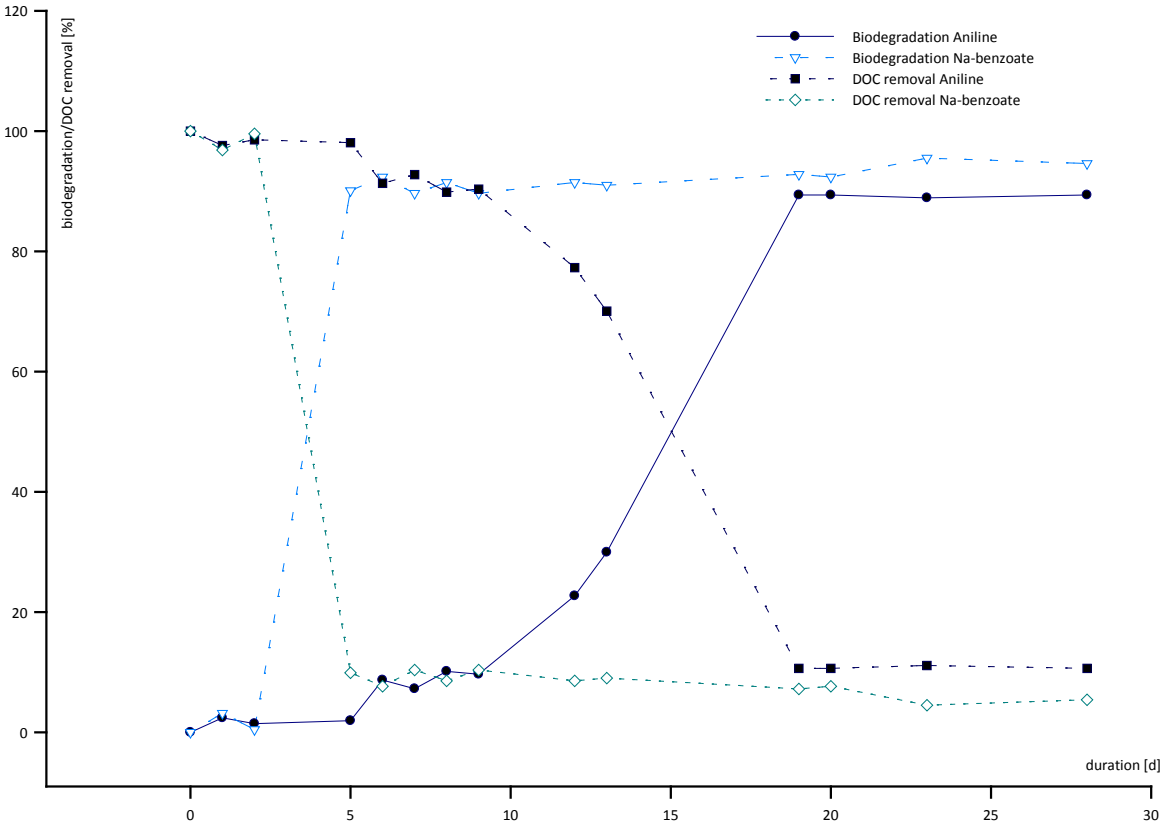


Figure 30 - Comparison of aniline and Na-benzoate in marine biodegradation test (OECD 306)

Further evaluation was done with the data collected from all other biodegradation tests. Dissolved organic carbon and CO_2 evolution was plotted for each reference substance and test, respectively. From the obtained graphs regression models were calculated to normalize and compare the data between different studies because generally samples were not taken always on the same days after the test was started. After each curve was fitted using a sigmoid regression model (Equation 43). The calculated parameters (a, b, x, x_0 and y_0) from regression of each degradation curve were used to recalculate virtual graphs using steps of 0.25, 0.5, 0.75, …, as x value.

Equation 43 - Sigmoid regression model for reference substance evaluation

$$f_{(x)} = y_0 + \left(\frac{a}{\left(1 + e^{-\left(\frac{x - x_0}{b}\right)}\right)^c} \right)$$

This procedure leads to a set of normalized/standardized data for each graph with now the same x-values. The calculated data was then statistically evaluated. For each step (on the time axis, x) min, max, mean, median etc. can now be calculated resulting in a standardized curve describing the data that was originally measured. This

overview of the scope and deviation between the test setups is shown in Figure 31 for marine tests with Na-benzoate and in for WWTP tests with aniline in Figure 32. The deviation could be determined to be 10-15% for the biodegradation of the test substances, respectively. In marine media the deviation was generally observed to be around 15%. It can be seen, that aniline is degraded very fast in WWTP activated sludge (Figure 32). Also it is observed that there is almost no difference between dissolved organic carbon removal and evolved carbon dioxide. In contrast to that, Figure 31 shows the degradation of Na-benzoate in marine medium.

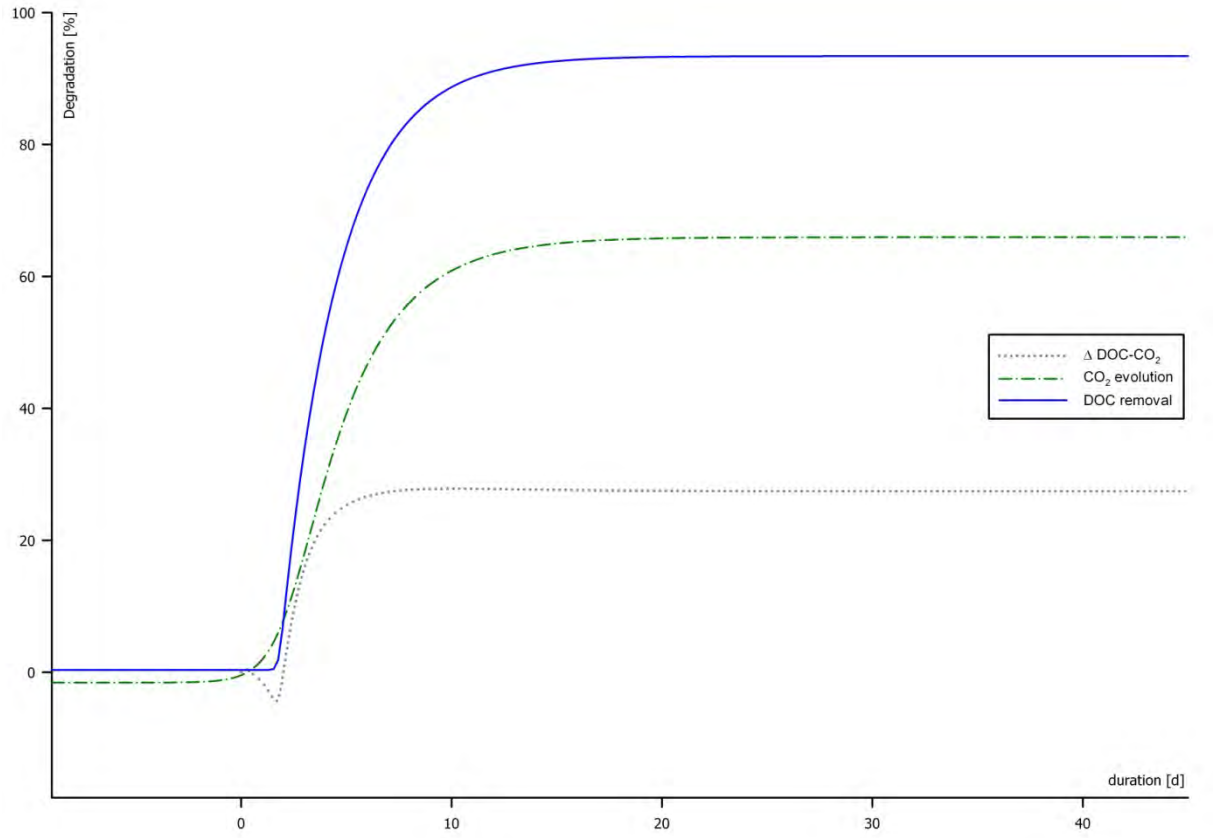


Figure 31 - Comparison of calculated mean biodegradation curves using data based on marine tests with Na-benzoate

It is very interesting that here, the biodegradation kinetic seems to be similar, but the difference between dissolved organic carbon and CO_2 parameters is significantly higher. The calculated mean dissolved organic carbon results are always approx. 20% higher than results generated from CO_2 evolution. The data used for this model was obtained from 11 reference substance test batches in WW and marine medium, respectively. When subtracting CO_2 evolution values from correlating dissolved organic carbon values, a comparison was made between different degradation processes (Figure 31 and Figure 32).

It can be clearly seen that for the used reference substances the differences are somewhat small but when using marine media, the gap between dissolved organic carbon removal and CO_2 evolution widens. It has also been observed that Na-benzoate degrades in WWTP activated sludge in the same way as observed for aniline (data not shown). Testing aniline in marine environment, one could observe the same kinetics in general as described for Na-benzoate (Figure 30) but a much longer lag phase of about 9-10 days in contrast to 2-3 days for benzoate.

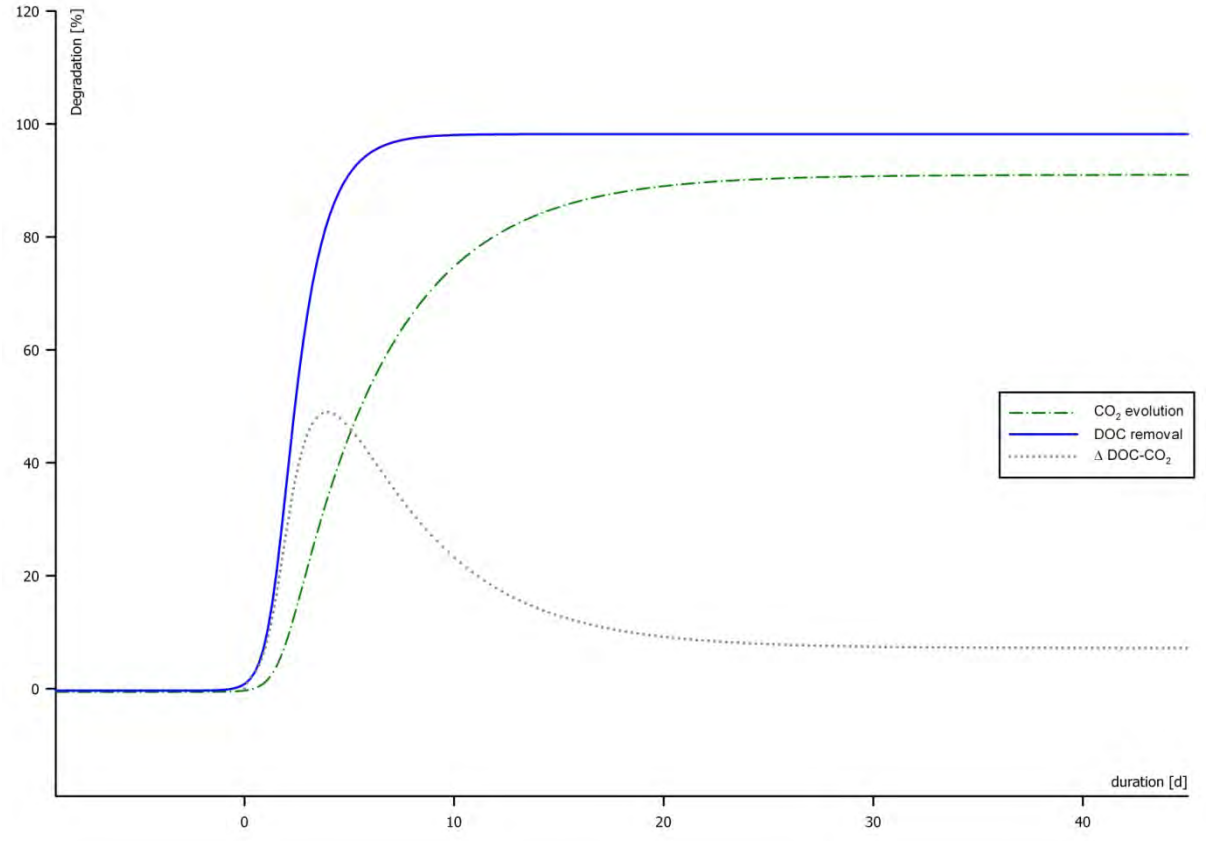


Figure 32 - Comparison of calculated mean biodegradation curves using data based on WWTP tests with Aniline

4.7 Biodegradation Tests with poly(vinyl pyrrolidone)

Within this research an important point was to compare different behavior of polymers in aquatic biodegradation tests. Therefore one focus was set on PVP biodegradation in the marine environment. PVP was prior reported to not biodegrade in freshwater environments [3] which was also confirmed later [208]. Figure 7 shows known biodegradation behavior of PVP. Only tests using UV or photochemical tools result in biodegradation of these polymers. PVP was submitted to marine biodegradation tests to compare the results and to confirm also that marine tests can be extended much longer and results are still reproducible.

Figure 33 - Marine dissolved organic carbon Die-Away test

PVP samples ranging from 2'000 to 1'500'000 g·mol^{-1} were tested in marine biodegradation tests. The medium was prepared using synthetic marine medium and inoculum suspension from the sea-water aquarium. 1L inoculum was used per 10L medium as described before and biodegradation tests were set up (e.g. such as the Die-Away test in Figure 33). As can be seen in Figure 34, PVP type polymers show almost no biodegradation in marine medium during the test period. The slight increase in the observed dissolved organic carbon removal in these experiments might possibly be due to adsorption to glass surface or biomass but most certainly not to biodegradation since no significant CO_2 evolution was detected (CO_2 evolution data not shown).

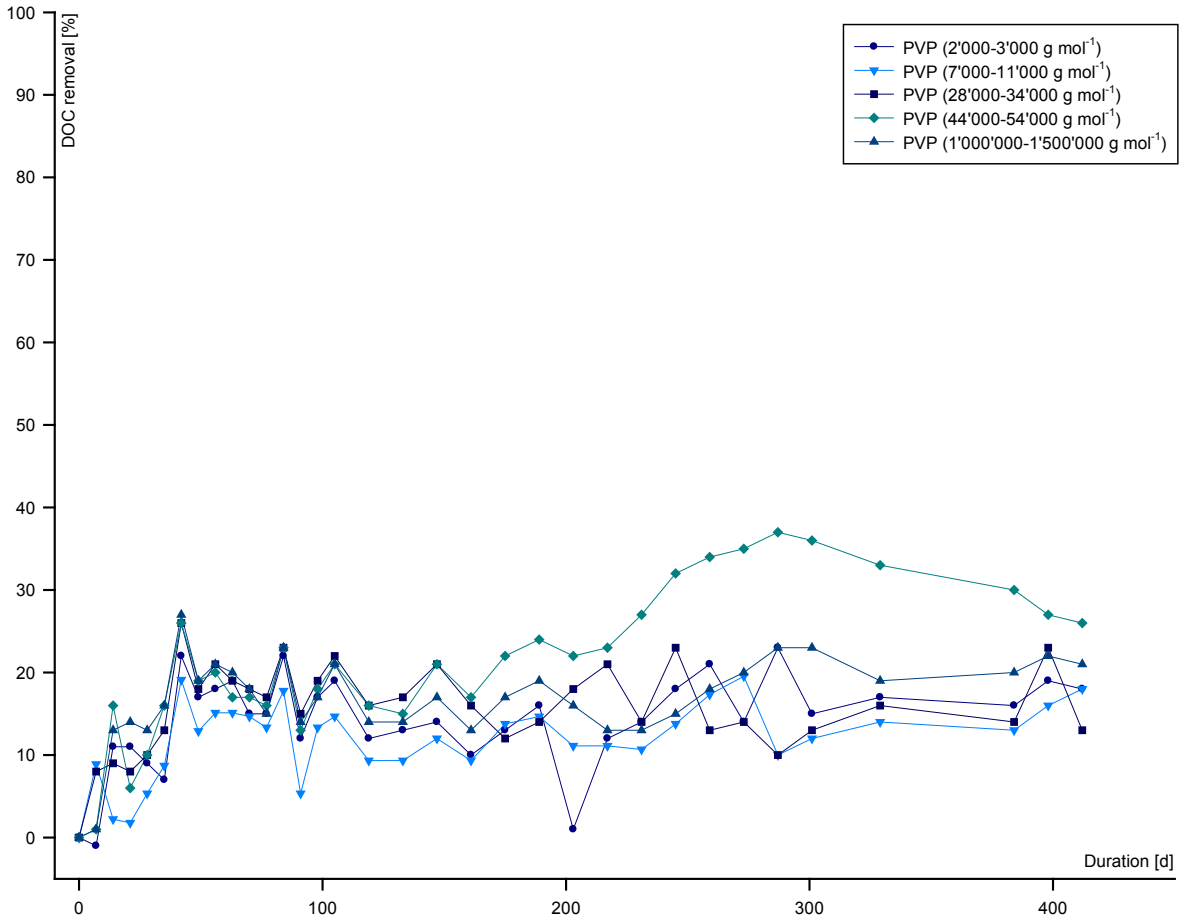


Figure 34 - Biodegradation of poly(vinyl pyrrolidone) samples in marine medium (two replicates for each polymer)

4.8 Biodegradation Tests with poly(ethylene glycol)

The aerobic biodegradation of PEGs having molecular weights from 250 to 57,800 g·mol^{-1} under simulated conditions in both freshwater and seawater media was tested (Table 38).

Table 38 - Biodegradation data for poly(ethylene glycols)

Experiment / TS / Medium	lag phase [d]	total experimental time [d]	Biodegradation phase (total time - lag phase) [d]	% biodegradation degree	% DOC removal degree	Mol weight (confirmed) [g·mol^{-1}]	Amount of substance at TC=20 mg·L^{-1} [mol·L^{-1}]	Time (10% DOC removal) [d]	Time (50% DOC removal) [d]	Time (90% DOC removal) [d]	Time (10% biodegradation) [d]	Time (50% biodegradation) [d]	Time (70% biodegradation) [d]	# Repeating units per molecule
Biodegradation experiments in freshwater medium with WWTP inoculum (activated sludge)														
250	4	30	26	80	100	230	1.6E-04	3.3	5.1	8.7	5.5	9.5	15.3	5.4
250	2	37	35	64	98	255	1.5E-04	1.6	4.8	14.5	3.8	13.0	--	5.4
930	2	29	27	90	100	981	3.8E-05	0.3	3.1	6.0	4.1	6.6	9.2	21
930	2	37	35	84	96	981	3.8E-05	1.4	2.7	10.5	1.8	6.4	12.2	21
2’000	4	29	25	90	100	2009	1.8E-05	3.3	4.2	7.5	4.3	7.3	9.7	45
2’000	2	37	35	51	97	2009	1.8E-05	1.1	2.1	5.3	3.3	5.5	9.0	45
4’500	4	29	25	85	100	4519	8.2E-06	3.4	5.9	6.9	4.9	8.9	13.2	102
4’500	3	37	34	74	90	4519	8.2E-06	1.9	3.4	4.2	4.7	8.9	7.1	102
7’400	4	29	25	90	100	7426	5.0E-06	3.5	5.9	6.9	5.3	8.9	12.2	168
10’300	6	29	23	80	100	10310	3.6E-06	4.0	6.3	12.0	6.5	11.0	7.5	233
14’600	6	29	23	80	100	14629	2.5E-06	4.1	6.2	12.5	5.7	9.9	6.5	332
26’700	22	70	48	100	100	26642	1.4E-06	20.0	26.0	30.0	19.0	27.0	31.0	605
57’800	22	70	48	100	100	56695	6.5E-07	19.0	34.0	54.0	22.0	38.0	45.0	1288
Biodegradation experiments in marine medium with inoculum from Luisenpark Mannheim. Germany														
250	4	28	24	45	93	230	1.6E-04	5	14	23	13	n.d.	n.d.	5.4
250	4	44	40	90	100	255	1.5E-04	4	14	30	4.5	23	44	5.4
250	5	60	55	80	98	230	1.6E-04	n.d.	n.d.	n d.	8	20	45	5.4
930	8	28	20	75	95	981	3.8E-05	4	12	17	9	15	n.d.	21
930	3	36	33	72	98	981	3.8E-05	n.d.	n.d.	n.d.	8	15	n.d.	21
930	8	28	20	72	93	981	3.8E-05	n.d.	n.d.	n d.	9	15	n.d.	21
2’000	10	30	20	70	90	2009	1.8E-05	8	15	23	10	20	n.d.	45
2’000	12	64	52	78	99	2009	1.8E-05	n.d.	n.d.	n d.	12	19	n.d.	45

Experiment / TS / Medium	lag phase [d]	total experimental time [d]	Biodegradation phase (total time - lag phase) [d]	% biodegradation degree	% DOC removal degree	Mol weight (confirmed) [g·mol^{-1}]	Amount of substance at TC=20 mg·L^{-1} [mol·L^{-1}]	Time (10% DOC removal) [d]	Time (50% DOC removal) [d]	Time (90% DOC removal) [d]	Time (10% biodegradation) [d]	Time (50% biodegradation) [d]	Time (70% biodegradation) [d]	# Repeating units per molecule
4'500	30	180	150	75	100	4519	8.2E-06	28	45	81	37	75	150	102
4'500	28	60	32	60	72	4519	8.2E-06	n.d.	n.d.	n d.	33	53	120	102
7'400	40	180	140	100	100	7426	5.0E-06	28	48	110	41	67	170	168
7'400	25	60	35	13	40	7426	5.0E-06	n.d.	n.d.	n d.	48	n.d.	n.d.	168
7'400	28	64	36	59	80	7426	5.0E-06	n.d.	n.d.	n d.	31	43	n.d.	168
10'300	40	180	140	94	100	10310	3.6E-06	28	35	53	37	52	190	233
14'600	36	180	144	10	45	14629	2.5E-06	27	130	n.d.	67	n.d.	n.d.	332
26'700	--	412	no biodegradation			26642	1.4E-06	40	no biodegradation					605
57'800	--	412	no biodegradation			56695	6.5E-07	40	no biodegradation					1288

Further on, Figure 35 shows the results (n = 2) of the combined CO_2/DOC Test obtained from degradation studies in freshwater with WWTP sludge inoculum. All PEGs with a molecular weight of up to 57,800 g·mol^{-1} are biodegradable in the given conditions. The PEGs can be divided into two groups: one group, the PEGs with molecular weight from 250 to 14'600 g·mol^{-1}, was fully biodegraded within 20 d. They had a lag-phase of up to 5 d; their graphs in the combined CO_2/DOC Test are similar in evolution. No differences concerning the biodegradation could be seen for these PEGs. The second group includes the PEGs 26'600 and 57'800, which had a longer lag-phase of approx. 22 d and were completely degraded within 45 d and 65 d, respectively. Evidently, the biodegradation of PEGs having an molecular weight > 14'600 g·mol^{-1}required much more time than that of PEGs with shorter chains but even PEG 57'800 was entirely biodegradable.

For PEGs up to molecular weight 57'800 g·mol^{-1} in freshwater media, the time taken for PEG degradation to reach the plateau phase generally increases with increasing molecular weight because the biodegradation rate (per time) decreases. The biodegradability (the part of the test substance, which is completely mineralized and characterized by the height of the plateau) is not affected by this. This is a new result because it is generally accepted that the biodegradation degree in freshwater decreases with increasing molecular weight [188;200].

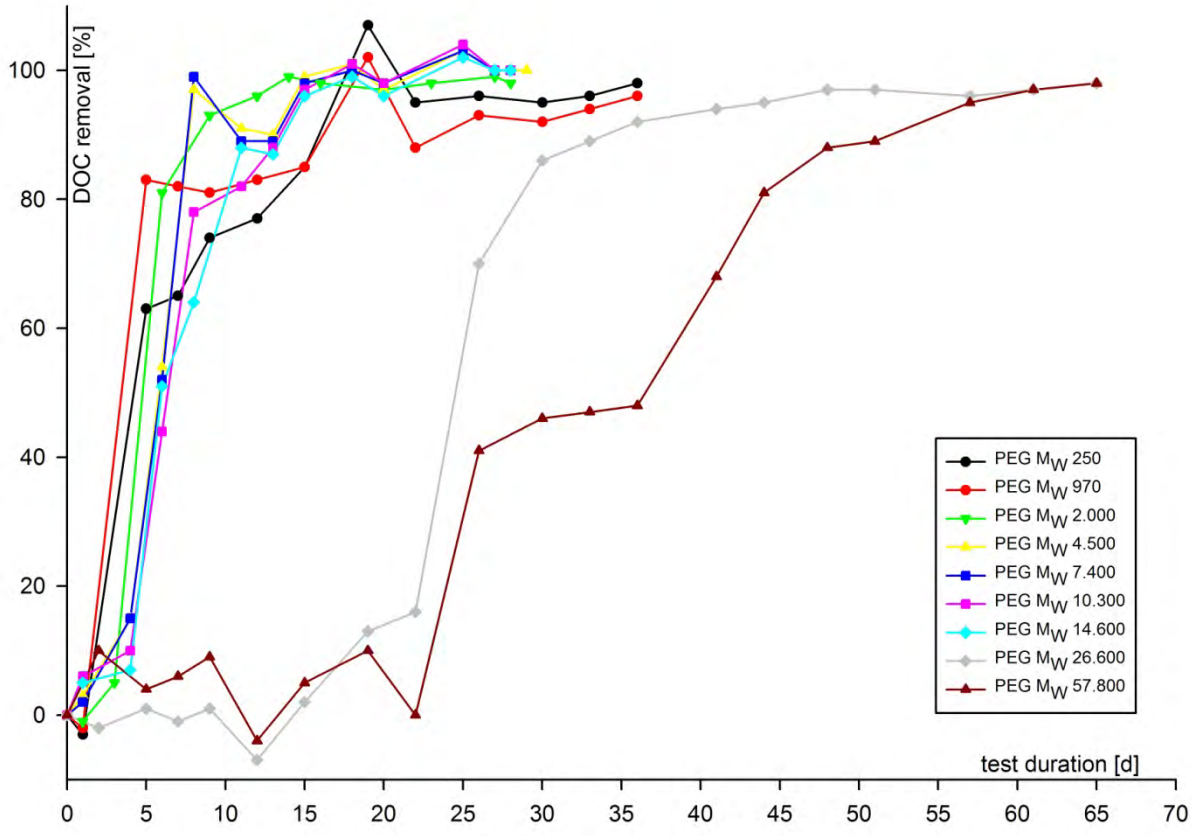


Figure 35 - Aerobic biodegradation (expressed as DOC removal in %) of PEGs in freshwater using WWTP sludge inoculum, 0 - 65 d (n = 2). Results were obtained from the combined CO_2/DOC Test

Aerobic biodegradation of PEGs in artificial seawater using marine bacteria is possible (Figure 36) but there are differences compared to freshwater media. The graphs of PEG 250, 970 and 2'000 have the same trend with a lag-phase of not more than 6 d, which is similar to freshwater media. These short-chain PEGs were fully biodegraded within 37 d. The PEGs with longer chains, PEG 4'500, 7'400, 10'300 and 14'600, all had a lag-phase of around 20 d until the biodegradation started. Their graphs are quite similar initially but then they vary, leading to different results for biodegradation. PEG 4'500 is completely degraded after approx. 100 d whereas PEG 7'400 needs around 130 d. PEG 10'300 has reached only 80 % dissolved organic carbon removal after 180 d and the degradation of PEG 14'600 has terminated after 50 d when dissolved organic carbon removal exceeded 40 %. Thus PEG 10'300 and 14'600 are not completely degraded in seawater media after 180 d, which is in complete contrast to the freshwater media in which they were entirely biodegraded within 20 d. PEG 26'600 and 57'800 were not degraded in seawater for a period of 135 d. Neither significant dissolved organic carbon removal nor CO_2 production was observed (Figure 36). This is contrary to freshwater media in which a full biodegradation of these PEGs could be seen. The reference compound sodium benzoate in the reference test systems of PEG 26'600 and 57'800 was degraded within 16 d indicating an active microbial population at the beginning of the test series.

As a result of PEG degradation in seawater media, the time required for it increases whereas the biodegradability decreases with increasing molecular weight. This was found for freshwater media [188;200] and can be transferred to seawater media.

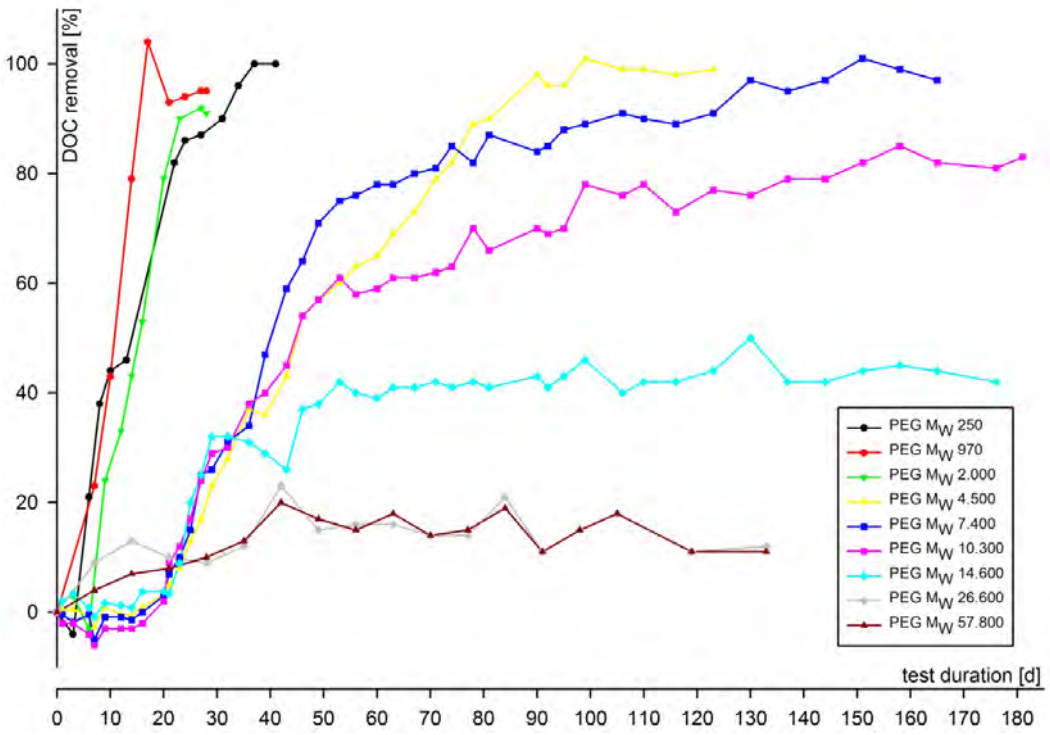


Figure 36 - Aerobic biodegradation (expressed as DOC removal in %) of PEGs in artificial seawater using marine inoculum, 0 - 180 d (n = 2). Results were obtained from the combined CO_2/DOC Test

The results based on evolved CO_2 from CO_2 evolution test (data not shown) and combined CO_2/DOC Test was similar for both test systems. The evolved CO_2 production did not reach 100 % of calculated theoretical CO_2 production, but was in a range between 70 and 95 %. The lag-times of graphs based on CO_2 Evolution were always longer than those of graphs created by dissolved organic carbon removal with differences being in the region of 1 to 3 d. Figure 37 is given as an example for these findings and this "discrepancy" should be considered in tests of water-insoluble polymers when no dissolved organic carbon can be measured.

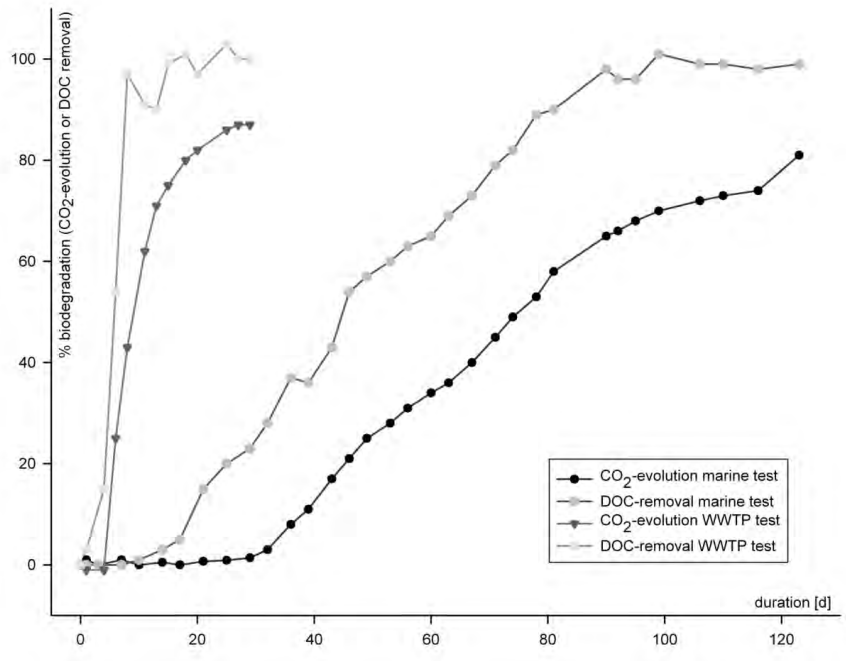


Figure 37 - Comparison of Measurement Parameters DOC and CO_2 in PEG 4500 g·mol^{-1} biodegradation tests using marine and activated sludge inoculum

The biodegradation tests are based on the determination of sum parameters. With the results obtained, it is possible to draw conclusions about the differences in biodegradation of PEGs in freshwater and seawater media in terms of time and chain length. It is impossible to compare the degradation pathways in both media of PEGs having the same molecular weight distribution, which may help to understand the difference between the biodegradation processes in the two media [289].

The results obtained from the biodegradation tests were confirmed using sophisticated analytical techniques [289]. In summary, the fate of each homologue of polydisperse PEG 250 and PEG 970 for freshwater and seawater media is shown in Figure 38 and Figure 39.

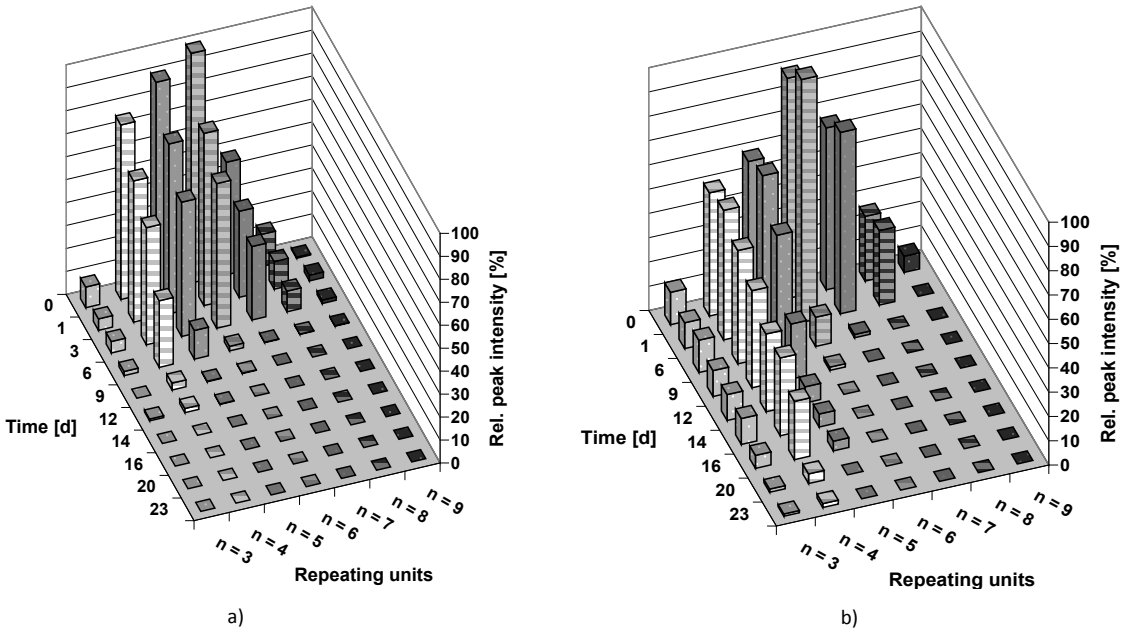
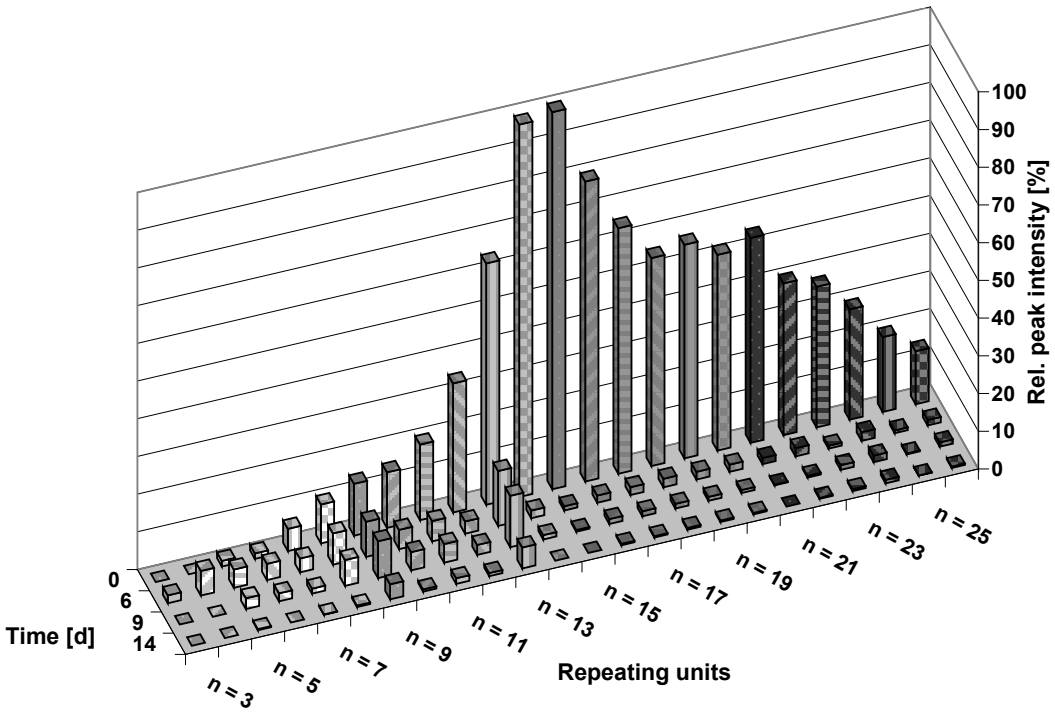
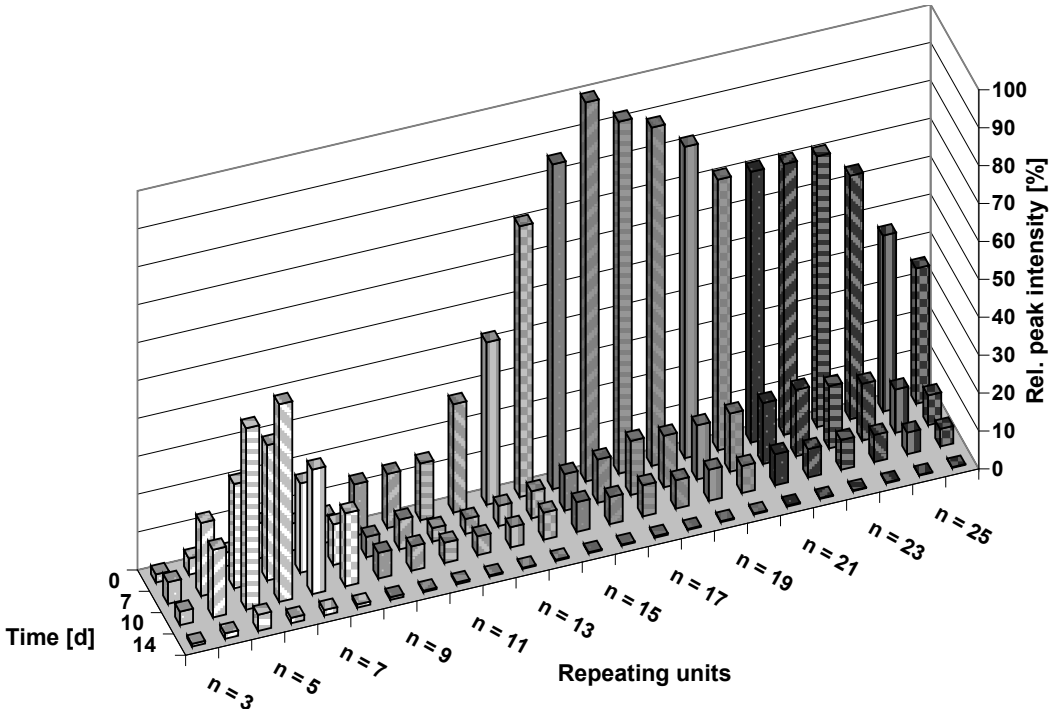


Figure 38 - Fate of individual homologues during aerobic biodegradation (0 - 23 d) of polydispersed PEG 250, measured by (+)-ESI-LC-MS. (a) freshwater media, (b) Artificial seawater media.

The peak intensity of each PEG homologue, measured as PEG-Na adduct by (+)-ESI-LC-MS, was set in relation to the peak intensity of the PEG homologue with the highest intensity. This illustration is limited due the different detector responses for the homologues of PEGs. Depending on the time, the change in intensities of each PEG homologue can be observed. At the beginning of the dissolved organic carbon removal test, the intensities of all homologues show the typical pattern of a polydispersed mixture. As can be seen for both media, the degradation of PEG 250 (Figure 38) and PEG 970 (Figure 39) started simultaneously with all PEG chains leading to a shift in the molecular weight distribution towards the short-chain homologues. The long-chain PEGs are completely degraded by gradual splitting of C_2-units off the chain. This is a common accepted pathway of aerobic PEG degradation in freshwater media [84;192], which now can be transferred to seawater media for PEG 250 and PEG 970. During degradation, short-chain PEG homologues are generated from the long-chain PEGs (Figure 38 and Figure 39). The amount of short-chain PEG increases in both media and decreases again when degradation proceeds (Figure 39). The aerobic biodegradation of polydispersed PEG 300 in freshwater with formation of short homologues is known [196]. However we found out not only the biodegradation of PEG 250 occurs in the same way for freshwater and seawater media but also PEG 970.



a)



b)

Figure 39 - Fate of individual homologues during aerobic biodegradation (0 - 14 d) of polydispersed PEG 970, measured by (+)-ESI-LC-MS. (a) freshwater media, (b) Artificial seawater media

Although the microorganisms are assumed to be different since we have not defined the microbial population, the biodegradation of PEG 250 and PEG 970 in seawater is the same as in freshwater with the only difference the biodegradation in freshwater media being faster. This result coincides with that reported for biodegradation of alkyl phenol ethoxylates and alkyl benzene sulfonates in seawater [434].

When searching for metabolites of PEG 250 and PEG 970, molecules having repeating units of 44 g·mol^{-1} were found. The molecules had an m/z of either 2 less or of 14 more than the m/z of corresponding PEG-Na adducts. The intensities of these metabolites increased during biodegradation and then decreased again (data not shown). This suggests the oxidation of one terminal OH-group to the corresponding aldehyde has occurred, which is the initial step of the biodegradation of PEG 250 and PEG 970 [84;192]. Further oxidation leads to the carboxylic acid derivative [84;192]. Figure 40 shows the results of the aerobic biodegradation of PEG 2000 in freshwater media measured by MALDI-TOF-MS.

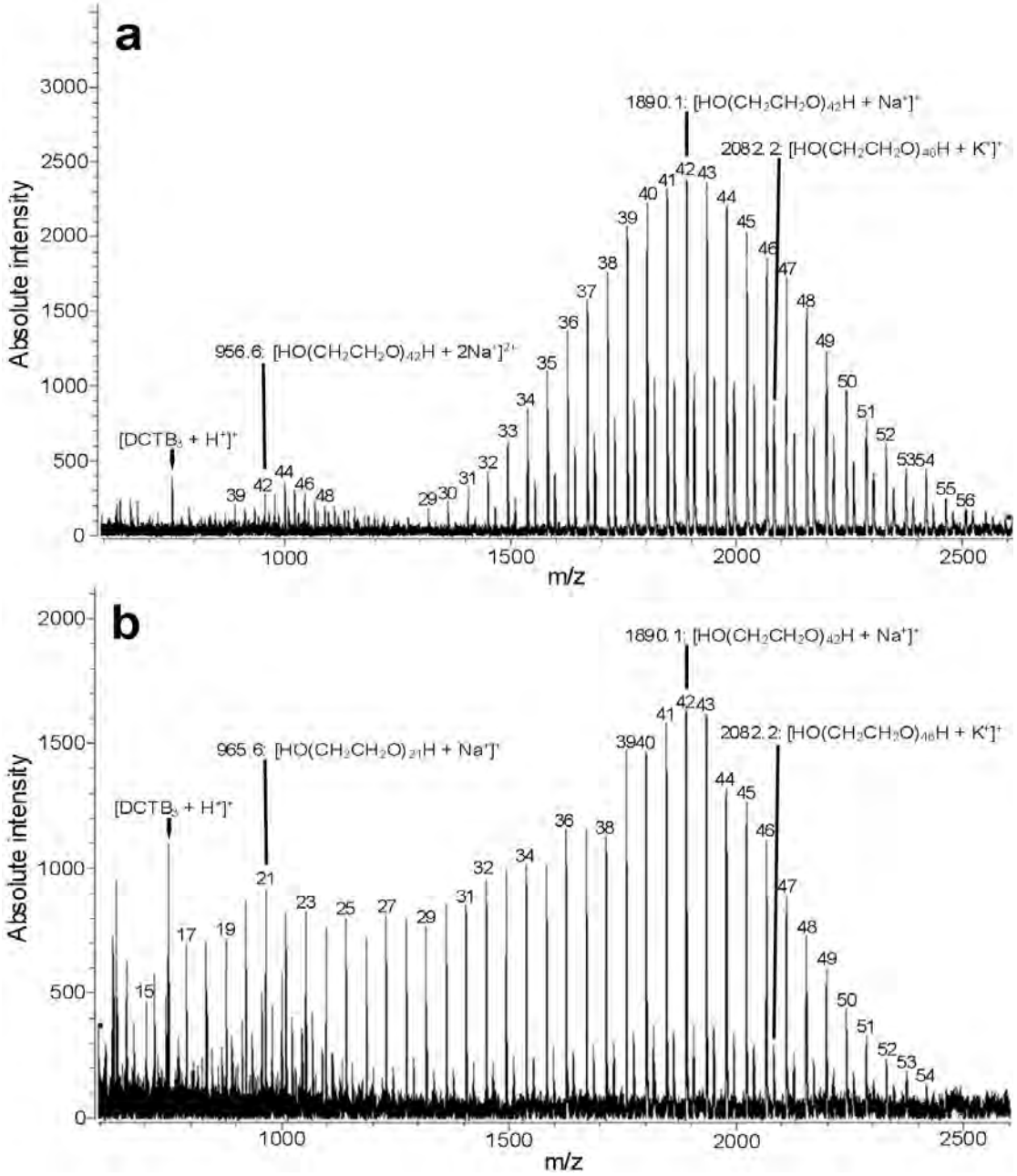


Figure 40 - MALDI-TOF-MS spectra of polydispersed PEG 2,000 during aerobic biodegradation in freshwater media with WWTP sludge inoculum, matrix was DCTB: (a) sample from day 1, (b) sample from day 6. Numbers indicate repeating units

On day 1 (Figure 40a), each individual PEG-Na-homologue (beside PEG-K-adducts and doubly charged PEG-2Na-adducts) of the polydispersed PEG 2000 can be seen in the spectrum, covering an m/z range from 1300 to 2600. When the biodegradation is in process on day 6 (Figure 40b), a shift in the chain length and molecular

weight of the former PEG 2000 has occurred. Individual PEG homologues cover an m/z range from 600 to 2500, indicating a loss of long-chain PEGs and a formation of short-chain PEGs. On day 9 (data not shown), PEG could no longer be detected. Obviously, the biodegradation of PEG 2000 is similar to that of PEG 250 and 970 for the freshwater media with formation of short-chain homologues.

In artificial seawater, a similar spectrum (Figure 41a) to that for freshwater media (Figure 40a) on day 1 was obtained for PEG 2000, with the difference that only singly and doubly charged PEG-Na-adducts were detected due the desalting of the sample and adding of NaTFA as cationization agent.

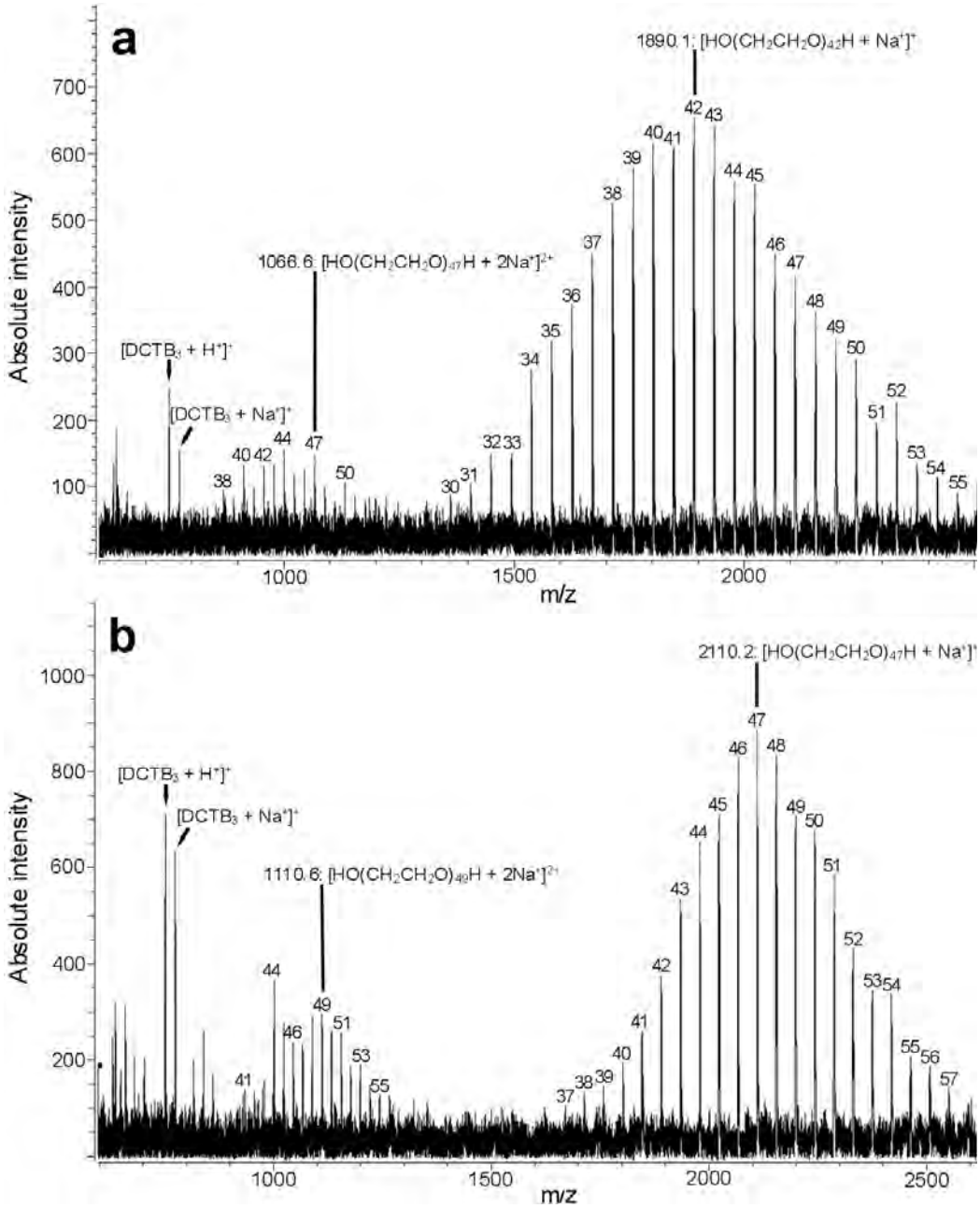


Figure 41 - MALDI-TOF-MS spectra of polydispersed PEG 2,000 during aerobic biodegradation in artificial seawater media with marine inoculum, matrix was DCTB: (a) sample from day 1, (b) sample from day 14. Numbers indicate repeating units

Although the dissolved organic carbon removal has reached some 50% on day 14 (Figure 36), no short-chain PEGs were detected at this time (Figure 41b). This is in contrast to the degradation of PEG 2000 in freshwater where short-chain homologues were found (Figure 40b). Comparing Figure 41b with Figure 41a, a loss of some

individual PEG homologues in the m/z range 1300 to 1600 can be observed but the long-chain PEGs are still present. Other samples of PEG 2000 (day 12 and day 16, data not shown) were analyzed, showing the same distribution of long-chain homologues. On day 20, only PEGs having a molecular weight between 1900 and 2700 $g \cdot mol^{-1}$ were present (data not shown), short-chain PEGs could not be detected. Evidently, PEG homologues with molecular weight <1900 $g \cdot mol^{-1}$ are degraded preferentially prior to long-chain homologues. The degradation of PEG 2000 is as fast as that of PEG 250 and PEG 970 in seawater media and was complete after 27 d (Figure 36). A different biodegradation pathway of PEG 2000 in seawater can only explain the difference. This pathway of PEG homologues having a molecular weight >1900 Da is characterized by a stable molecular weight pattern during degradation.

MALDI-TOF-MS spectra of PEG 4500, PEG 7400, PEG 10'300 and PEG 14'600 were recorded for freshwater media. All PEGs mentioned show a shift towards short-chain PEGs when the biodegradation is in progress (data not shown), meaning that PEGs from molecular weight 250 to 14'600 all have a similar degradation pathway in freshwater, which is characterized by gradual loss of one oxyethylene group resulting in formation of short-chain PEGs. During degradation of PEGs 4500 to 10'300 in artificial seawater media, no short-chain PEGs were formed and detected using MALDI-TOF-MS (data not shown) what suggests a similar degradation pathway as that of PEG 2000 in seawater. The long-chain PEGs were present until the biodegradation has come to an end, no change in the distribution pattern was observed. On day 90 of the biodegradation of PEG 10'300, the long-chain PEGs have disappeared (data not shown), indicating a complete primary degradation of this PEG in artificial seawater under the simulated conditions.

4.8.1 Adaptation of marine microorganisms

To gain an insight on the biodegradation process two sorts of additional experiments have been performed. First, the same PEG sample (all PEGs in this study were tested) was spiked at the same concentration (DOC ≡ 20 $mg \cdot L^{-1}$) after it was degraded completely by microorganisms in the first (and non-adapted) setup. It could be observed that all PEGs from 250 to 58'000 $g \cdot mol^{-1}$ are degraded right away without a lag-phase and biodegradation is completed within a few days (<6 d) in freshwater media (data not shown). In marine media the same experiment could not be done due to the lack of complete biodegradation of PEGs >2000 $g \cdot mol^{-1}$. Thus it was investigated if, after a "smaller" PEG molecule was degraded, a PEG with an exceptionally increase in molecular weight would be degraded more easily than before.

PEG 14'600 $g \cdot mol^{-1}$ was spiked after 120 d in a marine test where PEG 4'500 $g \cdot mol^{-1}$ was biodegraded first. But even after 50 additional days no biodegradation of the second PEG could be observed (data not shown). The same was done with a PEG 10'300 $g \cdot mol^{-1}$, which was spiked in a test after 50 days where PEG 250 $g \cdot mol^{-1}$ had been degraded. In this case degradation could be observed (data not shown) but not as quick as after spiking the same PEG in an adapted medium.

Another experiment with pre-adaptation of marine microorganisms for biodegradation study of poly(ethylene glycols) with molecular weight distribution above 4000 $g \cdot mol^{-1}$ was performed. Therefore 5L of marine water was taken from filter units from a sea-water aquarium at Luisenpark. The amount of water was squeezed gent-

ly out of the filter units. About 10-15 units were needed to recover 5L of water with an enriched population of microorganisms. The water samples were transported to the Laboratory within one hour after sampling. The water was filtered using a sieve with a mesh size of 70µm. For dry mass determination 1200mL of the water sample was centrifuged at 0°C and 12500xg for 20min. The supernatant was discarded and the pellet was washed twice with artificial sea-water and centrifuged again after each washing step. The pellet was afterwards transferred to weighing bottles with a previously determined weight. After drying the samples overnight at 110°C the final weight was determined and the dry mass calculated. The sea-water sample was aerated until further use for about 7 days. The colony forming units were determined with marine agar plates and an appropriate dilution. Then the complete water sample was centrifuged in aliquots of about 400mL at 0°C and 12'500xg for 20min. The supernatant was discarded and the pellet was re-suspended in artificial sea-water and made up to a total volume of 8.5L. The appropriate amount to obtain a final concentration of 20 $mg \cdot L^{-1}$ total carbon of PEG 2000 was added. PEG has a TOC of 540 $mg \cdot g^{-1}$ and therefore 37 $mg \cdot L^{-1}$ are needed. The substance dissolves quickly in water. The medium was aerated with carbon dioxide free air and stirred on a magnetic stirrer. To prevent too much loss in water by evaporation the bottle was covered with Parafilm® and kept in the dark at 22±2°C. A sample was taken and dissolved organic carbon was determined to confirm that the calculated dissolved organic carbon content. Samples were taken in intervals for dissolved organic carbon determination and plating to get colony counts. After the dissolved organic carbon had decreased to less than 4 $mg \cdot L^{-1}$ (after 28 days), the complete amount of medium was centrifuged again in aliquots of about 400mL at 0°C and 12'500xg for 20min. The supernatant was discarded and the pellet was re-suspended in freshly prepared synthetic sea-water and made up to 15L. The obtained marine water was used for degradation studies PEG 7'400 and 10'300 $g \cdot mol^{-1}$

As shown in Figure 22 (p.117) the pre-adaptation of microorganisms with PEG 2'000 $g \cdot mol^{-1}$ was successful. With increasing number in colony forming units a decrease of the dissolved organic carbon from the removal of PEG can be observed. After adaptation and spiking the new medium with PEG 7'400 and 10'300 $g \cdot mol^{-1}$, it was discovered that the adaptation phase does not seem to have any effect on the biodegradation of the PEG 7'400 and 10'300 $g \cdot mol^{-1}$ (data not shown).

4.8.2 Experiments with 10-fold increased PEG concentration in marine medium

In order to investigate possible threshold levels in biodegradation properties of the PEGs in marine tests, the concentration was increased by a factor of 10 and it was observed that the biodegradation rate decreased drastically by the same factor. The degradation rate during the degradation phase (per day) was calculated for PEG 2000 at approx. ~5.0% (at 20mg dissolved organic carbon per liter) and ~0.50% (at 200mg dissolved organic carbon per liter). For PEG 7400 the degradation rate was estimated to around ~2.3% (at 20mg dissolved organic carbon per liter) and ~0.23% (at 200mg dissolved organic carbon per liter).

When PEG 2000 and PEG 7400 are compared the 3.7 fold increase in molecular weight (from 2000 to 7400) decreases the biodegradation rate by approx. half for both test concentration in marine medium.

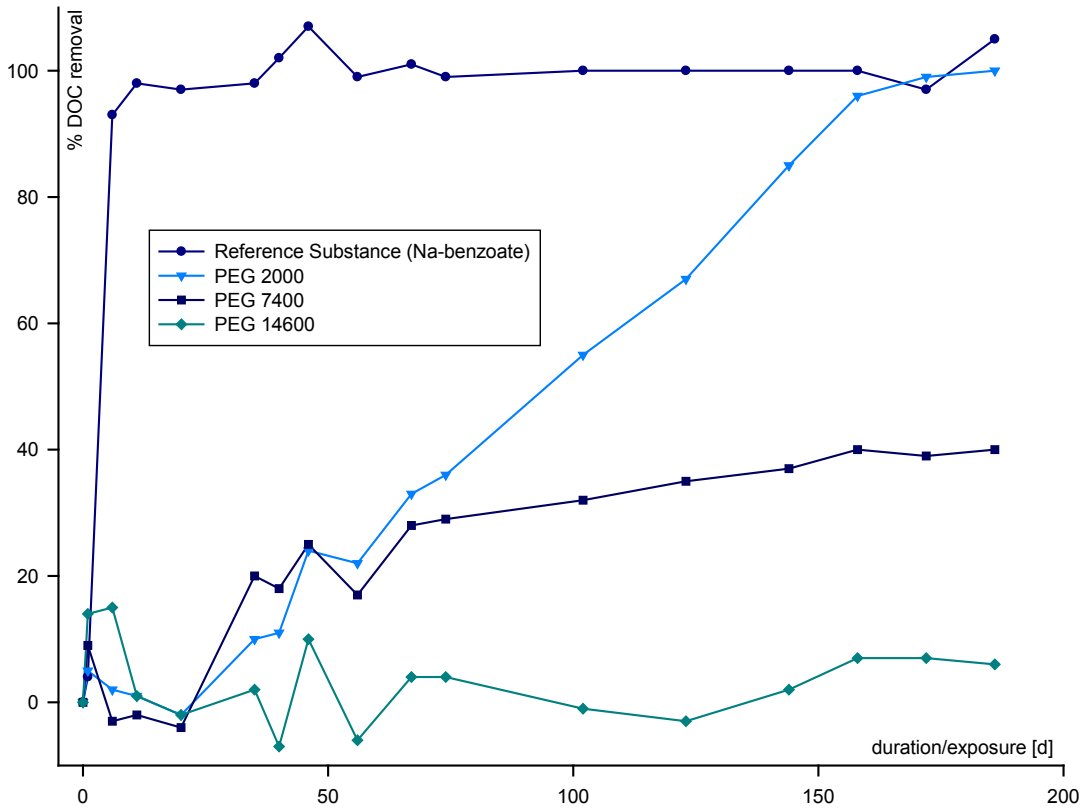


Figure 42 - Marine biodegradation of poly(ethylene glycol) samples at 10-fold increased test concentration (200mg·L^{-1})

4.8.3 Results from molecular analysis of marine PEG degradation tests

After sequencing, the data was analysed automatically. The forward and reverse sequence was compared to NCBI nBLAST library and the closest hits were than confirmed manually: if the corresponding forward and reverse sequences did not give same results, sequence alignment was performed and then again compared to the database. The results are given in Figure 43.

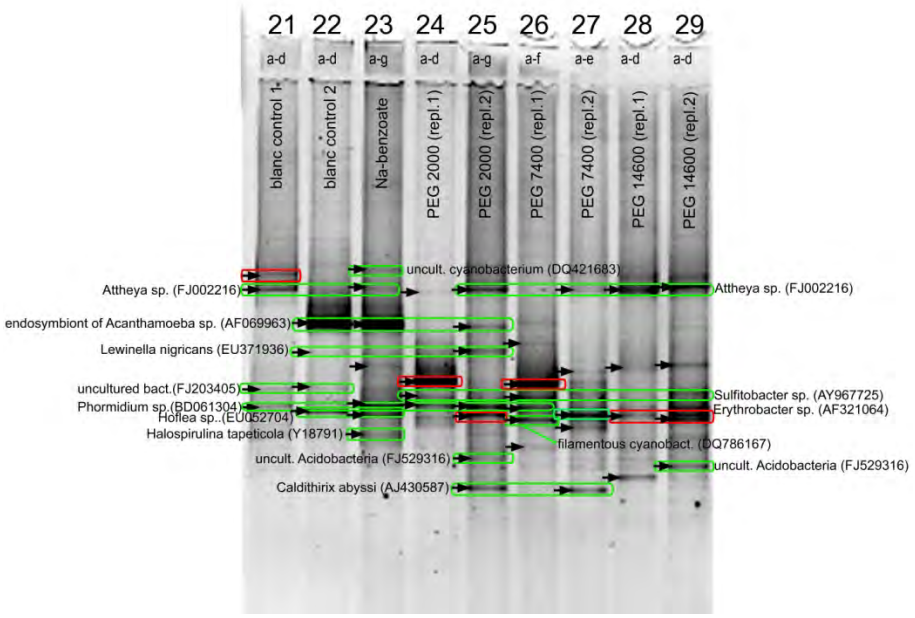


Figure 43 - Results of sequencing of samples from marine poly(ethylene glycol) biodegradation experiments

Green frames in the picture are used to highlight sequences where probability for the identification of the organism was higher than 98%. Red frames show DGGE bands were organisms were not accurately identified. Arrows show bands which have been sequenced but no hits were found in NCBI databases. The samples analysed were taken from biodegradation tests at the end of exposure after 206 days of a special experiment using 10-fold increased concentration of test substance (Figure 42).

It is very interesting to see that biodegradation can in this case not be linked to the data obtained by molecular analysis. The pattern of isolated strains seems random and it would be anticipated to have similar microorganisms in each of the corresponding test replicates. Also it would be anticipated that some organisms in the blank controls would be found in test replicates, since each of the replicates and blank controls was prepared from the same medium. The pattern of the strains shows that probably the microorganisms responsible for biodegradation of the PEGs and also the control substance Na-benzoate have not been detected. It also shows that marine microorganisms are able to survive for a long time even without nutrients (as is the case in blank controls). This may be due to the carbon storage capacity of marine water and the fact that at least some small amount of CO_2 will always be carried into the vessel and the medium by aeration.

Along with samples from biodegradation experiments marine water and synthetic marine medium were analysed at begin of exposure to show the conditions prior to the experiment Figure 44. Samples 30-33 were taken from marine synthetic water prepared as described in materials and methods section and samples 34-36 were taken from Sylt, Westerland from the surface layer of the North Sea. When these patterns are compared to the results obtained after the biodegradation test shown above it is very interesting that originally only few strains can be identified and no corresponding data can be established.

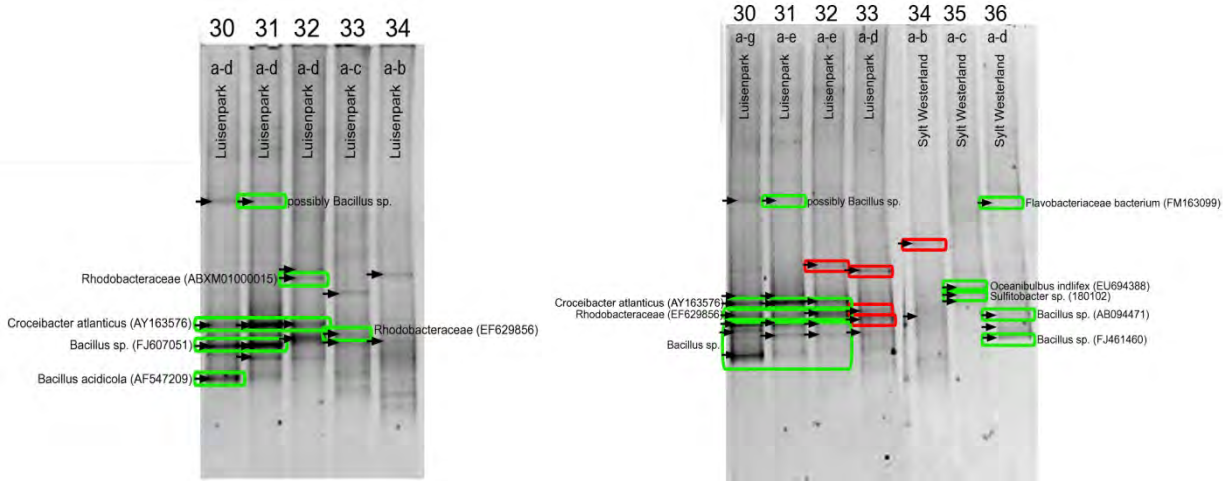


Figure 44 - Results of sequencing of samples from marine water (34-36, left photograph) and marine synthetic medium (30-33, right photograph)

4.9 Biodegradation Tests with Ecoflex

This is the first extensive study of aquatic biodegradability and the first trial of investigating pathways. The duration of the experiments is extremely long. In marine biodegradation tests this does not seem too much of a problem, since the microorganisms are used to live and survive in nutrient limited environment but it poses a

problem for WWTP biodegradation tests. In biodegradation tests the aerobic biodegradation of aliphatic-aromatic polyester was tested using 5 replicates and synthetic marine medium with inoculum from Luisenpark Mannheim as well as 3 replicates in WWTP inoculum (freshwater tests).

4.9.1 Aromatic aliphatic polyester biodegradation

The biodegradation in freshwater/WWTP medium was observed for about 500 days. The results are shown in Figure 45. Even though inoculum suspension was added during this test, no biodegradation could be observed. The decrease and sharp increase at the end of the test may be possibly due to fluctuations in aeration between blank controls and test replicates.

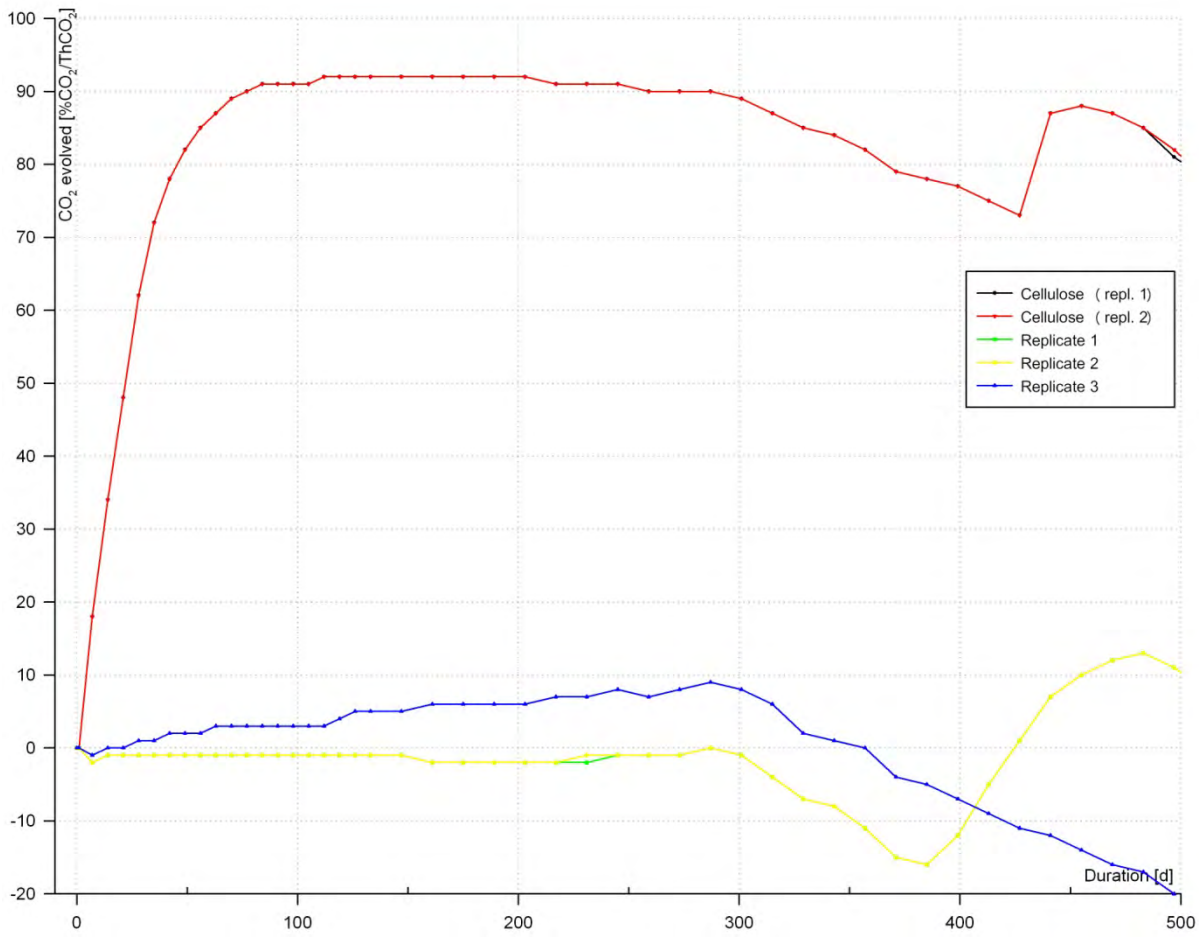


Figure 45 - Waste water treatment plant/freshwater biodegradation test results of Ecoflex

In the experiments with freshwater/WWTP inoculum, additional inoculum suspension was added once a month (5mL, prepared as described before) to ensure continuous availability of microorganism consortia.

The results of the marine biodegradation test are given in Figure 46. In marine tests also inoculum suspension was added in two blank controls and two test replicates. The blank control assays 3 and 4 as well as test substance assays 4 and 5 have been spiked with 10 mL of freshly taken sea water from the filter units of the aquarium. The suspension was added on day 475, 538, 594 and 652. Nevertheless it could not be determined that better or enhanced degradation derived from this procedure when the data of the spiked assays is compared

to the others. The fourth replicate shows falling carbon dioxide evolution after 300-400 days of exposure. This assay should be excluded from further evaluation; however substance specific analysis may reveal valuable results.

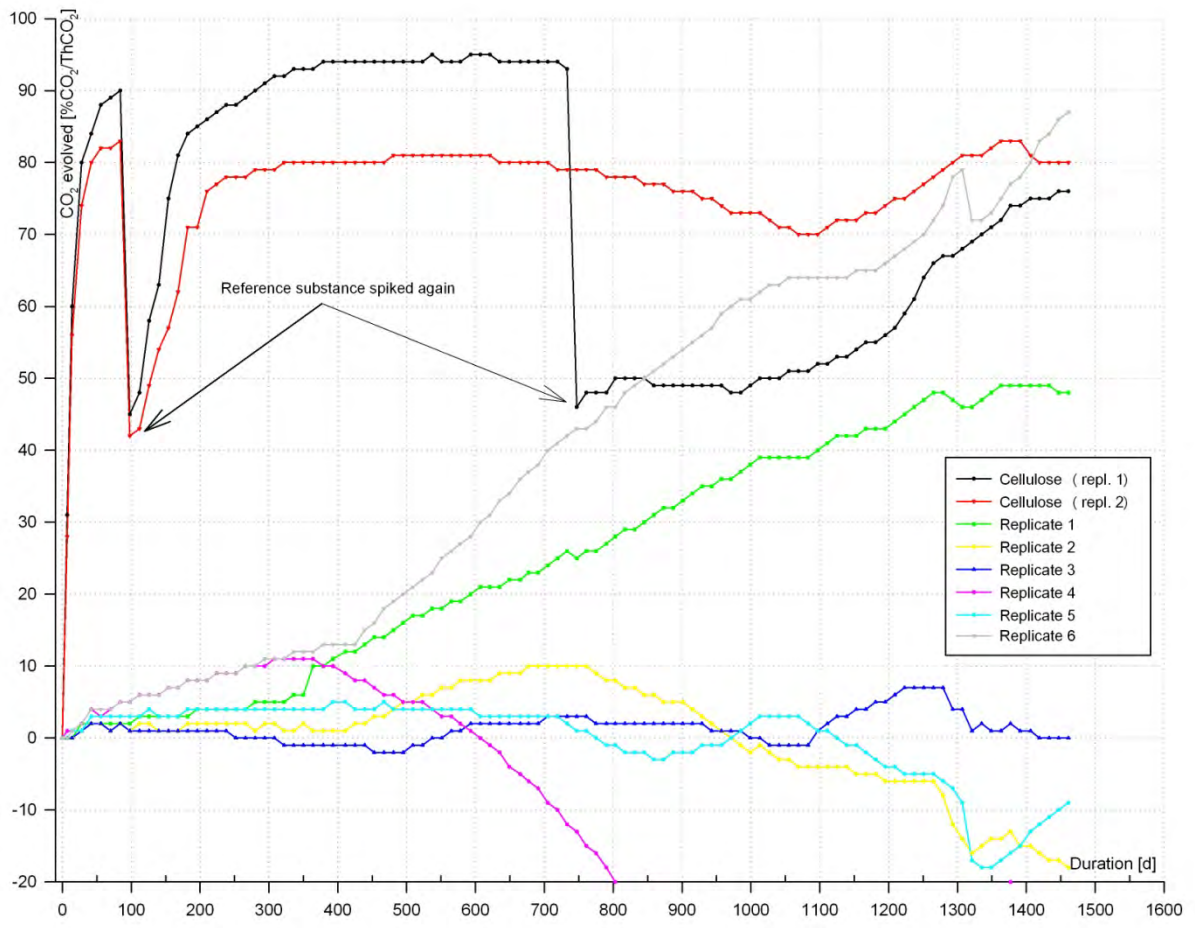


Figure 46 - Marine biodegradation test results of Ecoflex

Although biodegradation was only observed in two of the six replicates the others show because of their constant progress that the test system basically can operate even for long-time studies. Aliphatic-aromatic types of polymers have been extensively studied in soil or compost environment before which represents their main application area. We have observed that marine microorganisms can last very long without additional carbon sources because of their high surface area to volume ratio and their ability to survive in oligotrophic environment. These observations led to investigation of long-term biodegradability in order to establish metabolic pathways of the polymer tested. It was also shown by the data generated from blank controls, control substance and measurement statistics that these tests can be kept quite stable. The presence of microorganisms was confirmed as was the presence and change in polymers in the specific test assays using substance specific analysis [1] and molecular tools. During the process of analyzing these samples the whole test batches were filtered through 0.45µm filters and analyzed at the end of exposure. The residues from the different test assays are given in Figure 47. It is very interesting to find much difference in the residues and also in analytical data [1]. The residues show that there has been lots of algae growth in some of the test vessels even thought the flasks/bottles were kept mostly in the dark. The high variations found using the different parameters determined can lead to the conclusion that the differing biodegradation rates may be due to effects in population

changes. One option would be a predator-prey-dynamic that could result in major changes in biodegradation rates or even in the effect that no biodegradation is observed or some kind of steady state is reached.

It could be found when GPC data on the extracts was evaluated that in all test assays some breakdown and/or biodegradation had occurred since a shift in molecular weight distribution was observed when compared to the Ecoflex extract form synthetic marine medium without inoculum (standard). However, this is not consistent with the results obtained by biodegradation tests. The influence of loss of mineral medium and different amounts of test substance can be discarded because all test batches were treated in the same way and same amounts of samples were taken.

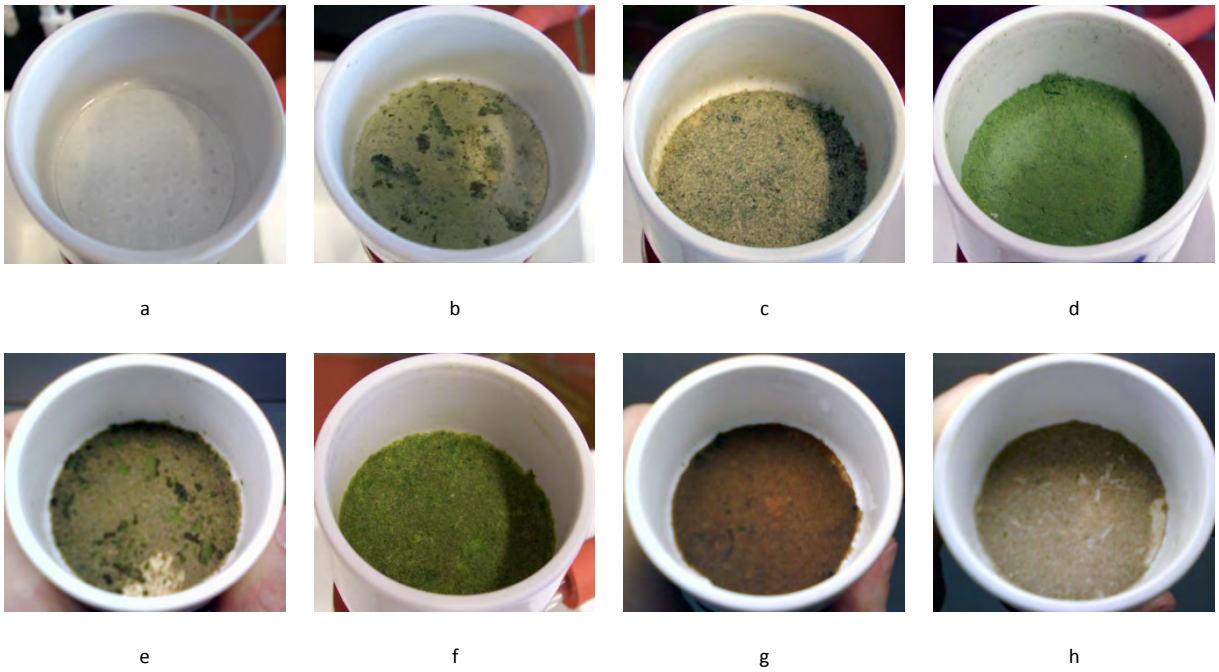


Figure 47 - Residues of the filtered test assay from the marine Ecoflex long term biodegradation test (a: synthetic test medium with Ecoflex powder (without inoculum) as verification of extraction procedures; b: blank control 1; c: test assay 1; d: test assay 2; e: test assay 3; f: test assay 4; g: test assay 5; h: test assay 6

The amount of medium has been very constant which was confirmed by weighing of the bottles. At the end of the test when samples for molecular analysis were taken, the amount of medium ranged from 1140-1207 mL in the test vessels (about 400mL were taken for molecular analysis). It was also confirmed that the correct amount of polymer was added [1]. GPC showed similar data and also the weighing scoops used to add the polymer samples were recovered afterwards confirming correct test conditions [1].

4.9.2 Results from molecular analysis of marine Ecoflex degradation tests

Since biodegradation of Ecoflex samples showed quite different kinetics for the replicates in the test, it was decided to analyze medium samples with molecular tools. The intention was to confirm different microorganism communities in the replicates as well as in blank controls and control substance (positive control) and maybe also to show similarities in those replicates where no biodegradation was observed and those which show some biodegradation at least.

The results are given in Figure 48. It is very interesting, that no prediction can be made based on this data to identify possible microorganisms that are able to degrade Ecoflex in marine medium. However it is also interesting, that there can still be found viable microorganisms in blank and positive controls after a very long time. Some organisms such as *Synedra sp.* were found in many test assays as well as *Haslea sp*.

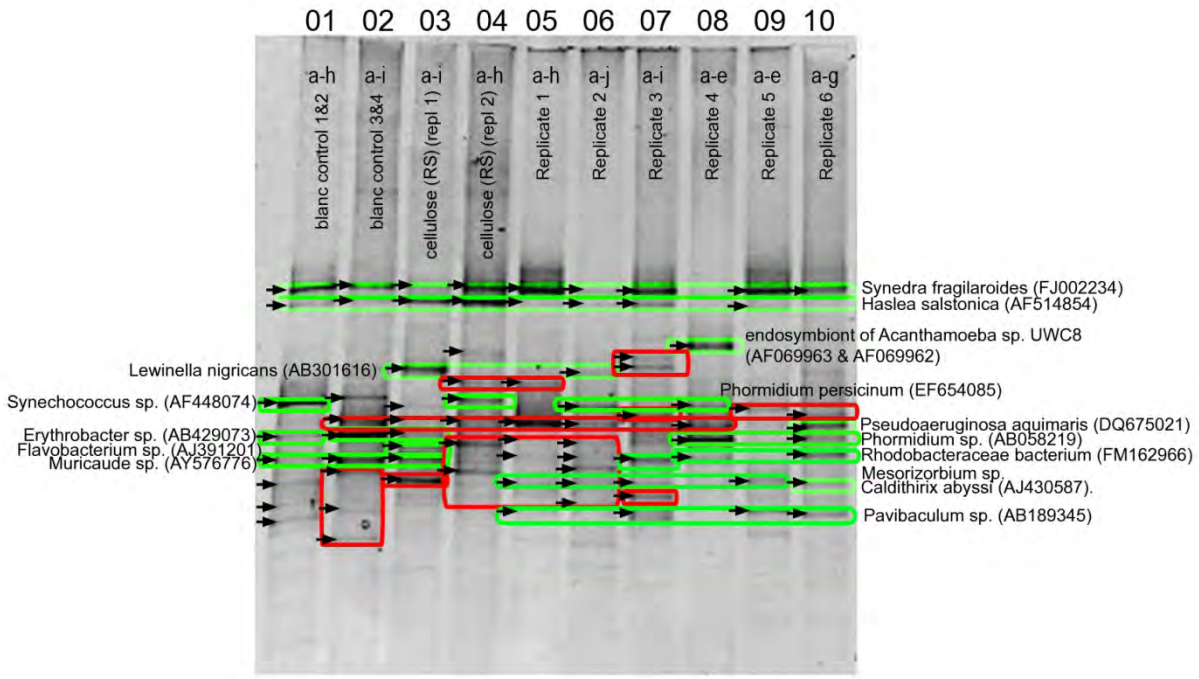


Figure 48 - Results of sequencing of samples from marine Ecoflex biodegradation experiments

When the sequencing data is compared to biodegradation test data, it seems obvious that the organisms that have been detected cannot be clearly correlated with the biodegradation data. This however unfortunate fact from this first trial shows that in order to identify biodegrading microorganisms in marine environment more detailed research might be necessary. On the other hand, industry and contracting parties doing tests for customers need much easier and faster methods to be useful an provide valuable data. It is also very interesting that even in blank and positive controls some microorganisms can be detected but not as many different ones as were found in the test substance batches. This could mean that there are probable more than one species that is able to grow on Ecoflex as a substrate even though biodegradation is very low and only notable in two of the replicates.

4.10 Biodegradation Tests with Ecovio

Two different types of Ecovio were submitted to freshwater (WWTP) and marine biodegradation tests. The tests were observed for 500 days. Two replicates were set up with Ecoflex again and three replicates were set up with Ecovio 2099 L BX 8145 and Ecovio 2129-2 L BX 8180, respectively. In freshwater/WWTP tests inoculum was added once per month using 5mL of the inoculum suspension that is regularly put in OECD 301 A & B tests. The polymers and the reference substance (Cellulose) were added to give a total organic carbon concentration of 100 $mg \cdot L^{-1}$ in the test. The results of Ecoflex were discarded. No Biodegradation was observed either in freshwater/WWTP nor in marine water during this 500 day period. The results for Ecovio are given in Figure 49

for freshwater and in Figure 50 for seawater. Both Ecovio samples are based on Ecoflex and PLA blends. The part of PLA is higher in Ecovio 2129-2 L BX 8180 than in the other Ecovio type.

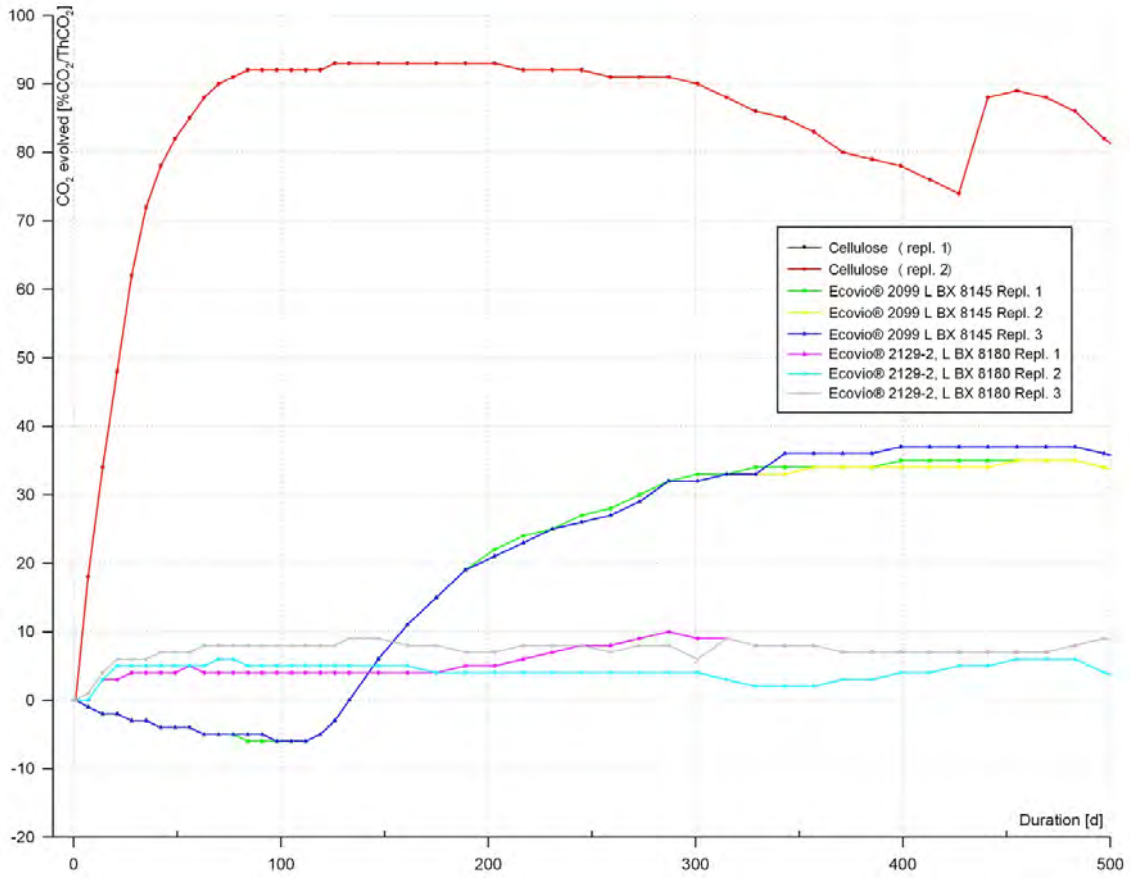


Figure 49 - WWTP/freshwater biodegradation test results of Ecovio

For Ecovio 2099 L BX 8145 it can be seen that in both test systems at the end of exposure almost the same biodegradation degree is obtained even though characteristics are much different. For Ecovio 2129-2 L BX 8180 no biodegradation is observed in both test systems. This is a very interesting observation and assuming that the Ecoflex part of the blend might be biodegraded it would fit almost the content of Ecoflex in the blends (45 and 19% respectively). Since PLA is known to hydrolyze but not to biodegrade very easily, this would explain why the biodegradation degree is lower in the second Ecovio type. This could also explain why Ecoflex has not been easily degraded in the first place. If PLA hydrolyzes and therefore breaks the polymer down to smaller parts, the Ecoflex part may be much more susceptible to microbial attack than in the pure Ecoflex grade.

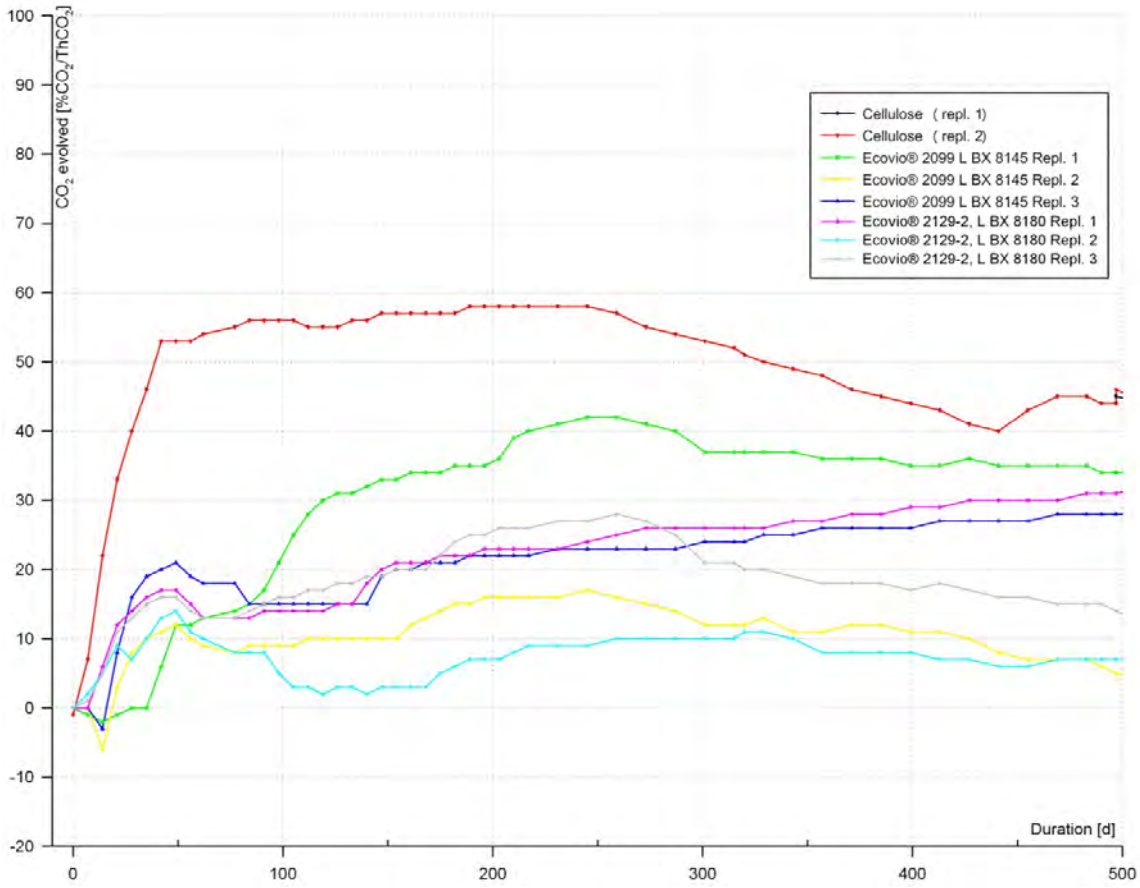


Figure 50 - Marine biodegradation test results of Ecovio

In marine tests it can be observed that the distribution is broader than in the corresponding freshwater test. This effect can maybe be attributed to the different conditions and the capacity to store carbon dioxide and buffer the complete system much more than freshwater. Since all these test replicates from both tests were set up parallel and on the same aeration line, fluctuations in aeration should have the same effect on each replicate and can be excluded as cause for this observation.

4.10.1 Results from molecular analysis of marine Ecovio degradation tests

Similar to the experiments with Ecoflex, molecular analysis was performed at the end of exposure of the experiments. The results are also similar (Figure 51) and answer not many questions. No clear identification of possible degraders correlates to the data from biodegradation tests described above. It is interesting that Rhodococcus sp. where found in all Ecovio replicates and estimated to occur in more or less similar amounts but in no other test assays or blank or positive controls.

Also a very interesting fact can be seen when blank and positive controls are compared to the data obtained from samples of marine medium or native se water given in Figure 44. Only few bands are mostly detected for different blank controls (at the end of exposure) and for the medium and native sea water (at the beginning of exposure). After the experiment different microorganisms are detected than before and also more precise bands result from the DGGE experiment. In the test assays this effect seems even more improved.

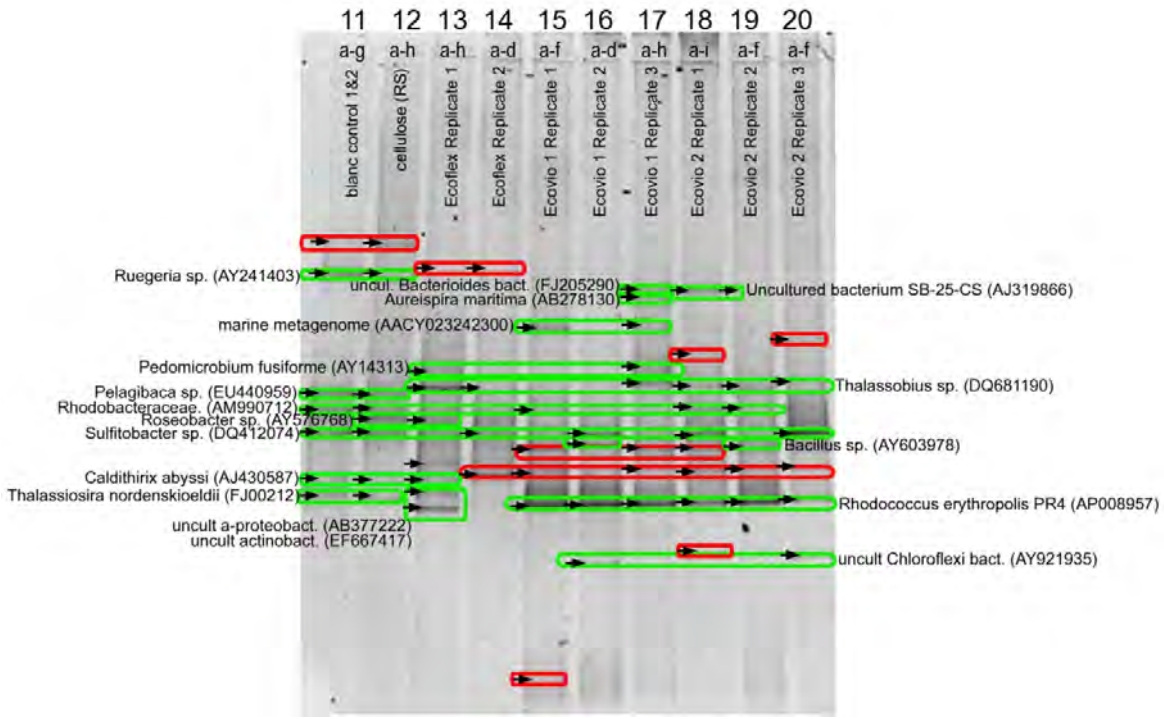


Figure 51 - Results of sequencing of samples from marine Ecovio biodegradation experiments

The conclusion could be drawn that generally the density of microorganisms is quite low for each species in the tested sample and after a certain time some of the organisms have survived and multiplied while others have not. In the replicates with polymers at least some degradation may occur even though it might be very low and not detectable with the used test methods but enough for more organisms to survive and for the growth of colonies. However, in another investigation on PU biodegradation, DGGE showed that communities on PU surface were less diverse than in soil and only few species found on the PU surface were detectable in the soil. Also the soil type influences the composition of microorganism communities depending on the pH and organic carbon. The interesting point is that PU is highly susceptible to soil biodegradation and independently degraded almost completely in both soil types but by different communities [202]. If this observation is transferred it could mean that the polymer might help to “enrich” more microorganisms that could not be found before using the same method of analysis.

4.11 Results on biomass effects on biodegradation tests

4.11.1 Determination of biomass

The results of the different methods for biomass determination are shown in Figure 52. The mean values of biomass concentration for centrifugation, filtration and Biuret assay are in close correspondence but due to a possible outlier in the centrifugation data set, the actual median value shows an actual lower distribution. Comparing the methods, filtration is the more accurate one with a relative standard deviation of about 3%. The most precise method of all determination techniques is the Biuret assay with a relative standard deviation of only 2%. The mean and median values are both a bit higher as in the first two methods. The reason for this effect could be the underestimation of biomass in the dry mass determination when filtration and centrifuga-

tion is applied. The abscission of biomass is less complemented in case of the centrifugation than at the filtration. Some cells may be retained in the supernatant of the centrifuge tubes. Both methods of biomass determination are underestimating the biomass concentration as well because of the cells volatile components could be lost during the process. It appears that the determination of living bacteria using plating techniques is the most inadequate an underestimating method to ascertain the biomass in municipal activated sludge. This is due to the fact that nutrient agars are first of all selective. Bacteria with complex nutrient requirements are may not form colonies. Second , only viable and cultivable (1-15% in WWTP activated sludge [435]) cells will be detected.

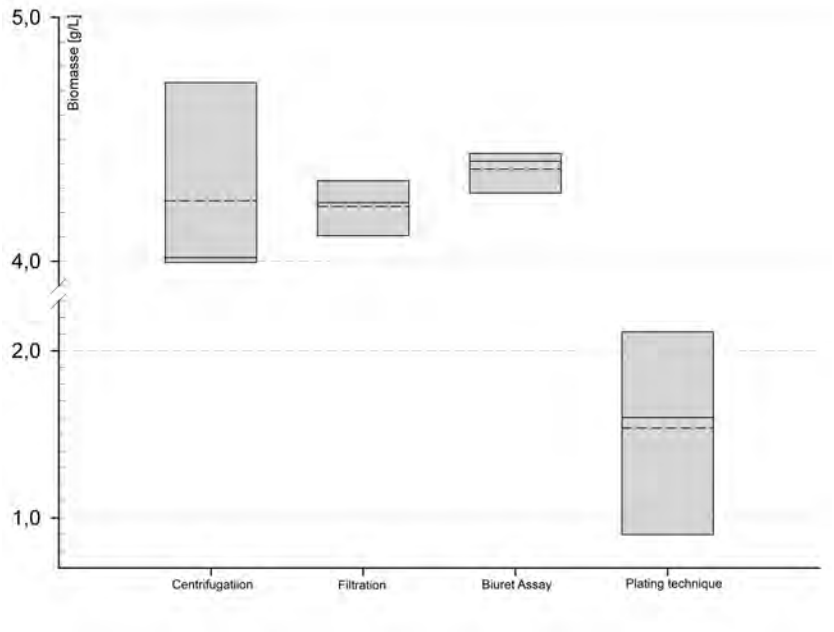


Figure 52 - Statistical comparison of different methods for biomass determination in WWTP activated sludge suspension (box = 25-75 percentile (50% confidence interval); drawn line = median; dashed line = mean value (n=4 replicates per determination method)

The Biuret assay is the most accurate method to determine biomass in municipal activated sludge. But this method is associated with a high complexity and also costly in terms of time and material. In practice it appears that the determination of dry mass using filtration is the most suitable method tested for this type of inoculum suspension because of its fast and accurate method and low-cost applicability. The results of the different determination methods are summarized in Table 39.

Table 39 - Comparison of the results on different methods for biomass determination

Method of determination	Centrifugation	Filtration	Biuret assay	Plating technique
mean value	4.25 $g \cdot L^{-1}$	4.23 $g \cdot L^{-1}$	4.38 $g \cdot L^{-1}$	1.54 $g \cdot L^{-1}$
median	4.015 $g \cdot L^{-1}$	4.24 $g \cdot L^{-1}$	4.41 $g \cdot L^{-1}$	1.6 $g \cdot L^{-1}$
rel. Standard deviation	11%	3%	2%	42%
25% percentile	4.73 $g \cdot L^{-1}$	4.33 $g \cdot L^{-1}$	4.44 $g \cdot L^{-1}$	2.11 $g \cdot L^{-1}$
75% percentile	4.00 $g \cdot L^{-1}$	4.11 $g \cdot L^{-1}$	4.28 $g \cdot L^{-1}$	0.90 $g \cdot L^{-1}$

The analysis of different methods for the determination of biomass shows, that the most suitable method is highly dependent from the kind of analyzed biomass suspension. We have also investigated Cell-counter-based methods [436] and the Bradford-Assay biochemical assay [437]. The results have shown that both are not applicable to WWTP activated sludge suspensions. Because of flocculation in the suspension to a high degree, a cell count using electronic cell counting techniques in flow through systems did result in high variation of the results and a significant underestimation of cells and biomass, respectively. The Bradford-assay could not be performed because the cells did not dissolve in the colour-reagent and coarse particles and flocculation remained in solution. Also sonication or treatment with Ultraturrax did not improve the situation.

For the assay of suspensions with a low amount of biomass such as often found in fresh or marine water systems, the cell counter and also the determination of living bacteria using plate technique are the most adequate methods to destine the biomass. This is shown by the determination of biomass in surface water of a freshwater lake (Kräppelweiher, near Frankenthal, Germany) (Figure 53).

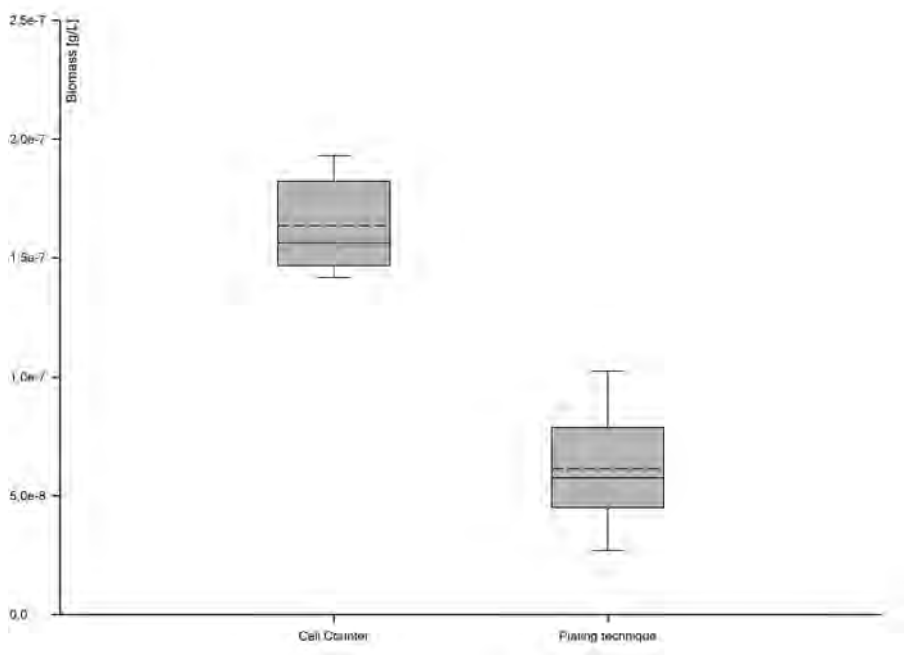


Figure 53 - Statistical comparison of different methods for biomass determination in surface water (box = 25-75 percentile (50% confidence interval), whisker caps = 10-90 percentile (80% confidence interval)); drawn line = median; dashed line = mean value (n=16 replicates per determination method)

Again, the plating technique underestimates the results for the already described reasons. The methods described for WWTP activated sludge suspension are not suitable for detecting low amounts of biomass such as found in freshwater.

4.11.2 Dependence of biodegradation of di(ethylene glycol) on biomass

The biodegradation results for diethylene glycol in the online CO_2 evolution tests with different biomass concentrations are shown in Figure 54.

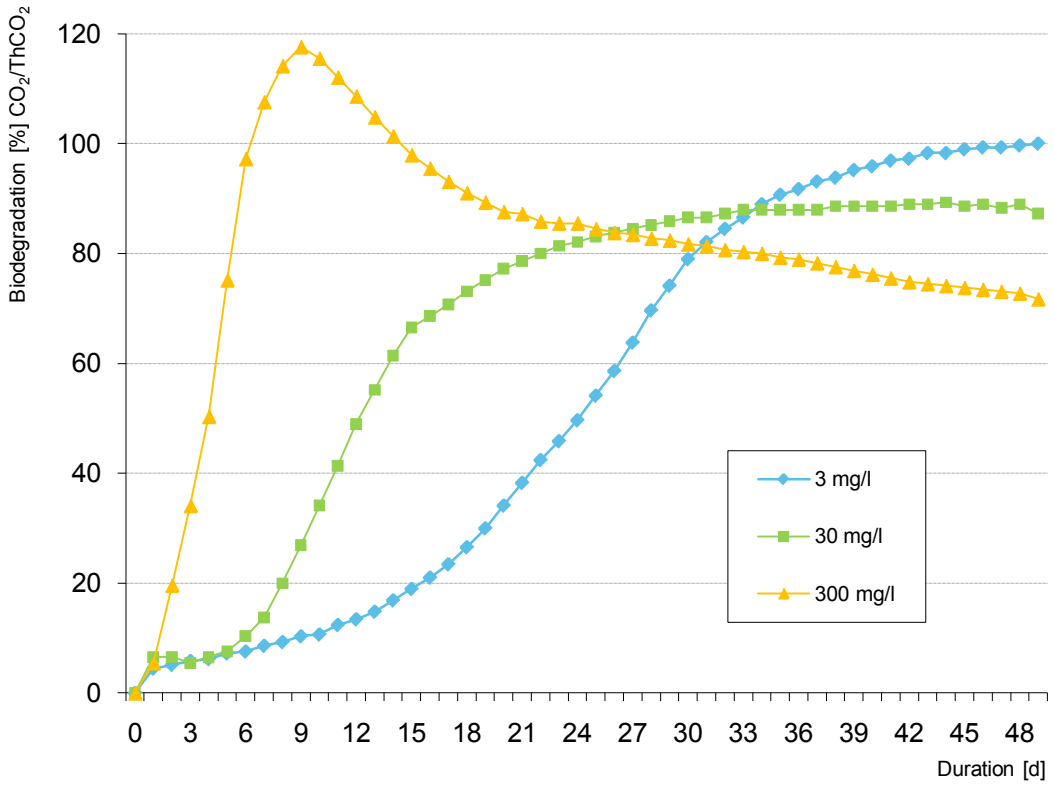


Figure 54 - Degradation of di-ethylene glycol in online CO_2 evolution test system with different biomass concentrations.

The characteristics, namely the duration of each phase of the degradation curves are changing with the different biomass concentrations and also with different test substances. The lag phase, where the adaption of the inoculum to the test substance occurs and the degradation phase in which the bacteria use the substances as carbon source, extend with declining biomass concentration. The results are summarized in Table 40.

Table 40 - Biodegradation parameters for di-ethylene glycol with different biomass concentrations

Test substance	di-ethylene glycol		
biomass concentration	3 mg·L^{-1}	30 mg·L^{-1}	300 mg·L^{-1}
lag-phase [d]	11	5	1
degradation phase [d]	25	14	8
plateau phase [d]	13	30	40
DOC-removal	100%	99%	100%
Biodegradation degree	Completely mineralized (80-100%) obtained within the test duration of 48 days		
degradation (OECD)	biodegradable	biodegradable	ready biodegradable

4.11.3 Dependence of biodegradation of poly(vinyl alcohol) on biomass

The biodegradation results for PVA in the online CO_2 evolution tests with different biomass concentrations are shown in Figure 55.

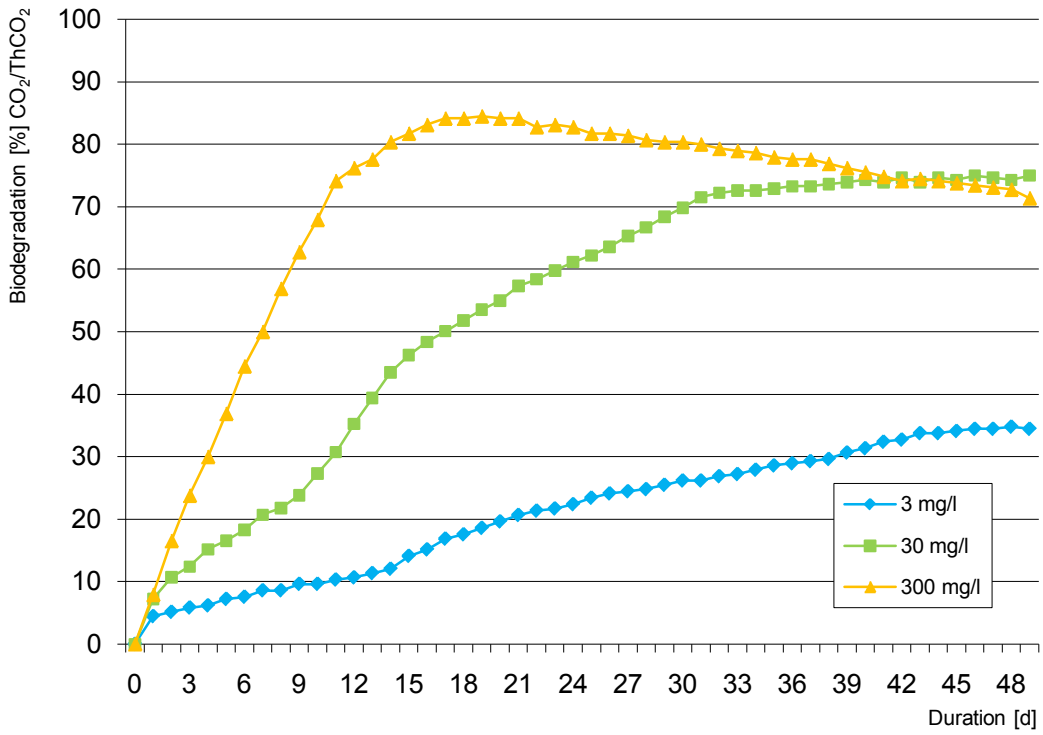


Figure 55 - Biodegradation of polyvinyl alcohol in the online CO_2 evolution test system with different biomass concentrations.

As can be seen, the results generally agree with the degradation of diethylene glycol but one can clearly determine a shift in the three phases that is different than in the diethylene glycol experiments although the medium and inoculum used were the same. This is very interesting because this effect would most certainly be attributed to the substance itself. The results are summarized in Table 41.

Table 41 - Biodegradation parameters for polyvinyl alcohol with different biomass concentrations

Test substance	Polyvinyl alcohol		
biomass concentration	3 mg·L^{-1}	30 mg·L^{-1}	300 mg·L^{-1}
lag-Phase [d]	14	6	1
degradation phase [d]	30	24	14
plateau-phase [d]	5	19	34
Biodegradation degree after 48 days (plateau partially not reached yet)	30 - 40%	60-70%	60 - 70%
degradation (OECD)	moderate biodegradable	biodegradable	biodegradable

5 Discussion, importance and suggestions for future impact

The issues described in this thesis are derived from a very complex and immense field of new research where especially when it comes to aquatic biodegradation, not much is known. Up to know only little work has been directed in a systematic way to complete some of the gaps in biodegradation research. Many new fields are tapped and an increasing amount of new developments broadens the field of research in this area extremely.

The objective of this thesis was the investigation of the biodegradation of polymers in the marine environment. Appropriate methods should be established to evaluate marine biodegradation in screening laboratory tests. For this purpose the biodegradation under marine conditions was compared with the biodegradation in standard WWTP-tests. Furthermore specific characteristics of the biodegradation under marine conditions should be identified to enable an estimation of the biodegradation potential based on molecular characteristics. For this purpose a specific analysis of the biodegradation pathway for polymers was performed.

The analytical work on biodegradation pathways was part of another dissertation and is described in another thesis [1]. Nevertheless the results are presented also here, since they are the basis for conclusions regarding the biodegradation under marine conditions.

The literature shows that up today most investigations focused on only a few products namely PHA, PLA, starch and starch based polymers and lately different polyesters. All other polymers are mainly discussed very shortly. The review on the current literature also shows that much effort was done in investigating biodegradation in soil and abiotic degradation of polymers (because recalcitrance was the real focus of research and necessary for many product developments). It is also demonstrated by this review that information is often limited to some molecular weight areas. Not much has been reported on biodegradation with polymers of high molecular weight over 100'000 $g \cdot mol^{-1}$ and even more. Also no data on the aquatic biodegradation of polymers are available, which include investigations on the microbial community and the analysis of the biodegradation pathway.

5.1 Marine versus freshwater biodegradation tests

The tests that have been performed were focused on comparing results between freshwater (WWTP) and sea water tests. Polymers with different characteristics and different water solubility were used. First of all, it was discovered that marine tests can be extended much longer than WWTP standard tests and valid results may still be obtained, since MO survived over longer time periods in media with low nutritional value. The second topic of interest was that experiments using native marine water and those using synthetic marine water with sea water inoculum did not differ significantly. The results of tests with native marine water are thus not further described in this study. The experiments in this thesis demonstrated that the results of biodegradation tests within test duration of 30-100 days were not significantly different for native and synthetic sea water. In conclusion synthetic marine water can be used as an alternative for laboratory tests without missing too much information.

Both test media where carefully characterized using different tools. The determined parameters showed that the test systems provided reproducible results. The standardized graphs (see Figure 31 and Figure 32) have shown a difference between biodegradation data from marine and WWTP tests for different measurement parameters. It is interesting that a difference of about 20% between biodegradation degree (CO_2 evolution) and dissolved organic carbon removal was generally observed in marine tests but not in WWTP tests. This effect might be explained by the much higher carbon storage capacity of sea water. This is basically one of the major differences in the tests that have been performed. The ability of marine water to store carbon is also providing an environment for organisms to survive.

Also a very interesting observation was made when the sea water samples (native and from the sea water aquarium) were analyzed. Determination of the microorganism communities showed that the organisms characterized in the medium were different and less diverse as in the test vessels and also surprisingly in the blank control vessels after a certain time span of the test (e.g. see Figure 43 and Figure 44). What was also very interesting is the fact that there is almost no overlapping data resulting from the molecular analysis that would give an insight on the degraders of the polymers. Since this analysis is only a picture of one single moment within the complete test duration, the development of the microbial community during the biodegradation process remains unknown??? This is certainly a weakness in this specific case because one cannot observe any interactions within the colony or the population dynamics with a single shot. Also virus-host interactions could influence the population dynamic dramatically and it would not be explained by this one picture of the complete process.

From the gained experience and data in this study, it can be concluded, that the marine biodegradation tests are mostly easy to carry out but some technical aspects need to be regarded. The tests work generally fine even though long term tests such as the one with Ecoflex may not provide reliable results as discussed later on. Reference substances can be used as indicated (Na-benzoate, aniline, cellulose or even a polymer such as PEG or PVA). The reference substance should be selected with regard to the duration of the test but can also be spiked a few times to the test vessels after biodegradation has reached the plateau phase. Measurement as done by NIR-spectroscopy works very well and reliable. Special care needs to be taken when the test setup is installed. Especially when “Sturm”-Tests are done, it must be carefully checked that the complete system is airtight and the air used to aerated should be CO_2-free. This is much more important than in WWTP tests. This is also the most crucial point where errors may occur that cannot easily be detected and that ruin the whole test. If possible, two measurement parameters should be used in studies that run for a longer time than usual OECD tests recommend.

Another interesting aspect is that the differences between substance biodegradation rate and degree seem to be larger in marine tests than in standard freshwater tests. When aniline and Na-benzoate were compared in WWTP and marine tests, it was observed that both degraded very fast in WWTP tests but aniline degraded much slower in marine tests than Na-benzoate. Marine tests react much slower on spiked substances and also varying concentrations of the test substance result in markedly more different results. This can be seen in the experiments where the concentration of PEG was increased by a factor of ten. Biodegradation can be observed

but much slower than at lower concentrations. Inhibition by the test substance is unlikely since the concentration is not known to be toxic. The difference is probably due to the fact that microorganisms in marine media have no need to metabolize carbon sources that require complicated breakdown and assimilation processes. Since there is often much carbon present in marine water and microorganisms having generally a huge surface area/volume ratio (SA/V) they are able to utilize smallest amounts of available carbon.

5.1.1 Biodegradation of poly(vinyl pyrrolidone)

The tests on PVP have been described in detail in chapter 4.7. This test design was especially used to confirm the stability of measurements (DOC and CO_2) during long-term biodegradation tests with marine medium. Also it was intended to confirm the recalcitrance of PVP also in marine tests while at the same time prove that long term biodegradation tests can be performed in marine medium and also more easily than in standard WWTP activated sludge tests.

It could be observed that dissolved organic carbon measurement was possible while CO_2 evolution can be very tricky and sometimes complicated to determine accurately in this special test (data not shown). On the contrary it is known that standardized tests following OECD guidelines do not cause that many variations. Most certainly the observed variations in CO_2 evolution during long term tests can be due to leaking systems or problems with the carbon dioxide free air but also it could be possible that due to pH shifts in the medium fluctuations can occur. Since pH could not be measured online in the system this still requires clarification. Normally the influences by pH shift are only very small and also marine medium is able to buffer changes in pH to certain degree. Other factors that can influence the variation of the results e.g. by analytical measurement were excluded because statistical evaluation on the carbon determination shows the validity of those results.

This experiment tells a lot about biodegradation tests in marine medium, because two parameters were used here and a polymer not known to be biodegradable in WWTP tests was spiked showing the weakness in this test setup. On the other hand the experiments using PEGs show in contrast that both parameters can be measured accurately when at least some biodegradation occurs in a reasonable time frame in marine tests. From the results obtained on both PVP and PEG biodegradation tests it can be derived that probably around 200 days after starting the experiments at least something should be biodegraded otherwise it seems that at least CO_2 evolution does not necessarily work as measurement parameter and should not be used as single one. In contrast using only dissolved organic carbon as parameter can lead to false positive results when polymers are adsorbed to glass or biomass surface or especially to marine particulates in the medium (e.g. marine snow).

In a nutshell this is still an important part to work on because PVP is used in huge amounts and was also reported lately to be found in the environment [208]. It will enter freshwater and salt water compartments mostly without notice. However no adverse effects are known today and PVP is a rather convenient and safe polymer this topic should be addressed further and observations of any biodegradation might be helpful.

5.1.2 Biodegradation of poly(ethylene glycol)

PEGs have played a major role in recent decades and are still very important in many applications. They are also the main focus of this work, because they close the gap between polymers such as PVP that are water soluble but not biodegradable (up to now!) and water insoluble polymers that have been shown to be biodegradable at least in certain environmental compartments (e.g. aliphatic aromatic polyesters such as Ecoflex). Because of that fact the results provided by the tests are very important. Since this is the first systematic study of a group of polymers in two freshwater compartments and using a broad range of molecular weight distribution some interesting topics were confirmed and some surprises were found. First, biodegradation is not static. PEGs that were originally not thought to be biodegradable in wastewater in the standardized tests maybe 5-10 years ago (including PEGs with a molecular weight larger than around 8000-10'000 $g \cdot mol^{-1}$) are biodegradable today as shown. Second, marine biodegradation is also possible to quite some extent. The process is very similar but somewhat slower. The kinetics is not completely different as was presumed before. Third, it was also shown that biodegradation of the PEGs can be influenced by increasing the concentrations especially in marine water while in WWTP no effects were observed in the range tested.

All of the selected PEGs in a range from molecular weight 250 up to 58'000 $g \cdot mol^{-1}$ were biodegradable in freshwater media under aerobic conditions using microorganisms obtained from WWTP sludge. PEGs from molecular weight 250 to 14'600 were fully biodegraded within 28 d and no differences concerning the biodegradation could be seen. PEGs 26'600 and 57'800 were fully degraded within 45 d and 65 d, respectively. The biodegradation of PEGs having a M_W > 14'600 required more time than that of PEGs with shorter chains but even PEG 57'800 is fully biodegradable within reasonable time. The effect seen in the biodegradation curve of PEG 57'800 reaching a first plateau after approximately 26 days of exposure and then continuing after a short time and reaching complete mineralization could be due to diauxie (see Figure 35). The growth phases of a microorganism in batch culture as it metabolizes a mixture of two substrates show a stepwise biodegradation rate. Rather than metabolizing the two available substrates simultaneously, microbial cells commonly consume them in a sequential pattern, resulting in two separate growth phases. During the first phase, cells preferentially metabolize the substrate on which it can grow faster (in this case probably PEG chain homologues of shorter chain lengths). This is due to the fact that the substrate on which the organism can grow faster suppresses formation of the enzymes that are required to degrade the second substrate. Only after the first part of substrate has been exhausted, the cells switch to the second. At the time of the "diauxic shift", there is often a lag period during which cells produce the exo-enzymes needed to metabolize the second sugar (second accumulation phase).

In seawater media with marine microorganisms, PEGs up to 7400 $g \cdot mol^{-1}$ are entirely biodegradable whereas PEGs having higher molecular weights are only partially degradable and persistent to microbial attack, respectively. The results show that the biodegradability decreases with increasing molecular weight but only for the seawater media. Further studies for freshwater media may examine if PEGs with higher molecular weight than 58'000 $g \cdot mol^{-1}$ show a decrease in biodegradability when molecular weight increases. Both media have in common that the time required for degradation generally increases with increasing molecular weight.

The increase in molecular weight causes an increase in time required for fully or partly biodegradation (PEGs 4'500, 7'400, 10'300, 14'600). PEG 26'600 and 57'800 were not degraded in saltwater for a period of 250 d what is a strong contrast to the freshwater media where they were fully biodegraded. If the biodegradation process follows the assumed path:

$$HO(CH_2CH_2O)nCH_2CH_2OH \rightarrow HO(CH_2CH_2O)nCH_2CHO \rightarrow HO(CH_2CH_2O)nCH_2COOH \rightarrow HO(CH_2CH_2O)_{n-1}CH_2CH_2OH \rightarrow \dots$$

Equation 44 - Assumed path of biodegradation of poly(ethylene glycols)

The observation why no biodegradation occurs or why the process stops at some point could be, that for example biodegradation process within a certain range of molecular weight occurs more easy than with the next higher range of molecular weight. If a polymer would be investigated (such as the PEG 14'600) and the molecular weight distribution would overlap with two areas were different biodegradation rates are observed, part of the polymer (the chains of lower molecular weight) would possibly biodegrade while the part containing longer chains would not or at least much slower. This could be an explanation for the biodegradation graph of the PEG 14'600 as shown in Figure 36. Another reason might be that a threshold level in the amount of substance has not been reached since the test substance was used in regard to carbon content and not amount of substance, which means that a longer chain results in a decrease in the amount of substance and number of end groups at the same carbon concentration Also it can be assumed, that because of the sheer size of the molecule at some point the microorganisms cannot assimilate the molecules but rather need to degrade the substance into smaller parts using exo-enzymes. Another reason for the changes in biodegradation rates and for the steady state observed could be effects on the population level. This could also explain why high variation is observed when samples are taken.

In terms of inocula it can be observed in these studies and also in others that the lag-phase can be very long and still biodegradation may start at some point. Marine organisms are known to live on very few nutrients and even in nutrient rich environments these organisms do not significantly increase their metabolic rate.

Also, the observed effect when the concentration of PEGs was increased by a factor of 10 from 20mg $\cdot L^{-1}$ to 200mg$\cdot L^{-1}$ in regard to TOC content in the test has been very interesting. The increase in molecular weight by a factor of 4 (from PEG 2000 to PEG 8000) led to a biodegradation rate decreased by a factor of 0.5. In future experiments this should be more closely investigated in order to find more correlating data.

As provided by analytical determination [1;289], the investigation of the degradation pathways shows the same degradation pathway for PEGs with molecular weight from 250 to 14'600 g$\cdot mol^{-1}$ in freshwater media, which is characterized by formation of shorter homologues. A similar pathway is seen for PEGs having an molecular weight <1900 g$\cdot mol^{-1}$ in seawater media, with the only difference the biodegradation in freshwater media being faster. Future studies may investigate if the microorganisms involved in the biodegradation in freshwater media are different from those in seawater media although the degradation pathway is similar. If the molecular weight of PEG exceeds 1900 g$\cdot mol^{-1}$, the pathway in seawater media will change. PEGs with a molecular weight from 2000 to 10'300 g$\cdot mol^{-1}$ show a completely different biodegradation pathway, with a stable molecular weight pattern during degradation. The degradation of PEGs with higher molecular weights is limited in seawater media, no pathway can be given. Taking into account that no report has been found on the accumulation of

PEGs in nature, it can be assumed the degradation of high molecular PEGs in seawater may occur by abiotic processes or simply by taking more time. It could also be possible, that when biodegradation is partly observed as shown in Figure 36, it might be due to the fact that only the lower molecular weight chains of the complete molecular weight distribution is biodegraded, but the rest is not.

It was also very interesting when samples were analyzed on molecular level (Figure 43 and Figure 44) to find that very different DGGE patterns were found for all the PEGs. Also there are on one hand similarities and on the other differences between blank controls, reference substance and different PEGs. That contradicts the assumption that the microorganism communities would adapt that much that only some species would be able to grow more than others and therefore would dominate in the medium. It is also interesting that if compared to the results of the pure medium prior to any biodegradation tests, the pattern and intensities of the DGGE bands are much different. In the medium, intensity is much lower, as is the number of bands found.

From this it can be assumed, that microorganisms do grow while a substrate is present, but also there is growth observed in blank controls to a certain extent after the experiment. It can also be reckoned that biodegradation seems to be possible by more than one or two species, since different DGGE bands and intensities are observed for even two replicates of a PEG sample with the same molecular weight distribution.

Even though no significant differences between the DGGE Bands of the 3 different PEGs tested was found, a slight shift is indicated showing that the patterns of the PEGs above 2000 $g \cdot mol^{-1}$ have more similarities and the DGGE bands of PEG 2000 together with reference substance (Na-benzoate) and the blank controls also shows more similarity (Figure 43). This slight indication could be due to the fact that biodegradation of PEGs with a molecular weight around 2000 $g \cdot mol^{-1}$ and lower may be degraded by other organisms than those with higher molecular weight distribution.

These findings indicate very much again, that biodegradation is never a static parameter such a physical constants. The investigation of PEG biodegradation was also necessary and successful because these intermediates are used very much in literally thousands of products. It was very interesting to find that biodegradation phases in WWTP and marine tests on PEGs are very much alike.

5.1.3 Biodegradation of Ecoflex and Ecovio

The experiments using Ecoflex were on one hand successful, because it could be demonstrated that the tests used, are not exactly appropriate for the purpose they were originally intended in the frame of this project. Ecoflex being generally biodegradable in compost was shown to also biodegrade in marine medium (Figure 46) but not in freshwater (WWTP) tests (Figure 45). The data obtained on biodegradation of Ecoflex was based only on CO_2 evolution and the test was prolonged for over three years. It is because of its water insolubility dissolved organic carbon could not be measured and also other analytical tools were limited because sampling of the test assays was limited by amount of samples and by the fact the homogenous samples would be necessary for any substance specific analysis. This is a provocative task because for such a long experiment all influences from the environment beyond the test vessel should be excluded.

Biodegradation in freshwater (WWTP) (Figure 45) shows that generally no biodegradation was observed but after a time of about 200 to 300 days, something happens to the tests. Even though all biodegradation tests were checked regularly for visible alterations, leakage, air-flow and appropriate test setup, changes seem to appear after this period in all test assays and also in the reference assay because biodegradation curves are going down. The test was ended after around 500 days because no biodegradation was observed and if assumptions are correct, biodegradation should start somewhere between tests that have been applied in compost and the test in marine medium discussed in this work.

The fact that no biodegradation is observed can be due to missing microorganisms and biomass in the test system. Either not enough microorganisms are present because the microorganisms in this medium are not able to live on the same small amounts of carbon available (as marine microorganisms can) and/or the microorganisms present are not able to degrade Ecoflex.

When looking at the data of the marine tests (Figure 46) it is conspicuous that only in two out of the six replicates containing Ecoflex biodegradation is observed. In contrast, the reference substance was spiked in the two test assays at around 100 and 750 days of exposure and shows that in the first assay, biodegradation is easily triggered again while in the second assay after 750 days it takes much more time. This can tell that maybe biodegradation of the much larger molecules from the polymer as observed in replicate #1 & #6 might not have made that much progress as indicated by the data. Also test replicate #4 shows that interestingly CO_2 seems to be removed by the system rather than evolved, meaning, blank values were higher than the values measured in this replicate. At first, this would be interpreted by a leak in the test system, but even when all relevant valves, tubes, fittings and sodium hydroxide traps were changed, this kept going on. A second reason could be that blank values would be fed with air richer in carbon dioxide, which can be excluded, since the data on these blank values is in the normal range and also the other test assays would be influenced by this effect in the same way. The CO_2-free air was generated by molecular sieves and special conditioning and was also used in the same way for not only all other parallel tests in this thesis, but also for tests performed in contract for customers and therefore it can also be excluded that errors may result from this.

However, one can see that also in this experiment after a certain time span of around 300 days, the pattern of the biodegradation graphs tends to broaden much more. This is an interesting point as this was already observed in PEG and PVP biodegradation as described before.

On the molecular level, the results are also very interesting. Again, it was not possible to prove the assumption indicating that special microorganisms would significantly be observed in the test assays where Ecoflex was degraded when compared to reference substance, blank values or the plain medium. There are again overlapping results in DGGE bands in both reference substance and blank values and test replicates. Also some DGGE bands are observed more in test replicates, but often not in all of the test replicates. Also the intensity of the bands is sometimes very low indicating that probably not many microorganisms of this species were present.

In conclusion, it is not possible at this time to distinguish where the observed effects originate from but it is interesting that biodegradation was observed in all test assays by substance specific analysis [1]. With GPC it

was found after the exposure of the Ecoflex experiments had been stopped, that biodegradation did occur in all test replicates to some extent. Because a shift to smaller chains and smaller molecular weight distribution was detected when THF extracts of Ecoflex from the replicates were analyzed this can be regarded as indication for some change. The recovery for the procedure has been determined and the amounts that have been found after the biodegradation test are given in Table 42.

At the beginning of the experiments the concentration of Ecoflex particles had been set to approximately 160mg·L^{-1}. For molecular analysis about 400 mL were taken and some samples were also withdrawn before. The amount of medium in the test was determined to be around 1168 mL and 156 mg Ecoflex were found approximately (recovery rate included in calculation). This leaves two possibilities on what could have happened:

- If no or at least not much Ecoflex was taken with the samples withdrawn during the exposure this would mean, that the original 240 mg·1500mL^{-1} were left in the remaining 1168 mL medium. This would then result in a concentration of about 204.9 mg·L^{-1} and therefore in a biodegradation rate of about 35%.
- If Ecoflex was homogeneously distributed in the medium while samples were taken during exposure it would mean, that from the original 240 mg only 186.6 mg were left in the amount of 1168 mL medium. Hence the biodegradation rate would only be around 16.4%.

Table 42 - Results of analytical determination of Ecoflex biodegradation test in marine medium

Sample	m (Ecoflex) from extract [mg]	including 79.7% recovery rate [mg]	amount of medium extracted [mL]	concentration [mg $^{-1}$] ·L	start concentration	assumed concentration of Ecoflex (100%) with no loss from sampling [mg $^{-1}$] -	assumed concentration of Ecoflex (100%) with maximum loss from sampling [mg $^{-1}$]	biodegradation if no loss by sampling [%]	biodegradation if maximum loss by sampling [%]
Ecoflex replicate 1	125.0	157.0	1185	132.5	241 2	203.5	160.8	34.9	17.6
Ecoflex replicate 2	128.0	161.0	1207	133.4	238 9	197.9	159.3	32.6	16.2
Ecoflex replicate 3	137.0	172.0	1140	150.9	240 3	210.8	160.2	28.4	5.8
Ecoflex replicate 4	125.0	157.0	1149	136.6	238.1	207.2	158.7	34.1	13.9
Ecoflex replicate 5	117.0	147.0	1178	124.8	239 6	203.4	159.7	38.6	21.9
Ecoflex replicate 6	112.0	141.0	1147	122.9	237.0	206.6	158.0	40.5	22.2
mean	124.0	155.8	1167.7	133.5	239 2	204.9	159.5	34.9	16.3
std dev of mean	3.6	4.4	10.8	4.1	0.6	1.8	0.4	1 8	2.5
standard deviation	8.7	10.9	26.4	10.0	1.5	4.4	1.0	4 3	6.1

Most certainly, the true biodegradation degree lays somewhere in-between 16% and 35%. This is due to the fact that one cannot assure accurately that when aliquots of the medium were taken, the corresponding amount of polymer was also taken with the sample.

However, biodegradation was observed in this test the degree is not corresponding to what can be seen by CO_2 evolution test data. With this experiment it was shown that this test setup has its flaws for such long-term tests when only one parameter for measurement is available.

In the performed biodegradation test with Ecovio samples it was observed that one of the samples partially biodegraded in WWTP medium. The test worked quite well, equilibrium is reached after around 350 days at around 30-40% biodegradation degree while the other Ecovio type did show no biodegradability. It is very interesting that the Ecovio containing lower PLA content was degraded while the other was not.

In the marine biodegradation test biodegradation rates were similar for both Ecovio types reaching from 0% to around 30%. However, in this test the reference substance biodegradation was somewhat lower than usual, so this could indicate that the medium used was not completely comparable to other marine media that were used in the other tests. Maybe this would explain the higher variety in the degradation rates along with structural differences when compared with Ecoflex. It the assumed biodegradation rate of Ecoflex in the other test is nearly correct, and the information of the WWTP test on the Ecovio types is regarded, it could explain that PLA has not been degraded while Ecoflex has to some extent. Then, Ecovio containing high PLA amount (80%) would probably not be biodegraded, or at least much slower, in marine water.

The results from molecular analysis show similar patterns as in the other tests. It seems impossible to indicate clear patterns that would be related to special organisms one would only detect in certain samples from the same polymer, blank or reference sample at significantly increased concentrations. These results are very interesting as a first basis. The following steps would be to find possibilities to shorten marine test systems and also to determine possibilities to conduct biodegradation test in small scale systems, such Microresp™ or similar setups [356;438]. These could possibly help to do better biodegradation research in marine environment and at the same time save space in the laboratory and provide fast and efficient test designs.

5.2 Biomass

It could be demonstrated that the kinetics of the degradation phases from biodegradation tests are changing with different biomass concentrations whereas the final degree of biodegradation was in the same range for all biomass concentrations tested with each substance. From the experiments performed it can be stated that it is basically possible to accelerate biodegradation tests when the biomass is increased. It was also demonstrated in this study that in the case of WWTP sludge suspension, Filtration and dry mass determination was the best, fastest and cheapest method to determine the biomass content. We have also shown that an influence of biomass concentration has been observed on the biodegradability of the model test substances during the different phases, but not in the final biodegradation degree, which can most certainly attributed to the fact that the tested substances were known to be biodegradable. It is interesting though, that biomass and test substance show both an influence on the biodegradation kinetics. An overview of the biodegradation phases is given in Figure 56 and it can be seen, that especially the biodegradation phase changes when diethylene glycol and PVA are compared.

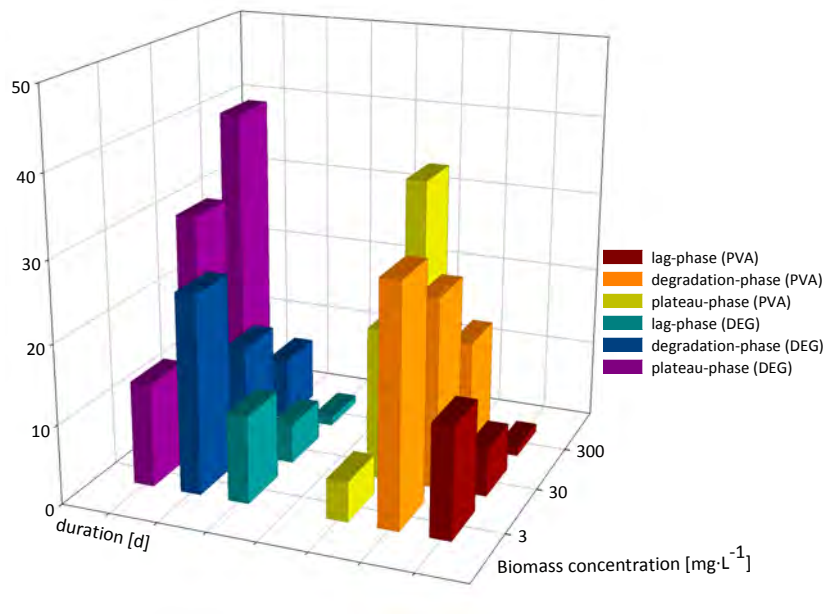


Figure 56 - Phase distribution for diethylene glycol and poly(vinyl alcohol) degradation

The importance of the biomass concentration demonstrated in this study also shows the problems posed by the information gap between standard and simulation tests especially for general risk assessments. The down-side of the ready biodegradation tests is that biomass is of little importance. Guidelines often do not include recommendations on biomass concentration and their effect. It was also shown that the methods applied to determine the biomass concentration and/or protein content and/or cell count need to be specifically appropriate for the cell suspension of interest.

In order to improve a future test system that is suitable to close the gap between OECD 301 (“ready-tests”) and OECD 307/308 (“simulation tests”) it would be necessary to enhance and confirm the results that this study has shown with other substances that especially have with the following criteria:

- Pass-level „ready biodegradable“ not fulfilled
- Many different chemicals structures
- Valid and complete data set of previous “ready-tests” and other biodegradation tests
- Degree of biodegradation should be ideally in the range 0-70% in valid OECD 301 tests
- Only pure substances and no formulations should be used for tests.

For future investigations tests should also be performed using inoculum suspensions from other environmental compartments such as WWTP effluent, freshwater and maybe sea water. This is important because simulation test are performed with freshwater and/or freshwater sediment systems. Also in these tests generally two different water/sediment systems are required with separate properties. Since the influence may be different regarding different environmental systems and when different substances are tested this should be carefully investigated and compared to WWTP test results. Another point would be to address if there are lower/higher threshold levels for the effect of biomass concentration on the biodegradation kinetics [439]. And last, the

influence of biodiversity might be included in such an investigation especially for freshwater and other native environments since there is a huge influence on diversity and biomass in the natural environment posed by many factors such as light, temperature, seasonal changes etc.

If such an enhanced test as proposed in this study should be used to close the gap between OECD 301 ("ready-tests") and OECD 307/308 ("simulation tests") one needs to also carefully select the methods and the biomass concentrations for the reasons described above. The determination of biomass in activated sludge is fairly easy possible and gives high reproducibility and good results while the procedure is fast and effective. When environmental aqueous media are used the methods become somewhat more difficult. The influence of nutrients available in the environment and annual fluctuations caused by seasons, day time, temperature and others are immense and will have an impact on the results. Nevertheless it would be useful to a) investigate the influence of biomass in environmental compartments on substance biodegradation as well as b) to use varying biomass concentrations for standard tests to provide a closer step between standard tests and environmental simulation tests.

5.3 Analytical and bioanalytical approaches

This discussion on biodegradation tests can be separated in to two areas. The first contains fast screening biodegradation tests using one or maybe two measurement parameters on substances that are either biodegradable or those that are not biodegradable, using no analytical or bioanalytical methods. The other group consists of sophisticated methods especially in substance specific analysis being able to determine already small changes on substances such as polymers that are partially biodegradable or degrade only very slow especially in marine medium. The challenge for analytical methods is to measure polymers with huge molecular weights in small and representative amounts in complex media. Up to now this can only be done to a certain extent. GPC requires relatively high concentrations while MALDI MS cannot be used as quantitative method.

As it was discovered, bioanalytical methods based on molecular determination were not of much use with this project, because the main focus was on different biodegradation behavior of the different test systems and polymers in different environmental media. Since microorganism communities from WWTP are not comparable to marine ones or other consortia, comparisons could only be made within one test system and the results were surprising because they contradict the assumption that similar organisms would be found in similar test replicates.

5.4 "The traveling ducks" - a connected world

The visual parts of polymers in the environment have been reported in public newspapers, magazines and news feeds all around the world for many times as well as in scientific journals [440]. The following example shows literally that the world is connected and that other effects described later on are based on this connection.

A freight container with plastic ducks fell into the Pacific Ocean in January 1992, during heavy weather. The container opened and the animals started floating around. The plastic ducks (Figure 57) have been observed floating around the world. This example clearly indicates that everything once introduced to aquatic systems may end up everywhere in this world. This relates to water insoluble materials as well as to water soluble ones and to large particles as well as micro or nano particles in a similar way.

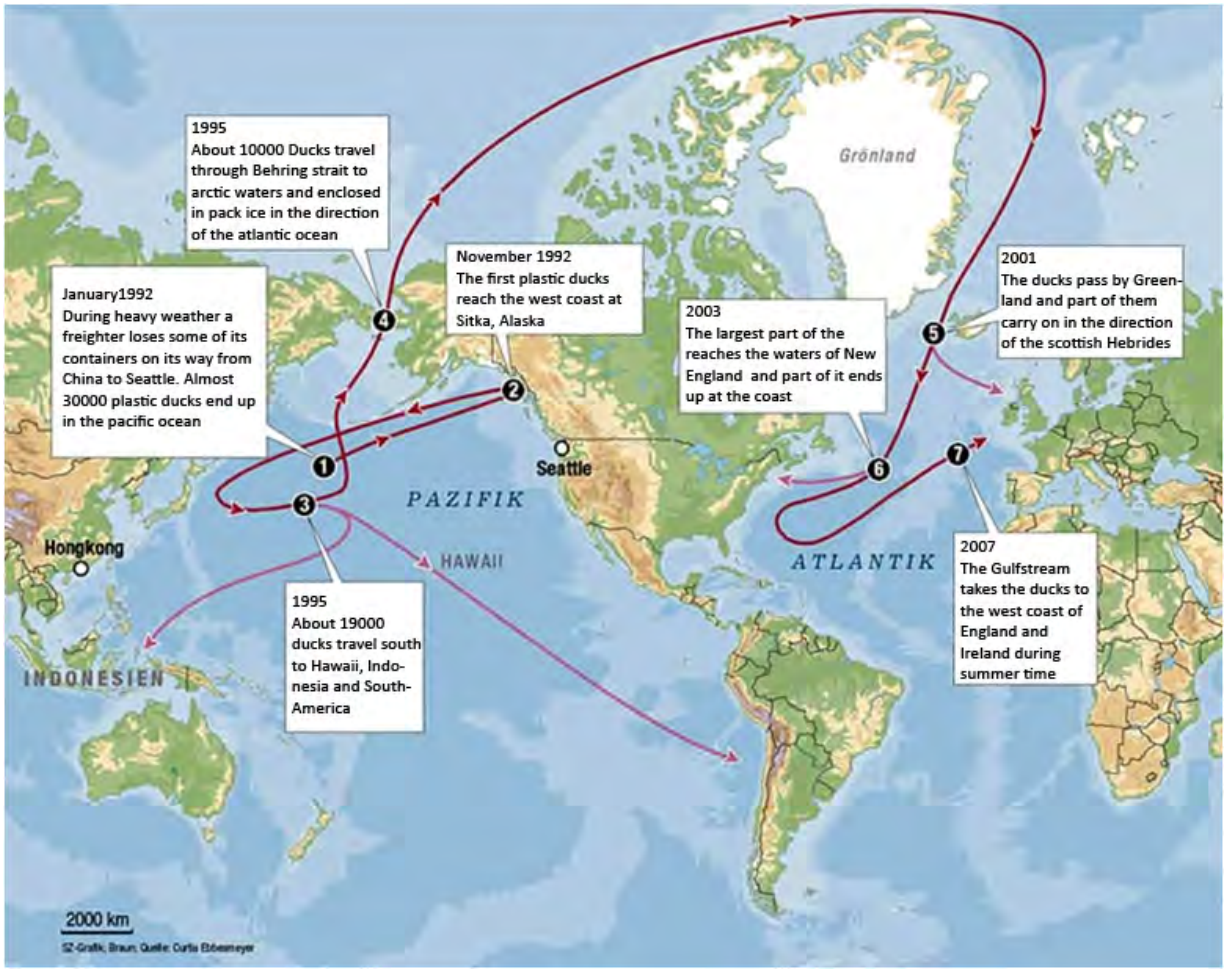


Figure 57 - The route of the travelling ducks shows how well the world's water resources are connected

5.5 Polymer wastes and disposal

Many people have heard of the negative effects of polymers in the environment. The obvious ones that are mainly based on bad habit rather than bad material are discussed now for many years in scientific, industrial and political bodies. The not so obvious effects, the long term and invisible effects are mainly not known today. It is very important not to confuse these topics scientifically and politically because different approaches are required for both.

It is known, that today only less than 5% of all produced plastics undergoes recycling procedure. Several 100'000 tons of polymers and polymer composites are being discarded intentionally or accidentally into the (marine) environment per year. This is estimated to results in over 1'000'000 deaths of marine animals by chocking or becoming entangled in polymer debris. The main products of today's polymer producing industries, polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC) and polystyrene (PS) are not generally biode-

gradable by microorganisms (MOs) in the first place. Table 43 gives an overview about the production of polymers.

Table 43 - Distribution of polymer classes on the market

Thermoplastic mass polymers (~60% of all polymers processed)		
PE	polyethylene	25.2%
PP	polypropylene	15.1%
PVC	polyvinylchloride	14.6%
PS	polystyrene	4.0%
Thermoplastic construction polymers (~11% of all polymers processed)		
PA	polyamide	
PET	polyethyleneterephthalate	
SAN	styrene-acrylonitrile (styrene copolymer)	
ABS	acrylonitrile-butadiene-styrene (styrene copolymer)	
Other polymers used		
PVA	poly vinyl acetate	
SBR	styrene-butadiene rubbers	
PUR	polyurethanes	

When thinking of biodegradation, this property is often equalled with “good” in public and political discussions. But biodegradability requires responsibility in the same way than all other properties of materials we use and require for our daily life. Biodegradability is not necessary beneficial because it may break closed life cycles. It may break and disrupt recycling chains or complicate processes that have been established to reuse the material. Therefore biodegradability must be applied with care. Polymers are very important but also their reuse and recycling is to make sustainable products. At the beginning of the 1990’s about 25 billion tons of plastics household wastes were discarded in the US. The most important part is sorting of different types of plastic when submitted for recycling, since already low content of different types may alter product properties beyond processability [210]. In 2001 about 15.6 million tons of different polymers were manufactured in Germany. About 10.3 million tons were processed to plastics the rest was utilized for manufacturing adhesives, resins, varnish and paints [441]. Today, plastics production increases at about 4% per year in Western Europe. Polymer wastes originate during production of plastics molding material and their processing to semi-finished as well as finished products and finally when or after using plastic goods [441]. In 2001 approx. 3.85 million tons of polymer wastes accumulated in Germany of which about 2.25 million tons were further utilized (62.5% recycled, 13% exploited as resource and 24.5% waste incineration with thermal dissipation) and 1.6 million tons were disposed of [441]. About 45% of all Polymer wastes in Germany are thermoplastics like PE and PP. Furthermore PVC (12.6%), PS/EPS (8.6%) and PUR (8.4%) are main factors in waste accumulation and therefore to be considered in waste management [441]. The ratio of utilization of plastics varies quite a lot. Manufacturing and

processing industry have with 88% and 91% the highest processing rates. Private households and commercial end-consumer show far lower processing rates (49% and 47%) and therefore higher waste output [441]. When looking at worldwide recycling and reuse, the rate of recycling of plastic material is down to approximately 3%. This shows that however biodegradation of polymers may not be as useful for central Europe (where recycling rates are high) but for most of the world it may solve or at least decelerate a still increasing problem. It is interesting, that certain countries today ban or will ban non-degradable polymers used in at least packaging but also often completely from being sold or used in the future. Only degradable polymers are allowed to be used in these countries. Examples for those countries are: (Argentine, Brazil, Hungary, Mexico, Morocco, Slovenia, Barbados, Gabon, Mauritius, Montenegro, Romania, United Arab Emirates, and Yemen)

In regard to the increasing amounts of polymers used in manifold applications in everyday life these facts show how important it is for the future to develop more intelligent products with completely closed life cycles. With rising costs for resources, production and waste disposal, biodegradability is just one small piece of the puzzle. This requires a broad knowledge about material development, processing, marketing strategies, recovering of used products and recycling as well as waste management, environmental assessment and eco-efficiency evaluation [211;442] and scientific input on different mechanisms especially in environmental chemistry, biology and ecotoxicology. Possibilities to re-use polymeric materials are material/physical recycling, chemical/biological [80;443] and energetic recycling as well as biodegradation [444]. Water soluble polymers were examined more detailed in this work because they are used in manifold applications and have a huge potential to enter the environment without being noticed. There are several disposal pathways for water soluble polymers. The most important ones are shown in Figure 58. Generally, those substances are discarded directly into the aquatic environment or in public or private sewage treatment plant or WWTP's. In form of packaging materials or hydrogels, they are discarded mostly via land filling or composting or incineration [89]. At the same time, recycling, reuse should also be improved further and the public must be taught to be more aware of the surrounding world and the effects we have in a long term on this planet if we continue down our actual path. Real biodegradability is an absolute beneficial property if put in the right product but not to encourage carelessness.

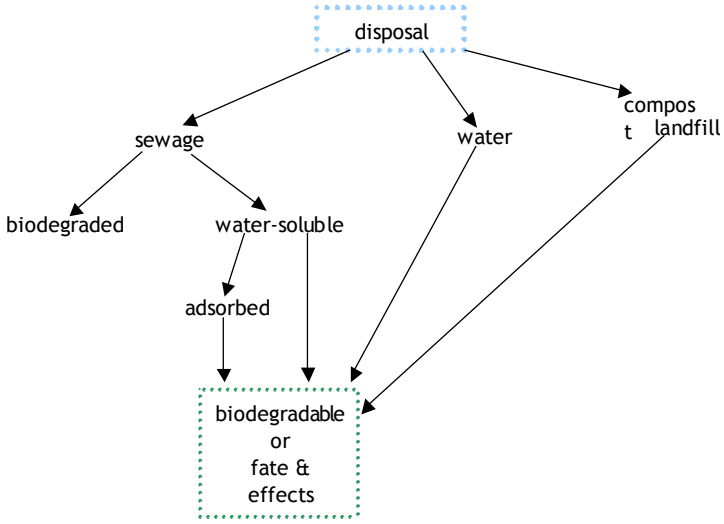


Figure 58 - Disposal pathways for water soluble polymers

Entering sewage treatment plant or WWTP's, water soluble polymers may remain in solution or adsorb completely or partly to the sludge/biomass. The solved fraction will enter the environment eventually and distribute through aqueous compartments. Adsorbed polymers will be transferred to a compartment where the sludge is disposed, such as land filling, incineration, compost etc. In summary, the following tasks need to be achieved for development of biodegradable water soluble polymers [89].

- Preferably the biodegradation should be within the residence time of the disposal site
- Good evidence of biodegradation and a careful risk assessment is necessary for those not being completely removed within the disposal site
- Suitable sites are:
    - WWTP's with residence times of
    - A few hours for non-adsorptive polymers
    - About two weeks for adsorptive polymers
    - Composting facilities with residence times of several weeks

6 Summary

In this work, initially the actual state of research on biodegradation and biodegradability of polymers was presented. The standard biodegradation tests were applied to polymer biodegradation studies and have been evaluated under these new and special conditions. Downsides and problems have been shown as well as it was observed that both marine and freshwater tests were applicable with certain but small changes. The downside is definitely the long time of exposure but here especially marine tests can be applied up to a certain limit of about a year.

It was shown that there are major differences between freshwater (WWTP activated sludge, OECD 301) tests and those in marine (synthetic and native marine water) tests. The differences reached from the time of biodegradation of the same substances to differences in the biodegradation graphs of reference substances and also to differences in the metabolic pathway as was shown with sophisticated analytical techniques [1;289]. The potential of biodegradation freshwater and marine tests was shown for the first time systematically for the group of poly(ethylene glycols) ranging from 200 to almost 60'000 $g \cdot mol^{-1}$. This data has shown differences in the applied systems but also the possibilities of the marine tests.

It was observed that specific parameters such as molecular or structural properties of the polymers do have influence on the biodegradation in different environments such as an influence by molecular weight, number of hetero atoms in the chain, specific behavior of groups that hydrolyze but do not biodegrade etc. similar to known data [67;86], and possible pathways of biodegradation were confirmed. Similarities and differences were identified.

From the data obtained it can be concluded that marine tests up to 200 or 300 days can produce reliable data but longer tests should not be carried out, since the variability of results increases much more significantly with

progressing time. However, the biodegradation under marine conditions is slower and marine tests require much longer than tests on ready biodegradability.

An increase in biomass or change in test substance concentration did not result in an acceleration of the test. Because of the nature of the microorganism communities being able to utilize smallest available carbon sources it seems that biodegradation is much more dependent on the availability of the polymer. This again proves that structure deterioration (by abiotic process) plays an important role. Some final statements may be suitable for further research:

- When using marine medium at this time around 300 days seems to be the upper limit for those tests.
- The test conditions using CO_2 free air in closed systems give stable conditions and variations can be kept low during the first 200-300 days.
- If possible more than one parameter should be measured to confirm test results, especially when such long tests are done.
- Adding medium/inoculum and taking samples can be performed easily and without too much disturbance of the test. One needs to be careful and test media should be thoroughly homogenized by agitating/stirring prior to sampling
- Marine tests show mainly far lower biodegradation when compared to freshwater/WW and soil or compost.
- Marine medium can be prepared synthetically in the lab or native sea water can be used. Transportation (for a few days) does not cause any known negative effect on the biodegradation of a test substance later on. The water samples should be stored at constant temperature and at room temperature at the highest.
- Tests using native water may have more impact and are closer to natural conditions but no significant differences were observed compared to synthetic medium in this study.
- Marine medium can be prepared using sea water aquaria inoculum suspension from filter units. A dilution of 1:10 (v/v) of the inoculum suspension with synthetic medium seems to give proper results and smooth tests.

As demonstrated, the conditions of the test were kept stable and were controlled throughout the tests. Nevertheless sometimes it still happens that results show high variation.

It was shown in this thesis that PEG biodegrades in freshwater and marine environment after certain time to full extent up to 60 kDa (freshwater and 15 kDa marine water). PEG biodegradation was investigated for the first time to this extend and biodegradation pathways were postulated as shown in Figure 59 [54]. It seems interesting, that there are obviously two different pathways in marine water for PEGs <1600 $g \cdot mol^{-1}$ and for those >1600 $g \cdot mol^{-1}$. It also seems interesting that some microorganisms prefer lower substrate concentration and the biodegradation degree is lower when an increased substance concentration is used, as was demonstrated for PEGs. The effect was reported a few times from different microbiologists on conferences of the water chemical society.

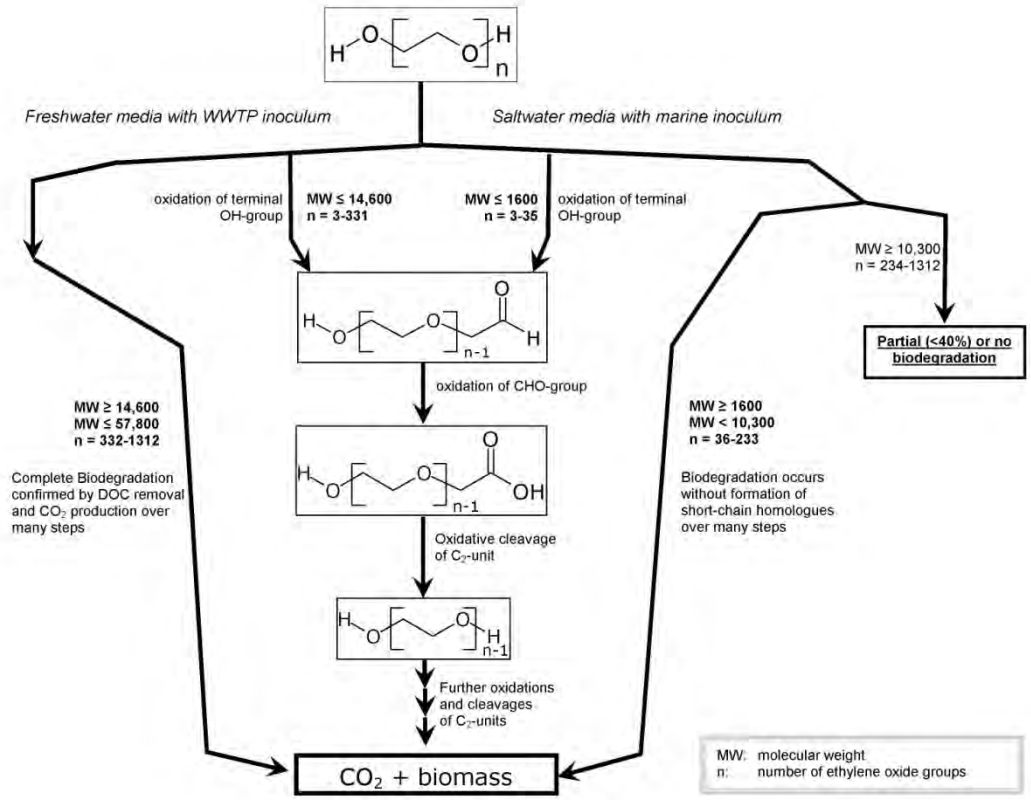


Figure 59 - Pathways for poly(ethylene glycol) degradation in freshwater and seawater

Ecoflex may degrade very slowly in marine water. For freshwater no evidence was found yet which may be due to the fact that the test systems are not suitable. This is also very interesting because Ecovio (with the PLA content of 32%) was degraded in the same system but the other Ecovio type was not. This is a very interesting observation.

It was hypothesized that the PLA part breaks down by hydrolysis (which is already known) but does not biodegrade further on. This breakdown may help to provide more access for the microorganisms to the Ecoflex and may therefore promote the biodegradation of Ecoflex in the test because of better susceptibility. It would then explain why biodegradation was observed with the Ecovio 8180. With about 80% PLA breakdown by hydrolysis could occur, but the little amount of Ecoflex would be degraded possibly without notice because the background noise maybe too much.

Ecovio degrades partially in freshwater and marine water. The biodegradation degree may be dependent on the content of Ecoflex in the polymer blend but also on PLA or other polymers that may promote and enhance biodegradation.

The results from different biodegradation studies in synthetic marine media using inoculum from sea water aquaria as well as native sea water have provided first insights in polymer biodegradation and the differences to freshwater environment. The experiments provide a basis to improve the tests. The biodegradation results especially with synthetic polymers have shown that:

- Biodegradation in standard test systems and marine test systems can differ in kinetics and pathway of biodegradation.
- Because of the immense buffer capacity of marine sea water generally higher blank control values as well as much more variation has been observed in the tests when compared with OECD 301 standard tests.
- The desired type of analytical procedure (DOC/DIC; BOD, CO_2) determines the length of the study and needs to be considered because carbon content in the blank controls may show higher deviation and variation than in freshwater media.

According the experience from the tests a dilution of 1:10 of inoculum suspension from filter units of sea water aquaria led to the most constant and best biodegradation tests. The colony forming units count for the medium would then be in the range of the upper 10^3 and lower 10^4 areas at the beginning of an experiment. This also suggests that an increased cell density does not necessarily lead to a higher degree in biodegradation. It can be observed visually that after a while biomass begins to form in the glass bottles. In contrast to that, increasing biomass concentration results often in better biodegradation rates in WWTP Tests as was shown with degradation and PVA

When comparing data obtained from biodegradation tests using different measurement techniques such as biological oxygen demand determination, dissolved carbon and inorganic carbon measurement, it could be seen that the treatment of the inoculum prior to the test and the mineral medium had some influence on the quality of the results. It was observed that the TOC content of the inoculum suspension must be reduced especially when using biological oxygen demand as the measured parameter of choice. But also for dissolved carbon and inorganic carbon determination it is useful. Therefore the inoculum suspension should be aerated for at least 7 days with CO_2-free air prior to the experiment. It was observed that the inorganic carbon content increases to some extent when using pressurized air because at least some of the CO_2 from the air forms CO_3^{2-} or HCO_3^- in marine water (data not shown). Especially the oxygen measurement (BOD) is very sensitive and may be influenced a lot through biodegradation of small amounts of carbon.

In terms of inocula it can be observed in these studies and also in others that the lag-phase can be very long and still biodegradation may start. Marine organisms are known to live on very few nutrients and even in nutrient rich environments these organisms do not significantly increase their metabolic rate.

Two aspects should be considered for the future. First, biodegradation of polymers can be specifically used for certain applications. Today there are many valuable ideas on the market. These include products that require fast degradation but also some where biodegradability should be more than one or two years because the product has a lifecycle within this time.

Second, biodegradation of polymers needs to be further researched and methods need to be developed and enhanced especially to qualitatively and quantitatively analyze different polymers in environmental matrices and also determine microorganism communities more easily. Also enhanced biodegradation tests should be developed that use abiotic ageing procedures prior to biodegradation experiments to provide more rapid techniques along standard tests. Further on it would be appropriate to find small scale tests that can be done with-

out much lab space as pre screening tests or even simulation tests in a small scale system. Today sensors are available to measure parameters online in closed test systems without any connection to cables and lines. Also it has been demonstrated that microbial activity can be measured using electronic equipment [445].

Finally, the results described and discussed in this work have given one of the first systematic impressions in the biodegradation process of synthetic polymers in the aquatic environment with a focus of marine environment. They show how difficult an approach to investigate biodegradation correctly is especially for the aquatic environment. Nevertheless, this is a serious topic that requires much more attention but certainly in a carefully directed and planned way because of the political and social discussion going on today.

7 Annex

7.1 List of figures

7.2 List of tables

7.3 List of equations

References

[1] Bernhard,M. **(2010)**. Dissertation, Working Title: "Method development for analytical determination of synthetic and natural polymers in aquatic media by gel permeation chromatography and mass spectrometry to analyze biodegradation pathways". to be submitted , University of Bremen and University of Applied Science Fresenius.

[2] Singh,B. & Sharma,N. **(2008)**. Mechanistic implications of plastic degradation. *Polymer Degradation and Stability* 93(3), 561-584.

[3] Trimpin,S., Eichhorn,P., Rader,H.J., Mullen,K. & Knepper,T.P. **(2001)**. Recalcitrance of poly(vinylpyrrolidone): evidence through matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. *Journal of Chromatography A* 938(1-2), 67-77.

[4] Ab-Malek,K. & Stevenson,A. **(1986)**. The effect of 42 year immersion in sea-water on natural rubber. *Journal of Materials Science* 21(1), 147-154.

[5] Carpenter,E.J., Anderson,S.J., Harvey,G.R., Miklas,H.P. & Peck,B.B. **(1972)**. Polystyrene Spherules in Coastal Waters. *Science* 178(4062), 749-750.

[6] Carpenter,E.J. & Smith,K.L., Jr. **(1972)**. Plastics on the Sargasso Sea Surface. *Science* 175(4027), 1240-1241.

[7] Gellert,E.P. & Turley,D.M. **(1999)**. Seawater immersion ageing of glass-fibre reinforced polymer laminates for marine applications. *Composites Part A: Applied Science and Manufacturing* 30(11), 1259-1265.

[8] Gonda,K.E., Jendrossek,D. & Molitoris,H.P. **(2000)**. Fungal degradation of the thermoplastic polymer poly-Î²-hydroxybutyric acid (PHB) under simulated deep sea pressure. *Hydrobiologia* 426(1), 173-183.

[9] Gonsalves,K.E., Patel,S.H. & Chen,X. **(1991)**. Development of potentially degradable materials for marine applications. II. Polypropylene-starch blends. *J. Appl. Polym. Sci.* 43(2), 405-415.

[10] Ikada,E. **(1999)**. Electron Microscope Observation of Biodegradation of Polymers. *Journal of Polymers and the Environment* 7(4), 197-201.

[11] Kasuya,K.i., Takagi,K.i., Ishiwatari,S.i., Yoshida,Y. & Doi,Y. **(1998)**. Biodegradabilities of various aliphatic polyesters in natural waters. *Polymer Degradation and Stability* 59(1-3), 327-332.

[12] Kootsookos,A. & Mouritz,A.P. **(2004)**. Seawater durability of glass- and carbon-polymer composites. *Composites Science and Technology* 64(10-11), 1503-1511.

[13] Mergaert,J., Wouters,A., Anderson,C. & Swings,J. **(1995)**. In situ biodegradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in natural waters. *Can. J. Microbiol.* 41(13), 154-159.

[14] Calmon-Decriaud,A., Bellon-Maurel,V.Ã., & Silvestre,F. **(1998)**. Standard Methods for Testing the Aerobic Biodegradation of Polymeric Materials. Review and Perspectives. In Advances in Polymer Science pp. 207-226. Springer Berlin / Heidelberg.

[15] Chiellini,E., Corti,A. & D'Antone,S. **(2007)**. Oxo-biodegradable full carbon backbone polymers - biodegradation behaviour of thermally oxidized polyethylene in an aqueous medium. *Polymer Degradation and Stability* 92(7), 1378-1383.

[16] Musat,F., Galuschko,A., Jacob,J., Widdel,F., Kube,M., Reinhard,R., Wilkes,H., Schink,B. & Rabus,R. **(2008)**. Anaerobic degradation of naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. *Environmental Microbiology* in press.

[17] Das,P., Mukherjee,S. & Sen,R. **(2008)**. Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. *Chemosphere* 72(9), 1229-1234.

[18] Melcher,R J., Apitz,S.E. & Hemmingsen,B.B. **(2002)**. Impact of Irradiation and Polycyclic Aromatic Hydrocarbon Spiking on Microbial Populations in Marine Sediment for Future Aging and Biodegradability Studies. *Appl. Environ. Microbiol.* 68(6), 2858-2868.

[19] Bayona,J. & Albaigés,J. **(2006)**. Sources and Fate of Organic Contaminants in the Marine Environment. In Marine Organic Matter: Biomarkers, Isotopes and DNA pp. 323-370.

[20] Stales,C A., Peterson,D.R., Parkerton,T.F. & Adams,W.J. **(1997)**. The environmental fate of phthalate esters: A literature review. *Chemosphere* 35(4), 667-749.

[21] Laist,D.W. **(1987)**. Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Marine Pollution Bulletin* 18(6, Supplement 2), 319-326.

[22] Rios,L.M., Moore,C. & Jones,P.R. **(2007)**. Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Marine Pollution Bulletin* 54(8), 1230-1237.

[23] Endo,S., Takizawa,R., Okuda,K., Takada,H., Chiba,K., Kanehiro,H., Ogi,H., Yamashita,R. & Date,T. **(2005)**. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: Variability among individual particles and regional differences. *Marine Pollution Bulletin* 50(10), 1103-1114.

[24] Mato,Y., Isobe,T., Takada,H., Kanehiro,H., Ohtake,C. & Kaminuma,T. **(2001)**. Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. *Environ. Sci. Technol.* 35(2), 318-324.

[25] Teuten,E.L., Rowland,S.J., Galloway,T.S. & Thompson,R.C. **(2007)**. Potential for Plastics to Transport Hydrophobic Contaminants. *Environ. Sci. Technol.* 41(22), 7759-7764.

[26] The European Parliament & The Council of the European Union. **(2006)**. Regulation (EC) No. 1907/2006 OF.

[27] Munn,C.B. **(2004)**. *Marine Microbiology - Ecology & Applications*, 1 edn. BIOS Scientific Publishers, New York and London.

[28] OSPAR Commission. **(1992)**. Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention).

[29] Lucas,N., Bienaime,C., Belloy,C., Queneudec,M., Silvestre,F. & Nava-Saucedo,J.E. **(2008)**. Polymer biodegradation: Mechanisms and estimation techniques - A review. *Chemosphere* 73(4), 429-442.

[30] Beek,B., Böhling,S., Franke,C., Jöhncke,U., Studinger,G., & Thumm,E. **(2001)**. The Assessment of Biodegradation and Persistence. In Biodegradation and Persistance pp. 291-320. Springer, Berlin / Heidelberg.

[31] Bosma,T., Harms,H., & Zehnder,A. **(2001)**. Biodegradation of Xenobiotics in Environment and Technosphere. In Biodegradation and Persistance pp. 163-202. Springer, Berlin / Heidelberg.

[32] Petrovic,M. & Barcelo,D. **(2004)**. Fate and Removal of Surfactants and Related Compounds in Wastewaters and Sludges. In Water Pollution - Emerging Organic Pollution in Waste Waters and Sludge, Vol. 1 pp. 1-28. Springer, Berlin / Heidelberg.

[33] Mezzanotte,V., Castiglioni,F., Todeschini,R. & Pavan,M. **(2003)**. Study on anaerobic and aerobic degradation of different non-ionic surfactants. *Bioresource Technology* 87(1), 87-91.

[34] Pagga,U. & Beimborn,D.B. **(1993)**. Anaerobic biodegradation test for organic compounds. *Chemosphere* 27(8), 1499-1509.

[35] Strevett,K., Davidova,I. & Suflita,J.M. **(2002)**. A comprehensive review of the screening methodology for anaerobic biodegradability of surfactants
25. *Reviews in Environmental Science and Biotechnology* 1(2), 143-167.

[36] Ying,G.G. **(2006)**. Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment International* 32(3), 417-431.

[37] Peschka,M., Frömel,T., Fichtner,N., Hierse,W., Kleineidam,M., Montenegro,E. & Knepper,T.P. **(2008)**. Mechanistic studies in biodegradation of the new synthesized fluorosurfactant 9-[4-(trifluoromethyl)phenoxy]nonane-1-sulfonate. *Journal of Chromatography A* 1187(1-2), 79-86.

[38] Mueller,K., Magesan,G.N. & Bolan,N.S. **(2007)**. A critical review of the influence of effluent irrigation on the fate of pesticides in soil. *Agriculture, Ecosystems & Environment* 120(2-4), 93-116.

[39] Giacomazzi,S. & Cochet,N. **(2004)**. Environmental impact of diuron transformation: a review. *Chemosphere* 56(11), 1021-1032.

[40] Petit,V., Cabridenc,R., Swannell,R.P J. & Sokhi,R.S. **(1995)**. Review of strategies for modelling the environmental fate of pesticides discharged into riverine systems. *Environment International* 21(2), 167-176.

[41] Honing,M., Barcelo,D., van Baar,B.L.M., & Brinkman,U.A.T. **(1995)**. 22. Sample handling and determination of carbamate pesticides and their transformation products in various matrices. In Techniques and Instrumentation in Analytical Chemistry
Quality Assurance for Environmental Analysis - Method Evaluation within the Measurements and Testing Programme (BCR) (Quevauviller,E.A.M., ed), pp. 535-562. Elsevier.

[42] White,F. **(1995)**. Multiple interactions in riverine biofilms -- surfactant adsorption, bacterial attachment and biodegradation. *Water Science and Technology* 31(1), 61-70.

[43] Mulbry,W. & Kearney,P.C. **(1991)**. Degradation of pesticides by micro-organisms and the potential for genetic manipulation. *Crop Protection* 10(5), 334-346.

[44] Benotti,M.J. & Brownawell,B.J. **(2009)**. Microbial degradation of pharmaceuticals in estuarine and coastal seawater. *Environmental Pollution* 157(3), 994-1002.

[45] Heberer,T. **(2002)**. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters* 131(1-2), 5-17.

[46] Fatta,D., Achilleos,A., Nikolaou,A. & Meriτ,S. **(2007)**. Analytical methods for tracing pharmaceutical residues in water and wastewater. *TrAC Trends in Analytical Chemistry* 26(6), 515-533.

[47] Kümmerer,K. **(2001)**. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - a review. *Chemosphere* 45(6-7), 957-969.

[48] Halling-Sörensen,B., Nors Nielsen,S., Lanzky,P.F., Ingerslev,F., Holten Lötzhoft,H.C. & Jörgensen,S.E. **(1998)**. Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere* 36(2), 357-393.

[49] Imfeld,G., Braeckevelt,M., Kuschk,P. & Richnow,H.H. **(2009)**. Monitoring and assessing processes of organic chemicals removal in constructed wetlands. *Chemosphere* 74(3), 349-362.

[50] Martinkova,L., Uhnakova,B., Patek,M., Nesvera,J. & Kren,V. **(2009)**. Biodegradation potential of the genus Rhodococcus. *Environment International* 35(1), 162-177.

[51] Raymond,J.W., Rogers,T.N., Shonnard,D.R. & Kline,A.A. **(2001)**. A review of structure-based biodegradation estimation methods. *Journal of Hazardous Materials* 84(2-3), 189-215.

[52] Jones,W.R. **(1997)**. Biosurfactants, bioavailability and bioremediation. In Studies in Environmental Science
Global Environmental Biotechnology, Proceedings of the Third Biennial Meeting of the International Society for Environmental Biotechnology (Wise,D.L., ed), pp. 379-391. Elsevier.

[53] Young,L.Y. & Häggblom,M.M. **(1991)**. Biodegradation of toxic and environmental pollutants. *Current Opinion in Biotechnology* 2(3), 429-435.

[54] Eubeler,J.P., Bernhard,M., Zok,S. & Knepper,T.P. **(2009)**. Environmental biodegradation of synthetic polymers I. Test methodologies and procedures. *TrAC Trends in Analytical Chemistry* 28(9), 1057-1072.

[55] Eubeler,J.P., Bernhard,M. & Knepper,T.P. **(2010)**. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. *TrAC Trends in Analytical Chemistry* 29(1), 84-100.

[56] Klemchuk,P.P. **(1990)**. Degradable plastics: A critical review. *Polymer Degradation and Stability* 27(2), 183-202.

[57] Chandra,R. & Rustgi,R. **(1998)**. Biodegradable polymers. *Progress in Polymer Science* 23(7), 1273-1335.

[58] Tharanathan,R.N. **(2003)**. Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology* 14(3), 71-78.

[59] Kyrikou,I. & Briassoulis,D. **(2007)**. Biodegradation of Agricultural Plastic Films: A Critical Review. *Journal of Polymers and the Environment* 15(2), 125-150.

[60] Mueller,R.-J. & Deckwer,W.-D. **(2000)**. Biologisch abbaubare Polymere. Berichte aus der Forschung. Gesellschaft fuer Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig. GBF Ergebnisbericht.

[61] Gross,R.A. & Kalra,B. **(2002)**. Biodegradable Polymers for the Environment. *Science* 297(5582), 803-807.

[62] Asrar,J. & Gruys,K.J. **(2003)**. Biodegradable Polymer (Biopol®). In Biopolymers (Steinbuechel,A., ed), pp. 53-68. Wiley VCH, Weinheim.

[63] Jung,C. and Steinbuechel,A. **(2001)**. Bioplastik aus Nutzpflanzen: Palette der nachwachsenden Rohstoffe erweitert. (bioplastics from economic plants). *Biologie in unserer Zeit* 31(4), 250-258.

[64] Mecking,S. **(2004)**. Nature or petrochemistry? – Biologically degradable materials. *Angew. Chem. Int. Ed.* 43 1078-1085.

[65] Scott,G. **(2003)**. *Degradable Polymers: Principles and Applications*. Kluwer Academic Publishers.

[66] Witt,U., Yamamoto,M., Seeliger,U., Mueller,R.-J. & Warzelhan,V. **(1999)**. Biologisch abbaubare Polymerwerkstoffe - nicht die Rohstoffquelle, sondern die chemische Struktur entscheidet über die Abbaubarkeit. *Angew. Chem.* 111(10), 1540-1544.

[67] Swift,G. **(1993)**. Directions for environmentally biodegradable polymer research. *Acc. Chem. Res.* 26 105-110.

[68] Gandini,A. **(2008)**. Polymers from Renewable Resources: A Challenge for the Future of Macromolecular Materials. *Macromolecules* 41(24), 9491-9504.

[69] Lenz,R.W., Kim,Y.B. & Fuller,R.C. **(1992)**. Production of unusual bacterial polyesters by Pseudomonas oleovorans through cometabolism. *FEMS Microbiol.* 103(2-4), 207-214.

[70] Braunegg,G., Lefebrve,G. & Genser,K.F. **(1998)**. Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects. *J. Biotechnol.* 65 127-161.

[71] Funke,U., Bergthaller,W. & Lindhauer,M.G. **(1998)**. Processing and characterization of biodegradable products based on starch. *Polymer Degradation and Stability* 59(1-3), 293-296.

[72] Simon,J., Muller,H.P., Koch,R. & Muller,V. **(1998)**. Thermoplastic and biodegradable polymers of cellulose. *Polymer Degradation and Stability* 59(1-3), 107-115.

[73] Bastioli,C. **(1998)**. Properties and applications of Mater-Bi starch-based materials. *Polymer Degradation and Stability* 59(1-3), 263-272.

[74] Slomkowski,S., Sosnowski,S. & Gadzinowski,M. **(1998)**. Polyesters from lactides and [e]-caprolactone. Dispersion polymerization versus polymerization in solution. *Polymer Degradation and Stability* 59(1-3), 153-160.

[75] Wang,L., Ma,W., Gross,R.A. & McCarthy,S.P. **(1998)**. Reactive compatibilization of biodegradable blends of poly(lactic acid) and poly([e]-caprolactone). *Polymer Degradation and Stability* 59(1-3), 161-168.

[76] Lunt,J. **(1998)**. Large-scale production, properties and commercial applications of polylactic acid polymers. *Polymer Degradation and Stability* 59(1-3), 145-152.

[77] Ajioka,M., Suizu,H., Higuchi,C. & Kashima,T. **(1998)**. Aliphatic polyesters and their copolymers synthesized through direct condensation polymerization. *Polymer Degradation and Stability* 59(1-3), 137-143.

[78] Duda,A., Biela,T., Libiszowski,J., Penczek,S., Dubois,P., Mecerreyes,D. & Jerome,R. **(1998)**. Block and random copolymers of [e]-caprolactone. *Polymer Degradation and Stability* 59(1-3), 215-222.

[79] Hu,Y., Zhang,L., Cao,Y., Ge,H., Jiang,X. & Yang,C. **(2004)**. Degradation Behavior of Poly(e-caprolactone)-b-poly(ethylene glycol)-b-poly(e-caprolactone) Micelles in Aqueous Solution. *Biomacromolecules* 5(5), 1756-1762.

[80] Ward,P.G., Goff,M., Donner,M., Kaminsky,W. & O'Connor,K.E. **(2006)**. A Two Step Chemo-biotechnological Conversion of Polystyrene to a Biodegradable Thermoplastic. *Environ. Sci. Technol.* 40(7), 2433-2437.

[81] Scott,G. & Wiles,D.M. **(2001)**. Programmed-Life Plastics from Polyolefins: A New Look at Sustainability. *Biomacromolecules* 2(3), 615-622.

[82] Swift,G. **(1994)**. Biodegradable plastics and polymers. Doi, Y. and Fukuda, K. Proceedings of the Third International Scientific Workshop on Biodegradable Plastics and Polymers, Osaka, Japan, Nove. *Proceedings of the Third International Scientific Workshop on Biodegradable Plastics and Polymers* New York, Elsevier Science B.V.

[83] Heyde,M. **(1998)**. Ecological considerations on the use and production of biosynthetic and synthetic biodegradable polymers. *Polymer Degradation and Stability* 59(1-3), 3-6.

[84] Gu,J.D. **(2003)**. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. *International Biodeterioration & Biodegradation* 52(2), 69-91.

[85] Kalbe,J., Koch,R., Rafler,G., Moosheimer,U. & Bach,H.-J. **(2000)**. Bioabbaubare Kunststoffe. *Spektrum der Wissenschaft Digest: Moderne Chemie II* 1 84-93.

[86] Scott,G. **(2000)**. `Green´ polymers. *Polymer Degradation and Stability* 68(1), 1-7.

[87] Pagga,U. **(1999)**. Compostable packaging materials: test methods and limit values for biodegradation. *Applied Microbiology and Biotechnology* 51(2), 125-133.

[88] De Wilde,B. & Boelens,J. **(1998)**. Prerequisites for biodegradable plastic materials for acceptance in real-life composting plants and technical aspects. *Polymer Degradation and Stability* 59(1-3), 7-12.

[89] Swift,G. **(1998)**. Requirements for biodegradable water-soluble polymers. *Polymer Degradation and Stability* 59(1-3), 19-24.

[90] Grima,S., Bellon-Maurel,V.Ã., Feuilloley,P. & Silvestre,F.o. **(2000)**. Aerobic Biodegradation of Polymers in Solid-State Conditions: A Review of Environmental and Physicochemical Parameter Settings in Laboratory Simulations. *Journal of Polymers and the Environment* 8(4), 183-195.

[91] Reineke,W. **(2001)**. Aerobic and Anaerobic Biodegradation Potentials of Microorganisms. In Biodegradation and Persistance pp. 1-161. Springer, Berlin / Heidelberg.

[92] Pagga,U. **(1997)**. Testing biodegradability with standardized methods. *Chemosphere* 35(12), 2953-2972.

[93] Mueller,R.-J., Witt,U., Rantze,E. & Deckwer,W.-D. **(1998)**. Architecture of biodegradable copolyesters containing aromatic constituents. *Polymer Degradation and Stability* 59(1-3), 203-208.

[94] Mueller,R.-J. **(2001)**. Biologisch abbaubare Kunststoffe. *Biologie in unserer Zeit* 30(4), 218-224.

[95] Witt,U., Mueller,R.J. & Deckwer,W.D. **(1996)**. Evaluation of the biodegradability of copolyesters containing aromatic compounds by investigations of model oligomers. *Journal of Polymers and the Environment* 4(1), 9-20.

[96] Witt,U., Muller,R.J. & Deckwer,W.D. **(1995)**. New biodegradable polyester-copolymers from commodity chemicals with favorable use properties. *Journal of Polymers and the Environment* 3(4), 215-223.

[97] Urstadt,S., Augusta,J., Mueller,R.-J. & Deckwer,W.-D. **(1995)**. Calculation of carbon balances for evaluation of the biodegradability of polymers. *Journal of Polymers and the Environment* 3(3), 121-131.

[98] Deckwer,W.-D. **(2003)**. Mechanismus des mikrobiellen Abbaus von polyestern (Mechanism of microbial degradation of polyesters). Technische Universitaet Braunschweig, Mascheroder Weg 1, 38124 Braunschweig. Bericht zum Forschungsvorhaben der MBFSt, Kennziffer 2290, Technische Universitaet Braunschweig, Fachbereich 4 Biowissenschaften: Bioverfahrenstechnik, GBF/TU-BCE.

[99] Faber,M.D. **(1979)**. Microbial degradation of recalcitrant compounds and synthetic aromatic polymers. *Enzyme and Microbial Technology* 1(4), 226-232.

[100] Howard,G.T. **(2002)**. Biodegradation of polyurethane: a review. *International Biodeterioration & Biodegradation* 49(4), 245-252.

[101] Schirmer,A., Jendrossek,D. & Schlegel,H.G. **(1993)**. Degradation of poly(3-hydroxyoctanoic acid) [P(3HO)] by bacteria: purification and properties of a P(3HO) depolymerase from Pseudomonas fluorescens GK13. *Appl. Environ. Microbiol.* 59(4), 1220-1227.

[102] Mabrouk,M.M. & Sabry,S A. **(2001)**. Degradation of poly (3-hydroxybutyrate) and its copolymer poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by a marine Streptomyces sp. SNG9. *Microbiological Research* 156(4), 323-335.

[103] Leathers,T.D., Govind,N.S. & Greene,R.V. **(2000)**. Biodegradation of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by a Tropical Marine Bacterium, Pseudoalteromonas sp. NRRL B-30083. *Journal of Polymers and the Environment* 8(3), 119-124.

[104] Jendrossek,D. **(2001)**. Microbial Degradation of Polyesters. In Biopolyesters (Babel,W. & Steinbuchel,A., eds), pp. 293-325. Springer Berlin / Heidelberg.

[105] Gu,J.G. & Gu,J.D. **(2005)**. Methods Currently Used in Testing Microbiological Degradation and Deterioration of a Wide Range of Polymeric Materials with Various Degree of Degradability: A Review. *Journal of Polymers and the Environment* 13(1), 65-74.

[106] Mueller,R.J., Kleeberg,I. & Deckwer,W.D. **(2001)**. Biodegradation of polyesters containing aromatic constituents. *Journal of Biotechnology* 86(2), 87-95.

[107] Jang,J.C., Shin,P.K., Yoon,J.S., Lee,I.M., Lee,H.S. & Kim,M.N. **(2002)**. Glucose effect on the biodegradation of plastics by compost from food garbage. *Polymer Degradation and Stability* 76(1), 155-159.

[108] Mezzanotte,V., Bertani,R., Innocenti,F.D. & Tosin,M. **(2005)**. Influence of inocula on the results of biodegradation tests. *Polymer Degradation and Stability* 87(1), 51-56.

[109] Scott,G. **(1997)**. Abiotic control of polymer biodegradation. *Trends Polym. Sci.* 5(11), 361-368.

[110] Kunioka,M. & Choi,H.J. **(1998)**. Hydrolytic degradation and mechanical properties of hydrogels prepared from microbial poly(amino acid)s. *Polymer Degradation and Stability* 59(1-3), 33-37.

[111] Rashkov,I., Manolova,N., Li,S.M., Espartero,J.L. & Vert,M. **(1996)**. Synthesis characterization, and hydrolytic degradation of PLA/PEO/PLA triblock copolymers with short poly(L-lactic acid) chains. *Macromolecules* 29(1), 50-56.

[112] Shin,J. & Yeh,K.N. **(1999)**. Hydrolytic degradation of poly(1,4-butylene terephthalate-co-tetramethylene oxalate) copolymer. *J. Appl. Polym. Sci.* 74(4), 921-936.

[113] Davies,P., Pomies,F. & Carlsson,L.A. **(1996)**. Influence of water and accelerated aging on the shear fracture properties of glass/epoxy composite. *Applied Composite Materials* 3(2), 71-87.

[114] Karbhari,V.M. & Zhang,S. **(2003)**. E-Glass/Vinylester Composites in Aqueous Environments I: Experimental Results. *Applied Composite Materials* 10(1), 19-48.

[115] Andrady,A.L., Pegram,J.E. & Song,Y. **(1993)**. Studies on enhanced degradable plastics. II. Weathering of enhanced photodegradable polyethylenes under marine and freshwater floating exposure. *Journal of Polymers and the Environment* 1(2), 117-126.

[116] Andrady,A.L. **(2000)**. Plastics and Their Impacts in the Marine Environment. Proceedings of the International Marine Debris Conference on Derelict Fishing Gear and the Ocean Environment. *Plastics and Their Impacts in the Marine Environment.Proceedings of the International Marine Debris Conference on Derelict Fishing Gear and the Ocean Environment*

[117] Gonsalves,K.E., Patel,S.H. & Trivedi,D.H. **(1992)**. Development of potentially degradable materials for marine applications. III. Polyethylene-polyethylene oxide blends. *J. Appl. Polym. Sci.* 45(2), 217-225.

[118] Breslin,V.T. and Li,B. **(1993)**. Weathering of starch-polyethylene composite films in the marine environment. *J. Appl. Polym. Sci.* 48(12), 2036-2079.

[119] Leonas,K.K. & Gorden,R.W. **(1993)**. An accelerated laboratory study evaluating the disintegration rates of plastic films in simulated aquatic environments. *Journal of Polymers and the Environment* 1(1), 45-51.

[120] Kim,H S. & Kim,H.J. **(2008)**. Enhanced hydrolysis resistance of biodegradable polymers and bio-composites. *Polymer Degradation and Stability* 93(8), 1544-1553.

[121] Guillet,J.E., Regulski,T.W. & McAneney,T.B. **(1974)**. Biodegradability of photodegraded polymers. II. Tracer studies of biooxidation of Ecolyte PS polystyrene. *Environ. Sci. Technol.* 8(10), 923-925.

[122] Jones,P.H., Prasad,D., Heskins,M., Morgan,M.H. & Guillet,J.E. **(1974)**. Biodegradability of photodegraded polymers. I. Development of experimental procedures. *Environ. Sci. Technol.* 8(10), 919-923.

[123] Boberic,G., Bajt,O., Sket,B. & Faganeli,J. **(1998)**. Photochemical degradation of butyl acrylate in different aqueous media. *Chemosphere* 37(1), 33-40.

[124] Arnaud,R., Dabin,P., Lemaire,J., Al-Malaika,S., Chohan,S., Coker,M., Scott,G., Fauve,A. & Maaroufi,A. **(1994)**. Photooxidation and biodegradation of commercial photodegradable polyethylenes. *Polymer Degradation and Stability* 46(2), 211-224.

[125] Gallet,G., Carroccio,S., Rizzarelli,P. & Karlsson,S. **(2002)**. Thermal degradation of poly(ethylene oxide-propylene oxide-ethylene oxide) triblock copolymer: comparative study by SEC/NMR, SEC/MALDI-TOF-MS and SPME/GC-MS. *Polymer* 43(4), 1081-1094.

[126] Ma,J., Ma,W., Song,W., Chen,C., Tang,Y., Zhao,J., Huang,Y., Xu,Y. & Zang,L. **(2006)**. Fenton Degradation of Organic Pollutants in the Presence of Low-Molecular-Weight Organic Acids: Cooperative Effect of Quinone and Visible Light. *Environ. Sci. Technol.* 40(2), 618-624.

[127] Kremer,M.L. **(2003)**. The Fenton Reaction. Dependence of the Rate on pH. *J. Phys. Chem. A* 107(11), 1734-1741.

[128] Buda,F., Ensing,B., Gribnau,M.C.M. & Baerens,E.J. **(2003)**. O_2 Evolution in the Fenton Reaction. *Chem. Eur. J.* 9(14), 3436-3444.

[129] Southworth,B.A. & Voelker,B.M. **(2003)**. Hydroxyl Radical Production via the Photo-Fenton Reaction in the Presence of Fulvic Acid. *Environ. Sci. Technol.* 37(6), 1130-1136.

[130] Christenson,E.M., Anderson,J.M. & Hiltner,A. **(2004)**. Oxidative mechanisms of poly(carbonate urethane) and poly(ether urethane) biodegradation: Invivo and invitro correlations. *J. Biomed. Mater. Res. A* 70(A)(2), 245-255.

[131] Arantes,V. & Ferreira Milagres,A.M. **(2005)**. Degradation of cellulosic and hemicellulosic substrates using a chelator-mediated Fenton reaction. *Journal of Chemical Technology & Biotechnology* 81(3), 413-419.

[132] Giroto,J.A., Teixeira,A.C S.C., Nascimento,C A.O. & Guardani,R. **(2008)**. Photo-Fenton removal of water-soluble polymers. *Chemical Engineering and Processing: Process Intensification* 47(12), 2361-2369.

[133] Berkowski,K.L., Potisek,S.L., Hickenboth,C.R. & Moore,J.S. **(2005)**. Ultrasound-Induced Site-Specific Cleavage of Azo-Functionalized Poly(ethylene glycol). *Macromolecules* 38(22), 8975-8978.

[134] The European Parliament & The Council of the European Union. **(2006)**. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. (EC) No 1907/2006

[135] European Commission,H.&.C.P.D.-G. **(2002)**. Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC. Sanco/3268/2001 rev.4 (final)

[136] European Commission,H.&.C.P.D.-G. **(2002)**. Draft Working Document Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 (final)

[137] Ahtiainen,J., Aalto,M. & Pessala,P. **(2003)**. Biodegradation of chemicals in a standardized test and in environmental conditions. *Chemosphere* 51(6), 529-537.

[138] Organisation for Economic Co-operation and Development (OECD). OECD Guidelines OECD 301 A-F, OECD 302 A-C, OECD 303, OECD 304, 306 & 309. Organisation for Economic Co-operation and Development (OECD), 2, Rue André Pascal, F-75775 Paris Cedex 16, France, Main Switchboard, tel.: +33 1.45.24.82.00 fax: +33 1.45.24.85.00.

[139] Guhl,W. & Steber,J. **(2006)**. The value of biodegradation screening test results for predicting the elimination of chemicals' organic carbon in waste water treatment plants. *Chemosphere* 63(1), 9-16.

[140] Reuschenbach,P., Pagga,U. & Strotmann,U. **(2003)**. A critical comparison of respirometric biodegradation tests based on OECD 301 and related test methods. *Wat. Res.* 37(7), 1571-1582.

[141] Strotmann,U.J., Schwarz,H. & Pagga,U. **(1995)**. The combined CO2/DOC test - a new method to determine the biodegradability of organic compounds. *Chemosphere* 30(3), 525-538.

[142] Li,J.L. & Chen,B.H. **(2009)**. Effect of nonionic surfactants on biodegradation of phenanthrene by a marine bacteria of Neptunomonas naphthovorans. *Journal of Hazardous Materials* 162(1), 66-73.

[143] Peschka,M., Frömel,T., Fichtner,N., Hierse,W., Kleineidam,M., Montenegro,E. & Knepper,T.P. **(2008)**. Mechanistic studies in biodegradation of the new synthesized fluorosurfactant 9-[4-(trifluoromethyl)phenoxy]nonane-1-sulfonate. *Journal of Chromatography A* 1187(1-2), 79-86.

[144] SCHER. **(2005)**. Opinion on Environmental Risk Assessment of non biodegradable Detergent Surfactants under Anaerobic Conditions.

[145] Schöberl,P. **(1994)**. Die Bedeutung fehlender anaerober biologischer Abbaubarkeit = Importance of missing anaerobic biodegradability. *Tenside, surfactants, detergents* 31(3), 157-162.

[146] The European Parliament & The Council of the European Union. **(2008)**. Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents. 648/2004/EC

[147] EMEA - European Medicines Agency. **(2006)**. Guideline on Environmental Impact Assessment for Medicinal Products for Human Use (CHMP). Doc.Ref.EMEA/CHMP/SWP/4447/00

[148] EMEA - European Medicines Agency. **(2005)**. Guideline on Environmental Impact Assessment for Veterinary Medicinal Products Phase II. Doc.Ref.CVMP/VICH/790/03-Final

[149] European Commission,H.&.C.P.D.-G. **(1991)**. Council Directive concerning the placing of plant protection products on the market. 91/414/EEC

[150] Vaishnav,D.D., Babeu,L. & Korthals,E.T. **(1989)**. Effect of microbial concentration on biodegradation rates of phenols. *Journal of Industrial Microbiology and Biotechnology* 4(4), 307-313.

[151] Lerchner,J., Wolf,A., Buchholz,F., Mertens,F., Neu,T.R., Harms,H. & Maskow,T. **(2008)**. Miniaturized calorimetry -- A new method for real-time biofilm activity analysis. *Journal of Microbiological Methods* 74(2-3), 74-81.

[152] T.R de Kievit **(2008)**. Quorum sensing in Pseudomonas aeruginosa biofilms. *Environmental Microbiology* in press.

[153] Flemming,H.C. **(1998)**. Relevance of biofilms for the biodeterioration of surfaces of polymeric materials*. *Polymer Degradation and Stability* 59(1-3), 309-315.

[154] Pagga,U. **(1998)**. Biodegradability and compostability of polymeric materials in the context of the European packaging regulation. *Polymer Degradation and Stability* 59(1-3), 371-376.

[155] International Organization for Standardization. ISO Methods ISO 7827(1994), ISO 9439(1999), ISO 9408(1999), ISO 9888(1999), ISO 10707(1994), ISO 10708(1997), ISO 14592(2002), ISO 14593(1999), ISO 15462(1997), ISO 16221(2001), ISO 14855-1 (2005), ISO 14851(1999), ISO 14852(1999), ISO 14855(1999), ISO 11734(1995), ISO 10634(1995), ISO 11733(2004), Iso 9887(1992). ISO, 1, rue de Varembé, Case postale 56, CH-1211 Geneva 20, Switzerland, tel. +41 22 749 01 11; fax +41 22 733 34 30.

[156] ASTM International. ASTM Test Methods for testing of Chemicals: D5209-92; D5210-92; D5247-92; D5271-02; D6003-96; D6340-98; D6691-01; D6692-01; D7081-05. ASTM International, West Conshohocken, PA, United States.

[157] De Baere,L., De Wilde,B., & Tillinger,R. **(1994)**. Biodegradable Plastics and Polymers. In Studies in Polymer Scienc (Doi,Y. & Fukuda,K., eds), pp. 323-330. Elsevier, Amsterdam.

[158] Pagga,U., Beimborn,D.B., Boelens,J. & De Wilde,B. **(1995)**. Determination of the aerobic biodegradability of polymeric material in a laboratory controlled composting test. *Chemosphere* 31(11-12), 4475-4487.

[159] Tosin,M., gli-Innocenti,F. & Bastioli,C. **(1996)**. Effect of the composting substrate on biodegradation of solid materials under controlled composting conditions. *Journal of Polymers and the Environment* 4(1), 55-63.

[160] Day,M., Shaw,K., Cooney,D., Watts,J. & Harrigan,B. **(1997)**. Degradable polymers: The role of the degradation environment. *Journal of Polymers and the Environment* 5(3), 137-151.

[161] Yabannavar,A.V. & Bartha,R. **(1994)**. Methods for Assessment of Biodegradability of Plastic Films in Soil. *Appl. Environ. Microbiol.* 60(10), 3608-3614.

[162] Mohee,R. & Unmar,G. **(2007)**. Determining biodegradability of plastic materials under controlled and natural composting environments. *Waste Management* 27(11), 1486-1493.

[163] Orhan,Y. & Buyukgungor,H. **(2000)**. Enhancement of biodegradability of disposable polyethylene in controlled biological soil. *International Biodeterioration & Biodegradation* 45(1-2), 49-55.

[164] Dibble,J.T. & Bartha,R. **(1979)**. Effect of environmental parameters on the biodegradation of oil sludge. *Appl. Environ. Microbiol.* 37(4), 729-739.

[165] Mergaert,J., Webb,A., Anderson,C., Wouters,A. & Swings,J. **(1993)**. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. *Appl. Environ. Microbiol.* 59(10), 3233-3238.

[166] Sang,I. **(2002)**. Fungal contribution to in situ biodegradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) film in soil. *Applied Microbiology and Biotechnology* 58(2), 241-247.

[167] Yang,H.S., Yoon,J.S. & Kim,M.N. **(2004)**. Effects of storage of a mature compost on its potential for biodegradation of plastics. *Polymer Degradation and Stability* 84(3), 411-417.

[168] Imam,S.H., Gordon,S.H., Shogren,R.L., Tosteson,T.R., Govind,N.S. & Greene,R.V. **(1999)**. Degradation of Starch-Poly(beta -Hydroxybutyrate-Co-beta -Hydroxyvalerate) Bioplastic in Tropical Coastal Waters. *Appl. Environ. Microbiol.* 65(2), 431-437.

[169] Kasuya,K., Mitomo,H., Nakahara,M., Akiba,A., Kudo,T. & Doi,Y. **(2000)**. Identification of a Marine Benthic P(3HB)-Degrading Bacterium Isolate and Characterization of Its P(3HB) Depolymerase. *Biomacromolecules* 1(2), 194-201.

[170] Greene,J. **(2007)**. Biodegradation of Compostable Plastics in Green Yard-Waste Compost Environment. *Journal of Polymers and the Environment* 15(4), 269-273.

[171] Kawai,F., Watanabe,M., Shibata,M., Yokoyama,S., Sudate,Y. & Hayashi,S. **(2004)**. Comparative study on biodegradability of polyethylene wax by bacteria and fungi. *Polymer Degradation and Stability* 86(1), 105-114.

[172] Pandey,J.K. & Singh,R.P. **(2001)**. UV-Irradiated Biodegradability of Ethylene-Propylene Copolymers, LDPE, and I-PP in Composting and Culture Environments. *Biomacromolecules* 2(3), 880-885.

[173] Pometto,A.L., III, Lee,B.T. & Johnson,K.E. **(1992)**. Production of an extracellular polyethylene-degrading enzyme(s) by Streptomyces species. *Appl. Environ. Microbiol.* 58(2), 731-733.

[174] ASTM D6003-96. **(1996)**. ASTM D6003-96; Standard test method for determining weight loss from plastic materials exposed to simulated municipal solid-waste (MSW) aerobic compost environment. ASTM International, West Conshohocken, PA, United States.

[175] Agamuthu,P. & Faizura,P.N. **(2005)**. Biodegradability of degradable plastic waste. *Waste Management Research* 23(2), 95-100.

[176] Watanabe,M. & Kawai,F. **(2003)**. Numerical simulation for enzymatic degradation of poly(vinyl alcohol). *Polymer Degradation and Stability* 81(3), 393-399.

[177] Chiellini,E., Corti,A., D'Antone,S. & Baciu,R. **(2006)**. Oxo-biodegradable carbon backbone polymers - Oxidative degradation of polyethylene under accelerated test conditions. *Polymer Degradation and Stability* 91(11), 2739-2747.

[178] Reddy,M.M., Deighton,M., Gupta,R.K., Bhattacharya,S.N. & Parthasarathy,R. **(2008)**. Biodegradation of oxo-biodegradable polyethylene. *Journal of Applied Polymer Science* 111(3), 1426-1432.

[179] Ojeda,T.F.M., Dalmolin,E., Forte,M.M.C., Jacques,R.J.S., Bento,F.M. & Camargo,F.A.O. **(2009)**. Abiotic and biotic degradation of oxo-biodegradable polyethylenes. *Polymer Degradation and Stability* In Press, Accepted Manuscript.

[180] Gouda,M.K., Kleeberg,I., Vanden Heuvel,J., Muller,R.J. & Deckwer,W.D. **(2002)**. Production of a Polyester Degrading Extracellular Hydrolase from Thermomonospora fusca. *Biotechnol. Prog.* 18(5), 927-934.

[181] Kleeberg,I., Welzel,K., VandenHeuvel,J., Mueller,R.J. & Deckwer,W.D. **(2005)**. Characterization of a New Extracellular Hydrolase from Thermobifida fusca Degrading Aliphaticâ˜Aromatic Copolyesters. *Biomacromolecules* 6(1), 262-270.

[182] Kleeberg,I., Hetz,C., Kroppenstedt,R.M., Muller,R J. & Deckwer,W.D. **(1998)**. Biodegradation of Aliphatic-Aromatic Copolyesters by Thermomonospora fusca and Other Thermophilic Compost Isolates. *Appl. Environ. Microbiol.* 64(5), 1731-1735.

[183] Mueller,R.J. **(2006)**. Biological degradation of synthetic polyesters--Enzymes as potential catalysts for polyester recycling. *Process Biochemistry* 41(10), 2124-2128.

[184] Kaplan,D.L., Hartenstein,R. & Sutter,J. **(1979)**. Biodegradation of polystyrene, poly(metnyl methacrylate), and phenol formaldehyde. *Appl. Environ. Microbiol.* 38(3), 551-553.

[185] Gusse,A.C., Miller,P.D. & Volk,T J. **(2006)**. White-Rot Fungi Demonstrate First Biodegradation of Phenolic Resin. *Environmental Science & Technology* 40(13), 4196-4199.

[186] Shimao,M. **(2001)**. Biodegradation of plastics. *Current Opinion in Biotechnology* 12(3), 242-247.

[187] Schink,B. & Stieb,M. **(1983)**. Fermentative degradation of polyethylene glycol by a strictly anaerobic, gram-negative, nonsporeforming bacterium, Pelobacter venetianus sp. nov. *Appl. Environ. Microbiol.* 45(6), 1905-1913.

[188] Dwyer,D.F. & Tiedje,J.M. **(1983)**. Degradation of ethylene glycol and polyethylene glycols by methanogenic consortia. *Appl. Environ. Microbiol.* 46(1), 185-190.

[189] Frings,J., Schramm,E. & Schink,B. **(1992)**. Enzymes Involved in Anaerobic Polyethylene Glycol Degradation by Pelobacter venetianus and Bacteroides Strain PG1. *Appl. Environ. Microbiol.* 58(7), 2164-2167.

[190] Dwyer,D.F. & Tiedje,J.M. **(1986)**. Metabolism of polyethylene glycol by two anaerobic bacteria, Desulfovibrio desulfuricans and a Bacteroides sp. *Appl. Environ. Microbiol.* 52(4), 852-856.

[191] Schmid,A., Benz,R. & Schink,B. **(1991)**. Identification of two porins in Pelobacter venetianus fermenting high-molecular-mass polyethylene glycols. *J. Bacteriol.* 173(16), 4909-4913.

[192] Kawai,F., Kimura,T., Fukaya,M., Tani,Y., Ogata,K., Ueno,T. & Fukami,H. **(1978)**. Bacterial oxidation of polyethylene glycol. *Appl. Environ. Microbiol.* 35(4), 679-684.

[193] Obradors,N. & Aguilar,J. **(1991)**. Efficient biodegradation of high-molecular-weight polyethylene glycols by pure cultures of Pseudomonas stutzeri. *Appl. Environ. Microbiol.* 57(8), 2383-2388.

[194] Pan,L. & Gu,J.D. **(2007)**. Characterization of Aerobic Bacteria Involved in Degrading Polyethylene Glycol (PEG)-3400 Obtained by Plating and Enrichment Culture Techniques. *Journal of Polymers and the Environment* 15(1), 57-65.

[195] Gu,J.D. & Pan,L. **(2006)**. Comparing the Growth Characteristics of Three Bacteria Involved in Degrading Rubbers. *Journal of Polymers and the Environment* 14(3), 273-279.

[196] Zgola-Grzeskowiak,A., Grzeskowiak,T., Zembrzuska,J. & Lukaszewski,Z. **(2006)**. Comparison of biodegradation of poly(ethylene glycol)s and poly(propylene glycol)s. *Chemosphere* 64(5), 803-809.

[197] Schramm,E. & Schink,B. **(1991)**. Ether-cleaving enzyme and diol dehydratase involved in anaerobic polyethylene glycol degradation by a new Acetobacterium sp. *Biodegradation* 2(2), 71-79.

[198] Corti,A., D'Antone,S., Solaro,R. & Chiellini,E. **(1998)**. Degradation of Poly(Ethylene Glycol)-Based Nonionic Surfactants by Different Bacterial Isolates from River Water. *Journal of Polymers and the Environment* 6(3), 121-131.

[199] Suzuki,J., Hukushima,K. & Suzuki,S. **(1978)**. Effect of ozone treatment upon biodegradability of water-soluble polymers. *Environ. Sci. Technol.* 12(10), 1180-1183.

[200] Otal,E., Mantzavinos,D., Delgado,M.V., Hellenbrand,R., Lebrato,J., Metcalfe,I.S. & Livingston,A.G. **(2007)**. Integrated wet air oxidation and biological treatment of polyethylene glycol-containing wastewaters. *Journal of chemical technology and biotechnology* 70(2), 147-156.

[201] Nakajima-Kambe,T., Shigeno-Akutsu,Y., Nomura,N., Onuma,F. & Nakahara,T. **(1999)**. Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. *Applied Microbiology and Biotechnology* 51(2), 134-140.

[202] Cosgrove,L., McGeechan,P.L., Robson,G.D. & Handley,P.S. **(2007)**. Fungal Communities Associated with Degradation of Polyester Polyurethane in Soil. *Appl. Environ. Microbiol.* 73(18), 5817-5824.

[203] Friedrich,J., Zalar,P., Mohorcic,M., Klun,U. & Krzan,A. **(2007)**. Ability of fungi to degrade synthetic polymer nylon-6. *Chemosphere* 67(10), 2089-2095.

[204] Gu J.D., Mitton D.B., Ford T.E. & Mitchell,R. **(1998)**. Microbial degradation of polymeric coatings measured by electrochemical impedance spectroscopy. *Biodegradation* 9(1), 39-45.

[205] Bode,H.B., Zeeck,A., Pluckhahn,K. & Jendrossek,D. **(2000)**. Physiological and Chemical Investigations into Microbial Degradation of Synthetic Poly(cis-1,4-isoprene). *Appl. Environ. Microbiol.* 66(9), 3680-3685.

[206] Sutherland,G.R.J., Haselbach,J. & Aust,S.D. **(1997)**. Biodegradation of cross-linked acrylic polymers by a white-rot fungus. *Environ. Sci. Pollut. R.* 16(1), 1-83.

[207] El-Mohdy,H.L A. & Ghanem,S. **(2009)**. Biodegradability, antimicrobial activity and properties of PVA/PVP hydrogels prepared by gamma-irradiation
1. *Journal of Polymer Research* 16(1), 1-10.

[208] Schwarzbauer,J. **(2008)**. Entwicklung einer analytischen Methode zur Erfassung von poly(vinyl pyrrolidon) PVP in Umweltproben auf Basis pyrolytischer Verfahren. SETAC and GDCH. SETAC GLB & German Society of Chemists, Environmental Chemistry and Ecotoxicology Group - Annual Conference. *Neue Problemstoffe in der Umwelt - Erfassung, Wirkungen, Lösungen* , 77 24-9-2008.

[209] Anderson,A.J. & Dawes,E A. **(1990)**. Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. *Microbiol. Mol. Biol. Rev.* 54(4), 450-472.

[210] Hankermeyer,C.R. & Tjeerdema,R.S. **(1999)**. Polyhydroxybutyrate: plastic made and degraded by microorganisms. *Rev. Environ Contam Toxicol.* 159 1-24.

[211] Schroeter,J. **(1998)**. Creating the framework for a widespread use of biodegradable polymers (standardization, labelling, legislation, biowaste management). *Polymer Degradation and Stability* 59(1-3), 377-381.

[212] Bastioli,C. **(2005)**. *Handbook of biodegradable polymers*, 1 edn. Smithers Rapra Technology..

[213] Tokiwa,Y. & Calabia,B. **(2007)**. Biodegradability and Biodegradation of Polyesters. *Journal of Polymers and the Environment* 15(4), 259-267.

[214] Reddy,C.S.K., Ghai,R., Rashmi & Kalia,V.C. **(2003)**. Polyhydroxyalkanoates: an overview. *Bioresource Technology* 87(2), 137-146.

[215] Tsz-Chun,M., Chan,P., Lawford,H., Chua,H., Lo,W. & Yu,P. **(2005)**. Microbial synthesis and characterization of physiochemical properties of polyhydroxyalkanoates (PHAs) produced by bacteria isolated from activated sludge obtained from the municipal wastewater works in Hong Kong. *Applied Biochemistry and Biotechnology* 122(1), 731-739.

[216] Law,K.H., Chan,P.L., Lau,W.S., Cheng,Y.C., Leung,Y.C., Lo,W.H., Lawford,H. & Yu,H.F. **(2004)**. Construction of recombinant Escherichia coli strains for production of poly-(3-hydroxybutyrate-co-3-hydroxyvalerate). *Appl. Biochem. Biotechnol.* 114(1-3), 361-372.

[217] van der Walle,G., de Koning,G., Weusthuis,R., & Eggink,G. **(2001)**. Properties, Modifications and Applications of Biopolyesters. pp. 263-291.

[218] Luzier,W.D. **(1992)**. Materials Derived from Biomass/Biodegradable Materials. *Proceedings of the National Academy of Sciences* 89(3), 839-842.

[219] Bucci,D Z., Tavares,L.B.B. & Sell,I. **(2007)**. Biodegradation and physical evaluation of PHB packaging. *Polymer Testing* 26(7), 908-915.

[220] Wang,Y.W., Mo,W., Yao,H., Wu,Q., Chen,J. & Chen,G.Q. **(2004)**. Biodegradation studies of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). *Polymer Degradation and Stability* 85(2), 815-821.

[221] Doi,Y., Kitamura,S. & Abe,H. **(1995)**. Microbial Synthesis and Characterization of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). *Macromolecules* 28(14), 4822-4828.

[222] Molitoris,H.P., Moss,S.T., de Koning,G J.M. & Jendrossek,D. **(1996)**. Scanning electron microscopy of polyhydroxyalkanoate degradation by bacteria. *Applied Microbiology and Biotechnology* 46(5), 570-579.

[223] Manna,A. & Paul,A.K. **(2000)**. Degradation of microbial polyester poly(3-hydroxybutyrate) in environmental samples and in culture. *Biodegradation* 11(5), 323-329.

[224] Kim,M.N., Lee,A.R., Yoon,J.S. & Chin,I.J. **(2000)**. Biodegradation of poly(3-hydroxybutyrate), Sky-Green(R) and Mater-Bi(R) by fungi isolated from soils. *European Polymer Journal* 36(8), 1677-1685.

[225] Reischwitz,A., Stoppok,E. & Buchholz,K. **(1998)**. Anaerobic degradation of poly-3-hydroxybutyrate and poly-3-hydroxybutyrate-co-3-hydroxyvalerate. *Biodegradation* 8(5), 313-319.

[226] Abou-Zeid,D.M., Muller,R.J. & Deckwer,W.D. **(2001)**. Degradation of natural and synthetic polyesters under anaerobic conditions. *Journal of Biotechnology* 86(2), 113-126.

[227] Abou-Zeid,D.M., Muller,R.J. & Deckwer,W.D. **(2004)**. Biodegradation of Aliphatic Homopolyesters and Aliphatic-Aromatic Copolyesters by Anaerobic Microorganisms. *Biomacromolecules* 5(5), 1687-1697.

[228] Dawes,E.A. **(1988)**. Polyhydroxybutyrate: An intriguing biopolymer. *Bioscience Reports* 8(6), 537-547.

[229] Matavulj,M. & Molitoris,H.P. **(1992)**. Fungal degradation of polyhydroxyalkanoates and a semiquantitative assay for screening their degradation by terrestrial fungi. *FEMS Microbiol.* 103(2-4), 323-331.

[230] Briese,B.H., Jendrossek,D. & Schlegel,H.G. **(1994)**. Degradation of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by aerobic sewage sludge. *FEMS Microbiol.* 117(1), 107-111.

[231] Mergaert,J., Anderson,C., Wouters,A., Swings,J. & Kersters,K. **(1992)**. Biodegradation of polyhydroxyalkanoates. *FEMS Microbiol.* 103(2-4), 317-321.

[232] Yamada,K., Mukai,K. & Doi,Y. **(1993)**. Enzymatic degradation of poly(hydroxyalkanoates) by Pseudomonas pickettii. *International Journal of Biological Macromolecules* 15(4), 215-220.

[233] Ramsay,B.A., Langlade,V., Carreau,P.J. & Ramsay,J.A. **(1993)**. Biodegradability and mechanical properties of poly-(beta-hydroxybutyrate-co-beta-hydroxyvalerate)-starch blends. *Appl. Environ. Microbiol.* 59(4), 1242-1246.

[234] Choi,G.G., Kim,H.W. & Rhee,Y.H. **(2004)**. Enzymatic and non-enzymatic degradation of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) copolyesters produced by Alcaligenes sp. MT-16. *J. Microbiol.* 42(4), 346-352.

[235] Numata,K., Kikkawa,Y., Tsuge,T., Iwata,T., Doi,Y. & Abe,H. **(2005)**. Enzymatic Degradation Processes of Poly[(R)-3-hydroxybutyric acid] and Poly[(R)-3-hydroxybutyric acid-co-(R)-3-hydroxyvaleric acid] Single Crystals Revealed by Atomic Force Microscopy: Effects of Molecular Weight and Second-Monomer Composition on Erosion Rates. *Biomacromolecules* 6(4), 2008-2016.

[236] Doi,Y., Kanesawa,Y., Kunioka,M. & Saito,T. **(1990)**. Biodegradation of microbial copolyesters: poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate). *Macromolecules* 23(1), 26-31.

[237] Brandl,H., Gross,R.A., Lenz,R.W., & Fuller,R.C. **(1990)**. Plastics from bacteria and for bacteria: Poly(ß-hydroxyalkanoates) as natural, biocompatible, and biodegradable polyesters. In Microbial Bioproducts pp. 77-93. Springer, Berlin / Heidelberg.

[238] El-Hadi,A., Schnabel,R., Straube,E., Muller,G. & Henning,S. **(2002)**. Correlation between degree of crystallinity, morphology, glass temperature, mechanical properties and biodegradation of poly (3-hydroxyalkanoate) PHAs and their blends. *Polymer Testing* 21(6), 665-674.

[239] Kim,D.Y., Lutke-Eversloh,T., Elbanna,K., Thakor,N. & Steinbuchel,A. **(2005)**. Poly(3-mercaptopropionate): A Nonbiodegradable Biopolymer? *Biomacromolecules* 6(2), 897-901.

[240] Yamamoto,M., Witt,U., Skupin,G., Beimborn,D., & Mueller,R.-J. **(2003)**. Biodegradable aliphatic-aromatic polyesters: "Ecoflex®". In Biopolymers (Steinbuechel,A., ed), pp. 299-305. Wiley VCH, Weinheim.

[241] Witt,U., Einig,T., Yamamoto,M., Kleeberg,I., Deckwer,W.-D. & Muller,R.-J. **(2001)**. Biodegradation of aliphatic-aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. *Chemosphere* 44(2), 289-299.

[242] Hakkarainen,M. & Albertsson,A.C. **(2008)**. Degradation Products of Aliphatic and Aliphaticâ€"Aromatic Polyesters. In Chromatography for Sustainable Polymeric Materials pp. 85-116.

[243] Nagarajan,V., Singh,M., Kane,H., Khalili,M. & Bramucci,M. **(2006)**. Degradation of a Terephthalate-containing Polyester by a Thermophilic Microbial Consortium. *Journal of Polymers and the Environment* 14(3), 281-287.

[244] Tan,F.T., Cooper,D.G., Maric,M. & Nicell,J.A. **(2008)**. Biodegradation of a synthetic co-polyester by aerobic mesophilic microorganisms. *Polymer Degradation and Stability* In Press, Accepted Manuscript.

[245] Pepic,D., Zagar,E., Zigon,M., Krzan,A., Kunaver,M. & Djonlagic,J. Synthesis and characterization of biodegradable aliphatic copolyesters with poly(ethylene oxide) soft segments. *European Polymer Journal* In Press, Corrected Proof.

[246] Soni,R.K., Soam,S. & Dutt,K. **(2008)**. Studies on biodegradability of copolymers of lactic acid, terephthalic acid and ethylene glycol. *Polymer Degradation and Stability* In Press, Accepted Manuscript.

[247] Yang,H S., Yoon,J.S. & Kim,M.N. **(2005)**. Dependence of biodegradability of plastics in compost on the shape of specimens. *Polymer Degradation and Stability* 87(1), 131-135.

[248] Massardier-Nageotte,V., Pestre,C., Cruard-Pradet,T. & Bayard,R. **(2006)**. Aerobic and anaerobic biodegradability of polymer films and physico-chemical characterization. *Polymer Degradation and Stability* 91(3), 620-627.

[249] Kale,G., Auras,R., Singh,S.P. & Narayan,R. **(2007)**. Biodegradability of polylactide bottles in real and simulated composting conditions. *Polymer Testing* 26(8), 1049-1061.

[250] Li,S.M., Rashkov,I., Espartero,J.L., Manolova,N. & Vert,M. **(1996)**. Synthesis, Characterization, and Hydrolytic Degradation of PLA/PEO/PLA Triblock Copolymers with Long Poly(l-lactic acid) Blocks. *Macromolecules* 29(1), 57-62.

[251] Tsuji,H., Echizen,Y. & Nishimura,Y. **(2006)**. Enzymatic Degradation of Poly(l -Lactic Acid): Effects of UV Irradiation. *Journal of Polymers and the Environment* 14(3), 239-248.

[252] Karjomaa,S., Suortti,T., Lempiainen,R., Selin,J.-F. & Itavaara,M. **(1998)**. Microbial degradation of poly-(-lactic acid) oligomers. *Polymer Degradation and Stability* 59(1-3), 333-336.

[253] Lostocco,M.R., Murphy,C.A., Cameron,J.A. & Huang,S.J. **(1998)**. The effects of primary structure on the degradation of poly([var e]-caprolactone)/poly(-lactide) block copolymers. *Polymer Degradation and Stability* 59(1-3), 303-307.

[254] Tuominen,J., Kylma,J., Kapanen,A., Venelampi,O., Itavaara,M. & Seppala,J. **(2002)**. Biodegradation of Lactic Acid Based Polymers under Controlled Composting Conditions and Evaluation of the Ecotoxicological Impact. *Biomacromolecules* 3(3), 445-455.

[255] Tilstra,L. & Johnsonbaugh,D. **(1993)**. A test method to determine rapidly if polymers are biodegradable. *Journal of Polymers and the Environment* 1(4), 247-255.

[256] Tilstra,L. & Johnsonbaugh,D. **(1993)**. The biodegradation of blends of polycaprolactone and polyethylene exposed to a defined consortium of fungi. *Journal of Polymers and the Environment* 1(4), 257-267.

[257] Haschke,H., Tomka,I. & Keilbach,A. **(1998)**. Systematische Untersuchungen zur biologischen Abbaubarkeit von Verpackungsmaterial, 1. Mitt. Zur tatsaechlichen biologischen Abbaubarkeit von sogenannten bioabbaubaren Kunststoffolien. *Monatshefte für Chemie / Chemical Monthly* 129(3), 253-279.

[258] Haschke,H., Tomka,I. & Keilbach,A. **(1998)**. Systematische Untersuchungen zur biologischen Abbaubarkeit von Verpackungsmaterial, 2. Mitt. Zur biologischen Abbaubarkeit von auf Polyvinylalkohol basierenden Verpackungsfolien. *Monatshefte fÃ¼r Chemie / Chemical Monthly* 129(4), 365-386.

[259] Haschke,H., Tomka,I. & Keilbach,A. **(1998)**. Systematische Untersuchungen zur biologischen Abbaubarkeit von Verpackungsmaterial, 3. Mitt. Neue Polyvinylalkohol-StÃ¤rke-Acetal-Folien. *Monatshefte fÃ¼r Chemie / Chemical Monthly* 129(5), 487-507.

[260] Li,S., Garreau,H., Pauvert,B., McGrath,J., Toniolo,A. & Vert,M. **(2002)**. Enzymatic Degradation of Block Copolymers Prepared from Caprolactone and Poly(ethylene glycol). *Biomacromolecules* 3(3), 525-530.

[261] O'Sullivan,C.A., Burrell,P.C., Clarke,W.P. & Blackall,L.L. **(2008)**. Structure of a cellulose degrading bacterial community during anaerobic digestion. *Biotechnol. Bioeng.* 92(7), 871-878.

[262] Pérez,J. & Muños-Dorado,J. **(2002)**. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *Int. Microbiol.* 5(2), 53-63.

[263] Benner,R., Maccubbin,A.E. & Hodson,R.E. **(1984)**. Preparation, Characterization, and Microbial Degradation of Specifically Radiolabeled [14C]Lignocelluloses from Marine and Freshwater Macrophytes. *Appl. Environ. Microbiol.* 47(2), 381-389.

[264] Benner,R. & Hodson,R.E. **(1985)**. Thermophilic Anaerobic Biodegradation of [14C]Lignin, [14C]Cellulose, and [14C]Lignocellulose Preparations. *Appl. Environ. Microbiol.* 50(4), 971-976.

[265] Pelaez,F., Martinez,M.J. & Martinez,A.T. **(1995)**. Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation. *Mycological Research* 99(Part 1), 37-42.

[266] van der Zee,M., Stoutjesdijk,J.H., Van der Heijden,P.A.A.W. & De Wit,D. **(1995)**. Structure-biodegradation relationships of polymeric materials. 1. Effect of degree of oxidation on biodegradability of carbohydrate polymers. *Journal of Polymers and the Environment* 3(4), 235-242.

[267] Gartiser,S., Wallrabenstein,M. & Stiene,G. **(1998)**. Assessment of Several Test Methods for the Determination of the Anaerobic Biodegradability of Polymers. *Journal of Polymers and the Environment* 6(3), 159-173.

[268] Roldan-Carrillo,T., Rodriguez-Vazquez,R., az-Cervantes,D., Vazquez-Torres,H., Manzur-Guzman,A. & Torres-Dominguez,A. **(2003)**. Starch-based plastic polymer degradation by the white rot fungus Phanerochaete chrysosporium grown on sugarcane bagasse pith: enzyme production. *Bioresource Technology* 86(1), 1-5.

[269] Oppermann,F.B., Pickartz,S. & Steinbuchel,A. **(1998)**. Biodegradation of polyamides. *Polymer Degradation and Stability* 59(1-3), 337-344.

[270] Tabata,K., Kasuya,K.i., Abe,H., Masuda,K. & Doi,Y. **(1999)**. Poly(Aspartic Acid) Degradation by a Sphingomonas sp. Isolated from Freshwater. *Appl. Environ. Microbiol.* 65(9), 4268-4270.

[271] Hoffmann,J., Reznickova,I., Kozakova,J., Ruzicka,J., Alexy,P., Bakos,D. & Precnerova,L. **(2003)**. Assessing biodegradability of plastics based on poly(vinyl alcohol) and protein wastes. *Polymer Degradation and Stability* 79(3), 511-519.

[272] Reeve,R.N. **(2002)**. *Introduction to Environmental Analysis*, 1 edn. John Wiley & Sons.

[273] Hesselmann,R.P.X., Fleischmann,T., Hany,R. & Zehnder,A.J.B. **(1999)**. Determination of polyhydroxyalkanoates in activated sludge by ion chromatographic and enzymatic methods. *Journal of Microbiological Methods* 35(2), 111-119.

[274] Weller,M.G. **(2000)**. Immunchemische Methoden in der Umweltanalytik. Technische Universität München.

[275] Köster,W., Egli,T., Ashbolt,N., & et al. **(2003)**. Analytical methods for microbiological water quality testing. In Assessing microbiological safety of drinking water: improving approaches and methods (Dufour,A., Snozzu,M., Köster,W., & et al., eds), pp. 237-242. IWA Publishing.

[276] Tomer,K.B. **(2001)**. Separations Combined with Mass Spectrometry. *Chem. Rev.* 101(2), 297-328.

[277] Anderson,D.G. **(2001)**. Coatings. *Anal. Chem.* 73(12), 2701-2704.

[278] Anderson,D.G. **(1991)**. Coatings. *Anal. Chem.* 63(12), 87-99.

[279] Peacock,P.M. & McEwen,C.N. **(2006)**. Mass Spectrometry of Synthetic Polymers. *Anal. Chem.* 78(12), 3957-3964.

[280] Peacock,P.M. & McEwen,C.N. **(2004)**. Mass Spectrometry of Synthetic Polymers. *Anal. Chem.* 76(12), 3417-3428.

[281] Przybilla,L., Brand,J.-D., Yoshimura,K., Räder,H.J. & Müllen,K. **(2000)**. MALDI-TOF Mass Spectrometry of Insoluble Giant Polycyclic Aromatic Hydrocarbons by a New Method of Sample Preparation. *Analytical Chemistry* 72(19), 4591-4597.

[282] Przybilla,L., Francke,V., Räder,H.J. & Mülen,K. **(2001)**. Block Length Determination of a Poly(ethylene oxide)-*b*-poly(*p*-phenylene ethynylene) Diblock Copolymer by Means of MALDI-TOF Mass Spectrometry Combined with Fragment-Ion Analysis. *Macromolecules* 34(13), 4401-4405.

[283] Hoteling,A J., Erb,W.J., Tyson,R.J. & Owens,K.G. **(2004)**. Exploring the Importance of the Relative Solubility of Matrix and Analyte in MALDI Sample Preparation Using HPLC. *Anal. Chem.* 76(17), 5157-5164.

[284] Vreeland,W.N., Desruisseaux,C., Karger,A.E., Drouin,G., Slater,G.W. & Barron,A.E. **(2001)**. Molar mass profiling of synthetic polymers by free-solution capillary electrophoresis of DNA-polymer conjugates. *Anal Chem* 73(8), 1795-1803.

[285] Hoshino,A., Tsuji,M., Momochi,M., Mizutani,A., Sawada,H., Kohnami,S., Nakagomi,H., Ito,M., Saida,H., Ohnishi,M., Hirata,M., Kunioka,M., Funabash,M. & Uematsu,S. **(2007)**. Study of the Determination of the Ultimate Aerobic Biodegradability of Plastic Materials Under Controlled Composting Conditions. *Journal of Polymers and the Environment* 15(4), 275-280.

[286] Solaro,R., Corti,A. & Chiellini,E. **(1998)**. A new respirometric test simulating soil burial conditions for the evaluation of polymer biodegradation. *Journal of Environmental Polymer Degradation* 6 203-208.

[287] ASTM D6340-98. **(1998)**. ASTM D6340-98; Standard test method for determining aerobic biodegradation of radiolabeled plastic materials in an aqueous or compost environment. ASTM International, West Conshohocken, PA, United States.

[288] ASTM D6692-01. **(2001)**. ASTM D6692-01; Standard test method for determining the biodegradability of radiolabeled polymeric plastic materials in seawater. ASTM International, West Conshohocken, PA, United States.

[289] Bernhard,M., Eubeler,J., Zok,S. & Knepper,T.P. **(2008)**. Aerobic biodegradation of polyethylene glycols of different molecular weights in wastewater and seawater. *Wat. Res.* 42(19), 4791-4801.

[290] Lee,S., Winnik,M.A., Whittal,R.M. & Li,L. **(1996)**. Synthesis of Symmetric Fluorescently Labeled Poly(ethylene glycols) Using Phosphoramidites of Pyrenebutanol and Their Characterization by MALDI Mass Spectrometry. *Macromolecules* 29(9), 3060-3072.

[291] Se,K., Sakakibara,T. & Ogawa,E. **(2002)**. Molecular weight determination of star polymers and star block copolymers using GPC equipped with low-angle laser light-scattering. *Polymer* 43(20), 5447-5453.

[292] Drimal,P., Hoffmann,J. & Druzbik,M. **(2007)**. Evaluating the aerobic biodegradability of plastics in soil environments through GC and IR analysis of gaseous phase. *Polymer Testing* 26(6), 729-741.

[293] Drimal,P., Hrncirik,J. & Hoffmann,J. **(2006)**. Assessing Aerobic Biodegradability of Plastics in Aqueous Environment by GC-Analyzing Composition of Equilibrium Gaseous Phase. *Journal of Polymers and the Environment* 14(3), 309-316.

[294] Gallet,G., Erlandsson,B., Albertsson,A.C. & Karlsson,S. **(2002)**. Thermal oxidation of poly(ethylene oxide-propylene oxide-ethylene oxide) triblock copolymer: focus on low molecular weight degradation products. *Polymer Degradation and Stability* 77(1), 55-66.

[295] Zhu,W.W. & Allaway,J.R. **(2004)**. Gel permeation chromatography followed by pyrolysis-gas chromatography-mass spectrometry via a solvent-evaporative interface. *Journal of Chromatography A* 1055(1-2), 191-196.

[296] Liu,X.M., Maziarz,E.P., Heiler,D.J. & Grobe,G.L. **(2003)**. Comparative studies of poly(dimethyl siloxanes) using automated GPC-MALDI-TOF MS and on-line GPC-ESI-TOF MS. *Journal of the American Society for Mass Spectrometry* 14(3), 195-202.

[297] Terrier,P., Buchmann,W., Cheguillaume,G., Desmazieres,B. & Tortajada,J. **(2005)**. Analysis of Poly(oxyethylene) and Poly(oxypropylene) Triblock Copolymers by MALDI-TOF Mass Spectrometry. *Anal. Chem.* 77(10), 3292-3300.

[298] Wetzel,S.J., Guttman,C.M., Flynn,K.M. & Filliben,J.J. **(2006)**. Significant Parameters in the Optimization of MALDI-TOF-MS for Synthetic Polymers+. *Journal of the American Society for Mass Spectrometry* 17(2), 246-252.

[299] Iwata,T. & Doi,Y. **(1998)**. Morphology and Enzymatic Degradation of Poly(L-lactic acid) Single Crystals. *Macromolecules* 31(8), 2461-2467.

[300] Heldal,M., Norland,S., Fagerbakke,K.M., Thingstad,F. & Bratbak,G. **(1996)**. The elemental composition of bacteria: A signature of growth conditions? *Marine Pollution Bulletin* 33(1-6), 3-9.

[301] Luyt,A.S. & Bruell,R. **(2004)**. Investigation of polyethylene-wax blends by CRYSTAF and SEC-FTIR. *Polymer Bulletin* 52(2), 177-183.

[302] Hsu,H J., Kuo,T.L., Wu,S.H., Oung,J.N. & Shiea,J. **(2005)**. Characterization of Synthetic Polymers by Electrospray-Assisted Pyrolysis Ionization-Mass Spectrometry. *Anal. Chem.* 77(23), 7744-7749.

[303] Fabbri,D., Tartari,D. & Trombini,C. **(2000)**. Analysis of poly(vinyl chloride) and other polymers in sediments and suspended matter of a coastal lagoon by pyrolysis-gas chromatography-mass spectrometry. *Anal. Chim. Acta.* 413 3-11.

[304] Fabbri,D. **(2001)**. Use of pyrolysis-gas chromatography/mass spectrometry to study environmental pollution caused by synthetic polymers: a case study: the Ravenna Lagoon. *Journal of Analytical and Applied Pyrolysis* 58-59 361-370.

[305] Hanton,S.D. & Liu,X.M. **(2000)**. GPC Separation of Polymer Samples for MALDI Analysis. *Anal. Chem.* 72(19), 4550-4554.

[306] Saucy,D.A., Ude,S., Lenggoro,I.W. & FernandezdelaMora,J. **(2004)**. Mass Analysis of Water-Soluble Polymers by Mobility Measurement of Charge-Reduced Ions Generated by Electrosprays. *Anal. Chem.* 76(4), 1045-1053.

[307] Ku,B.K., FernandezdelaMora,J., Saucy,D.A. & Alexander,J.N. **(2004)**. Mass Distribution Measurement of Water-Insoluble Polymers by Charge-Reduced Electrospray Mobility Analysis. *Anal. Chem.* 76(3), 814-822.

[308] Skelton,R., Dubois,F. & Zenobi,R. **(2000)**. A MALDI Sample Preparation Method Suitable for Insoluble Polymers. *Anal. Chem.* 72(7), 1707-1710.

[309] Trimpin,S., Keune,S., Rader,H J. & Mullen,K. **(2006)**. Solvent-Free MALDI-MS: Developmental Improvements in the Reliability and the Potential of MALDI in the Analysis of Synthetic Polymers and Giant Organic Molecules. *Journal of the American Society for Mass Spectrometry* 17(5), 661-671.

[310] Byrd,H.C.M. & McEwen,C.N. **(2000)**. The Limitations of MALDI-TOF Mass Spectrometry in the Analysis of Wide Polydisperse Polymers. *Anal. Chem.* 72(19), 4568-4576.

[311] Fei,X. & Murray,K.K. **(1996)**. On-Line Coupling of Gel Permeation Chromatography with MALDI Mass Spectrometry. *Anal. Chem.* 68(20), 3555-3560.

[312] Bahr,U., Deppe,A., Karas,M., Hillenkamp,F. & Giessmann,U. **(1992)**. Mass spectrometry of synthetic polymers by UV-matrix-assisted laser desorption/ionization. *Anal. Chem.* 64(22), 2866-2869.

[313] Chaudhary,A.K., Critchley,G., Diaf,A., Beckman,E.J. & Russell,A J. **(1996)**. Characterization of Synthetic Polymers Using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry. *Macromolecules* 29(6), 2213-2221.

[314] Montaudo,G., Montaudo,M.S., Puglisi,C. & Samperi,F. **(1995)**. Characterization of Polymers by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. End Group Determination and Molecular Weight Estimates in Poly(ethylene glycols). *Macromolecules* 28(13), 4562-4569.

[315] Williams,J.B., Gusev,A.I. & Hercules,D.M. **(1996)**. Use of Liquid Matrices for Matrix-Assisted Laser Desorption Ionization of Polyglycols and Poly(dimethylsiloxanes). *Macromolecules* 29(25), 8144-8150.

[316] Montaudo,G., Garozzo,D., Montaudo,M.S., Puglisi,C. & Samperi,F. **(1995)**. Molecular and Structural Characterization of Polydisperse Polymers and Copolymers by Combining MALDI-TOF Mass Spectrometry with GPC Fractionation. *Macromolecules* 28(24), 7983-7989.

[317] Gies,A.P. & Nonidez,W.K. **(2004)**. A Technique for Obtaining Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectra of Poorly Soluble and Insoluble Aromatic Polyamides. *Anal. Chem.* 76(7), 1991-1997.

[318] Mahoney,C.M., Yu,J. & Gardella,J A.Jr. **(2005)**. Depth Profiling of Poly(l-lactic acid)/Triblock Copolymer Blends with. *Anal. Chem.* 77(11), 3570-3578.

[319] Anderson,D.G. **(1999)**. Coatings. *Anal. Chem.* 71(12), 21-32.

[320] Anderson,D.G. **(1997)**. Coatings. *Anal. Chem.* 69(12), 15-28.

[321] Anderson,D.G. **(1993)**. Coatings. *Anal. Chem.* 65(12), 1-11.

[322] Anderson,D.G. **(1989)**. Coatings. *Anal. Chem.* 61(12), 33-45.

[323] Anderson,D.G. & Vandeberg,J.T. **(1987)**. Coatings. *Anal. Chem.* 59(12), 31-46.

[324] Mahadevan,R. & Smith,L. **(2007)**. A Mechanistic Model Describing the Degradation of Polymers. *Journal of Polymers and the Environment* 15(2), 75-80.

[325] Gordon,S.H., Imam,S.H., Shogren,R.L., Govind,N.S. & Greene,R.V. **(2000)**. A semiempirical model for predicting biodegradation profiles of individual polymers in starch-poly- (-hydroxybutyrate-*co*--hydroxyvalerate) bioplastic. *J. Appl. Polym. Sci.* 76(12), 1767-1776.

[326] Metters,A.T., Anseth,K S. & Bowman,C.N. **(2001)**. A Statistical Kinetic Model for the Bulk Degradation of PLA-b-PEG-b-PLA Hydrogel Networks: Incorporating Network Non-Idealities. *J. Phys. Chem. B* 105(34), 8069-8076.

[327] Metters,A.T., Bowman,C.N. & Anseth,K.S. **(2000)**. A Statistical Kinetic Model for the Bulk Degradation of PLA-b-PEG-b-PLA Hydrogel Networks. *J. Phys. Chem. B* 104(30), 7043-7049.

[328] Dimitrov,S., Kamenska,V., Walker,J.D., Windle,W., Purdy,R., Lewis,M. & Mekenyan,O. **(2004)**. Predicting the biodegradation products of perfluorinated chemicals using CATABOL. *SAR and QSAR in Environmental Research* 15(1), 69-82.

[329] McDowell,R.M. & Jaworska,J.S. **(2002)**. Bayesian analysis and inference from QSAR predictive model results. *SAR QSAR Environ Res* 13(1), 111-125.

[330] Sakuratani,Y., Yamada,J., Kasai,K., Noguchi,Y. & Nishihara,T. **(2005)**. External validation of the biodegradability prediction model CATABOL using data sets of existing and new chemicals under the Japanese Chemical Substances Control Law. *SAR and QSAR in Environmental Research* 16(5), 403-431.

[331] Keller,V.D.J., Rees,H.G., Fox,K.K. & Whelan,M.J. **(2007)**. A new generic approach for estimating the concentrations of down-the-drain chemicals at catchment and national scale. *Environmental Pollution* 148(1), 334-342.

[332] Duesterberg,C.K. & Waite,T.D. **(2006)**. Process Optimization of Fenton Oxidation Using Kinetic Modeling. *Environ. Sci. Technol.* 40(13), 4189-4195.

[333] Li,X.y., Zhang,J.j. & Lee,J.H.W. **(2004)**. Modelling particle size distribution dynamics in marine waters. *Wat. Res.* 38(5), 1305-1317.

[334] Sartory,D.P., Gu,H. & Chen,C.M. **(2008)**. Comparison of a novel MPN method against the yeast extract agar (YEA) pour plate method for the enumeration of heterotrophic bacteria from drinking water. *Wat. Res.* In Press, Accepted Manuscript.

[335] Neu,T.R., Woelfl,S. & Lawrence,J.R. **(2004)**. Three-dimensional differentiation of photo-autotrophic biofilm constituents by multi-channel laser scanning microscopy (single-photon and two-photon excitation). *Journal of Microbiological Methods* 56(2), 161-172.

[336] Sizemore,R.K. & Stevenson,L.H. **(1970)**. Method for the Isolation of Proteolytic Marine Bacteria. *Appl. Environ. Microbiol.* 20(6), 991-992.

[337] Button,D.K., Schut,F., Quang,P., Martin,R. & Robertson,B.R. **(1993)**. Viability and Isolation of Marine Bacteria by Dilution Culture: Theory, Procedures, and Initial Results. *Appl. Environ. Microbiol.* 59(3), 881-891.

[338] Bolhuis,H., te Poele,E.M. & Rodriguez-Valera,F. **(2004)**. Isolation and cultivation of Walsby's square archaeon. *Environ Microbiol* 6(12), 1287-1291.

[339] Burns,D.G., Camakaris,H.M., Janssen,P.H. & Dyall-Smith,M.L. **(2004)**. Cultivation of Walsby's square haloarchaeon. *FEMS Microbiol.* 238(2), 469-473.

[340] Schut,F., DE Vries,E.J., Gottschal,J.C., Robertson,B.R., Harder,W., Prins,R.A. & Button,D.K. **(1993)**. Isolation of Typical Marine Bacteria by Dilution Culture: Growth, Maintenance, and Characteristics of Isolates under Laboratory Conditions. *Appl. Environ. Microbiol.* 59(7), 2150-2160.

[341] Schut,F., Gottschal,J.C. & Prins,R A. **(1997)**. Isolation and characterisation of the marine ultramicrobacterium Sphingomonas sp. strain RB2256. *FEMS Microbiology Reviews* 20(3-4), 363-369.

[342] Zengler,K., Toledo,G., Rappe,M., Elkins,J., Mathur,E.J., Short,J.M. & Keller,M. **(2002)**. Cultivating the uncultured. *Proceedings of the National Academy of Sciences* 99(24), 15681-15686.

[343] Rappe,M S., Connon,S.A., Vergin,K.L. & Giovannoni,S.J. **(2002)**. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* 418(6898), 630-633.

[344] Connon,S A. & Giovannoni,S.J. **(2002)**. High-Throughput Methods for Culturing Microorganisms in Very-Low-Nutrient Media Yield Diverse New Marine Isolates. *Appl. Environ. Microbiol.* 68(8), 3878-3885.

[345] Zoetendal,E.G. & Mackie,R.I. **(2005)**. Molecular methods in microbial ecology. In Probiotics and Prebiotics Scientific Aspects (Tannock,G.W., ed), Caister Academic Press.

[346] Hoffmann,S. & Hoffmann,A. **(2008)**. Is there a "true" diversity? *Ecological Economics* 65(2), 213-215.

[347] Quince,C., Curtis,T.P., & Sloan,W.T. **(2008)**. The rational exploration of microbial diversity. *ISME J* , International Society for Microbial Ecology.

[348] Clayton,P.D., Fielder,D.P., Howell,S., & Hill,C J. **(2006)**. Aquatic Biodiversity Assessment and Mapping Method (AquaBAMM): a conservation values assessment tool for wetlands with trial application in the Burnett River catchment. Environmental Protection Agency,Queensland Government.

[349] Dolmanondon,P. **(1996)**. Biodiversity: Measurement and estimation : Edited by David L. Hawksworth. The Royal Society. Chapman and Hall, London. 1995. 140 pp. ISBN 0 412 62480 X. Price: ú37.50. *Biological Conservation* 76(1), 94.

[350] Willis,K J., Gillson,L., Brncic,T.M. & Figueroa-Rangel,B.L. **(2005)**. Providing baselines for biodiversity measurement. *Trends in Ecology & Evolution* 20(3), 107-108.

[351] Fuhrman,J.A., McCallum,K. & Davis,A A. **(1993)**. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. *Appl. Environ. Microbiol.* 59(5), 1294-1302.

[352] Lipp,J S., Morono,Y., Inagaki,F. & Hinrichs,K.U. **(2008)**. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature*.

[353] Moyer,C.L. **(2001)**. Molecular phylogeny: Applications and implications for marine microbiology. In Methods in Microbiology Marine Microbiology (John,H.P., ed), pp. 375-394. Academic Press.

[354] Otton,S.V., Sura,S., Blair,J., Ikonomou,M.G. & Gobas,F.A.P.C. **(2008)**. Biodegradation of mono-alkyl phthalate esters in natural sediments. *Chemosphere* 71(11), 2011-2016.

[355] Sibila,M A., Garrido,M.C., Perales,J.A. & Quiroga,J.M. **(2008)**. Ecotoxicity and biodegradability of an alkyl ethoxysulphate surfactant in coastal waters. *Science of The Total Environment* 394(2-3), 265-274.

[356] Mehdi,H. & Giti,E. **(2008)**. Investigation of alkane biodegradation using the microtiter plate method and correlation between biofilm formation, biosurfactant production and crude oil biodegradation. *International Biodeterioration & Biodegradation* 62(2), 170-178.

[357] Powell,S.M., Harvey,P.M., Stark,J.S., Snape,I. & Riddle,M.J. **(2007)**. Biodegradation of petroleum products in experimental plots in Antarctic marine sediments is location dependent. *Marine Pollution Bulletin* 54(4), 434-440.

[358] Sudhakar,M., Doble,M., Murthy,P.S. & Venkatesan,R. **(2008)**. Marine microbe-mediated biodegradation of low- and high-density polyethylenes. *International Biodeterioration & Biodegradation* 61(3), 203-213.

[359] Sudhakar,M., Priyadarshini,C., Doble,M., Sriyutha Murthy,P. & Venkatesan,R. **(2007)**. Marine bacteria mediated degradation of nylon 66 and 6. *International Biodeterioration & Biodegradation* 60(3), 144-151.

[360] Danovaro,R., Corinaldesi,C., Luna,G., & Dell'Anno,A. **(2006)**. Molecular Tools for the Analysis of DNA in Marine Environments. In Marine Organic Matter: Biomarkers, Isotopes and DNA pp. 105-126.

[361] Dorigo,U., Volatier,L. & Humbert,J.F. **(2005)**. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. *Water Research* 39(11), 2207-2218.

[362] Peplies,J., Lachmund,C., Glockner,F.O. & Manz,W. **(2006)**. A DNA Microarray Platform Based on Direct Detection of rRNA for Characterization of Freshwater Sediment-Related Prokaryotic Communities. *Appl. Environ. Microbiol.* 72(7), 4829-4838.

[363] Rudolph,C., Wanner,G. & Huber,R. **(2001)**. Natural Communities of Novel Archaea and Bacteria Growing in Cold Sulfurous Springs with a String-of-Pearls-Like Morphology. *Appl. Environ. Microbiol.* 67(5), 2336-2344.

[364] Endo,H., Nakayama,J. & Hayashi,T. **(2000)**. Application of flow cytometry to environmental control in marine aquaculture. *Materials Science and Engineering: C* 12(1-2), 83-88.

[365] Gerdts,G. & Luedke,G. **(2006)**. FISH and chips: Marine bacterial communities analyzed by flow cytometry based on microfluidics. *Journal of Microbiological Methods* 64(2), 232-240.

[366] Hammes,F., Berney,M., Wang,Y., Vital,M., K÷ster,O. & Egli,T. **(2008)**. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Research* 42(1-2), 269-277.

[367] Lin,Y.H. & Lee,G.B. **(2008)**. Optically induced flow cytometry for continuous microparticle counting and sorting. *Biosensors and Bioelectronics* In Press, Corrected Proof.

[368] Rifà,T.G., Latatu,A., Ayo,B.a., Iriberri,J., Comas-Riu,J. & Vives-Rego,J. **(2002)**. Flow Cytometric Detection and Quantification of Heterotrophic Nanoflagellates in Enriched Seawater and Cultures. *Systematic and Applied Microbiology* 25(1), 100-108.

[369] Veal,D.A., Deere,D., Ferrari,B., Piper,J. & Attfield,P.V. **(2000)**. Fluorescence staining and flow cytometry for monitoring microbial cells. *Journal of Immunological Methods* 243(1-2), 191-210.

[370] Chen,F. & Hodson,R.E. **(2001)**. In situ PCR/RT-PCR coupled with in situ hybridization for detection of functional gene and gene expression in prokaryotic cells. In Methods in Microbiology
Marine Microbiology (John,H.P., ed), pp. 409-424. Academic Press.

[371] Wintzingerode,F., G÷bel,U.B. & Stackebrandt,E. **(1997)**. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Reviews* 21(3), 213-229.

[372] Beier,S., Witzel,K.P. & Marxsen,J. **(2008)**. Bacterial Community Composition in Central European Running Waters Examined by Temperature Gradient Gel Electrophoresis and Sequence Analysis of 16S rRNA Genes. *Appl. Environ. Microbiol.* 74(1), 188-199.

[373] Schäfer,H. & Muyzer,G. **(2001)**. Denaturing gradient gel electrophoresis in marine microbial ecology. In Methods in Microbiology
Marine Microbiology (John,H.P., ed), pp. 425-468. Academic Press.

[374] Smalla,K., Oros-Sichler,M., Milling,A., Heuer,H., Baumgarte,S., Becker,R., Neuber,G., Kropf,S., Ulrich,A. & Tebbe,C.C. **(2007)**. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbiological Methods* 69(3), 470-479.

[375] Dorigo,U., Volatier,L. & Humbert,J.F. **(2005)**. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. *Water Research* 39(11), 2207-2218.

[376] Peplies,J., Lachmund,C., Glockner,F.O. & Manz,W. **(2006)**. A DNA Microarray Platform Based on Direct Detection of rRNA for Characterization of Freshwater Sediment-Related Prokaryotic Communities. *Appl. Environ. Microbiol.* 72(7), 4829-4838.

[377] Rhee,S.K., Liu,X., Wu,L., Chong,S.C., Wan,X. & Zhou,J. **(2004)**. Detection of Genes Involved in Biodegradation and Biotransformation in Microbial Communities by Using 50-Mer Oligonucleotide Microarrays. *Appl. Environ. Microbiol.* 70(7), 4303-4317.

[378] Schadt,C.W., Liebich,J., Chong,S.C., Gentry,T J., He,Z., Pan,H., & Zhou,J. **(2004)**. Design and Use of Functional Gene Microarrays (FGAs) for the Characterization of Microbial Communities. In Methods in Microbiology
Microbial Imaging (and,T.S., ed), pp. 331-368. Academic Press.

[379] Wagner,M., Amann,R., Lemmer,H. & Schleifer,K.H. **(1993)**. Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. *Appl. Environ. Microbiol.* 59(5), 1520-1525.

[380] Wu,L., Thompson,D.K., Li,G., Hurt,R.A., Tiedje,J.M. & Zhou,J. **(2001)**. Development and Evaluation of Functional Gene Arrays for Detection of Selected Genes in the Environment. *Appl. Environ. Microbiol.* 67(12), 5780-5790.

[381] Zhou,J. **(2003)**. Microarrays for bacterial detection and microbial community analysis. *Current Opinion in Microbiology* 6(3), 288-294.

[382] Smalla,K., Oros-Sichler,M., Milling,A., Heuer,H., Baumgarte,S., Becker,R., Neuber,G., Kropf,S., Ulrich,A. & Tebbe,C.C. **(2007)**. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbiological Methods* 69(3), 470-479.

[383] Amann,R.I., Ludwig,W. & Schleifer,K.H. **(1995)**. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59(1), 143-169.

[384] Amann,R., Lemmer,H. & Wagner,M. **(1998)**. Monitoring the community structure of wastewater treatment plants: a comparison of old and new techniques. *FEMS Microbiology Ecology* 25(3), 205-215.

[385] Paul,J.H. **(2001)**. Gene expression by mRNA analysis. In Methods in Microbiology
Marine Microbiology (John,H.P., ed), pp. 395-408. Academic Press.

[386] Brosius,J., Palmer,M.L., Kennedy,P.J. & Noller,H.F. **(1978)**. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. *Proc Natl Acad Sci USA* 75(10), 4801-4805.

[387] Woese,C.R. **(1987)**. Bacterial evolution. *Microbiol. Mol. Biol. Rev.* 51(2), 221-271.

[388] Vandamme,P., Pot,B., Gillis,M., de Vos,P., Kersters,K. & Swings,J. **(1996)**. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol. Mol. Biol. Rev.* 60(2), 407-438.

[389] Santosa,D.A. **(2001)**. Rapid estraction and purification of environmental DNA for molecular cloning applications and molecular diversity studies. *Mol.Biotechnol.* 17(1), 59-64 , Humana Press.

[390] Anderson,D.G. & McKay,L.L. **(1983)**. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. *Appl. Environ. Microbiol.* 46(3), 549-552.

[391] Bostroem,K.H., Simu,K., Hagstroem,A. & Riemann,L. **(2004)**. Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. *Limnol. Oceanogr. Methods* 2 365-373.

[392] Mullis,K., Faloona,F., Scharf,S., Saiki,R., Horn,G. & Erlich,H. **(1986)**. Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction. *Cold Spring Harbor Symposia on Quantitative Biology* 51 263-273.

[393] Saiki,R.K., Scharf,S., Faloona,F., Mullis,K.B., Horn,G.T., Erlich,H A. & Arnheim,N. **(1985)**. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230(4732), 1350-1354.

[394] Mullis,K.B. & Faloona,F.A. **(1987)**. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155 335-350.

[395] Osborne,C A., Galic,M., Sangwan,P. & Janssen,P.H. **(2005)**. PCR-generated artefact from 16S rRNA gene-specific primers. *FEMS Microbiol.* 248(2), 183-187.

[396] Ercolini,D. **(2004)**. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods* 56(3), 297-314.

[397] Nübel,U., Engelen,B., Felske,A., Snaidr,J., Wieshuber,A., Amann,R.I., Ludwig,W. & Backhaus,H. **(1996)**. Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. *J. Bacteriol.* 178(19), 5636-5643.

[398] Klappenbach,J.A., Saxman,P.R., Cole,J.R. & Schmidt,T.M. **(2001)**. rrndb: the Ribosomal RNA Operon Copy Number Database. *Nucl. Acids Res.* 29(1), 181-184.

[399] Schmalenberger,A., Schwieger,F. & Tebbe,C.C. **(2001)**. Effect of Primers Hybridizing to Different Evolutionarily Conserved Regions of the Small-Subunit rRNA Gene in PCR-Based Microbial Community Analyses and Genetic Profiling. *Appl. Environ. Microbiol.* 67(8), 3557-3563.

[400] Dahllöf,I., Baillie,H. & Kjelleberg,S. **(2000)**. rpoB-Based Microbial Community Analysis Avoids Limitations Inherent in 16S rRNA Gene Intraspecies Heterogeneity. *Appl. Environ. Microbiol.* 66(8), 3376-3380.

[401] Qi,Y., Patra,G., Liang,X., Williams,L.E., Rose,S., Redkar,R J. & DelVecchio,V.G. **(2001)**. Utilization of the rpoB Gene as a Specific Chromosomal Marker for Real-Time PCR Detection of Bacillus anthracis. *Appl. Environ. Microbiol.* 67(8), 3720-3727.

[402] Dahllöf,I. **(2002)**. Molecular community analysis of microbial diversity. *Current Opinion in Biotechnology* 13(3), 213-217.

[403] Muyzer,G. **(1999)**. DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology* 2(3), 317-322.

[404] Muyzer,G. & Smalla,K. **(1998)**. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73(1), 127-141.

[405] Smalla,K., Oros-Sichler,M., Milling,A., Heuer,H., Baumgarte,S., Becker,R., Neuber,G., Kropf,S., Ulrich,A. & Tebbe,C.C. **(2007)**. Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbiological Methods* 69(3), 470-479.

[406] Calabria de Araujo,J. & Schneider,R.P. **(2008)**. DGGE with genomic DNA: suitable for detection of numerically important organisms but not for identification of the most abundant organisms. *Water Research* In Press, Accepted Manuscript.

[407] Muyzer,G. & Smalla,K. **(1998)**. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73(1), 127-141.

[408] Pruesse,E., Quast,C., Knittel,K., Fuchs,B.M., Ludwig,W., Peplies,J. & Glockner,F.O. **(2007)**. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucl. Acids Res.* 35(21), 7188-7196.

[409] Cole,J.R., Chai,B., Farris,R.J., Wang,Q., Kulam,S.A., McGarrell,D.M., Garrity,G.M. & Tiedje,J.M. **(2005)**. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucl. Acids Res.* 33(suppl_1), D294-D296.

[410] Ludwig,W., Strunk,O., Westram,R., Richter,L., Meier,H., Yadhukumar, Buchner,A., Lai,T., Steppi,S., Jobb,G., Forster,W., Brettske,I., Gerber,S., Ginhart,A.W., Gross,O., Grumann,S., Hermann,S., Jost,R., Konig,A., Liss,T., Lussmann,R., May,M., Nonhoff,B., Reichel,B., Strehlow,R., Stamatakis,A., Stuckmann,N., Vilbig,A., Lenke,M., Ludwig,T., Bode,A. & Schleifer,K.H. **(2004)**. ARB: a software environment for sequence data. *Nucl. Acids Res.* 32(4), 1363-1371.

[411] Abdo,Z., Schuette,U.M., Bent,S.J., Williams,C.J., Forney,L.J. & Joyce,P. **(2006)**. Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ Microbiol.* 8(5), 929-938.

[412] Kleeberg,I. **(2000)**. Untersuchung zum mikrobiellen Abbau von aliphatisch-aromatischen Copolyestern sowie Isolierung und Charakterisierung eines polyesterspaltenden Enzyms (Investigation on microbial degradation of aliphatic-aromatic copolyesters as well as isolation and chraecterization of a polyester cleaving enzyme). Technische Universität Carolo-Wilhemina zu Braunschweig.

[413] Organisation for Economic Co-operation and Development (OECD). OECD-Guideline for testing of Chemicals: Biodegradability in seawater. Organisation for Economic Co-operation and Development (OECD), 2, Rue André Pascal, F-75775 Paris Cedex 16, France, Main Switchboard, tel.: +33 1.45.24.82.00 fax: +33 1.45.24.85 00.

[414] Garthe,J.W. & Kowal,P.D. **(2001)**. The chemical composition of degradable plastics.

[415] van der Zee,M., Sijtsma,L., Tan,G.B., Tournois,H. & De Wit,D. **(1994)**. Assessment of biodegradation of water insoluble polymeric materials in aerobic and anaerobic aquatic environments. *Chemosphere* 28(10), 1757-1771.

[416] Itavaara,M. & Vikman,M. **(1995)**. A simple screening test for studying the biodegradability of insoluble polymers. *Chemosphere* 31(11-12), 4359-4373.

[417] Sawada,H. **(1998)**. ISO standard activities in standardization of biodegradability of plastics development of test methods and definitions. *Polymer Degradation and Stability* 59(1-3), 365-370.

[418] Pagga,U., Schafer,A., Muller,R.J. & Pantke,M. **(2001)**. Determination of the aerobic biodegradability of polymeric material in aquatic batch tests. *Chemosphere* 42(3), 319-331.

[419] Pagga,U., Schäfer,A., Müller,R.J. & Pantke,M. **(2001)**. Determination of the aerobic biodegradability of polymeric material in aquatic batch tests. *Chemosphere* 42(3), 319-331.

[420] Marten,E. **(2000)**. Korrelation zwischen der Struktur und der enzymatischen Hydrolyse von Polyestern. Technische Universitaet Braunschweig.

[421] Welzel,K., Mueller,R.-J. & Deckwer,W.-D. **(2002)**. Enzymatischer Abbau von Polyester-Nanopartikeln, enzymatic degradation of polyester nanoparticles. *Chem. Ing. Tech.* 74(10), 1496-1500.

[422] Welzel,K. **(2003)**. Einfluss der chemischen Struktur auf die enzymatische Hydrolyse von polyester-Nanopartikeln. Technische Universitaet Braunschweig.

[423] Strotmann,U., Reuschenbach,P., Schwarz,H. & Pagga,U. **(2004)**. Development and Evaluation of an Online CO2 Evolution Test and a Multicomponent Biodegradation Test System. *Appl. Environ. Microbiol.* 70(8), 4621-4628.

[424] Brüggemeier,C. **(2009)**. Untersuchung der Abhängigkeit des biologischen Abbaus von der Biomassekonzentration. FH Bingen, Fachbereich 1 - Life Sciences and Engineering.

[425] Eigenbetrieb Stadtentwässerung Mannheim. **(2008)**. Data of the wastewater treatment plant Mannheim (Germany).

[426] Bast,E. **(2001)**. *Mikrobiologische methoden (Microbiological methods)*, 2 edn. Spektrum Akademischer Verlag, Heidelberg, Berlin.

[427] Friedrich Lottspeich **(2006)**. *Bioanalytik (Bioanalytics)*, 2 edn. Spektrum Akademischer Verlag, Heridelberg, Berlin.

[428] Robinson,H.W. & Hogden,C.G. **(1940)**. The biuret reaction in the determination of serum proteins. I . A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. *J. Biol. Chem.* 135(2), 707-725.

[429] Friedrich Lottspeich **(2006)**. *Bioanalytik (Bioanalytics)*, 2 edn. Spektrum Akademischer Verlag, Heridelberg, Berlin.

[430] Strickland,L.H. **(1951)**. The determination of small quantities of bacteria by means of the biuret reaction. *Journal of General Microbiology* 5(4), 698-703.

[431] Balkwill,D.L., Leach,F.R., Wilson,J.T., McNabb,J.F. & White,D.C. **(1988)**. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. *Microbial Ecology* 16(1), 73-84.

[432] Sharma,R.V., Edwards,R.T. & Beckett,R. **(1993)**. Physical Characterization and Quantification of Bacteria by Sedimentation Field-Flow Fractionation. *Appl. Environ. Microbiol.* 59(6), 1864-1875.

[433] Balkwill,D.L., Leach,F.R., Wilson,J.T., McNabb,J.F. & White,D.C. **(1988)**. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. *Microbial Ecology* 16(1), 73-84.

[434] Jonkers,C.C.A. & de Voogt,P. **(2003)**. Nonionic surfactants in marine and estuarine environments. In Analysis and Fate of Surfactants in the Aquatic Environment (Knepper,T.P., Barcelo,D., & de Voogt,P., eds), pp. 719-748. Elsevier, Amsterdam.

[435] Amann,R.I., Ludwig,W. & Schleifer,K.H. **(1995)**. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59(1), 143-169.

[436] Coulter,W.H. **(1953)**. Means for counting particles suspended in a fluid. (United States Patent 2656508)

[437] Bradford,M.M. **(1976)**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1-2), 248-254.

[438] Campbell,C.D., Chapman,S.J., Cameron,C.M., Davidson,M.S. & Potts,J.M. **(2003)**. A Rapid Microtiter Plate Method To Measure Carbon Dioxide Evolved from Carbon Substrate Amendments so as To Determine the Physiological Profiles of Soil Microbial Communities by Using Whole Soil. *Appl. Environ. Microbiol.* 69(6), 3593-3599.

[439] Alexander,M. **(1986)**. Biodegradation of Chemicals at Trace Concentrations. ADA168922 , Cornell University, Ithaca, New York, USA Dept. of Agronomy.

[440] Thompson,R.C., Olsen,Y., Mitchell,R.P., Davis,A., Rowland,S J., John,A.W.G., McGonigle,D. & Russell,A.E. **(2004)**. Lost at Sea: Where Is All the Plastic? *Science* 304(5672), 838.

[441] Umweltbundesamt. **(2003)**. Kunststoffabfaelle. *http://www.umweltbundesamt.de/uba-info-daten/daten/kunststoffabfaelle.htm* , Umweltbundesamt, Postfach 1406, 06813 Dessau, Tel:+49-(0)340-2103-0 Fax: +49 (0)340 2103-2285.

[442] Cornell,D. **(2008)**. Biopolymers in the Existing Postconsumer Plastics Recycling Stream. *Journal of Polymers and the Environment*.

[443] Kaihara,S., Osanai,Y., Nishikawa,K., Toshima,K., Doi,Y. & Matsumura,S. **(2005)**. Enzymatic transformation of bacterial polyhydroxyalkanoates into repolymerizable oligomers directed towards chemical recycling. *Macromol. Biosci.* 5(7), 644-652.

[444] Storrer,J. & Rohrmann,S. **(2001)**. Abfallverwertung nach dem Vorbild der Natur. (Waste recycling based on the paradigm of nature). *Biologie in unserer Zeit* 31(2), 116-122.

[445] Holtmann,D. **(2005)**. Electro-Chemical Monitoring of Microbial Activity - Fundamentals and Application in Waste Water Treatment. Otto-von-Guericke-Universität Magdeburg, The Faculty for Process and Systems Engineering.