Ecotoxicity assessment of biodegradable plastics and sewage sludge in compost and in soil

Anu Kapanen

VTT

Ecotoxicity assessment of biodegradable plastics and sewage sludge in compost and in soil

Anu Kapanen

Academic dissertation in Microbiology

To be presented with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki for public criticism in Auditorium L3 at Latokartanonkaari 9, Helsinki on the 16th of November 2012, at 12 noon.

Ecotoxicity assessment of biodegradable plastics and sewage sludge in compost and in soil

Abstract

Biodegradable plastics, either natural or synthetic polymers, can be made from renewable or petrochemical raw materials. The most common applications for biodegradable plastics are packaging materials and waste collection bags. Other applications include catering products, wrappings, food containers, laminated paper, golf tees, hygiene products and agricultural applications. The one thing in common for all these biodegradable items is that at the end of their life cycle they should degrade into harmless end products, during a specified time frame. Depending on the target application and excluding medical applications, the degradation may take place in soil, in water, in an anaerobic digestion plant or in compost. In addition to its use as a waste treatment process for biodegradable plastics, composting can be used in the removal of organic contaminants from sewage sludge. Due to mixed contamination present in soil, in compost, or in sewage sludge the environmental impact of biodegradable materials is difficult to assess based only on concentrations of chemical constituents. Therefore, biotests are needed for detecting potential risks derived from the use of biodegradable materials in environmental applications.

In this study the biodegradation properties of bioplastics targeted for agricultural and compost applications were investigated. In addition, the potential of biotests were evaluated in ecotoxicity assessment of biodegradable plastics and their components during the biodegradation process in vermiculite, compost and soil. Acute toxicity of polymer components and degradation products was screened with a kinetic luminescent bacteria test (ISO 21338), and possible hazardous compounds could be identified and further studied. During the biodegradation of chain-linked lactic acid polymers and polyurethane-based plastic material in controlled composting conditions the release of toxic degradation products could be demonstrated by biotests. Clear toxic responses in the luminescent bacteria test and/or plant growth test (OECD 208) were observed. The fate of an endocrine disrupting plasticizer, diethyl phthalate (DEP) was also studied in a controlled composting test, in pilot composting scale and in plant growth media. A high concentration of DEP induced changes in the microbial community, gave a clear response in the biotest and its degradation was inhibited. However, in pilot scale composting toxicity was not detected and the degradation of DEP was efficient. The studied starch-based biodegradable mulching films showed good product performance, good crop quality and high yield in protected strawberry cultivation.

Furthermore, no negative effects on the soil environment, *Enchytraeidae* reproduction (ISO 16387) or amoA gene diversity were detected.

Biotests were also used to study compost quality during sewage sludge composting. If sewage sludge is used as a soil conditioner, many harmful substances can potentially end up in the environment. In our study, composting reduced efficiently the amount of organic contaminants such as DEHP, PAH, LAS, and NPs in sewage sludge. In addition, composting resulted in reduction in acute toxicity, genotoxicity and endocrine-disruption potential of the sewage sludge. The use of biotests is recommended as an indicator of potential risk when sewage sludge-based products are used in agricultural or landscaping applications.

Potentials and also limitations were recognized in the performances of different biotests when studying the ecotoxicity of biodegradable materials during biodegradation processes in compost and in soil environment. With biotests it was possible to identify potential hazardous polymer components or degradation products that might be released to the environment during the degradation. In addition, the biotest could be used to monitor the detoxification of sewage sludge during the composting process. However, soil, compost and sludge as testing environments do set limitations for the use of biotests. Colour, amounts of nutrients, additional carbon sources, presence of bark and peat, compost immaturity, and high microbial activity are some of the factors limiting the use of biotests or complicate interpretation of the results.

Biohajoavien muovien sekä jätevesilietteen ympäristömyrkyllisyyden arviointi kompostissa ja maaperässä

Tiivistelmä

Biohajoavat muovit voivat koostua luonnonpolymeeristä tai synteettisistä polymeereistä, joita valmistetaan joko uudistuvista tai uusiutumattomista raaka-aineista kuten petrokemikaaleista. Yleisimpiä biohajoavista muoveista valmistettuja tuotteita ovat pakkausmateriaalit ja jätepussit. Tämän lisäksi biohajoavia muoveja voidaan käyttää raaka-aineena hyvin erilaisissa sovelluksissa, joita ovat esimerkiksi aterimet, käärepaperit, säilytysrasiat, laminoitu paperi, golf-tii, hygieniatuotteet ja erilaiset sovellukset maatalouskäyttöön. Kaikkien näiden biohajoavien tuotteiden tulee käyttöikänsä jälkeen hajota haitattomiksi lopputuotteiksi. Biohajoamisen tulisi tapahtua tietyn ajan sisällä käyttöympäristön tai käytetyn jätteenkäsittelyteknologian asettamien vaatimusten mukaisesti. Sovelluskohteen ja käyttötarkoituksen mukaan hajoaminen voi tapahtua joko maassa, vedessä, anaerobiprosessissa tai kompostissa, jos ei lueta mukaan lääketieteen sovelluksia, joissa hajoaminen voi tapahtua myös ihmisen kehossa. Useat biohajoavista muoveista on suunniteltu täyttämään kompostoituville materiaaleille asetetut vaatimukset. Kompostointia hyödynnetään yleisesti myös jätevesilietteiden käsittelyssä. Kompostoinnilla on mahdollista alentaa lietteen sisältämien orgaanisten haitta-aineiden pitoisuuksia ja näin parantaa lietteen hyötykäyttömahdollisuuksia. Biohajoavat materiaalit sekä niiden biohajoamisympäristöt, kuten komposti, jätevesiliete tai maaperä, voivat sisältää monia erilaisia haitallisia yhdisteitä, joten ympäristövaikutusten arviointi ainoastaan kemiallisen analytiikan perusteella on haastavaa. Biohajoavien materiaalien käytön aiheuttamien ympäristöriskien arvioinnissa onkin suositeltavaa hyödyntää kemiallisten analyysien rinnalla biotestejä.

Tässä työssä tutkitut kompostoituvat ja maatalouskäyttöön tarkoitetut biohajoavat muovit biohajosivat tavoitteiden mukaisesti suhteessa käyttötarkoituksen asettamiin vaatimuksiin. Materiaalien biohajoavuusominaisuuksien lisäksi tarkasteltiin myös biotestien mahdollisuuksia biohajoavien muovien ja niiden komponenttien ympäristömyrkyllisyyden arvioinnissa biohajoavuusprosessin aikana sekä kompostissa, maaperässä että vermikuliitissa. Tutkittujen polymeerien komponenttien ja hajoamistuotteiden joukosta seulottiin mahdollisesti ympäristölle haitalliset yhdisteet kineettisellä valobakteeritestillä (ISO 21338) ja haitallisiksi tunnistettujen yhdisteiden ympäristövaikutuksia tarkasteltiin tutkimuksessa yksityiskohtaisemmin. Tutkimuksessa osoitettiin, että maitohappopohjaisten polymeerien ja polyuretaanipohjaisten biomuovien biohajoamisen aikana ympäristöön voi vapautua ympäristölle haitallisia yhdisteitä. Haittavaikutukset voitiin osoittaa sekä kineettisellä valobakteeritestillä että kasvitestillä (OECD 208). Tämän lisäksi tutkittiin muovin

pehmittimenä käytetyn ja hormonin kaltaisesti vaikuttavan dietyyliftalaatin (DEP) käyttäytymistä laboratorio- ja pilot-mittakaavan kompostiprosessissa sekä kompostista valmistetussa kasvualustassa. Laboratoriomittakaavan kokeissa korkeat DEP-pitoisuudet aiheuttivat muutoksia kompostikasvualustan mikrobidiversiteetissä, vaikuttivat kasvien kasvuun ja mikrobien toimintaan alentaen yhdisteen biohajoavuutta. Pilot-mittakaavan kompostointiolosuhteissa DEP hajosi tehokkaasti, eikä haittavaikutuksia havaittu biotesteillä. Tutkitut tärkkelyspohjaiset biohajoavat katekalvot toimivat hyvin kenttäolosuhteissa, materiaali oli kestävää eikä alentanut tuotetun mansikkasadon laatua tai määrää. Käytön jälkeen peltomaahan kynnetyn katekalvon hajoaminen kesti noin vuoden. Katekalvon hajoamisella ei havaittu olevan vaikutusta maaperän laatuun, kun maan laatua tarkasteltiin *Enchytraeidae*-jälkeläisten määrän (ISO 16387) ja amoA-geenin diversiteetin avulla.

Lietteen sisältämiä orgaanisia haitta-aineita voi päätyä ympäristöön, kun jätevesilietettä käytetään maanparannusaineena. Haitta-aineiden pitoisuuksia voidaan vähentää kompostoimalla. Pilot-mittakaavan kompostikokeessa tutkittavan jätevesilietteen sisältämät orgaaniset haitta-aineet, kuten DEHP, PAH, LAS ja NP, hajosivat tehokkaasti. Biotesteillä osoitettiin että kompostointi vähensi myös lietteen akuuttia myrkyllisyyttä, genotoksisuutta sekä hormonin kaltaisesti vaikuttavien yhdisteiden määrää lietteessä. Tämän tutkimuksen perusteella biotestien käyttöä voidaan suositella maatalouskäyttöön tai viherrakentamiseen tarkoitettujen jätevesilietteiden laadun tarkkailussa.

Biotesteillä voitiin osoittaa tutkittujen polymeerien ympäristölle haitalliset komponentit, niiden vapautuminen ympäristöön ja haitallisten hajoamistuotteiden syntyminen biohajoavuusprosessin aikana. Lisäksi kompostoinnin vaikutus kunnallisen jätevesilietteen laatuun osoitettiin biotesteillä. Tutkimuksessa havaittiin myös, että biohajoavat materiaalit sekä komposti ja maaperä testiympäristöinä asettavat biotesteille suuria haasteita. Maaperä, komposti tai liete testiympäristöinä rajoittivat biotestien hyödyntämismahdollisuuksia ja vähensivät hyödynnettyjen testien herkkyyttä. Tutkittavien näytteiden väri, orgaaninen hiili, kuorike, turve, kompostin kypsyysaste ja korkea mikrobiaktiivisuus ovat niitä tekijöitä, jotka vaikuttavat biotestien toimintaan ja tekevät biotestien tulosten tulkinnan haastavaksi.

Preface

This thesis consists of studies carried out at the VTT Technical Research Centre of Finland during the years 1998–2007. The research was funded by the Finnish Funding Agency for Technology and Innovation (TEKES), the Ministry of Agriculture and Forestry and VTT. Furthermore, the European Commission funded the research through two projects; Labeling biodegradable products (SMT4-CT97-2187) and Environmentally friendly mulching and low tunnel cultivation (QLK5-CT-2000-00044).

I cordially thank Professor Anu Kaukovirta-Norja and Dr. Raija Lantto for providing excellent research facilities at VTT and the possibility to finalize this thesis as well as for a working environment that encouraged taking professional challenges. I warmly thank Professor Kaarina Sivonen for her patience and help in organizing the final steps of the long journey to achieve this dissertation. I express my genuine appreciation to my pre-examiners Professor Jussi Kukkonen and Dr.ir. Maarten van der Zee for their valuable comments and criticism as well as for their interest in the research area covered in this thesis. To my supervisor Docent Merja Itävaara I will always be grateful for the fruitful collaboration and encouragement throughout my career at VTT. She provided me with the possibility to contribute to so many interesting research projects and showed with her example that anything is possible.

Studies included in this thesis were made possible through co-operation with several research groups. I warmly thank all of my co-authors at VTT, and all around the world. Especially I wish to thank Olli Venelampi and Minna Vikman for their contribution to composting experiments, Jukka Tuominen and Janne Kylmä for fascinating collaboration within polymer science, Evelia Schettini and Guiliano Vox for good and warm hearted teamwork in combining agricultural polymer applications and ecotoxicology, Francesco Degli-Innocenti for providing the perspective of the industry, John Stephen and Julia Brüggemann for introducing me to the world of molecular biology, and finally Marko Virta for offering me the possibility to learn more about bioreporters.

Dear VTT colleagues present and past, I don't know where to start. Especially I want to thank the "old" biodegradation gang Merja Itävaara, Minna Vikman, Olli Venelampi, Reetta Piskonen, and Sari Karjomaa for enthusiastic collaboration related to biodegradation of anything you can think of starting from biodegradable polymers, paper products, chemicals or nanoparticles etc. None of this would have

Academic dissertation

List of papers:

This thesis is based on the following original articles, referred to in the following text by their Roman numbers. In addition, some unpublished data is presented.

I   Degli-Innocenti, F. Bellia, G., Tosin, M, Kapanen, A. and Itävaara, M. 2001. Detection of toxicity released by a biodegradable plastics after composting in activated vermiculite. Polymer Degradation and Stability 73, 101–106.

II Tuominen, J., Kylmä, J., Kapanen, A., Venelampi, O., Itävaara M., and Seppälä, J. 2002. Biodegradation of lactic acid based polymers in controlled composting conditions and evaluation of the ecotoxicological impact. Biomacromolecules 3(3), 445–455.

III Kapanen, A., Stephen, J.R., Brüggeman, J., Kiviranta, A., White, D.C., Itävaara, M. 2007. Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community. Chemosphere, 67(11), 2201–2209.

IV Kapanen A., Schettini E., Vox G., and Itävaara M. 2008. Performance and environmental impact of biodegradable films in agriculture: a field study on protected cultivation. The Journal of Polymers and the Environment 16, 109–122.

V   Kapanen, A., Vikman, M., Rajasärkkä, J., Virta, M., and Itävaara M. 2011. Biotests for environmental quality assessment of composted sewage sludge. Submitted to Waste Management.

Author's contributions

    I   Anu Kapanen was responsible for the experimental work related to eco-toxicity testing and for interpreting the results. The manuscript was prepared in collaboration with Francesco Degli Innocenti.

    II   Jukka Tuominen, Janne Kylmä and Anu Kapanen were jointly responsible for preparation of the manuscript. The research plan, experimental work, and interpretation of the results concerning biodegradability and ecotoxicity were carried out by Olli Venelampi and Anu Kapanen, respectively.

    III   Anu Kapanen was responsible for planning and performing the experimental work and for interpreting the results of the biodegradation and ecotoxicity assessment. The manuscript was prepared by Anu Kapanen, Merja Itävaara and John Stephen.

    IV   Anu Kapanen, Evelia Schettini and Giuliano Vox were jointly responsible for preparation of the manuscript. Anu Kapanen was responsible for the research plan, experimental work and interpretation of the results concerning biodegradability, ecotoxicity testing and microbial diversity.

    V   Anu Kapanen had the main responsibility for preparing and writing the article. She planned the study and was responsible for the experimental work and for interpreting the results, except for the yeast cell assay performed by Johanna Rajasärkkä and composting by Minna Vikman.

Contents

Appendices

   Publications I–V

Paper IV is not included in the PDF version.
Please order the printed version to get the complete publication
(http://www.vtt.fi/publications/index.jsp).

List of symbols

AOB	Ammonia oxidizing bacteria
AOX	Adsorbable organic halogens
ASTM	American Society for Testing and Materials
BD	1,4-butanediol
BDI	1,4-butane di-isocyanate
BFR	Brominated flame retardant
BTA	Poly[(tetramethylene terephthalate)-co-(tetramethylene adipate)]
CA	Cellulose acetate
CAB	Cellulose acetate butyrate
CAP	Cellulose propionate
CEN	European Standardization Committee
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DGGE	Denaturing gradient gel electrophoresis
DIN	Deutsches Institut für Normung
dw	Dry weight
EC	Effective concentration
EEQ	Estradiol equivalency
EU	European Union
GC-MS	Gas chromatography-mass spectrometry
GI	Germination index
GWC	Garden waste compost
HBCD	Hexabromocyclododekane
HMDA	1,6-hexamethylene diamine
HMDI	1,6-hexamethylene diisocyanate
HPLC	High performance liquid chromatography
IF	Induction factor
ISO	International Organization for Standardization
LAS	Linear alkylbenzene sulphonate

LCA	Life cycle analysis
LDPE	Low density polyethylene
LWIR	Long wave infrared radiation
MDA	4,4´-diamino diphenyl methane
MDI	4,4´-diphenyl methane di-isocyanate
MSW	Municipal solid waste
MSWC	Municipal solid waste compost
NP	Nonylphenol
NPE	Nonylphenolethoxylate
OECD	Organisation for Economic Cooperation and Development
PAH	Polycyclic aromatic hydrocarbon
PBAT	poly(butylene adipate terephthalate)
PBDE	Polybrominated diphenylether
PBS	Poly(butylene succinate)
PBSA	Poly(polybutylene succinate adipate)
PBST	Poly(butylene adipate terephthalate)
PCB	Polychlorinated biphenyls
PCDD/F	Polychlorinated dibenzo-p-dioxins and -furans
PCL	Poly(caprolactone)
PCR-DGGE	Polymerase chain reaction – Denaturing gradient gel electrophoresis
PEA	Poly(esteramide)
PES	Poly(ethylene succinate)
PESA	Poly(ethylene succinate adipate)
PHA	Poly(hydroxyalkanoate)
PHB	Poly(hydroxybutyrate)
PHBV	Poly(hydroxybutyrate hydroxyvalerate)
PHV	Poly(hydroxyvalerate)
PLA/PLLA	Poly(lactide)
PNEC	Predicted no effect concentration
PTT	Poly(trimethylene perephthalate)
PU	Polyurethane
PVA	Poly(vinyl alcohol)
PVC	Poly(vinyl chloride)
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
RI	Root growth inhibition
SS	Sewage sludge
T_g	glass transition temperature (°C)
TBBPA	Tetrabromobisphenol A
TPS	Thermoplastic starch

1. Introduction

1.1 Biodegradable plastics

In 2009, annual plastics production was 230 million tons globally, of which about 60 million tons was produced in Europe. Polyethylene was the most abundant of the produced plastics. The share of bioplastics, including both bio-based plastics and biodegradable plastics, was only 0.1–0.2 per cent of the total amount of plastics produced in EU. However, the market share of bioplastics is increasing rapidly (Mudgal et al. 2011; Barker and Safford 2009).

Bioplastics can be either bio-based plastics derived from renewable resources and not necessary biodegradable, or biodegradable plastics that do meet the standards for biodegradability and compostability. Bio-based plastics can be made of natural polymers from renewable sources, polymers synthesised from monomers derived from renewable sources or polymers produced by microorganisms (Anonymous 2011; Mudgal et al. 2011). Biodegradable plastics can be divided into natural or synthetic polymers depending on the origin of their components from renewable or from petrochemical sources. Examples of biodegradable polymers are listed in Table 1. Many types of biodegradable polymers are already on the market and new materials are under development (Di Franco et al. 2004; Cho et al. 2011). Biodegradable products already on the market include packaging and catering products, wrappings, egg cartons, razor handles, toys, food containers, film wrapping, laminated paper, agricultural applications (mulching films, low tunnel films, seed film, planting pots), disposable non-wovens (engineered fabrics), golf tees and hygiene products (diaper back sheets, cotton swabs) (Gross and Kalra 2002; Barker and Safford 2009; Mudgal et al. 2011; Rudnik 2008). For example degradable polyesters are produced through chain linking for bulk applications such as packaging, agricultural and biomedical applications (Seppälä et al. 2004). In the EU, however, bioplastics are mainly used in packaging, loose fill packaging and waste collection bags (Mudgal et al. 2011). In addition, composite materials including natural fibers combined with biodegradable polymers are under development (Di Franco et al. 2004; Finkenstadt and Tisserat 2010).

Table 1. Biodegradable polymers from renewable and petroleum sources (Rudnik 2008).

Polymers from renewable sources	Primary feedstock/ synthesis	Trade names
PLA polylactide	Lactic acid by chemical synthesis or carbohydrate fermentation	Lacea, Lacty, Nature Works, Hycail
PHA poly(hydroxyalkanoates); PHB poly(3-hydroxybutyrate) PHV poly(3-hydroxyvalerate) PHBV poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	Synthesized by bacteria (Carbohydrates, alkanes, organic acids etc.)	Biopol®, Kaneka, Nodax®
TPS thermoplastic starch	Starch	Solanyl, Bioplast TPS, EverCorn, Plantic, Biopar, Placorn
Cellulose; CA cellulose acetate CAP cellulose propionate CAB cellulose acetate butyrate	Esterification of cellulose (wood, cotton, hemp, sugar cane, corn etc.)	Natureflex, Tenite, Bioceta, Cellidor
Chitosan	Deacetylation of chitin from shells of shellfish and sea-food processing wastes	Chitosan
Polymers from petroleum sources	**Primary feedstock/synthesis**	**Trade names**
Aliphatic polyesters and copolyesters; PBS poly(butylene succinate) PBSA poly(butylene succinate adipate) PES poly(ethylene succinate) PESA poly(ethylene succinate adipate)	Synthetic polyesters made by polycondensation with raw materials from petrochemical feed stock	Bionolle®, SkyGreen
PCL poly(caprolactone)	Linear polyester by ring opening polymerization of a ε-caprolactone	Tone, CAPA, Placcel
PEA poly(esteramide)	Polycondensation of α-amino acids, aliphatic dicarboxylic acids (or dichloride of dicarboxylic acids), and diols	BAK
PVA poly(vinyl alcohol)	Polymerization of vinyl acetate	Mowiol, Erkol, Sloviol, Polyvinol, Elvanol etc.

Polymers from petroleum sources	Primary feedstock/synthesis	Trade names
Aromatic copolyesters; PBST poly(butylene succinate terephthalate) PBAT poly(butylene adipate terephthalate) PTT poly(trimethylene terephthalate)	Polycondensation with raw materials from petrochemical feed stock	Biomax®, Eastar Bio®, Ecoflex®, Sorona™, Corterra®, PermaStat

Blends	Primary feedtock/ synthesis	Trade names
Cellulose derivative/starch blends	Mater-Bi, Ecostar, Ecofoam, Biograde, Biolex, Fasal, Cereplast	

1.1.1 Environmental fate of biodegradable plastics

Drivers favouring bioplastics production and consumption over conventional plastics are reduced landfill capacity, pressure from retailers, consumer demand, legislation-based concern over fossil-fuel dependence and greenhouse gas emissions, reduction in the environmental impact associated with disposal of oil-based polymers and decrease in plastic waste production by novel design of plastics and increased use of bioplastics (Mudgal et al. 2011; Song et al. 2009). Key considerations in biodegradable plastics production are availability of farming land for cultivation of feed stock for biopolymer production, competition with food crop production, possibilities for alternative feed stocks such as side streams from industry and algal biomass, and amount of renewable energy needed for their manufacturing (Anonymous 2011; Mudgal et al. 2011). It has been stated that life cycle analysis (LCA) assessment of the environmental impact of bioplastics and redesigned plastics should focus more on end-of-life analysis (Anonymous 2011). For example, greenhouse gas emissions during the degradation phase of biodegradable plastics have been criticized. However, CO_2 emission at the end of life of bioplastics is balanced during plant growth (Mudgal et al. 2011). The ideal closed life cycle of biodegradable plastics in presented in Figure 1.
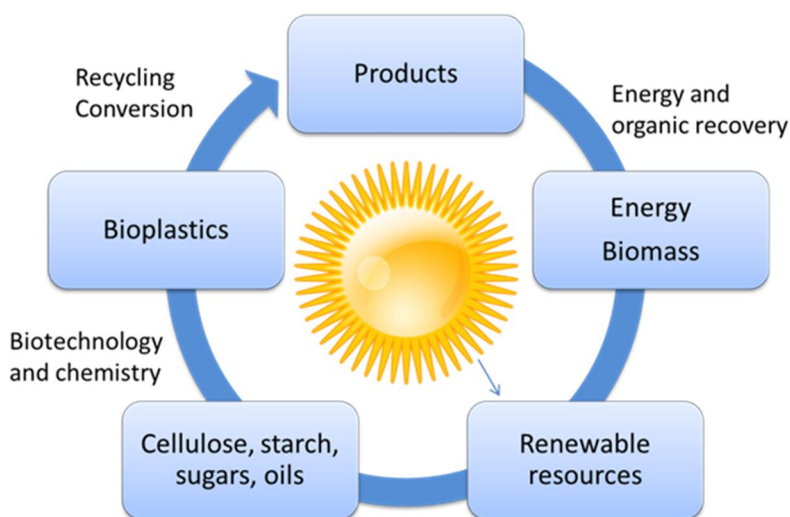
Figure 1. Idealized closed life cycle for biodegradable plastics (Based on Anonymous 2011).

Increasing production and utilization of biodegradable polymers creates pressure for acquiring knowledge on suitable disposal options for biodegradable polymer wastes (Cho et al. 2011). Landfill directive (1999/31/EC) includes targets for e.g. reduction of the amount of biodegradable waste in landfills, and promoting recycling and composting. This also applies to the recycling/composting and disposal of biodegradable polymers. In addition, Thematic Strategy on the Prevention and Recycling of Waste in EU (COM [2005]

666) focuses on prevention of waste production and reducing the environmental impact. Use of biodegradable polymers instead of conventional plastics may reduce the costs of waste management and the risk of accumulation of plastic materials in the environment. Most essential is that suitable waste treatment options and collection systems are available for different types of materials (Favoino 2005).

Disposal options for bioplastics after their use include anaerobic digestion, composting, gasification, incineration, landfilling, mechanical biological treatment, pyrolysis, waste-to-energy approach and recycling (Barker and Safford 2009; Mudgal et al. 2011). In some cases biodegradable plastics may be recycled to monomers or oligomers after use (Siotto et al. 2011). In addition, in agricultural applications biodegradable polymers can be degraded in soil. The actual degradation rate of biodegradable plastics under different environmental conditions, especially when present in large quantities, has been discussed and is becoming a concern due to lack of data (Cho et al. 2011). Biodegradation properties of biodegradable polymers in a particular environment determine the possible applications and suitability of different disposal technologies for managing biodegradable polymer waste.

Plastics contain additives to enhance their performance. Additives can be inorganic fillers to strengthen the plastic material, thermal stabilizers to improve processability at high temperatures, plasticizers to give flexibility, fire retardants, and UV stabilizers to hinder degradation when exposed to sunlight (Andrady and Neal 2009). Millions of tonnes of plasticizers are incorporated into conventional and biodegradable plastics every year. Today, plasticizers such as phthalates are found in many mass-produced products including medical devices, food packages, perfumes, cosmetics, children's toys, flooring materials, computers, CDs, etc. Currently plasticizers are ubiquitous in the environment. For example the fact that phthalates, which are not classified as persistent, can be found widely in the environment argues against their rapid biodegradation in the environment (Fromme et al. 2002; Staples et al. 1997b; Horn et al. 2004; Oehlmann et al. 2009).

1.1.2 Biodegradation

Materials can be defined as biodegradable if they fulfill the criteria for biodegradability set in standardization organizations such as ISO, ASTM, DIN or OECD. A (non-exclusive) overview of standard tests for biodegradability assessment of biodegradable plastics in aquatic, soil and compost environments is presented in Table 2. Bio/degradability can be defined in several different ways. The definition for biodegradability and compostability of polymeric materials in the context of the European packaging regulation is described in Table 3. Biodegradability criteria require that the organic carbon of the material should decompose to harmless end products during a specified time frame set in standards (Itävaara and Vikman 1996).

Biodegradability of a polymer is affected by the structure of the polymer and by prevailing environmental conditions. Environmental conditions affecting degradation rate include e.g. temperature, moisture, amount of oxygen, acidity, and number and diversity of microbes (Briassoulis 2007; Kyrikou and Briassoulis 2007). Biodegradable polymers can be degraded by microorganisms such as bacteria, fungi, and algae (Gross and Kalra 2002; Müller 2005a; van der Zee 2005; Cho et al. 2011; Luckachan and Pillai 2011). In the environment polymer degradation is often a combination of chemical and biological hy-

drolysis. In addition, the polymer chain can be broken down by photo degradation or wearing due to environmental stress. Final degradation products of biodegradable plastics are water, CO_2, CH_4, humic substances, biomass and other natural substances.

Table 2. Standards for biodegradability assessment of biodegradable plastics in aquatic, soil and compost environments.

Environment	Standard for biodegradability assessment	
Aquatic	ISO 14852:1999	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by analysis of evolved carbon dioxide
	ISO 14851:1999	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by measuring the oxygen demand in a closed respirometer
	ISO 14853:2005	Plastics – Determination of the ultimate anaerobic biodegradation of plastic materials in an aqueous system – Method by measurement of biogas production
	EN 14047:2003	Packaging. Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium. Method by analysis of evolved carbon dioxide
	EN 14048:2003	Packaging. Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium. Method by measuring the oxygen demand in a closed respirometer
	ASTM D6691-01	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium
Compost	ISO 14855-1: 2005	Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide
	EN 14046:2003	Packaging. Evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions. Method by analysis of released carbon dioxide
	EN 14045:2003	Packaging. Evaluation of the disintegration of packaging materials in practical oriented tests under defined composting conditions
	EN 14806:2005	Packaging. Preliminary evaluation of the disintegration of packaging materials under simulated composting conditions in a laboratory scale test

Environ-ment	Standard for biodegradability assessment	
	ASTM D5338-98	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions
	ASTM D5929-96	Standard Test Method for Determining Biodegradability of Materials Exposed to Municipal Solid Waste Composting Conditions by Compost Respirometry
	ASTM D5951-96	Standard Practice for Preparing Residual Solids Obtained After Biodegradability Standard Methods for Plastics in Solid Waste for Toxicity and Compost Quality Testing
	ASTM D6340-98	Standard Test Methods for Determining Aerobic Biodegradation of Radiolabeled Plastic Materials in an Aqueous or Compost Environment
Soil	ISO 17556:2003	Plastics – Determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved
	ASTM D5988-96	Standard Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residual Plastic Materials After Composting
Sewage sludge	ASTM D5210-92	Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge

Table 3. Definitions for biodegradability and compostability of packaging materials (EN 13193; Pagga 1998; Müller 2005a).

Target function	Definition
Degradation	Irreversible process leading to a significant change in the structure of a material, typically characterized by a loss of properties (e.g. integrity, molecular weight or structure, mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions, proceeds over a period of time and may involve one or more steps
Degradable	A material is called degradable in specific environmental conditions if it undergoes degradation to a specified extent within a given time measured by specific standard test methods.
Biodegradation	Degradation caused by biological activity, especially by enzymatic action, leading to a significant change in the chemical structure of a material.
Inherent biodegradability	The potential of a material to be biodegraded, established under laboratory conditions.
Ultimate biodegradability	The breakdown of an organic chemical compound by microorganisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and new biomass or in the absence of oxygen to carbon dioxide, methane, mineral salts and new biomass.
Compostability	Compostability is the potential of a packaging to be biodegraded in a composting process. In order to claim compostability it must have been demonstrated by standard methods that a packaging can be biodegraded in a composting system. The end product must meet the relevant compost quality criteria.

Polymer surface plays an important role in the degradability of polymers. Both hydrophilic nature and crystallinity of the polymer influence its degradation behaviour and accessibility of the polymer surface. Semicrystalline nature of a polymer limits the accessibility and restricts the degradation in the amorphous region of the polymer, although highly crystalline starch and bacterial polyester have been reported to hydrolyse rapidly (van der Zee 1997). Important chemical properties affecting degradation are chemical linkages in the polymer backbone, position and activity of the pendant groups and chemical activity of end-groups of the polymer chain (van der Zee 1997). Furthermore, chemical hydrolysis may be affected by the type of copolymer composition, temperature and pH (van der Zee 2005; Luckachan and Pillai 2011).

In the environment, biodegradation is always accompanied by abiotic degradation. Hakkarainen (2002) discussed the degradation of polylactide, polycaprolactone, poly(3-hydroxybutyrate) and their copolymers in different abiotic and biotic environments. For example the molecular weight of PLA and PLA oligomers was decreased initially by abiotic hydrolysis. However, after the initial abiotic hydrolysis PLA degraded faster in biotic

medium than in abiotic medium. In their study, there were also differences detected in biotic and abiotic degradation products. Temperatures higher than glass transition temperature increase the flexibility and water adsorption of PLA polymer matrix. In addition, chemical hydrolysis and the attachment of enzymes and microbes is improved (Itävaara et al. 2002a).

Biodegradation processes have been found to occur more slowly in real life conditions, and therefore the real environmental fate of materials should also be studied. For example, criteria for compostability include in addition to the biodegradability test, a disintegration test of the material in composting conditions followed by phytotoxicity test of the end product (EN 13432; EN 14995; Venelampi et al. 2003). Field tests have many limitations when biodegradability of polymers is studied. Weather conditions such as temperature and rainfall may vary locally and geographically during the experiments, resulting in high variation in results. Therefore controlled laboratory tests measuring intrinsic biodegradability and simulating natural environments are also needed (Müller 2005b).

1.1.2.1 Biodegradable plastics in compost

Composting of solid waste is commonly considered as a suitable treatment method for green waste from gardens or biowaste from kitchens. In addition, many materials such as waste from the food industry, packaging materials made from paper, cardboard and wood, and biodegradable plastics of natural origin or synthesized, are suitable for composting when not recyclable in other ways (Bastioli 1998; Kapanen and Itävaara 2001; Pagga 1997; Degli-Innocenti and Bastioli 1997). Basic requirements for compostability are biodegradation, disintegration and compost quality (De Wilde 2005) (Table 4). DIN 54900 (1998), EN 13432 (2000) and EN 14995 (2006) include the requirement that introduction of man-made polymers into the organic waste recovery must not influence negatively the quality of the final product. Quality criteria for compost after degradation of biodegradable material include an ecotoxicity test with two plant species in EN 13432 (2000) and EN 14995 (2006), or in DIN 54900 (1998) ecotoxicity with summer barley. According to EN13432 (2000) and EN 14995 (2006), ecotoxicity of biodegradable plastics should be tested after three months of composting. Concentration of the material in compost sampled for ecotoxicity assessment should be very high, 10% of the total wet weight of the organic waste to be composted.

PLA, PHB, TPS, cellulose, chitosan, proteins, aliphatic polyesters and copolyesters (e.g. PBS and PBSA), aromatic copolyesters (e.g. PBAT), PCL, PEA, and PVA are examples of compostable polymers (Rudnik 2008). Temperature is the main factor affecting the biodegradation behaviour of PLA during composting (Itävaara et al. 2002a; Kunioka et al. 2006; Kale et al. 2007a). Itävaara et al. (2002a) studied biodegradability of poly-L-lactide in bench scale composting reactors in which the maximum temperature increased over 70 ºC. Thermophilic conditions accelerated the biodegradation of PLLA probably due to enhanced hydrolysis followed by enhanced biodegradation. Kale et al. (2007a) observed some residues of PLA bottles after 30 days of composting with maximum temperature over 60 ºC, but deli containers made of PLA were fully degraded. According to Rutkowska et al. (2001), degradation of PHB and PHBV in compost takes about 4 to 5 weeks. In addition, thermoplastic starch and different starch blends are widely targeted for applications in which one of the waste treatment options is composting (Vikman et al.

1995; Degli Innocenti et al. 1998). The degradability of PBSA, PCL, PEA and PBAT is high, between 90% and 100% (Rudnik 2008; Eldsäter 2000; Yang et al. 2005). Zhao et al. (2005) reported that PBS in powder form degraded at best 72% in 90 days of composting. Starch–polyester blend (Bionolle) was totally mineralized in a compost environment during 45 days (Jayasekara et al. 2003). Biodegradability of paper products in composting has been demonstrated to be affected by the lignin content and process temperature. There was a strong correlation between lignin content and biodegradability at a temperature of 58°C. High lignin content reduced the biodegradation of paper products (Vikman et al. 2002). Furthermore materials with high lignin content, such as mechanical pulp (27 wt% lignin), degraded better in lower temperatures of 35°C and 50°C than at the temperature of 58°C required by the European standard EN 14046.

Table 4. Standards related to composting of biodegradable plastics (Rudnik 2008; De Wilde 2005).

Standard	Biodegradation	Disintegration	Safety
EN 13432:2000 Packaging – Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging	Biodegradation should be 90% of the degradation of a positive control within six months	10% residues larger than 2 mm allowed	Limit for heavy metals. Physical/chemical analysis. Ecotoxicity assessment includes plant growth assay with two plant species.
EN 14995:2006 Plastics. Evaluation of compostability. Test scheme and compostability	Biodegradation should be 90% of the degradation of a positive control within six months	10% residues larger than 2 mm allowed	Limit for heavy metals. Physical/chemical analysis. Ecotoxicity assessment includes plant growth assay with two plant species.
ISO 17088:2008 Specifications for compostable plastics	60% mineralization for homopolymers in 180 days. For other polymers 90% mineralization during 180 days	10% of original dry mass allowed in size larger than 2 mm after 84 days in a controlled composting test	Heavy metals Volatile solids Ecotoxicity assessment includes plant growth assay with two plant species.
ASTM 6400-04 Standard specifications for compostable plastics	60% mineralization for homopolymers in 180 days. For other polymers 90% mineralization during 180 days	10% of original dry mass allowed in size larger than 2 mm after 84 days in a controlled composting test	No adverse effect on compost as a growth medium. Heavy metals.

There are several labeling or certification systems based on standardization of compostability of biodegradable plastics. These labels ensure the suitability of packaging materials to be disposed of through composting processes. DIN CERTCO is based on

DIN EN 13432, AIB Vincotte OK Compost on EN 13432, Compostable on ASTM D6400, and GreenPla on JIS K 6953 (Biodegradable Plastics Society, Japan). In Finland the Finnish Solid Waste Association FSWA gives a compostability logo for materials that fulfil the requirements set in EN 13432 for compostable packaging materials (Figure 2) (Kale 2007b; Rudnik et al. 2008).

Figure 2. Logos in compostability certificates a) Compostable (FSWA/EN) b) DIN CERTCO (DIN, EN) c) AIB Vincotte (EN) d) US composting council (ASTM).

Quality of compost is related to agronomical quality and commercial value. Quality requirements for compost product include both analytical and biological criteria. Volumetric weight, total dry solids, volatile solids, salt content, amount of inorganic nutrients (total nitrogen, phosphorus, magnesium or calcium and ammonium nitrogen) are analytical parameters to be measured. In addition, hygienic quality of the compost product must be ensured. Chemical safety of the compost product is characterized by chemical analysis. There are limit values set only for heavy metals and no criteria for ecotoxicological analysis. However, it is very difficult to evaluate the quality of compost based on solely chemical analysis (Kapanen and Itävaara 2001). Biotests are needed for quality assessment of the compost products.

1.1.2.2 Biodegradation in soil

Biodegradable plastics can enter soil intentionally or unintentionally through the use of compost as a soil improver, littering, from mulching or other agricultural applications (Degli Innocenti 2005). Type of soil, pH, organic matter etc. have a major influence on the biodegradation rate of polymers in soil. In addition, many factors such as irradiation, heat, rainfall, irrigation, macroorganisms and activity of microorganisms affect biodegradation in soil. Irradiation may reduce polymer molecular weight and increase biodegradability. At the same time, irradiation may also induce crosslinks resistant to degradation. On the other hand, heat from sunlight may locally increase temperature, thus increasing microbial activity or melting the polymer. Available water increases hydrolysis and leaching of plasticizers that enable active biodegradability. Macroorganisms may make polymers brittle by mechanical stress (Degli Innocenti 2005).

Biodegradable products used in agriculture, such as low tunnel films, mulching films, bale wraps and irrigation tubes, are targeted to control weeds, conserve water, limit the use of fertilizers, pesticides, and herbicides, increase the yield and quality of horticultural products, advance harvesting time, limit erosion, control temperature, and protect plants

(Briassoulis 2006; Briassoulis 2007; Kyrikou and Briassoulis 2007). Biodegradable films used in agriculture should be fully biodegradable in soil after use and should not leave any harmful substances in soil after degradation. Concerns about the use of degradable agricultural plastics relate to the acceptable time for degradation of the films in soil (Kyrikou and Briassoulis 2007). Good evidence for adequate degradation properties in soil has been reported for e.g. poly(hydroxybutyrate) (PHB) and its copolymers, poly(caprolactone) (PCL), polybutylene succinate adipate (PBSA) and some starch blends such as Mater-Bi (Briassoulis 2007; Calmon et al. 1999; Kim et al. 2000; Ratto et al. 1999). Recently, poly(lactic acid) Osage Orange wood composite was introduced as mulching film with controlled release of phyto-active agents naturally present in Osage Orange wood (Finkenstadt and Tisserat 2010). Use of biodegradable mulching films in agriculture has increased. However, due to soil being a very complex matrix standardization of the test methods for biodegradability in soil is challenging (Müller 2005a; Siotto et al. 2011).

1.2 Sewage sludge

Annual production of sewage sludge in Europe is 10 million tons (dry matter), of which 160 000 tons is produced in Finland (EC, 2008; Laturnus 2007). Utilization of sewage sludge or composted sewage sludge as a fertilizer in agriculture or as a soil conditioner provides a sustainable way of recycling nutrients from sewage sludge back to soil (Ødegaard et al. 2002). In addition, applications on farmland increase the organic matter content and water retention capacity in soil, prevent soil erosion, improve soil structure and reduce plant diseases (Poulsen and Bester 2010). However, sewage sludge contains pathogens, heavy metals and organic pollutants that limit its applicability as a fertilizer in agriculture and as a basis for growth media (Laturnus et al. 2007; Eriksson et al. 2008). These risks are controlled by legislative means and EU is currently reviewing the Sewage Sludge Directive (86/278/EEC). A Working Document of Sludge and Biowaste is under discussion (WD 2010; WD 2000) and sets the frame for monitoring the quality of sludge in Europe. According to the legislation the use of sewage sludge should not create any risk for the health or safety of animals or humans. Currently there are limit values for heavy metals (As, Hg, Cd, Cr, Cu, Pb, Ni, Zn), certain pathogens (Salmonella, *Escherichia coli*, plant pathogens) and impurities (weed seeds etc.). Moreover, several European countries have limit values for organic contaminants. Limit values for organic contaminants are also proposed in Working Document of Sludge (WD 2000; WD 2010). Current and suggested limit values for organic contaminants in sewage sludge used as soil improvers are presented in Table 5.

In Finland, EU directives are implemented in national legislation. Utilisation of sludge is regulated by several acts and regulations. Act on Fertilizers (Lannoitevalmistelaki 539/2006) targets the quality of fertilisers used on land. The Finnish Environmental Protection Act (Ympäristönsuojelulaki 86/2000), Health protection Act (Terveydensuojelulaki 763/1994) and Waste Act (Jätelaki 1072/1993) focus on reduction of negative health and environmental impacts related to the utilisation of sludge. A Council of State decision on the use of sewage sludge in agriculture (Valtioneuvoston päätös puhdistamolietteen käytöstä maanviljelyksessä 282/1994) regulates sludge treatment and especially removal of pathogens and odour and contains limit values for metal concentrations in sludge.

Table 5. Limit values and suggested limit values for organic contaminants in sewage sludge used as soil improver (WD 2000; WD 2010; Andersen 2001; Laturnus et al. 2007; Gawlik and Bidoglio 2006; EC 2008).

mg kg^{-1} dw	EU (WD 2000)	Gawlik and Bidoglio (2006)	Option 2 (EC 2008)	Option 3 (EC 2008)	EU (WD 2010)	Denmark	Sweden	Germany	France	Austria
AOX	500	400–600				–	–	500	–	500 a,b,f
LAS	2600	–		5000		1300	–	–	–	–
DEHP	100	[1]				50	–	–	–	–
NPE[3]	50	50–100		450		10	100	–	–	–
PAH	6	1–3[2]	6	6 (PAH-3)	6 (2[4])	3	3	–	2–5 n 1.5–4 o	6 f
PCB	0,8	0.6–1	0.8	0.8		–	0.4	0.2s	0.8 m	0.2 a,b,d 1 f
Toluene	–	–				–	5	–	–	–
PBDE		[1]								
PCDD/F ng TE/kg dw	100	50–200		100		–	–	100	–	100 a,b,d 50 f

[1] more information needed; [2] benzo[a]pyrene, analysis of other PAHs needs discussion; [3] includes nonylphenol; [4] or benzo[a]pyrene
a = lower Austria; b = upper Austria; c = Burgenland; d = Vorarlberg; e = Steiermark; f = Carinthia
m = Sum of seven principal PCBs (PCB 28, 52, 101, 118, 138, 153, 180)
n = fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene
o = When used on pasture land, s = For each one of the six congeners

1.2.1 Organic contaminants in sewage sludge

There are over 500 organic compounds which could potentially be present in sewage sludge (Harrison et. al. 2006; Eriksson et al. 2008). In the latest draft for sewage sludge and biowaste legislation there is limit value proposed for the total amount of PAH or alternatively for benzo[a]pyrene. Currently heavy metals do not raise concern in most European countries or in the USA (Poulsen and Bester 2010). In the EU, the importance and risks of several organic pollutants in sewage sludge have been acknowledged. Organic contaminants of concern are adsorbable organic halogens (AOX), linear alkylbenzene sulphonates (LAS), nonylphenols and nonylphenolethoxylates (NP and NPE), diethylhexylphthalate (DEHP), polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F), which are listed in Working Document on Sludge 3rd draft (WD 2000) (Amlinger et al. 2004; Andersen, 2001; Brändli et al. 2004; Erhardt and Pruess 2001; Gawlik and Bidoglio 2006; EC 2008). In addition, polybrominated diphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD), known as brominated flame retardants (BFR), are some of the emerging contaminant groups (de Wit 2002; Hale et al. 2003; Hale et al. 2006). The presence of several pharmaceuticals, antioxidants, fragrances and flavours is also one of the concerns when sludge is used as a fertilizer or soil conditioner (Eriksson et al. 2008). The number of potentially hazardous compounds is high, and Eriksson et al. (2008) identified 99 hazardous compounds in sludges. When micropollutants originate from small waste water streams such as private households or small enterprises, the emissions are very difficult to eliminate (Poulsen and Bester 2010). More information is needed concerning the degradation behaviour and toxicity of these hazardous compounds identified in sludge. According to Eriksson et al. (2008), lack of toxicity data is most critical in the case of soil-dwelling organisms.

Disposal routes for sewage sludge include application to land, composting, landfill disposal, incineration and sea disposal (Ødegaard et al. 2002). Quality of sewage sludge can be improved by composting, which reduces the amount of biodegradable contaminants in the end product (Moeller and Reeh 2003; Amir et al. 2005; Jensen and Jepsen 2005; Ramirez et al. 2008; Marttinen et al. 2004; Cheng et al. 2008; Poulsen and Bester 2010). Successful reduction of linear alkylbenzene sulphonates (LAS), nonylphenols (NP), nonylphenolethoxylates (NPE), diethylhexylphthalate (DEHP) and some polycyclic aromatic hydrocarbons (PAHs) by composting has been reported (Stamatelatou et al. 2011) and some of the reported levels of organic pollutant reductions through sewage sludge composting are presented in Table 6.

Table 6. Reduction of LAS, PAH, NPE and DEHP by composting.

Compound	Reduction	Composting	Reference
LAS	> 95%	Laboratory scale (10 l)	Moeller and Reeh 2003
	75%	Full scale	Amundsen et al. 2001
	Half-life from 7 to 33 days	Sludge-amended soil	Ying 2005
PAH	70% in 25 days	Laboratory scale (10 l)	Moeller and Reeh 2003
	15.8% to 48.6% in 76 days	Compost bin (30 L)	Oleszczuk 2007
	88% in 180 days	Pilot scale compost pile	Amir et al. 2005
NPE	50%	Full scale	Amundsen et al. 2001
DEHP	69% (55 °C) in 25 days	Laboratory scale (10 l)	Moeller and Reeh 2003
	91% (65 °C) in 25 days		
	> 85% in 18 days	Compost reactor (110 l)	Cheng et al. 2008
	93% in 24 days	Full scale compost pile	Poulsen and Bester 2010
Phthalates	from 34% to 64%	Full scale	Amundsen et al. 2001
	from 34% to 58% in 85 days	Compost bin (220 l)	Marttinen et al. 2004
	32% in 28 days	Rotary drum (5 m^3)	

With composting both the amount and the bioavailability of organic pollutants can be reduced. Test duration and composting conditions have an effect on the removal efficacy of organic contaminants. Amir et al. (2005) observed that the starting concentration of DEHP as well as process temperature had an effect on the DEHP removal efficacy. For example PAHs and DEHP have high affinity for organic matter (Jensen and Jepsen 2005). Low concentration or low bioavailability might lead to slow degradation rate. Reduction of PAHs has been reported to be efficient in the maturation phase of composting processes (Amir et al. 2005). Oleszczuk et al. (2007) observed that the share of mutagenic and carcinogenic five- and six-ring PAHs decreased during the 76 days of composting of municipal sewage sludge. Many of the surfactants are reported to be biodegradable (Ying 2005). Efficient biodegradation rates for surfactants such as LASs and NPEs during composting have been described (Amundsen et al. 2001). If the composted sewage sludge is used as a soil amendment, the remaining organic contaminants in compost may undergo further processes of degradation, stabilization and adsorption into the organic matter in soil.

1.3 Ecotoxicity assessment

The effect of a compound or mixed contamination on an organism can be assessed with a biotest. The goal of ecotoxicity assessment is to understand the link between the concentrations of chemicals and effects on organisms in the environment (Rudnik 2008). In addition, ecotoxicity assessment provides tools to conduct risk assessment of chemicals (Pessala 2008). Using ecotoxicity data, environmentally relevant concentrations of chem-

icals, e.g. predicted no-effect concentration (PNEC) can be estimated. In the EU, REACH regulation (Registration, Evaluation, Authorisation and Restrictions of Chemicals) (EC 1907/2006) requires chemical safety assessment measures that include ecotoxicological evaluation of chemicals. While REACH regulations cover the chemical safety aspect in Europe, European norms EN 13432 and EN 14995 regulate material compostability criteria including a requirement for plant growth assay as a compost quality measure.

Ecotoxicity tests can be classified according to the duration (acute or chronic), the mode of effect (death, growth, or reproduction), the effective response (lethal or sublethal) or specific response (carcinogenicity, mutagenicity, immunotoxicity, and neurotoxicity) (Landis and Yu 1995; Kroes 1995). Ecotoxicity of solid or poorly water soluble samples such as polymers, soil, compost and sewage sludge can be measured from aqueous extracts or directly from the solid sample. There are many ASTM, EU, ISO, OECD and DIN standard methods available that can be applied when ecotoxicity of biodegradable materials such as compost products, biodegradable packaging materials, biodegradable plastics and paper products is evaluated. Some of the standards for soil ecotoxicity assessment are listed in Table 7. However, there is no established way of applying these methods for evaluating the ecotoxicity of compost and soil amendments such as sludges and mixtures of these (Kapanen and Itävaara 2001). Only the phytotoxicity test is included in the standards on degradability of polymers or packaging materials. By contrast, no ecotoxicity testing is included in legislation related to sludge quality. There are only a few recommendations for suitable ecotoxicity tests to be used in evaluation of ecotoxicity of biodegradable polymers, compost or soil samples. Itävaara et al. (1998) recommended the acute test with Collembola (ISO 11267) and ammonium oxidation test (ISO 15685) for compost toxicity assessment. In addition, Daphnia test was used and also recommended for testing ecotoxicity of solid samples by Fritz (1999). However, the evaluation of ecotoxicity test results requiring an extraction step is challenging (Joutti and Ahtiainen, 1994).

Evaluation of ecotoxicity of biodegradable materials at the stage of material or product development ensures the environmental safety of products released to the market. Polymers designed to be biodegradable during composting or in soil should degrade to harmless end products. Correspondingly, sewage sludge should be treated with the best available technology to reduce the chemical burden on the environment. The biodegradability and ecotoxicity of the materials should be verified with standardized methods. Furthermore, the biodegradability methods applied should be chosen according to the intended end use of the product (Itävaara et al. 2002a). If there is a need for analysing the concentration of a specific metabolite or residue, it is recommended to use an inert mineral matrix, e.g. vermiculite, for testing the biodegradation and to perform chemical analysis (Tosin et al. 1998). The environmental impact of biodegradable materials is difficult to assess based only on the concentration of chemical constituents; biotests are necessary to complement the evaluation (Fritz 2005; Kapanen and Itävaara 2001).

Table 7. Standardized ecotoxicity tests available for soil samples.

Trophic level	Standard
Plants	ASTM E 1598, Standard practice for conducting early seedling growth tests (1994)
(Included in EN 13432 and ISO 17088)	ISO 11269 Soil Quality – Determination of the effects of pollutants on soil flora. Part 1. Method for the measurement of inhibition of root growth. Part 2. Effects of chemicals on the emergence and growth of higher plants
	OECD 208. Guidelines for testing of chemicals. Terrestrial Plant Test, Seedling emergence and seedling growth
Soil Fauna	ASTM E 1676, Standard guide for conducting a laboratory soil toxicity test with the lumbricid earthworm *Eisenia fetida*
	ISO 11267 Soil Quality – Effects of pollutants on collembola (*Folsomia candida*) – methods for the determination of effects on reproduction
	ISO 11268 Soil Quality – Effects of pollutants on earthworms (*Eisenia fetida*). Part 1. Method for the determination of acute toxicity using artificial soil substrate. Part 2. Determination of effects on reproduction
	OECD Guidelines for testing of chemicals
	207, Earthworm, Acute Toxicity Tests
	220, Enchytraeid Reproduction Test
	222, Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*)
	232, Collembolan Reproduction Test in Soil
Microorganisms	ISO 21338 Water Quality – Kinetic determination of the effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri** (kinetic luminescent bacteria test)
	ISO 14238 Soil Quality – Determination of nitrogen mineralisation in soils and influence of chemicals on the process
	ISO 14240 Soil Quality – Determination of soil microbial biomass. Part 1. Respiration method. Part 2. Fumigation extraction method
	ISO 15685, Soil Quality – Ammonium oxidation, a rapid method to test potential nitrification in soil
Other	ISO 15799 Soil Quality – Guidance on the ecotoxicological characterisation of soils and soil materials

*Vibrio fischeri is currently reclassified as *Aliivibrio fischeri* (Urbanczyk et al. 2007). In this thesis *Vibrio fischeri* is used in order to be consistent with the articles cited.

In order to obtain reliable results with ecotoxicity tests, the tests applied should be repetitive and measure the desired parameter under variable test environments (Kapanen and Itävaara 2001). It is important to distinguish indirect toxicity resulting from the sample environment such as soil or compost from chemical toxicity originating from chemical, polymer, polymer components or metabolites. In order to avoid the false interpretation of ecotoxicity response when biodegradable materials are studied with biotests, the follow-

ing issues should be taken into account (Fritz et al. 2003; Fritz 2005; Itävaara et al. 2002b; Kapanen and Itävaara 2001):

- active degradation (high microbial activity) resulting in production of organic acids, nutrient deficiency, oxygen deficiency
- storage of microbiologically active samples; accumulation of alcohol, methane, acetic acid
- release of e.g nitrogen from polymer material
- presence of high amounts of undegraded polymer serves as nutrient for e.g. soil fauna
- high ammonium concentration in compost or in sludge
- salinity
- effect of humic substances on toxicity, colour
- compost immaturity.

Active biodegradation of polymers in soil may inhibit the growth of higher plants and sometimes also affect daphnia and bacteria. High microbial activity can lead to anaerobic conditions in soil and inhibit plant growth significantly However, the inhibition has been demonstrated to turn to increased plant compatibility after a longer period of time (160d) (Fritz et al. 2003). Fritz (1999) showed with a set of biotests that toxicity caused by readily degradable substances derived from biodegradable polymers during biodegradation in soil decreases during the degradation. Furthermore, for example a high amount of NH_3 released during poly(esteramide) (BAK 1095) degradation in laboratory scale composting was shown to lead to toxic response in biotest (Fritz 1999). However, in field scale composting ammonia concentration would have decreased to non-toxic levels (Bruns et al. 2001).

When ecotoxicity of polymers is studied in a compost environment, natural characteristics of composting phase, e.g. high amount of ammonia, high microbial activity and presence of phytotoxic substances should be taken into account. Itävaara et al. (2002b) showed that immature field scale composts (compost age up to 3 months) induce a toxic response in seedling growth of barley and radish. Phytotoxic response correlated with acute toxicity measured as inhibition of light production of *Vibrio fischeri* (Figure 3). In order to minimize the false positive or false negative results in biotests, maturity of compost should always be determined before using biotest in compost quality assessment (Itävaara et al. 2010).
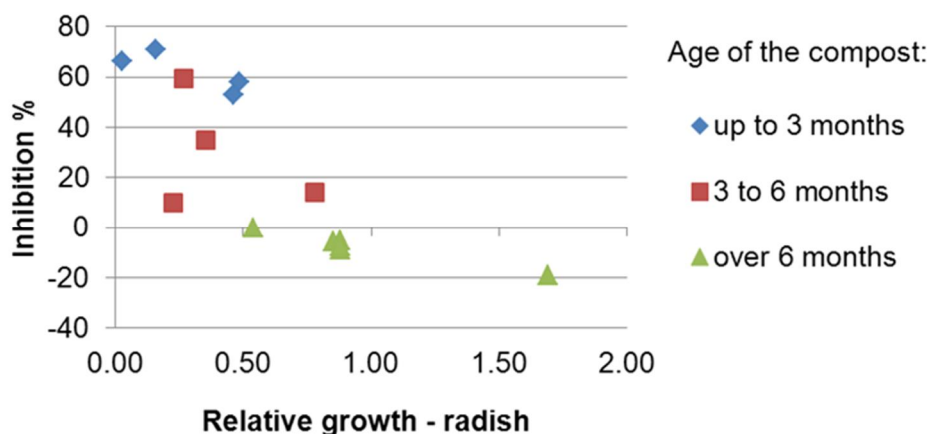
Figure 3. Toxicity of municipal biowaste compost with different stages of maturity (up to 3 months, from 3 to 6 months and over 6 months) measured as inhibition in light production in the kinetic luminescent bacteria test and relative growth in the seedling growth test with radish (Itävaara et al. 2002b).

1.3.1 Ecotoxicity of biodegradable plastics

It is difficult to predict the ecotoxicity of polymers or the presence of ecotoxic degradation products or metabolites from the chemical structure of the polymer alone. However , if the polymer contains heavy metals, or other known toxic or harmful compounds or aromatic monomers are part of the polymer, the probability of harmful metabolites or release of toxic substances increases (Fritz et al. 2003). Furthermore, it is difficult if not impossible to recover the toxic metabolites or residues of biodegradable plastics from compost or soil and therefore combining the biodegradation tests with ecotoxicity tests is recommended (Fritz 2005).

   Biodegradability of biodegradable polymers has been extensively studied but literature on ecotoxicity assessment of biodegradable polymers targeted for compost application or to be used in agricultural applications is scarce (Rudnik et al. 2007; Kapanen and Itävaara, 2001; Kapanen et al. 2002; Witt et al. 2001; Fritz et al. 2003). The available ecotoxicity tests for compost applications were reviewed by Kapanen and Itävaara (2001). The methods applied for the evaluation of the ecotoxicity of natural and synthetic biodegradable polymer materials are mainly based on the use of plants, soil fauna (earthworms), crustaceans (Daphnia), algae (green algae), and microbes (luminescent bacteria). Studies have been performed in different environments; aquatic, compost, and soil environments, depending on the targeted use of the designed polymer (Table 8).

   Luminescent bacteria test has been used as an ecotoxicity assay for several different types of polymers. Of the tested polymers and their degradation products e.g. modified starch cellulose fibre composites and aliphatic aromatic copolyester (Ecoflex®) did not give a toxic response in the luminescent bacteria test (Rudnik et al. 2007; Witt et al. 2001). Accordingly, when ecotoxicity of an aliphatic-aromatic copolyester was studied with a seedling growth test, *Daphnia magna* or luminescent bacteria test during the bio-

degradation process there was no indication of environmental risk (Witt et al. 2001; Rychter et al. 2010). However, Fritz (1999) reported untypical dose-response curves in the luminescent bacteria test due to highly colourful compost eluates, and furthermore dissolved humic substances may inhibit Daphnia mobility in high concentrations (Fritz 1999). By contrast, the presence of starch-polyester blend in compost increased the body weight of earthworms, and partially degraded starch-polyester blend increased the number of earthworm juveniles (Jayeskara et al. 2003). Fritz et al. (2003) also showed that increased amount of organic matter in soil due to addition of biodegradable polymers induces the growth and survival of earthworms. For this reason earthworms are not recommended for use in this type of test.

Additives in biodegradable plastics, if not degraded during the waste treatment process e.g. composting, may be released to the environment. The presence of plasticizer metabolites in the environment has already been reported. Degradation products of plasticizers DEHP and di(2-ethylhexyl) adipate (DEHA), 2-ethylhexanoic acid and 2-ethylhexanol can be resistant to further degradation and in addition cause acute toxicity in microorganisms, crustaceans and fish (Horn et al. 2004).

Table 8. Ecotoxicity assessment of biodegradable polymers.

Sample	Target	Specification/ Standard	Test Organism	End point	Environment	Reference
Aliphatic-aromatic copolyester Ecoflex®	Luminescent bacteria Crustacean	DIN 38412 part 34 and 30	*Vibrio fischeri* *Daphnia magna*	Light emission G_L Mobility	Biodegradation in aquatic environment by the actino-mycete *Termomonospora fusca.*	Witt et al. 2001
Modified starch-cellulose fibre composites	Luminescent bacteria	ISO 11348 /DIN 38412 (LumisTox)	*Vibrio fischeri*	Light emission EC50, EC10	Biodegradation in aqueous medium (ISO 9408).	Rudnik et al. 2007
Starch-polyethylene	Luminescent bacteria	Microtox	*Photobacterium phosphoreum (Vibrio fischeri)*	Light emission $EC50_{5\ and\ 15\ min}$	Aquatic, ASTM type I.	Johnson et al. 1993
Starch, Cellulose, Sawdust, Starch + wood (FASAL F129), Starch + sugar beet residues (ÖKOPUR), poly(esteramide) (BAK 1095), PHB, PLA, PLA-U (lactic acid-polyurethane), Polyesteramide, PBSA	Luminescent bacteria Plant growth Soil fauna Crustaceans Algae	DIN 38412 (LumisTox) ÖNORM S2023 OECD 207 OECD 202/DIN 38412 L30 OECD 201/DIN 38412 L33	*Vibrio fischeri* Cress, rape, millet Earthworm (*Eisenia fetida*) *Daphnia magna* *Scenedesmus subs.* *Chlorella*	Light emission 30 min Fresh weight Mortality Mobility Growth inhibi-tion	Bench scale biodegradation in soil, outdoors, no tempera-ture control, composting.	Fritz et al. 2003; Fritz et al. 1999; Fritz et al. 2001
Poly(esteramide) BAK 1095	Phytotoxicity Gaseous phytotoxic effects Crustaceans	BKG (1998) OECD 202	Barley (*Hordeum vulgare*) Cress seeds (*Lepidium sativum L.*) *Daphnia magna*	Fresh weight Fresh weight Mortality	Controlled composting test (DIN V 54900). Pilot scale composting. Effect of ammonium content.	Bruns et al. 2001
Wheat gluten with glycerol as a plasti-cizer	Microbial activity	ISO 14852 modified Sturm test	Activated sludge inoculum	Degradation rate and miner-alization	Aquatic biodegradation test with activated sludge inocu-lum.	Domenek et al. 2004

Sample	Target	Specification/ Standard	Test Organism	End point	Environment	Reference
Blend of Bionolle and starch	Soil fauna	Modified ASTM E 1676-97	*Eisenia fetida*	Weight, repro-duction and survival	Compost during and after degradation of polymer.	Jayasekara et al. 2003
Poly[(tetramethylen e terephthalate)-*co*-(tetramethylene adipate)] BTA	Seedling growth and germination	OECD 208 ISO 11269-2	Oat (*Avena sativa*) Radish (*Raphanus sativus L.subvar. raducula Pers.*) Cress (*Lepidium sativum L.*)	Dry and fresh weight	Biodegradation in standard soil (4, 10, 16 and 22 months).	Rychter et al. 2010
Mono- and diesters of o-phthalic esters	Luminescent bacteria Green algae Crustacean	ISO 11348-3 Miniscale algal growth inhibition tests ISO 6341	*Vibrio fischeri* *Pseudokirchneriel-la subcapitata* *Daphnia magna*	Light emission EC_{15min} Growth inhibi-tion Mobility	Range of phthalic acid mo-noesters along with their corresponding diesters and phthalic acid.	Jonsson and Baun 2003
Plasticizers: 2-ethylhexanol 2-ethylhexanoic acid	Crustaceans Fish Luminescent bacteria	Microtox	*Daphnia magna* Rainbow trout (*Oncorhynchus mykiss*) Fathead minnow (*Pimephales pro-melas*) *Vibrio fischeri*	Mortality Mortality Body weight Light emission $EC50_{5min}$	Ecotoxicity and presence of plasticizer metabolites in the environment.	Horn et al. 2004

1.3.2 Ecotoxicity of sewage sludge and compost

Sewage sludge and sewage sludge-derived products contain beneficial nutrients and organic matter but in addition they can contain heavy metals, pathogens and organic contaminants. Over 500 organic chemicals are known to be present in sewage sludge (Harrison et al. 2006, Eriksson et al. 2008), and therefore chemical analyses of all possible potential toxic compounds in the sludge and sludge-derived products is impossible. It is essential to combine chemical analysis with ecotoxicity assessment in order to be able to characterize sludge properties. Good characterization of the sludge enables for example the use of waste classification methods supporting the application of sludge on land (Mantis et al. 2005). However, natural characteristics of sewage sludge and compost, such as intense colour, high amounts of nutrients, high microbial activity and mixtures of contaminants presents challenges to performing biotests. Sample characteristics, such as maturity of the compost, affect the success of the ecotoxicity assessment. In order to be able to obtain reliable results from ecotoxicity assessment and to minimize the possibility of false positive results, maturity of the compost must be measured (Kapanen and Itävaara 2001, Itävaara et al. 2002b).

The quality of sewage sludge and composted sewage sludge has been studied with several biotests. Acute toxicity, genotoxicity, and phytotoxicity of sewage sludge as well as evaluation of the endocrine-disruption potential of sludge have been reported (Table 9). Luminescent bacteria test has been the most commonly applied biotest in sewage sludge ecotoxicity assessment (la Farré et al. 2001; Perez et al. 2001; Mantis et al. 2005; Fuentes et al. 2006). In some cases the amount of organic contaminants in sewage sludge could be linked to the toxic response. la Farré et al. (2001) reported sludge extracts containing NPs and NPEs to be toxic in the luminescent bacteria test. By using indirect exposure bioassays with luminescent bacteria and *D. magna* or direct bioassay applying the earthworm bioassay and phytotoxicity tests, Alvarenga et al. (2007) classified a sewage sludge sample to be highly ecotoxic (class 3) or significantly ecotoxic (class 2), respectively. This particular sample was considered unsuitable to be used as a fertilizer due to its high Zn concentration (7620 mg kg^{-1} dw). However, there are also studies in which this correlation between organic pollutants and toxic response was not confirmed. For example, PAH concentration in sewage sludge or compost fertilizers did not correlate with crustacean mortality and plant growth in a study made by Oleszczuk (2010). Park et al. (2005) reported different toxicity levels in luminescent bacteria test and in a test with rotifer for sludge samples derived from variable sources. However, the authors did not find significant correlation between toxicity response and the amount of pollutants measured. Based on ecotoxicological properties of organic pollutants and their estimated no-effect concentrations for soil-dwelling species, Jensen (2004) proposed that some cut-off values of organic contaminants in sludge could be raised. The suggested cut off values of 2500 mg kg^{-1} for LAS, 50 mg kg^{-1} for eight PAHs and 500 mg kg^{-1} for DEHP are considered appropriate to protect agro-ecosystems. However, unwanted accumulation of these contaminants should be avoided.

Luminescent bacteria test was also successfully used for monitoring ecotoxicity of composted sewage sludge, plant waste and municipal waste by Lopez et al. (2010). In their study measured light inhibition decreased during the composting process and mature compost inhibited light production of *Vibrio fischeri* less than 20%. In addition to

the luminescent bacteria test several other biotests have been used effectively for com-composted sewage sludge quality assessment (Table 9). Hamdi et al. (2006) recommended a direct solid-phase chronic toxicity test with ostracod (*Heterocypris incongruens*) for both sewage sludge and compost toxicity assessment. In their study, PAH concentration in composted sewage sludge correlated well with ostracod test response, whereas inhibition in root elongation test and *D.magna* did not correlate with measured PAH concentrations. However, root elongation was sensitive to high amounts of ammonia and soluble salts and *Daphnia magna* to heavy metals and other contaminants present in sewage sludge compost. If composted sewage sludge is used as a soil improver, phytotoxicity is one of the most important tests to be performed. There are several studies available concerning the phytotoxicity of composts and soils amended with sewage sludge (Dubova and Zarina 2004; Hamdi et al. 2006; Alvarenga et al. 2007; Ramirez et al. 2008; Oleszczuk 2008; Natal-da-Luz et al. 2009; Oleszczuk 2010). Plant growth tests with field mustard (*Brassica rapa),* ryegrass (*Lolium perenne)* and red clover (*Trifolium pratence)* revealed that composting effectively reduces the toxicity of digestate and thermally dried sludge measured by phytotoxicity assessment (Ramirez et al. 2008). Immature sewage sludge compost and released decomposition end products from therein have specific effects on different biotests; earthworm biomass and reproduction might be favoured and plant germination and collembola reproduction inhibited. (Domene et al. 2011).

Due to the presence of genotoxic chemicals such as benzo[a]pyrene, fluoranthene, fenantrene and crysene in sewage sludge it is important to evaluate the genotoxic potential of the samples (Klee et al. 2004). Genotoxicity of sewage sludge has been studied with Ames-test, Comet assay and SOS-Chromotest (Klee et al. 2004; Renoux et al. 2001). Klee et al. (2004) observed genotoxicity and mutagenicity in industrial sewage sludge derived from a wastewater treatment plant receiving influents containing pharmaceutical substances, chemical intermediates and explosives by a battery of *in vitro* biotests. Mutagenicity determined with the Ames test decreased after aerobic or anaerobic sludge treatment. Aerobic treatment also reduced the genotoxicity of the sludge, whereas anaerobic treatment was less effective in removing the genotoxicity. In this study the genotoxicity was evaluated by the Comet assay.

Sewage sludge contains many chemicals such as NP, bisphenol-A, dibutylphthalate (DBP) and diethyl hexyl phthalate (DEHP), that have endocrine-disruptive properties (Harrison et al. 2006; Fromme et al. 2002). Endocrine disruption potential (Leusch et al. 2006; Hernandez-Raguet et al. 2007) and the presence of dioxin-like compounds (Engwall et al. 1999; Suzuki et al. 2004; Suzuki et al. 2006) in sewage sludge and compost have been studied with biotests. When Murk et al. (2002) used three different assays, ER-Calux, YES and ER-binding, to predict the oestrogenic potencies in wastewater and sludge, ER-binding assay was the most sensitive of the applied tests. Hernandez-Raguet et al. (2007) demonstrated the reduction of oestrogenic activity of NP-spiked sewage sludge after aerobic sewage sludge treatment by MELN bioassay.

Table 9. Ecotoxicity assessment of sewage sludge (SS) and compost environments.

Sample	Target	Specification/ Standard	Test Organism	End point	Application	Reference
Sewage sludge (SS)	Luminescent bacteria Rotifer	Microtox® Rotoxkit M	*Vibrio fischeri* *Brachionus plicatilis*	Light emission EC50$_{15min}$ Mortality and population growth rate	Urban SS, industrial waste, rural sewage, and livestock waste	Park et al. 2005
SS	Luminescent bacteria	ToxAlert®100	*V. fischeri*	Light emission EC50$_{15min}$	SS from three WWTPs (one influent was 20% tannery waste water)	la Farré et al. 2001
SS	Luminescent bacteria	LUMIStox	*V. fischeri*	Light emission EC50$_{30min}$	Municipal and industrial waste water sludge	Mantis et al. 2005
SS	Luminescent bacteria Phytotoxicity	Microtox	*V. fischeri* Barley (*Hordeum vulgare L.*) Cress (*Lepidium sativum L.*)	Light emission EC50$_{15min}$ Germination index (GI) and root growth	SS from urban WWTP; aerobic, anaerobic or waste pond stabilisation	Fuentes et al. 2006
SS	Luminescent bacteria	ToxAlert®100	*V. fischeri*	Light emission EC50/TII50$_{(15\ and\ 30\ min)}$	Municipal waste water sludge, PAH extractions	Perez et al. 2001
SS	Dioxin-like compounds	EROD induction	Chicken embryo liver culture	bio-TEQ	Dioxin-like compounds in sewage sludge	Engwall et al. 1999
SS	Cytotoxicity Genotoxicity Mutagenicity	Comet assay Ames test	RTL-W1, rainbow trout liver *Salmonella* strains TA98, TA98NR and TA100	Cell viability IF IF	Anaerobic and aerobic treatment of industrial sludge	Klee et al. 2004
Composted SS	Soil fauna Phytotoxicity	ISO 11268-2 ISO 11267 OECD 208	Earthworm (*Eisenia andrei*) Collembola (*F. candida*) Mustard (*Brassica rapa* L var. *oleifera*) Ryegrass (*Lolium perenne* L. var. *Tove*)	Growth, reproduction, survival Reproduction and survival Plant emergence	Composted undigested sludge and digested sludge	Domene et al. 2011

Sample	Target	Specification/ Standard	Test Organism	End point	Application	Reference
SS Garden waste compost (GWC) Municipal solid waste compost (MSWC)	Luminescent bacteria	ISO 11348-2/ LUMIStox ISO 15799	*V. fischeri*	Light emission EC50$_{15/30min}$	Evaluation of a potential application of municipal SS in soil	Alvarenga et al. 2007
	Phytotoxicity	Fuentes et al. 2004	Barley (*H. vulgare L.*) Cress (*L. sativum L.*)	Relative growth (%) and germination index (GI)		
	Soil fauna	ASTM E 1676 (Modified)	*Eisenia fetida*	Mortality (%)		
	Crustaceans	ISO 6341	Daphnia magna	Mobility		
SS Compost	Luminescent bacteria	ISO 21338 ISO 11348-3	*V. fischeri*	Light emission EC50$_{30s}$	Composting SS, plant waste and MSW	Lopez et al. 2010
SS Compost Digestate	Phytotoxicity	OECD 208A	Mustard (*Brassica rapa*) Ryegrass (*Lolium perenne*) Red clover (*Trifolium pretence*)	Seed emergence shoot length	Composting municipal SS and pig slurry	Ramirez et al. 2008
SS and SS amended soils	Bacteria Protozoa Crustaceans Phytotoxicity	ISO 10812 SanPiN No 2.1.7.573	*Pseudomonas putida* *Paramecium caudatum* *D.magna* Radish (*Raphanus sativus*)	Growth inhibition Mortality Mobility Germination, root length	Grey forest soil and municipal SS	Selivanovskaya and Latypova 2003
SS and SS amended soil	Luminescent bacteria	Microtox	*V. fisheri* 15 min	EC50$_{15\ min}$	Bioleaching of metals from SS	Renoux et al. 2001
	Phytotoxicity	OECD	Barley (*H. vulgare L.*) Lettuce (*Lactuca sativa*)	Seed germination, sprout growth, root elongation		
	Soil fauna Crustaceans Genotoxicity	Environ. Canada SOS Chromot.	Earthworm *Eisenia andrei* *Daphnia magna* *Escherichia coli*	Mortality Mortality IF		
SS	Oestrogenic activity	MELN in-vitro bioassay	Human breast cancer cells, with an estrogen-responsive reporter-gene coding for luciferase (ERE-bGlob-Luc-SVNeo)	17-β estradiol equivalent estrogenic activity per kg of dw	Aerobic and anaerobic SS treatment (laboratory scale)	Hernandez-Raquet et al. 2007
Water and SS	Oestrogenic activity	Yeast estrogen screen (YES) ER-CALUX	Rat uterus cytosol Yeast cells T47D human breast adenocarcinoma cells	Estradiol equivalency (EEQ)	Influent, effluent and sewage sludge from industrial and municipal WTPs	Murck et al. 2002

| Sample	Target	Specification/ Standard	Test Organism	End point	Application	Reference
Soil amended with SS	Genotoxicity	M_2 Meiocytes of Zea plants	Corn (*Zea mays*)	Frequency of aberrant meiocytes/meiotic phase	Application of SS as fertilizer for *Zea mays*	Amin 2011
Soil amended with SS	Soil fauna	ISO 11268-2 ISO 11267, ISO 17512-1 and 2	Earthworm *(E. andrei)* Collembola *(F. candida)*	Reproduction and Avoidance	SSs from municipal wastewater treatment plant, olive and electroplating industry	Natal-da-Luz et al. 2009
	Phytotoxicity	ISO 11269-2	Turnips (*Brassica rapa*) Oat (*Avena sativa*)	Seed germination, biomass		
Composted SS, soil with or without SS compost	Crustaceans Phytotoxicity	DAPHTOXKIT™ Ostracodtoxkit F™	*Daphnia magna* *H. incongruens* Lettuce (*Lactuca sativa* var. Melbourne)	Mobility Mortality, growth inh. Root elongation	Spiked anthracene, pyrene and benzo[a]pyrene, 6- month simulated landfarming	Hamdi et al. 2006
SS compost	Phytotoxicity	Phytotoxkit® test Seedling growth	Cress (*L. sativum*)	Seed germination, root growth and biomass	Composted SS (30 L)	Oleszczuk 2008
Composted SS	Dioxin-like compounds	DR-CALUX	Rat hepatoma H4IIE cell line with AHR-regulated luciferase gene construct	CALUX-TEQ	Composted municipal SS (industrial scale)	Suzuki et al. 2004
Composted SS	Microorganisms Phytotoxicity Microbiotests (crustaceans, rotifer, ciliate)	OECD 208 Ostracodtoxkit F™ Protoxkit F™ Rotoxkit F™ Thamnotoxkit F™	Bacteria, fungi Cress (*L. sativum* L.) *H. incongruens* *Tetrahymena thermophile* *Brachionus calyciflorus* *Thamnocephalus platyurus*	Total number Seed germination Mortality and growth inhibition	Composted SS from wastewater treatment plant, cheese whey from a dairy factory, and coniferous and/or deciduous solid wastes– sawdust.	Dubova and Zarina 2004
Compost	Luminesc. bact. Phytotoxicity	ISO 21338 OECD 208	*V. fischeri* Barley (*H. vulgare*), Radish (*Raphanus sativus*)	Light emission $EC50_{30s}$ Seedling growth	MSWC in different maturity and in different process conditions.	Itävaara et al. 2002b

2. Aims of the study

The first aim of this study was to evaluate the ecotoxicity of biodegradable plastics, their components and degradation products during biodegradation in vermiculite, in compost and in soil. The second main goal was to reduce the amount of organic pollutants in sewage sludge by composting and to evaluate the quality of sewage sludge and composted sewage using biotests. The hypothesis of this dissertation was that during the biodegradation process in different environments it is possible to evaluate ecotoxicity of biomaterials and their metabolites with biotests.

The specific aims of the study were

- to evaluate the biodegradability of biodegradable polymers and organic contaminants in laboratory scale, pilot scale and field scale composting (I, II, III, V)

- to study the performance and limitations of ecotoxicity tests during the biodegradation studies in compost and soil environments (I–V)

- to evaluate the sensitivity of the kinetic luminescent bacteria test in measuring the acute toxicity of polymer components, polymer additives, or degradation products (I, II, III, IV)

- to evaluate the acute toxicity, phytotoxicity and effects on microbial diversity of biodegradable polymers, polymer components and their breakdown products during biodegradation assessment in compost or vermiculite (I, II, III, unpublished data).

- to evaluate the performance and the environmental impact of starch-based biodegradable films used for crop protection (IV)

- to evaluate the acute toxicity, genotoxicity and endocrine disruption activity of sewage sludge and composted sewage sludge containing mixtures of organic contaminants (V).

3. Materials and methods

Bio/degradation and ecotoxicity of chemicals, biodegradable polymers and organic contaminants were studied in laboratory, pilot and field scale applications (Figure 4). Experiments were designed to reveal the possible release or presence of harmful substances derived from the studied chemical, polymer, or product to the media under investigation, e.g. compost or soil.
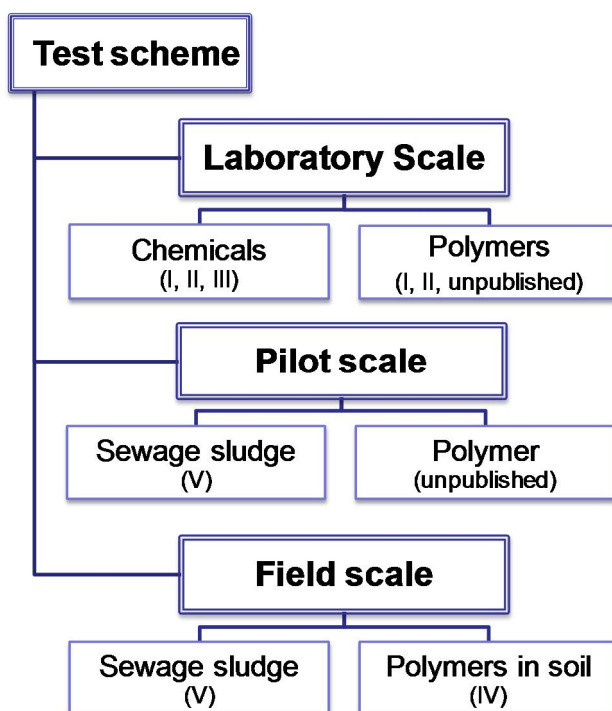
Figure 4. Test scheme for evaluation of biodegradation and ecotoxicity of biodegradable polymers and chemicals in compost and in soil.

3.1 Polymers and chemicals

The polymers and chemicals studied in papers I, II, III and IV are presented in Tables 10 and 11, respectively. In addition, the environment of the experiment is specified.

Table 10. Polymer samples and testing environment in biodegradability and ecotoxicity evaluation (Papers I, II, IV, unpublished).

Polymer /film	Composition and/or company	Environment	Paper
2030/483 Cellulose	Experimental product composed of starch, poly(caprolactone) and poly(urethane) Microcristalline cellulose Avicell (Merck)	Compost Vermiculite (Laboratory scale)	I
E4% PEU 1%H PEU 4%H PEU4% B PEA PLLA	Lactic acid oligomer, 4% BD HMDI -based poly(ester-urethane) HMDI -based poly(ester-urethane) BDI -based poly(ester-urethane) Poly(ester-amide) Polylactide (POLLAIT, Fortum Oil and Gas, Finland)	Compost (Laboratory scale)	II
M0 M1 L0 L1	Non-biodegradable black mulching film (50 μm), LPDE film Black mulching film (50 μm), destructurised starch complexed with biodegradable polyesters (Mater-Bi) Non-biodegradable transparent low tunnel film (60 μm), LPDE film Transparent low tunnel film (60 μm), destructurised starch complexed with biodegradable polyesters (Mater-Bi)	Soil (Field scale)	IV
CA-DEP	Cellulose acetate: milled, granular and film (Dexel S 200–25 + UV, DEP content 25%)	Compost (Laboratory and pilot scale)	un-published data

Table 11. Chemicals and ecotoxicity test environments in papers I, II, and III.

Chemical	Test environment	Paper
4,4´-diaminodiphenyl methane (MDA)	Tested as pure chemical and in compost and vermiculite	I
L-Lactic acid	Aquatic/compost	II
lactide	Aquatic/compost	II
1,4-butanediol (BD)	Aquatic/compost	II
stannous octoate	Aquatic/compost	II
1,6-hexamethylene di-isocyanate (HMDI)	Aquatic/compost	II
1,4-butane di-isocyanate (BDI)	Aquatic/compost	II
1,6-hexamethylenediamine	Aquatic/compost	II
1,4-butane diamine	Aquatic/compost	II
succinic acid anhydride	Aquatic/compost	II
succinic acid	Aquatic/compost	II
2,2´-bis(2-oxazoline)	Aquatic/compost	II
diethyl phthalate (DEP)	Compost based plant growth medium	III

3.2 Sewage sludge and composted sewage sludge

Sewage sludge and composted sewage sludge samples were collected from laboratory and field scale experiments. The ecotoxicities of two different types of sewage sludge samples were evaluated. The sewage sludge sample (A, Paper V) originated from a municipal waste water treatment plant (WWTP) receiving 17% industrial waste water with high organic contaminant concentrations. The other sewage sludge sample (B, Paper V) was from WWTP handling a lower load of industrial waste water (8%) and contained less organic contaminants. In addition, ecotoxicity of composted sewage sludge samples from a pilot scale composting experiment (A, Paper V) and compost samples with different stages of maturity from two municipal field scale composts (1, 2a and 2b, Paper V) was assessed.

3.3 Methods

Biodegradation and/or degradation of biodegradable polymers, chemicals and organic pollutants in municipal sewage sludge were studied in laboratory, pilot and field scale experiments (Figure 4).

In **laboratory scale**, controlled composting test biodegradation was determined as evolved carbon dioxide. In the controlled composting test either mature compost (Papers I and II, unpublished data) or activated vermiculite simulated the composting process (Paper I). In addition, degradation was determined as decrease in concentration of the added component in compost media (Paper III).

In the **pilot scale** composting experiment the activity of the composting process was followed by monitoring evolved carbon dioxide. Degradation of organic pollutants originating from municipal sewage sludge and plasticizer DEP in cellulose acetate film was determined as decrease in concentration (Paper V, unpublished data).

In the **field scale** study in soil, the degradation of mulching films was determined by sieving soil and collecting and weighing the residues of the film (Paper IV). In addition, microbial activity in soil was measured with Solvita ™Soil Life Tests (Paper IV).

Methods for studying biodegradability, ecotoxicity, microbial diversity, microbial activity and compost maturity are listed in Table 12.

Table 12. Methods related to measuring biodegradability and ecotoxicity of biodegradable polymers and organic contaminants in composts, soil and sludge (Papers I–V).

Method		Paper
Bio/degradability or removal		
Chemical analysis	HPLC	I, II, III, V
	GC-Electron Capture Detector	V
	GC-Mass Spectrophotometer (GC-MS)	V
	GC-High Resolution Mass Spectrometer	V
	GC-MS-negative ion chemical ionization detection (GC-NCI)	V
Controlled composting test	EN 14046, CO_2 evolution	I, II
Pilot composting	EN 13432, Disintegration	V, unpub
Degradation in soil	Weight loss	IV
Ecotoxicity		
Acute toxicity	ISO 11348-3 Water Quality - Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (luminescent bacteria test)	II, III
	ISO 21338 Water Quality – Kinetic determination of the effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)	I, II, III, IV, V and unpub
Phytotoxicity	Seedling emergence and growth, OECD 208 Cress (*Lepidium sativum),* Barley (*Hordeum vulgare*) Radish (*Raphanus sativus*)	II, III, and unpub
Soil fauna	ISO 16387 Soil Quality: Effects of pollutants on *Enchytraeidae* – determination of effects on reproduction and survival (*Echytraeus albidus*)	IV
Genotoxicity	Vitotox ™ 10Kit, *Salmonella typhimurium* TA104 *rec*N2-4	V
Endocrine disrupters and dioxin -like compounds	Yeast cell bioreporter, genetically modified *Saccharomyces cerevisiae*	V

Method		Paper
Microbial diversity		
	Total DNA extraction from compost and soil	III, IV
	PCR-DGGE targeting to the bacterial 16S rRNA gene, partial 16S rRNA sequencing (bacteria) and phylogenetic analysis	III
	PCR-DGGE targeting to ammonium oxidizers (amoA), sequencing of amoA	IV
Microbial activity		
Soil respiration	Solvita^{IM} Soil Life Tests	IV
Chemical and physical parameters		
	Dry weight (EN13040)	I–V
	pH (EN 13037)	I–V
	Conductivity (EN 13038)	II, IV, V
	Organic matter (EN 13039)	IV, V
	Water holding capacity (I.M.A 1999 Gazzetta Ufficiale n248)	IV
	N (ISO 11261), P (ISO 11263) and K (I.M.A. 248, 21/10/1999)	IV
Maturity		
Mineralization phase of organic N	nitrate-N/ammonium-N ratio	V
Microbial activity	Evolution of CO_2	V
Phytotoxicity	Germination index (Cress, *Lepidium sativum*) (Method book Itävaara et al. 2006)	V

4.   Results and discussion

4.1 Ecotoxicity of biodegradable plastics

Polymers designed to be biodegradable should decompose to harmless end products such as water, carbon dioxide, methane and biomass (Müller, 2005a; Pagga, 1997; De Wilde and Beolens, 1998). However, in some cases harmful compounds can be released during biodegradation of polymers. Biodegradability and ecotoxicity of biodegradable plastics, polyurethane starch blend 2030/489 and lactic acid polymers prepared by chain linking were evaluated in laboratory scale under controlled composting conditions (Papers I and II).

4.1.1 Acute toxicity of polymer components and degradation products

Acute toxicity of several polymer components and possible degradation products was evaluated by the luminescent bacteria test (Table 13; Papers I, II and III). We determined acute toxicity using two different luminescent bacteria tests, of which ISO 11348 is traditionally used for measuring acute toxicity of chemicals and environmental samples. Kinetic luminescent bacteria test (ISO 21338) was designed especially for coloured and solid samples (Lappalainen 2001). Therefore we used it for acute toxicity assessment of compost, soil and sewage sludge samples. Among the lactic acid -based polymer components, lactic acid, 1,4-butanediol (BD), stannous octoate, 1,6-hexamethylene di-isocyanate (HMDI), 1,4-butane di-isocyanate (BDI), 1,6-hexamethylenediamine (HMDA), 1,4-butane diamine, lactide, succinic anhydride, succinic acid and 2,2-bis(2-oxazoline), only the chain extenders HMDI and BDI showed high toxic response in the luminescent bacteria test with *Vibrio fischeri*, reducing the light production significantly (Paper II) . The $EC50_{30min}$ value for both HMDI and BDI in the traditional luminescent bacteria test (ISO 11348) was 0.02 µg l^{-1} and in the kinetic luminescent bacteria test (ISO 21338) $EC50_{30s}$ was 0.1 and 0.2 µg l^{-1}, respectively. EC50 values measured for the other tested chemicals were at concentration levels not expected to be present in the biodegradation test environment. Because HMDI is hydrolysed rapidly in an aquatic environment, the ecotoxicological tests conducted in aquatic environment also contained the hydrolysis product(s). The PNECaqua value of 77.4 µg l^{-1} for HMDI is derived from the EC50-value for algae using an assessment factor of 1000, and also includes the effect of the hydrolysis product HMDA (OECD/SIDS 2004). In our study, low EC50 value in the kinetic luminescent bacteria test (Table 13) may be due to the very short exposure time resulting

in a lower degree of HMDI hydrolysis to less toxic degradation products compared to other aquatic biotests reported. HMDA has been found to have minor acute toxicity towards freshwater fish species and moderate acute toxicity to *Daphnia magna* and some algal species. HMDA is biodegradable in the activated sludge process; degradation in soil is also possible (OECD/SIDS 1994).

Table 13. Acute toxicity of chemicals studied with the traditional (ISO 11348-3) and the kinetic luminescent bacteria test (ISO 21338) with *Vibrio fischeri.*

Compound	ISO 11348-3 $EC50_{30min}$	ISO 21338 $EC50_{30s}$	Paper
4,4´-diaminodiphenyl methane (MDA)		38 mg l^{-1}	I
lactid acid	11 g l^{-1}	65 g l^{-1}	II
lactide	5.5 g l^{-1}	64 g l^{-1}	II
1,4-butanediol (BD)	15 g l^{-1}	70 g l^{-1}	II
stannous octoate	0.2 g l^{-1}	0.6 g l^{-1}	II
1,6-hexamethylene di-isocyanate (HMDI)	0.02 µg l^{-1}	0.1 µg l^{-1}	II
1,4-butane di-isocyanate (BDI)	0.02 µg l^{-1}	0.2 µg l^{-1}	II
1,6-hexamethylenediamine	36 g l^{-1}	46 g l^{-1}	II
1,4-butane diamine	3.1 g l^{-1}	20 g l^{-1}	II
succinic acid anhydride	43 g l^{-1}	26 g l^{-1}	II
succinic acid	+++[a]	+++[a]	II
2,2´-bis(2-oxazoline)	15 g l^{-1}	51 g l^{-1}	II
diethylene phthalate (DEP)	0.92 g l^{-1}	0.5 g l^{-1}	III

[a] Increase in light production

In addition to the components of lactid acid -based polymers we also studied the possible release and toxicity of 4,4´-diaminodiphenyl methane (MDA) during the biodegradation of 4,4´-diphenyl methane di-isocyanate (MDI)-based thermoplastic polycaprolactone-type polyurethane 2030/489. We determined the $EC50_{30s}$ value for MDA, a degradation product of MDI, which was 38 mg l^{-1} in the kinetic luminescent bacteria test. This concentration level of MDA could potentially be present in compost media. However, toxicity was not detected from compost samples. In vermiculite test media acute toxicity could be detected by the kinetic luminescent bacteria test. The reported EC50 value for MDA in the traditional luminescent bacteria test (ISO 11348-3) after 30 minutes of exposure time is 6.6 mg l^{-1} (EU RAR 2001).

EC50 values for plasticizer DEP in the luminescent bacteria test in our experiments were relatively high, EC_{30s} 920 mg l^{-1} in the kinetic luminescent bacteria test and $EC50_{30min}$ 500 mg l^{-1} in the traditional test. Correspondingly, Jonsson and Baun (2003) reported an $EC50_{15min}$ value of 143 mg l^{-1} for DEP in the traditional luminescent bacteria test. These concentrations are high and can easily be detected by chemical analysis from compost media. DEP concentrations causing acute and chronic effects on other microorganisms, algae, invertebrates and fish are lower, ranging from about 10 to 130 mg l^{-1} and 3.65 to 25 mg l^{-1}, respectively (Staples et al. 1997b). Therefore luminescent bacteria cannot be considered sufficiently sensitive to measure acute toxicity of DEP in the environment.

In the following sections the fate of the identified potentially hazardous polymer chain extenders (HMDI, BDI) and polymer degradation products (HMDA, MDA) in compost environment during the degradation process will be discussed in more detail. Compost has a highly complex composition that leads to challenges in extraction and analytical detection of degradation products or toxic intermediates, as was seen when monitoring the amount of HMDA and MDA in compost (Paper I and II). This is also a challenge for other applied biotests in which bioavailability of target chemicals is essential for the sensitivity and applicability of the test method. Compost immaturity causes problems in ecotoxicity assessment and therefore the maturity of the composted materials or chemicals should be confirmed before toxicity assessment (Itävaara et al. 2010). Immature compost may give a toxic response e.g. in the luminescent bacteria test (Itävaara et al. 2002b). This must be taken into account when ecotoxicity of pilot scale or industrial scale composts is to be evaluated and evaluation should be focused only on mature compost samples.

4.1.2 Biodegradation and ecotoxicity of biodegradable plastics in compost and in vermiculite

Chain-linked lactic acid-based polymers, poly(ester-amide), polylactide and polyurethane-based plastic studied in the controlled composting test fulfilled the criteria for biodegradability set in EN Standards (EN 13432, EN 14995) for biodegradability of packaging materials and plastics (Papers I and II, Table 14). During biodegradation of MDI (4,4´-diphenyl methane di-isocyanate)-based thermoplastic polycaprolactone-type polyurethane 2030/489, a toxic degradation product, MDA may be released to the environment. Due to its good biodegradability, 2030/489 film is a good model for testing the toxicity of released harmful compounds (Bellia et al. 1999). Release of MDA was evident when we studied the biodegradation of 2030/489 film in controlled composting test, with vermiculite as biodegradation media (Paper I). Released MDA (87.8 µg g^{-1} dw) and acute toxicity in the kinetic luminescent bacteria test were detected in vermiculite extracts, but not when biodegradation was monitored in compost media. We observed that overnight extraction did not increase the toxic response but did activate light production of *Vibrio fischeri* in compost samples with and without 2030/489 film and vermiculite without the film. The phenomenon of induced light production by compost samples was reported previously by Kapanen et al. (2009). High nutrient concentration or available organic carbon source may activate the bacteria. Because sample matrix itself may cause interference in the luminescent bacteria test it is very important to include proper controls in the experiment in order to avoid false negative and false positive results.

Recovery of MDA from compost has been reported to be very low, only 10%, when compared to the 90% recovery from vermiculite (Tosin, et al. 1998). MDA may be adsorbed to the compost particles and thus be less extractable to water and less toxic to test organisms, especially in short term tests. This might explain why compost samples with 2030/489 film were not acutely toxic in our experiments (Paper I). MDA could also be more biodegradable in compost environment than in vermiculite. We determined that as a pure chemical MDA was toxic to *Vibrio fischeri* in all the tested concentrations of 0.8–205 mg l^{-1},

with an EC_{50} value after 30 s contact time of 38 mg l^{-1} (Table 13). Thus, it was possible to detect toxicity in vermiculite sample with 2030/489 film, which contained 2.3 mg l^{-1} MDA. In one EU Risk assessment report PNECs for MDA in aquatic and in soil environments were 3 μg l^{-1} and 128 μg kg^{-1}, respectively (EU RAR 2001). In our study the amount of released MDA in vermiculite was very high compared to the PNEC value for soil reported by European Commission. However, the amount of the sample used in the biodegradation experiment was higher than would be expected in real composting conditions.

Table 14. Biodegradability of polymers in controlled composting test EN 14046 (Papers I and II).

Sample	Biodegradation (%) CO_2 of $ThCO_2$	Time (d)	Environment	Biodegradation test	Paper
2030/489	90	56	Compost	Laboratory	I
	88	56	Vermiculite	scale/Controlled composting test	I
E4%	85	112	Compost	Laboratory	II
PEU1%H	87	112		scale/Controlled	II
EU4%H	95	112		composting test	II
PEA	100	112			II
PEU4%B	91	70			II
PLLA	92	150			II
Positive control	95	112			II
Positive control	94	70			II

We also studied the effect of the type and the amount of chain extender and the esterblock on biodegradability and ecotoxicity of lactic acid polymers prepared by chain linking (Paper II). We demonstrated that all the studied polymers, namely poly(ester-urethanes), poly(ester-amide), and a reference polymer polylactide biodegraded to over 90% of the positive control during the composting experiment, which is the limit set for biodegradability in the standard EN 13432 for packaging materials and EN 14995 for plastics (Table 14). The degradability of poly(ester-urethanes) is mainly based on degradation of polyester blocks rather than the cleavage of urethane bonds (Seppälä et al. 2004, Paper II). We observed that the length of the poly(lactic acid) chain and different amounts of chain extender, HMDI slightly affected the rate of biodegradability. Biodegradation of PEU4%H was slightly faster but only 8% higher than biodegradation of PEU1%H. This has also been detected earlier in aquatic environment (Hiltunen et al. 1997). Polymer PEU4%B with a different chain extender, 1,4-butane di-isocyanate (BDI), biodegraded faster than PEU4%H. The lag time was longer but 91% biodegradability was reached already in 70 days, when biodegradation of PEU4% was only about 80%. The chain-linking agent was the only difference between these polymers. Chain extenders do have an impact on polymer characteristics such as chain flexibility, crystallinity, mechanical properties, hydrolysis behaviour and degradation behaviour. Therefore it is important to select a suitable chain extender if degradation properties of produced polymers are tailored (Seppälä et al. 2004; Wang et al. 2011).

In PEA 2,2´-bis(2-oxazoline) was used as an chain extender, oxamine groups were introduced to the polymer structure and 1,4-butanediol was substituted with succinic acid in the ester chain. However, we found that the degradation behaviour of PEA was similar to that of PEU1%H and PEU4%H. PEA with a higher glass transition temperature (T_g) had a longer lag phase but reached or even exceeded 100% estimated level of biodegradation. In some cases long incubation or sample type might itself activate degradation of the compost matrix, resulting in overestimation of the biodegradability results. This phenomenon is called priming effect (Shen and Bartha 1996). Different glass transition temperatures (T_g) and degree of crystallinity of the polymers affected the lag phase and biodegradation rate in the controlled composting test. In our study high T_g and the semicrystalline nature of PLLA resulted in a long lag phase and slower biodegradation rate. PLLA degrades faster in bench-scale composting conditions in which the temperature increases higher than 58 ºC in the controlled composting test, resulting in faster hydrolysis followed by enhanced biodegradation (Itävaara et al. 2002a).

With biotests we could detect the release of the harmful compounds to the compost media when biodegradation was studied under controlled composting conditions. We detected acute toxicity and phytotoxicity derived from PEU1%H and PEU4%H polymers during and after the biodegradation in controlled composting test (Figure 5, Paper II). Toxic response was clearly related to the amount of HMDI in the polymer. In the kinetic luminescent bacteria test, the inhibition in light production due to PEU4%H was twofold or more, relative to PEU1%H. Furthermore, the other tested polymers such as PEU4%B, in which the chain-linking was carried out with BDI, did not show any indication of toxicity. In this case, the toxicity detected was evidently due to the breakdown of HMDI units. In addition, we observed that the effect on cress, radish and barley seedling growth was more distinct in growth media containing a 1:2 (v/v) mixture of compost with the higher concentration of HMDI. In addition, PEU4%H caused chlorosis and damaged the shoot tips of test plants.
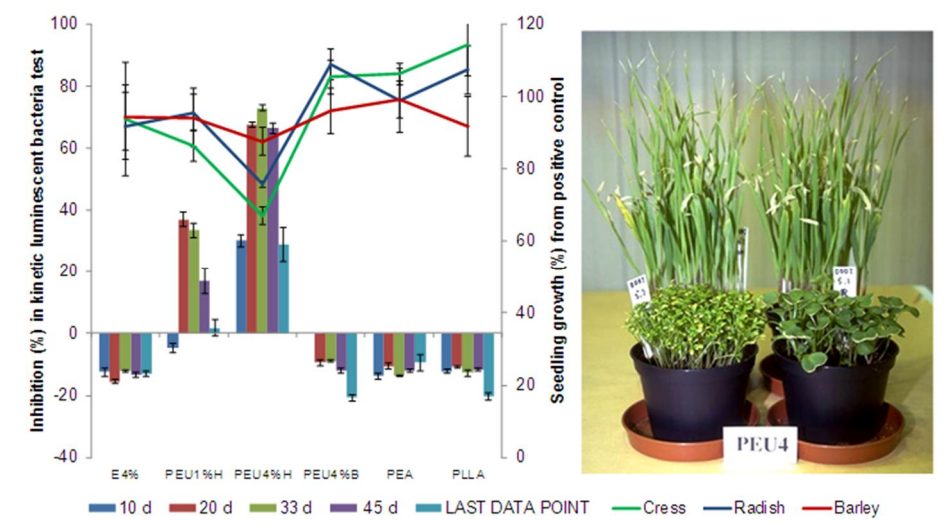
Figure 5. Inhibition of light production by *V. fischeri* in the kinetic luminescent bacteria test (last data point for E4%, PEU1%H, PEU4%H, PEA was 112 days, for PLLA 202 days, and for PEU4%B 70 days) and biomass (%) compared to positive control in the plant growth test. On the right, seedling growth of a) cress, b) radish, and c) barley in PEU4%H/compost medium.

We showed that HMDI was probably indirectly responsible for the toxic response in compost samples. However, the form of toxicity induced was not self-evident. Polyurethanes and especially degradation products derived from di-isocyanates may yield toxic compounds during degradation. In water, di-isocyanates are expected to be rapidly hydrolyzed to the corresponding amines and polyuria (Anonymous 1998). We identified traces of the degradation product of HMDI, 1,6-hexamethylenediamine (HMDA) in compost samples after degradation of PEU4%H. However, HMDA was not detected from the PEU1%H samples, in which HMDI-units were one-fourth of the amount in PEU4%H. The toxic response in the kinetic luminescent bacteria test could not be due only to the presence of HMDA in the studied compost samples, since the detected concentrations were at least 15 – fold lower than those needed for detecting the toxic response with the applied test (EC20$_{30s}$). Toxicity decreased during the composting test, indicating further degradation or modification of degradation products. In order to be able to detect potential toxic degradation products of materials targeted for composting, it is important to monitor the toxicity of compost not only after the composting but already during the degradation phase. Our results clearly showed that 1,6-hexamethylene di-isocyanate should not be used as a building block in biodegradable polymers because of its potential environmental risk. In polymer PEU4%B, in which no toxicity was detected during the composting, the degradation product of BDI is nontoxic 1,4-butane diamine, a compound that is also present in mammalian cells (de Groot et al. 1997).

4.1.3 Plasticizer DEP in compost environment

Phthalates are used to plasticize polymers such as polyvinyl chloride (PVC), polyvinyl acetates, cellulosics and polyurethanes. These polymers are widely used in food wraps, plastic tubing, furniture, toys, shower curtains, cosmetics etc. Phthalates when used as plasticizers are not chemically bound to the polymer and leach easily from polymer matrices into the environment. Environmental fate, toxicity and biodegradability of phthalates have been extensively studied (Staples 1997a, 1997b, and 2000, Cartwright et al. 2000a and 2000b; Fromme et al. 2002; Amir et al. 2005; Liang et al. 2008). One of the main concerns regarding phthalates is their endocrine -disrupting properties (Jobling et al. 1995; Zou and Fingerman 1997).

Ecotoxicity and biodegradability of plasticizer DEP was demonstrated in laboratory and pilot scale experiments (Paper III and unpublished data). In the laboratory scale experiment, biodegradability, ecotoxicity and microbial diversity were investigated in compost growth substrate to which DEP was added at different concentrations, 0.1–100 g kg^{-1} fw (Paper III). In addition, we evaluated the biodegradability of cellulose acetate (Dexel S 200–25 + UV) containing DEP as an additive in controlled composting conditions and its ecotoxicity was assessed. In pilot scale composting disintegration and ecotoxicity of the cellulose acetate film were studied (unpublished data).

We detected toxicity of compost-based plant growth media, spiked with DEP in concentrations from 1 to 100 g kg^{-1} fw, as inhibition in light production in the luminescent bacteria test with *Vibrio fischeri*, as reduction in seedling growth with radish (*Raphanus sativus*) and as change in microbial community (Figure 6, Table 15, Paper III). When added at 1 g kg^{-1} fw DEP reduced seedling growth of radish (*Raphanus sativus*). In addition to its effect on plant growth, Saarma et al. (2003) reported that the presence of DEP in plant cultivation media may lead to changes in protein synthesis and induce formation of several stress -related proteins. The presence of heat shock proteins (HSPs), as well as novel proteins possibly related to DEP in growth media was reported when the DEP concentration was 222 mg l^{-1} in liquid medium. Compared to other reported aquatic toxicity data, a relatively high concentration of DEP was needed in our study to induce acute toxicity in the kinetic luminescent bacteria test. Light inhibition starting from 51% in compost media samples (100 g l^{-1}) was detected with a concentration of 10 g DEP kg^{-1} fw; acute toxicity of DEP to microorganisms, algae, invertebrates and fish varies between 10 and 130 mg l^{-1} (Colborn et al. 1993; Staples et al. 1997a, b; Zou and Fingerman 1997). Short contact time in the kinetic luminescent test and the effect of compost matrix on bioavailability of DEP may be the reasons for low toxic response in the luminescent bacteria test.
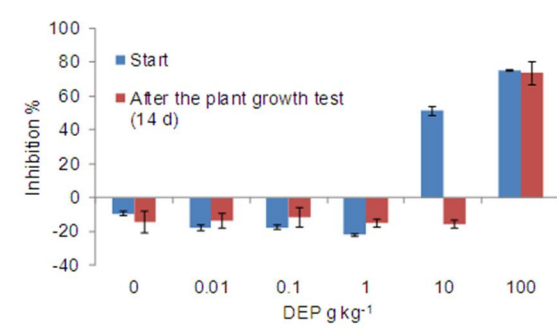
We observed that DEP degraded efficiently in plant growth media during the 14 day plant growth test (Table 15). The concentration decreased to 64 g DEP kg^{-1} even in the sample containing the highest DEP concentration (100 g kg^{-1}). Compost amendment has been shown to increase the degradation rate of phthalate acid esters such as di-n-butyl phthalate (DBP) and di-(2-ethyl hexyl) phthalate (DEHP) in soil (Chang et al. 2009). We demonstrated that composting also effectively reduces the amount of DEP used as a plasticizer in cellulose acetate film, as well as the amount of DEHP in sewage sludge (unpublished data, Paper V). There are many species within *Sphingomonas*, *Pseudomonas*, *Corynebacterium*, *Aureobacterium*, *Micrococcus*, *Actinomycetes*,

Arthrobacter, and *Flavobacterium* etc. which are capable of DEP degradation, and effi-efficient biodegradation of DEP has been detected for example in soil, sludge -amended soil, compost and activated sludge (Cartwright et al. 2000a; Jianlong and Xuan 2004; Liang et al 2008; Suemori et al. 1993; Chauret et al. 1995). We found in our study that *Actinomycetes*, *Sphingomonas* and *Pseudomonas* groups emerged in compost media spiked with high DEP concentrations (Paper III).

Table 15. Degradation and ecotoxicity of DEP in compost plant growth media (Paper III).

Compost plant growth medium spiked with DEP g kg^{-1} fw	Biodegradation (%) after 14 d	Germination (%) of the control	Plant growth (%) of the control
0.01	100%	90	97
0.1	100%	95	96
1	100%	88	76
10	99.8%	28	8
100	36%	0	0

Figure 6. Acute toxicity as inhibition (%) in light production (ISO 21338) in DEP-spiked compost medium before and after the plant growth test (Paper III).

In our experiment, cellulose acetate containing 25% DEP as a plasticizer (Dexel S 200–25 + UV, CA-DEP) was not biodegraded in controlled composting test (unpublished data). We tested the biodegradability of the material in two forms; milled and granular. Only 10% of granular CA-DEP and none of milled CA-DEP was biodegraded during a 70 day experiment. Milled CA-DEP reduced the microbial activity in compost; the amount of evolved CO_2 was about 5% below the control compost levels. With the biotest we could also detect toxicity induced by both milled and granular CA-DEP in compost (Figure 7). We detected clear response in acute toxicity by the kinetic luminescent bacteria test throughout the controlled composting test. In samples collected after the composting we observed that plant growth of radish (*Raphanus sativus*) and barley (*Hordeum vulgare*) was reduced especially in the compost with milled CA-DEP. Milled CA-DEP reduced the germination of radish to 70% of the control. Seedling growth of radish and barley were

reduced by 50% and 89%, respectively, by milled CA-DEP in the controlled composting test. Correspondingly, acute toxicity in the kinetic luminescent bacteria test decreases during composting in granular CA-DEP samples but not in milled CA-DEP samples.

a)

b)
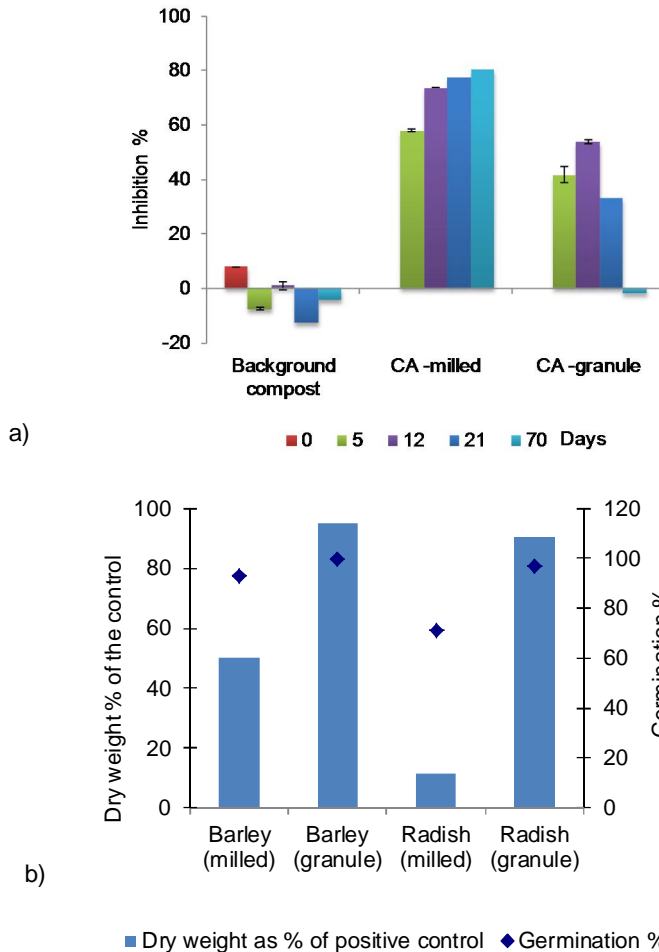
Figure 7. Ecotoxicity of milled and granular CA-DEP during the controlled composting test EN 14046. a) Inhibition in light production in the kinetic luminescent bacteria test (sample concentration in the test was 100 g fw l^{-1} b) Germination and seedling growth of barley and radish (1+1 compost and commercial growth media) after the biodegradation test.

When disintegration of CA-DEP film (1% of the fresh weight) was studied in a 200 litre pilot-scale composting test (fruit and vegetable waste, bark, peat and wood chips), no toxic response was detected in the kinetic luminescent bacteria test or in the plant growth test. After the pilot composting, weight loss of the film was 27% in 91 days and the

amount of DEP in CA-film decreased from 25% to 2.5%. After the pilot composting we measured the acute toxicity in the kinetic luminescent bacteria test of the following milled cellulose acetate samples; CA without DEP, CA-DEP film and CA-DEP film residues after the pilot composting (unpublished data). CA-DEP was acutely toxic, with 55% inhibition in light production of *Vibrio fischeri*. However, the toxicity disappeared during the pilot scale composting experiment (Figure 8). Decrease in toxicity was due to degradation or leaching of DEP from the cellulose acetate film. Weight loss of CA-DEP (27%) film was mostly due to DEP degradation, whereas the amount of DEP in the tested film decreased from 25% to 2.5% and the amount of DEP in compost was below the detection limit of 1 mg/kg. Non-toxic cellulose acetate served as a control in the toxicity test. This opens a possibility to evaluate the ecotoxicity directly from milled biodegradable materials. If toxicity emerges during biodegradation and is derived from biodegradation products, this approach in biotesting is not valid.
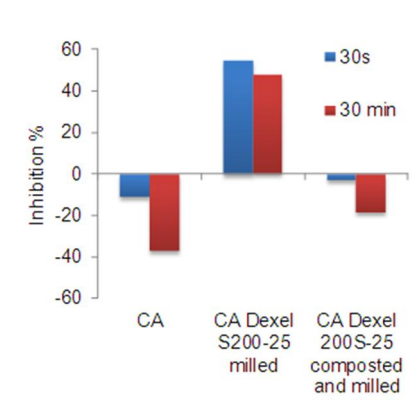
Figure 8. Inhibition% in light production of *Vibrio fischeri* in the kinetic luminescent bacteria test measuring acute toxicity of cellulose acetate (CA) and cellulose acetate film with 25% DEP (CA Dexel S 200–25 milled) before and after composting.

4.1.4 Performance and safety of biodegradable mulching films in agriculture

Low tunnel and mulching films are used in agriculture in order to control weeds, conserve water, limit the use of fertilizers and plant protection chemicals, provide a better microenvironment for plants to increase the yield and quality of horticultural products and to protect plants against adverse climate conditions (Briassoulis 2006 and 2007; Kyrikou and Briassoulis 2007). Low-density polyethylene (LDPE) films are commonly used in agriculture as coverings for greenhouses or low tunnels and for soil. However, compared to LDPE films, biodegradable films have many advantages in agricultural applications. In contrast to conventional mulching film that must be collected from the field after use, biodegradable mulching films can be ploughed into the soil at the end of the crop cycle. Due to the possibility to save working hours, farmers are highly motivated to use biodegradable materials such as mulching films for agriculture (Fritz et al. 2003). However, these materials should be compatible with conventional products in relation to

crop production and quality and in addition degradation residues and metabolites should not accumulate in the soil or be toxic. In our field study on biodegradable starch-based low tunnel and mulching films in strawberry cultivation, the studied materials showed good product performance, quality of the strawberries produced was good and the yield was high, with no detectable negative effects on the environment (Paper IV, Figure 9).

Figure 9. Field scale experiment of low tunnel and mulching film performance in protected strawberry cultivation in Italy.

The useful lifetime of the studied biodegradable films, 9 months for mulching films and 6 months for low tunnel films, was sufficient for strawberry (*Fragaria* sp.) cultivation in the Mediterranean area (paper IV). Previously, similar types of Mater-Bi-based films have lasted from 2 to 5 months in the field (Schettini et al. 2007). Kraft paper impregnated with vegetable oil-based resins withstood field conditions for shorter periods of time, only 8–12 weeks (Shogren 2000).

The mulching films studied in our field experiment were opaque to the PAR radiation (400–700 nm) necessary for photosynthesis and inhibited weed growth as well as LDPE film (Figure 3 in Paper IV). Transmissivity of the film affects the microclimate under low tunnels. In our study biodegradable films maintained a higher air temperature under low tunnels compared to LDPE films. Due to the very low long wave infrared radiation (LWIR) transmissivity coefficient of studied biodegradable low tunnel films, minimum temperatures during the night were more than 2 ºC higher under biodegradable low tunnel films (L1 and L3) than under LDPE film (L0). Thus, the microclimate established under biodegradable low tunnel films favoured strawberry plant growth, increased the yield and resulted in earlier harvesting when compared to LDPE film (L0) (Paper IV).

After the cultivation period all low tunnel films and LDPE mulching film were collected and transferred from the field. Biodegradable mulching films together with strawberry plants were tilled into the soil (Paper IV). Environmental conditions such as temperature,

soil water content and amount of oxygen as well as properties of the polymer affect the degradation rate of mulching films in soil (Briassoulis 2007; Kyrikou and Briassoulis 2007). We observed that microbial activity in soil was highest at the time of tillage, when the amount of polymer in the soil was also at its highest. Microbial activity decreased during degradation of the polymers, and after 12 months only low microbial activity was detected, and only 4% of the films were detectable in soil. Similar degradation rates in a laboratory scale soil burial test have been reported for mulching films made of poly(3-hydroxybutyrate) (PHB) and an aliphatic polyester Sky-Green® (Kim et al. 2000) and in field scale experiments with starch-based biodegradable film (Mater-Bi) (Briassoulis 2007; Schettini et al. 2007).

We did not observe any indication of acute toxicity in the kinetic luminescent bacteria test or in the survival and reproduction of *Enchytraeidae* during the degradation of mulching films in soil (Figures 7 and 8 in Paper IV). However, we detected an increase in light production of *V. fischeri* in soil samples taken one month after mixing the mulching film into the soil. At that time 44% of the initial weight of the polymer and plant residues was still present in the soil. This phenomenon has previously been reported in compost samples containing available nutrients and carbon source (Kapanen and Itävaara, 2001, Kapanen et al. 2009). In our test field, where the amount of organic matter was very low, buried plant residues and biodegradable film served as organic substrate for soil microorganisms and soil fauna and also provided better conditions for reproduction of *Enchytraeidae*. One month after tillage the reproduction of *Enchytraeidae* was slightly higher in the soil with biodegradable mulching film than in the control soil without the polymer. High organic matter content in soil might favour *Enchytraeidae* (Palojärvi et al. 2002). Fritz et al. (2003) demonstrated that earthworms are also capable of consuming and digesting undegraded organic matter and benefit from residues of biodegradable polymers in soil. This might mask the possible toxic response and the authors concluded that earthworms would not be a suitable test organism for testing the ecotoxicity of biodegradable polymers.

This study showed that the studied biodegradable films have the required functionalities for use in protective cultivation: good radiometric properties, prolonged lifetime, reasonable degradation properties after soil burial and no indication of ecotoxicity. However, in this field scale experiment the amount of the polymer mixed into the soil was relatively low. Therefore it is recommended in addition to the field scale tests to study the ecotoxicity of biodegradable polymers in laboratory scale experiments with higher polymer concentration.

4.1.5 Microbial diversity as an indicator of environmental health

Changes in biodiversity and microbial functional stability are good indicators of soil health (Bécaert and Deschênes 2006). In addition, ecotoxicity assessment of the compost environment may profit from the information gained from the bacterial community studies, as was suggested for soil remediation experiments (Stephen et al. 1999a, 1999b; MacNaughton et al. 1999). We applied a microbial ecological approach in two of the cases presented in this thesis. Ammonia oxidizing bacteria (AOB) diversity was used as a marker for soil quality in a field scale experiment with biodegradable mulching films

(Paper IV) and the effects of DEP in compost growth media (Paper III) were observed as the bacterial community response.

In our study buried biodegradable mulching films did not affect the diversity of ammonia oxidizing bacteria (AOB) in soil and did not have negative effects on microbial functions in soil (Paper IV). One major band (band 1 in Figure 10) dominated the PCR-DGGE profile of the functional amoA gene throughout the whole cultivation and mulching film degradation periods. We sequenced a total of seven bands but none of the sequenced bands could be identified to the species level. Accordingly, Phillips et al. (2000) found no change in community structure of ammonia oxidizing bacteria (AOB) in relation to agricultural practice. By contrast Norton et al. (2002) reported differences in AOB diversity among agricultural soils treated with nitrogen fertilizers, composted dairy waste or liquid dairy waste.
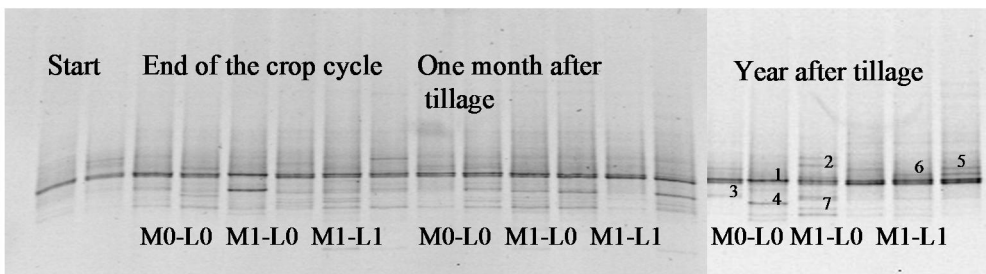
Figure 10. PCR-DGGE profiles of ammonium oxidizer communities in soil samples from M0-L0c (non-biodegradable black mulching film with transparent non-biodegradable low-tunnel film), M1-L0c (black biodegradable mulching film with transparent non-biodegradable low-tunnel film) and M1-L1c (black biodegradable mulching film with transparent biodegradable low-tunnel film) blocks at the start of the field experiment, 1 month after tillage and 1 year after tilling the films into the soil. Sequenced bands are marked as 1–7 (two replicates/test block) (Paper IV).

We observed through PCR-DGGE profiling that the bacterial community in the compost changed at the same concentrations of DEP that were found to be toxic in the single-species toxicity tests (Paper III). Based on the PCR-DGGE profile, it was shown that low DEP concentrations did not change the bacterial community. At a concentration of 10 g DEP kg^{-1} in compost growth medium, DEP did induce changes in bacterial community structure and several strong bands emerged. At very high DEP concentration, 100 g DEP kg^{-1}, the original broad diversity of bacteria was reduced to only about 10 major species, including those species which emerged in the 10 g DEP kg^{-1} sample (Figure 10). Several of these induced species, belonging to the genera *Actinomycetes*, *Sphingomonas* and *Pseudomonas*, are known to be able to degrade DEP (Liang et al 2008; Fang et al. 2007). In addition to the induction of species capable of degradation, suppression of diversity can also be driven from toxicity to other microorganisms. The same concentration level of DEP causing changes in microbial diversity was found to be acutely toxic in the kinetic luminescent bacteria test and also reduced plant growth (Table 6, Table 15). Most probably, in our test setup both activated degradation and toxicity of DEP had an effect on the microbial community dynamics. At a concentration of 10 g DEP kg^{-1},

diversity changed due to activated degradation and at 100 g DEP kg^{-1}, toxicity of DEP and its degradation products decreased the diversity. However, the degradation product of DEP, monoethyl phtahalate (MEP), has been reported to be less toxic than DEP (Jonsson and Baun 2003).

4.2 Biotests in ecotoxicity assessment of sewage sludge and sewage sludge-based compost

We studied the amounts of the following organic contaminant concentrations in municipal sewage sludge samples derived from waste water treatment plants in Finland and their reduction in a composting process: absorbable organic halogens (AOX), linear alkylbenzene sulphonates (LAS), nonylphenols and nonylphenolethoxylates (NP and NPE), di-ethylhexylphthalate (DEHP), polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) (Paper V). Compared to the previously reported concentrations in Finland, in Europe or draft limit values set in the Working Document on Sludge and Biowaste (WD 2010) presented in Table 16, the overall levels of organic pollutants in sewage sludges or composted sewage sludges in our study were not alarming. The level of organic pollutants present in Finnish sewage sludge was mainly influenced by the source of the waste water and especially the share of industrial waste water treated (Table 16, Vikman et al. 2006). A similar conclusion was reached by Natal-da-Luz et al. (2009) when they studied the toxicity of industrial and municipal sewage sludges amended with soil. In our study, the proposed limit values in WD 2000 and WD 2010 were exceeded in the case of DEHP, with a concentration of 110 mg kg^{-1} dw in one of the studied sewage sludge samples. In addition the PAH (SUM9) concentration (5.8 mg kg^{-1} dw) was very close to the limit value of 6 mg kg^{-1} dw set in WD 2000 and WD 2010.

Table 16. Average amounts of organic pollutants reported in sewage sludge and compost in Europe and in pilot scale and field scale composts in Paper V.

Organic pollutant mg kg^{-1} dw	Finland[1] Sewage sludge	EU Sewage sludge	Paper V Sewage sludge A/B	EU compost[5,7]	Finland Composted sewage sludge[8]	Paper V Pilot scale compost	Paper V Industrial scale compost
DEHP	39–70	20–660[2] 1–100[3,4,5]	57/110	0–70	1.2–21	11	2–16
LAS	360–1700	10–19 000[2] 50–15000[3,4,5] < 1000– 30000[5]	1500/1300	9–396	< 50–190	< 50	50–310
NP/NPE	2–35	8–22[2] 100–3000[3,4,5]	15.2/8.9	< 0.03–1.6	< 0.2–6	2.6	< 0.6–47
PAH (EPA-PAH 16)	1.0–11.6	0.02–12[2] 1–10[3,4,5]	8.0/0.5	0.08–13	0.4–3.6	0.6	< 0.5
PCB	0.03–0.08	< 0.005–21[2] 1–20[3,4,5]	1.4/0.04	0–0.6	0.01–0.08	0.2	0.02–0.06
PCDD/F ng TE kg^{-1} dw	0.34.4–55	0.006–192[2]	3.8/0.3	37 (Median)	< 8.9	nd	3.2

[1] Vikman et al. 2006; [2] Andersen 2001; [3] Smith 1996 and 2000; [4] Schowanek et al. 2004; [5] Amlinger et al. 2004; [6] Gawlik and Bidoglio 2006; [7] Brändli et al. 2004; [8] Priha et al. 2009

It is important to reduce the environmental load of organic contaminants through use of sewage sludge -based products targeted for example to agricultural or landscaping purposes. Successful reduction of organic contaminants in sewage sludge through composting has been reported by Amundsen et al. (2001), Moeller and Reeh (2003), Marttinen et al. (2004), Amir et al. (2005) and Oleszczuk (2010). Of the studied organic contaminants, PAH and DEHP have high affinity to organic matter, having nevertheless a good potential for biodegradation during the composting process (Jensen and Jepsen, 2005). In our pilot-scale composting experiment there was a substantial decrease in the amount of both PAH and DEHP after 124 days of composting, 84% and 56%, respectively (Figure 11). Moeller and Reeh (2003) reported a PAH removal rate of 70% after 25 days of composting. Compared to our pilot-scale composting, similar degradation levels for DEHP, from 34 to 64% have been published by Amundsen et al. (2001) and Marttinen et al. (2004). For DEHP even higher reduction rates of 85% and 93% have been reported (Poulsen and Bester 2010; Cheng et al. 2008). Degradation efficacy is affected by type of sludge, original concentration of organic contaminant, and temperature during the composting process (Amir et al. 2005; Oleszczuk 2010). For example increasing the temperature from 55 ºC to 65 ºC increased the DEHP degradation from 69% to 91% in a 25-day composting experiment by Moeller and Reeh (2003). In our study the maximum temperature detected during composting was 65 ºC. We measured corresponding or even lower concentrations of PAH and DEHP in industrial scale compost compared to those in our pilot composting experiment (Table 16).

Many anionic surfactants such as LAS, non-ionic surfactants such as NPE and cationic surfactants have been found to be biodegradable in the aerobic environment (Ying 2005). Compared to the degradation rate of PAH and DEHP, degradation of LAS and NP/NPE was very efficient in the beginning of our composting experiment. Similar reduction rates as well as concentration levels for LAS (92%, < 50 mg kg^{-1} dw) and NPE (61%, 2.6 mg kg^{-1} dw) to those reported by us have been published by Amundsen et al. (2001) and Moeller and Reeh (2003).
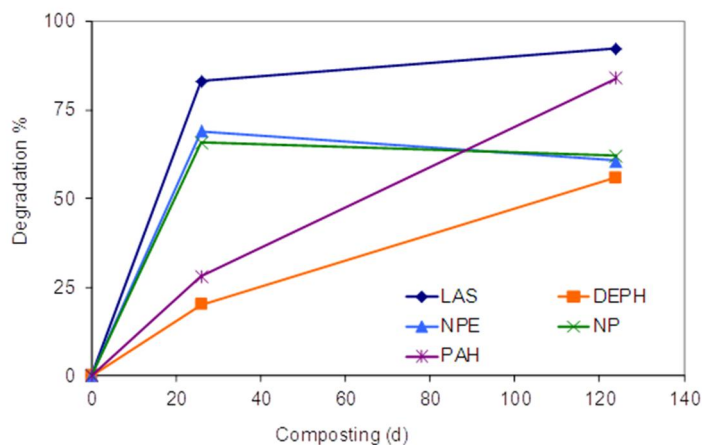
Figure 11. Removal (%) of LAS, NP, NPE, DEHP and PAH in pilot scale sewage sludge composting.

In our study we showed that biotests can be used in sewage sludge and composted sewage sludge quality assessment (Paper V). We evaluated the ecotoxicity of two different types of sewage sludge samples by measuring acute toxicity with the kinetic luminescent bacteria test with *Vibrio fischeri* (ISO 21338), genotoxicity by the Vitotox® test kit and endocrine-disruptive activity and dioxin-like activity with yeast cell bioreporters. The studied sewage sludges were treated by anaerobic digestion but differed from each other in the share of industrial waste water treated at the waste water treatment plant from which the samples originated from. This led to differences in the load of organic contaminants in the sewage sludge (Table 1 in paper V). It is challenging to link the amount of contaminants in sewage sludge or in compost to toxic response in biotest (la Farré et al. 2001; Perez 2001; Oleszczuk 2010). However, with the biotests applied in our study we could separate the studied sewage sludge samples into more and less toxic one and reduction of toxicity during composting could also be detected by biotests. Toxicity of sewage sludge is highly dependent on the source of the treated waste water (Natal-da-Luz et al. 2009). Accordingly we observed that acute toxicity in the luminescent bacteria test was higher and genotoxicity was more evident in sewage sludge with a higher organic contaminant load from the wastewater treatment plant (Table 4 and 5 in Paper V). In order to link the toxicity response to a specific compound, chemical fractionation is needed. After chemical fractionations sewage sludge toxicity revealed by the luminescent bacteria test has been linked to the presence of NPE and NP (la Farré et al. 2001) or PAH (Perez 2001). In our study the fractionation approach was not exploited. However, an additional DMSO extraction step before the kinetic luminescent bacteria test increased bioavailability of the contaminants and also increased the detected toxicity.

Many of the chemicals such as NP, bisphenol-A, dibutylphthalate (DBP) and DEHP also found in sewage sludge have endocrine disrupting properties (Harrison et al. 2006; Fromme et al. 2002). Using the yeast cell bioreporter we observed that both studied sewage sludge samples did have relatively high oestrogenic and androgenic activities, as well as dioxin-like activity (Table 5 in Paper V). Similar oestrogenic and androgenic potencies in sludge and sewage were detected with ER-Calux, yeast oestrogen screen and in vitro competitive receptor binding assay by Murk et al. (2002) and Leusch et al. (2006). Dioxin-like activity observed was related to the presence of polyhalogenated persistent organic pollutants (POP), such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs) and diphenyl ethers found in sewage sludge.

We demonstrated that acute toxicity, genotoxicity, endocrine disruption potential and dioxin-like activity of sewage sludge can be reduced by composting. Biodegradation of organic pollutants derived from municipal sewage sludge was in relation to the reduction in ecotoxicity response. Figure 12 demonstrates the decrease in the amount of PAHs (both according to EPA-PAH and WD 2010) during composting, along with the corresponding acute toxicity. The limit value, 2 mg kg^{-1} dw, proposed for benzo[a]pyrene in WD (2010) was not exceeded in our sewage sludge samples or in any of the compost samples studied. Acute toxicity of compost (slurry) decreased according to the decrease of the measured PAH concentrations during 124 d of composting. Compost samples extracted with DMSO induced high acute toxicity in the luminescent bacteria test. The observed toxicity could be due to the presence of some degradation products or humic substances extracted with DMSO. In agreement with the fact that we demonstrated with the yeast bioreporter that composting reduces the oestrogenic and androgenic potencies of sewage sludge, Hernandez-Raguet et al. (2007) showed a decrease in oestrogenic activity of NP-spiked sewage sludge by MELN bioassay after aerobic sewage sludge treatment. In addition, the activated sludge process has been shown to decrease the oestrogenic and androgenic potencies of waste water (Leusch et al. 2006).
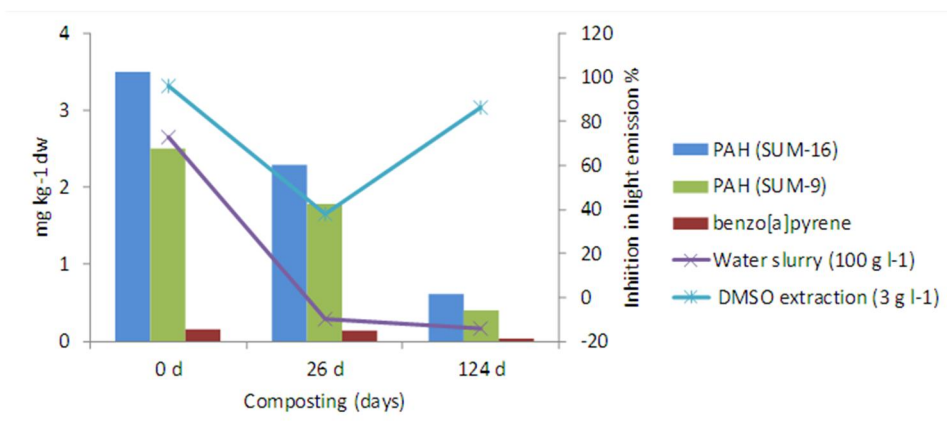
Figure 12. Changes in PAH concentrations (bars) during the pilot composting experiment and acute toxicity (ISO 21338) (lines) after 30 minutes exposure to water or DMSO extracts of sewage sludge (0 d) and compost after 26 d and 124 d of composting.

Based on our experience the luminescent bacteria test was a suitable biotest for sewage sludge and compost acute toxicity assessment. On the other hand, with the Vitotox™ test measuring genotoxicity we faced problems with sewage sludge and compost samples. The test organism *Salmonella typhimurium* TA104 *rec*N2-4 strain might lose its sensitivity in the presence of nutrients in the sample under study. Yeast bioreporter gave interesting results concerning the oestrogenic and androgenic potencies and dioxin-like activity present in sewage sludge and compost. However, the high toxicity of the bark/peat mixture (i.e. one of the compost components) to the yeast *S.cerevisiae* might bring constraints to the use of this test with compost samples. In addition, while the maturity of compost is known to affect the biotest response it is not feasible to measure the ecotoxicity of compost samples in very immature phase (Itävaara et al. 2002b). In order to avoid false positive responses in biotests, it is very important, in addition to the ecotoxicity evaluation, to measure the maturity state of the compost sample.

4.3 Feasibility of ecotoxicity tests for biodegradable materials in compost and in soil

In our study we demonstrated many successful approaches for ecotoxicological assessment of biodegradable materials in compost and in soil. It was shown that it is very beneficial to combine end point information from the ecotoxicity tests, biodegradability monitoring data and data on the released degradation products. In addition, combining observations of microbial community changes into the potential toxicity effects detected with biotest during degradation process, more in-depth representation of environmental impact of studied materials can be

recovered. Methods in molecular biology are developing rapidly and hopefully this progress will enable more extensive use of molecular methods in context of ecotoxicity assessment. It will be most interesting to see how microbial community responses to toxic substances will contribute to environmental impact assessment in the future. Furthermore, test environment and test scale have an effect on the final results. At the laboratory scale, the test concentrations of the material are high and enable the identification of potential degradation products and their ecotoxicity. However, when conducting field experiments, it is important to realize the link between the functionality and performance of biodegradable materials in relation to the amount of potential exposure and effects. For example biodegradable polymers in soil have to biodegrade in specified time frame or amount of sewage sludge applied on land is dependent on its nutrient composition.

The ecotoxicity test battery for compost, sewage sludge and soil toxicity assessment should include biotests with organisms from several trophic levels. There are several standard tests available for soil ecotoxicity assessment covering a variety of species from plants and soil fauna to soil microorganisms. However, there are no standard tests available specifically for evaluation of compost or sewage sludge ecotoxicity. Some of the soil ecotoxicity tests have also been recommended for assessing ecotoxicity of compost environment e.g. seedling emergence and growth (OECD 208), tests with soil fauna (e.g. ISO 11267, ISO 16387, ISO 11268), kinetic luminescent bacteria test (ISO 21338) and ammonium oxidation assay (ISO 15685) (Kapanen and Itävaara 2001; Juvonen et al. 2000; Domene et al. 2011). Due to the potential application of compost or sewage sludge as a soil improver or fertilizer it is self-evident that effect on plants plays on important role in their applicability. In our experiments compost samples tested in plant assays were mature (Papers II, III and unpublished data) and in the field scale experiment with biodegradable mulching materials, soil was well characterized. However, it is good to acknowledge, that immature compost or active degradation in soil may cause negative impacts on plant growth (Itävaara et al. 200b; Fritz et al. 2003). Furthermore, Juvonen et al. (2000) indicated in their study that soil animals and luminescent bacteria were also sensitive towards compost immaturity. In addition, compost matrix itself has shown to affect *Enchytraeidae* reproduction if the share of compost is too high in the test media. To exclude false positive results, the suitable mature compost standard soil ratio in test media in *Enchytraeidae* test has been reported to be 4:5 (v:v) (Kapanen et al. 2009). In our experiment the test environment for *Enchytraeidae* was agricultural soil with low organic matter content (Paper IV). However, an increase in organic matter due to introduced biodegradable material in soil might induce the growth of soil fauna and hide potential toxic response (Palojärvi et al. 2002, Paper IV). Luminescent bacteria test has been widely used for testing acute toxicity of chemicals and environmental samples including soils, sewage sludge and compost (Table 8 and 9). Kinetic modification of the luminescent bacteria test has improved the applicability of the test for solid and colourful samples. This method may be successfully used for compost, sewage sludge and soil ecotoxicity

assessment bearing in mind that with compost samples tested should be mature and samples with high nutrient and organic carbon concentration may induce the light production of the test organism (Papers I, II, III, IV and V).

In addition to the immaturity or high microbial activity, other compost characteristics such as a high amount of nutrients or the presence of bark and peat was shown to generate problems when biotests were applied in ecotoxicity assessment of compost matrix. Sensitive test organisms such as genetically modified Salmonella in applied genotoxicity assay lost its sensitivity due to high amount of nutrients in the sample (V). The presence of bark and peat in the tests with yeast bioreporter reduced viability of the test organisms and therefore reduced sensitivity and in the worst case prevented the use of the test (V). Based on our study recommendations for suitable tests and their limitations in assessing ecotoxicity of soil, compost and sewage sludge are presented in Table 17.

We have learned that it can be challenging to differentiate the toxicity caused by the compost matrix itself from toxicity derived from the treated material e.g. contaminated soil or biodegradable plastic. This challenge is due to the sensitivity of the test organisms to e.g. oxygen depletion, organic acids naturally present in compost, nutrients, and compost components such as bark and peat. Therefore it is recommended to use all available data e.g. compost maturity, microbial activity, amount of organic matter and nutrients of samples to be tested for ecotoxicity to ensure that the most suitable test is selected for environmental hazard assessment and possibility for false positive and false negative results are minimized. In addition, when possible, follow the degradation process, and do the sampling in course of the degradation process to be able to identify degradation products and study their potential ecotoxicity.

Table 17. Feasibility of biotests used in this thesis to assess ecotoxicity of compost, soil, and sewage sludge environment.

Method	Soil	Compost	Sewage sludge	Soil + biodegradable plastic	Compost + biodegradable plastic
Kinetic luminescent bacteria test (ISO 21338)	Recommended	Recommended with restrictions - Suitable for mature compost	Recommended	Recommended	Recommended with restrictions - Suitable for mature compost
Luminescent bacteria test (ISO 11348)	Not recommended for colourful samples	Not recommended for colourful samples	Not recommended for colourful samples	Not recommended for colourful samples	Not recommended for colourful samples
Enchytraeidae reproduction and survival (ISO 16387)	Recommended	Recommended with restrictions - Mature compost : standard soil ration in test media 4:5 (v:v) (Kapanen et al. 2009)	Not tested	Recommended - Note the potential of additional organic carbon to increase the biomass growth	Recommended with restrictions - Mature compost : standard soil ration in test media 4:5 (v:v) (Kapanen et al. 2009)
Seedling emergence and growth (OECD 208)	Recommended	Recommended with restrictions (Paper II) - Compost:commercial growth media 1:1 (v/v)	Not tested	Recommended	Recommended with restrictions (Paper II) - Compost:commercial growth media 1:1 (v/v)
Genotoxicity with Vitotox[TM] (Paper V)	Not tested	Not recommended - Nutrients reduce sensitivity	Recommended	Not tested	Not tested
Endocrine disrupters and dioxin-like compounds with Yeast bioreporter (Paper V)	Recommended	Recommended with restrictions - Note potential cytotoxicity due to presence of bark and peat	Recommended	Not tested	Not tested

5. Conclusions

Due to increased use and development of biodegradable materials there is increasing pressure to ensure their safety in recycling and waste treatment processes. Reliable methods are needed to assess the environmental safety of biodegradable materials. Chemical analysis of harmful compounds from the environment is challenging. Chemical analysis may fail in the detection of potentially harmful compounds in the studied environment due to restricted extent of the chemical analysis performed, lack of appropriate methods, or synergistic effects of mixed contamination. In the present work we demonstrated that the biotest can be used to evaluate environmental toxicity of compounds released or formed during biodegradation processes. However, the characteristics of the studied environment must be taken into account in order to be able to eliminate possible false positive signals of toxicity induced by the test environment, e.g. compost and sludge.

There are several standardized methods for evaluation of biodegradability of biodegradable plastics and chemicals in the environment and in waste treatment processes. In addition, necessary biodegradability characteristics can be tailored for materials targeted to different environmental applications or waste treatment options. We observed good degradation properties of bioplastics designed for agricultural applications as well as of materials targeted to compost applications. Biodegradability of chain-linked lactic acid-based polymers and polyurethane-based plastic material was confirmed in controlled composting conditions. The studied starch-based biodegradable mulching films showed good product performance, good crop quality and high yield in protected strawberry cultivation. Furthermore, in the case of starch-based biodegradble mulching films no negative effects on the soil environment were detected with the applied biotests. If ecotoxicity is studied in the field scale, it is recommended that laboratory scale experiments are also carried out with higher polymer concentrations than are possible to attain in real life conditions.

Whereas the evaluation of biodegradability is well covered, there is a need for more precise information on ecotoxicity of biodegradable polymers, their additives or degradation products. Biotests applied in this study indicated the potential risks due to release of toxic compounds during biodegradation processes. When the kinetic luminescent bacteria test was used as a screening test for ecotoxicity of

polymer components and their degradation products, HMDI, HMDA, MDA and DEP were identified as hazardous compounds that could potentially cause risks when released during polymer degradation processes in the environment. The kinetic luminescent bacteria test was sufficiently sensitive to identify toxicity of MDA released from an experimental biodegradable plastic in a controlled composting test performed in vermiculite, but not in compost media. Vermiculite as a testing environment enabled detection of the toxicity of the released toxic metabolites. With biotests we also verified that 1,6-hexamethylene di-isocyanate (HMDI) was indirectly responsible for toxicity detected during the degradation of poly(ester-urethanes). HMDI, which is frequently used in urethane chemistry and as a connecting agent in different polymers, should not be used as a structural unit in biodegradable polymers because of the environmental risk. In contrast, biotests clearly showed that poly(esterurethane), in which lactic acid prepolymers were chain linked with 1,4-butane di-isocyanate, did not exhibit any ecotoxicological effect, and neither did poly(ester-amide) or poly-(lactide).

DEP that is used as a plasticizer is an acknowledged endocrine disruptive substance and therefore it is important to understand its fate in the environment. In addition to its endocrine disruptive properties, DEP concentrations from 1 to 100 g kg^{-1} induce acute toxicity, reduce plant growth and also affect the microbial diversity when present in compost plant growth media. Changes observed in microbial community in DEP -spiked compost media originate from the role of DEP as a carbon source for microbes, or from the toxicity of DEP to microbes in the highest tested concentrations. During the composting of cellulose acetate with DEP as a plasticizer, DEP is released from the polymer to the compost. In controlled composting test conditions in which the concentration of the tested polymers is high, a clear toxic response can be detected by biotests. However, in a pilot scale composting experiment the released DEP degraded during the experiment and no toxicity was detected in the end product.

Sewage sludge may contain high amounts of different contaminants, both organic and inorganic. In addition to the chemical analysis, the benefit of biotests in quality assessment of sewage sludge and composted sewage sludge could be high. Biotest can be used as an indicator for potential risk for example when sewage sludge-based-products are targeted to agricultural or landscaping applications. We detected acute toxicity, genotoxicity and elevated endocrine disruption and dioxin-like activity in sewage sludges from plants treating higher amounts of industrial wastewaters, which also contained the highest loads of organic contaminants. With composting, the amounts of the organic contaminants DEHP, PAH, LAS, and NPs in sewage sludge can be reduced efficiently. In addition, composting results in reduction in acute toxicity, genotoxicity and endrocine-disruption potential of the sewage sludge.

It can be concluded that biotests do provide beneficial information concerning polymer safety during the design, recycling, and disposal life stages of the biodegradable materials. There are promising possibilities but also some limitations in the performance of different biotests when studying the ecotoxicity of biodegradable materials during the biodegradation processes in compost and soil

environments. Advantages of biotests in the examination of compost quality are clear. Even when there is no evidence based on chemical analysis of hazardous amounts of toxic chemicals, biotests may show evidence of toxicity as was observed in the laboratory scale composting test during the PEU1%H and PEU4%H biodegradation. Of the applied biotests in this study, the kinetic luminescent bacteria test and plant growth assay were good methods for measuring ecotoxicity of biodegradable polymers and sewage sludge and products derived therefrom in compost and in soil environments. The kinetic luminescent bacteria test is especially designed for colourful samples and is therefore well suited for testing the toxicity of compost, sewage sludge and soil samples. However, care must be taken in the interpretation of the ecotoxicity test results. With the luminescent bacteria test, additional carbon source and nutrients present in the sample may activate the light production of *Vibrio fischeri* and slight signals for toxicity could be hidden. Vitotox^{TM} 10Kit as an indicator of genotoxicity, and yeast bioreporter for detecting endocrine disruptive activity, were not ideal for evaluation of the quality of compost or sewage sludge samples due to high amounts of nutrients and bark/peat in the studied samples. In addition, when *Enchytraeidae* tests are conducted in soils during the active polymer degradation phase, reproduction of *Enchytraeidae* is favoured by high organic matter content during polymer degradation in soil. Furthermore, with compost samples it is most important to take into account the stage of maturity in order to be able to avoid false positive results in ecotoxicity testing. One biotest is never sufficient for evaluating the environmental toxicity of biodegradable materials; therefore we recommend using biotests covering more than one end point and trophy level, and carefully evaluating the suitability of biotests applied with regard to the studied environment.

References

86/278/EEC. Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture.

1999/31/EC. Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste.

Alvarenga, P., Palma, P., Gonçalves A.P., Fernandes, R.M., Cunha-Queda, A.C., Duarte, E., Vallini, G. 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33, 505–513.

Anonymous 1998. Toxicological profile for hexamethylene diisocyanate U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, August 1998.

Anonymous 2011. Science for Environment Policy, Future Briefs Issue No. 1, p. 7.

Amin, A.W. 2011. Evaluation of the Genotoxicity of Residual Repeated Applications of Sewage Sludge on M_2 Meiocytes of Zea Plants. Res. J. Environ. Toxicol. 5, 235–250.

Amir, S., Hafidi, M., Merlina, G., Hamdi, H., Jouraiphy, A., El Gharous, M., Revel, J.C. 2005. Fate of phthalic acid esters during composting of both lagooning and activated sludge. Process Biochem. 40, 2184–2190.

Amlinger, F., Pollack, M., Favoino, E. 2004. Heavy metals and organic compounds from waste used as organic fertilizers Ref.Nr.: Tend/AML/2001/07/20, NV.A.2./ETU/2001/0024, Summary Report. (Access date 26 June, 2011)

Amundsen, C.E., Eggen, T., Lystad, H. 2001. Stabilitet og nedbrytning av organiske forurensninger i avløpsslam ved stor-skala kompostering – Resultater fra rankekompostering ved Lindum Ressurs og Gjenvinning TemaNord, 588.

Andersen, A., 2001. Disposal and recycling routes for sewage sludge – Part 2 – Regulatory report and Part 3 – Scientific and technical sub-component report, 23 October 2001. European commission DG Environment – B/2. (Access date 23 Sept, 2010)

Andrady, A.L., Neal, M.A. 2009. Applications and societal benefits of plastics. Phil. Trans. R. Soc. B 364, 1977–1984.

ASTM D6400-12. Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities. American Society for Testing and Materials.

ASTM E1676-97. Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia Fetida* and the Enchytraeid Potworm *Enchytraeus albidus*. American Society for Testing and Materials.

Barker, M., Safford, R. 2009. Industrial uses for crops: markets for bioplastics. Project Report No. 450, HGCA, Caledonia House, London, p. 76.

Bastioli, C. 1998. Properties and applications of Mater-Bi starch-based materials. Polym. Deg. Stab. 59, 263–272.

Bécaert, V., Deschênes, L. 2006. Using soil health to assess ecotoxicological impacts of pollutants on soil microflora. Rev. Environ. Contam. Toxicol. 188, 127–148.

Bellia G, Tosin M, Floridi G, Degli-Innocenti, F. 1999. Activated vermiculite, a solid bed for testing biodegradability under composting conditions. Polym. Degrad. Stab. 66, 65–79.

Briassoulis, D. 2006. Mechanical behavior of biodegradable agricultural films under real field conditions. Polym. Degr. Stab. 91, 1256–1272.

Briassoulis, D. 2007. Analysis of the mechanical and degradation performances of optimised agricultural biodegradable films. Polym. Degrad. Stab. 92, 1115–1132.

Bruns, C., Gottschall, R., De Wilde, B. 2001. Ecotoxicological trials with BAK 1095 according to DIN V 54900. Orbit J. 1, 1–10.

Brändli, R., Kupper, T., Bucheli, T., Mayer, J., Stadelmann F.X., Tarradellas, J. 2004. Occurrence and relevance of organic pollutants in compost, digestate and organic residues – Literature review. Final report, 193. (Access date 6 Mar, 2006)

Calmon, A. Guillaume, S., Bellon-Maurel, V., Feuilloley, P., Silvestre1, F. 1999. Evaluation of Material Biodegradability in Real Conditions-Development

of a Burial Test and an Analysis Methodology Based on Numerical Vi-Vision. J. Environ. Polym. Degrad. 7, 157–166.

Cartwright, C.D., Owen, S.A., Thompson, I.P., Burns, R.G. 2000a. Biodegradation of diethyl phthalate in soil by a novel pathway. FEMS Microbiol. Lett. 186, 27–34.

Cartwright, C.D., Thompson, I.P., Burns, R.G. 2000b. Degradation and impact of phthalate plasticizers on soil microbial communities. Environ. Toxicol. Chem. 19, 1253–1261.

Chang, B.V., Lu, Y.S., Yuan, S.Y, Tsao, T.M, Wang, M.K. 2009. Biodegradation of phthalate esters in compost-amended soil. Chemosphere 74, 873–877.

Chauret, C., Mayfield, C.I., Inniss, W.E. 1995. Biotransformation of di-N-butyl phthalate by a psychrotrophic Pseudomonas fluorescens (Bgw) isolated from subsurface environment. Can. J. Microbiol. 41, 54–63.

Cheng, H-S, Kumar, M., Lin, J-G. 2008. Degradation kinetics of di-(2-ethylhexyl) phthalate (DEHP) and organic matter in sewage sludge during composting. J. Hazard. Mater. 154, 55–62.

Cho, H.S., Moon, H.S., Kim, M., Nam, K., Kim, J.Y. 2011. Biodegradability and biodegradation rate of poly(caprolactone)-starch blend and poly(butylene succinate) biodegradable polymer under aerobic and anaerobic environment. Waste Manage. 31, 475–480.

COM [2005] 666. Communication From the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions. Taking sustainable use of resources forward: A Thematic Strategy on the prevention and recycling of waste. Commission of the European Communities 2005.

Colborn, T., vom Saal, F., Sotto, A.M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Persp. 101, 378–384.

Degli Innocenti, F. 2005. Biodegradation behavior of polymers in the soil. In Handbook of Biodegradable Polymers (Ed. C.Bastioli), Rapra Technology Limited, UK, 57–102.

Degli-Innocenti, F., Bastioli, C. 1997. Definition of compostability criteria for packaging: initiatives in Italy. J. Environ. Polym. Degrad. 5, 183–189.

Degli-Innocenti, F., Tosin, M., Bastioli, C. 1998. Evaluation of the Biodegradation of Starch and Cellulose Under Controlled Composting Conditions. J. Environ. Polym. Degrad. 6, 197–202.

DIN 38412. German Standard Methods For the Examination of Water, Waste Water and Sludge; Bio Assays (group L), Deutsches Institut für Normung.

DIN 54900. Parts 1–4, Testing of the Compostability of Plastics. Deutsches Institut für Normung 1998.

Domene, X., Solà, L., Ramírez, W., Alcañiz, J.M., Andrés, P. 2011. Soil bioassays as tools for sludge compost quality assessment. Waste Manag. 31, 12–522.

Domenek, S., Feuilloley, P., Gratraud, J., Morel, M.-H., Guilbert, S. 2004. Biodegradability of wheat gluten based bioplastics. Chemosphere 54, 551–559.

Dubova, L., Zarina, Dz. 2004. Application of Toxkit microbiotests for toxicity assessment in soil and compost. Environ. Toxicol. 19, 274–279.

EC 1907/2006. 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).

EC 2008. Environmental, economic and social impacts of the use of sewage sludge on land. Milieu LTD, WRc and RPA for the European commission, DG Environment under the study contract DG ENV.G.4/ETU/2008/0076r. (Access date 10 June, 2010)

Eldsäter, C., Erlandsson, B., Renstad, R., Albertsson, A-C., Karlsson, S. 2000. The biodegradation of amorphous and crystalline regions in film-blown poly(e-caprolactone). Polymer 41, 1297–1304.

EN 13037. Soil improvers and growing media – Determination of pH. European Committee for Standardisation 1999.

EN 13038. Soil improvers and growing media – Determination of electrical conductivity. European Committee for Standardisation 1999.

EN 13039. Soil improvers and growing media – Determination of organic matter content and ash. European Committee for Standardisation1999.

EN 13040. Soil improvers and growing media – Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density. European Committee for Standardisation 1999.

EN 13432. Packaging – Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging. European Committee for Standardisation 2000.

EN 14045. Packaging. Evaluation of the disintegration of packag-ing materials in practical oriented tests under defined composting conditions. European Committee for Standardisation 2003.

EN 14046. Packaging. Evaluation of the ultimate aerobic biodegradability of packaging materials under controlled composting conditions. Method by analysis of released carbon dioxide. European Committee for Standardisation 2003.

EN 14995. Plastics. Evaluation of compostability. Test scheme and specifications. European Committee for Standardisation 2006.

Engwall, M., Brunström, B., Näf, C. Hjelm, K. 1999. Levels of dioxin-like compounds in sewage sludge determined with a bioassay based on erod induction in chicken embryo liver cultures. Chemosphere, 38, 2327–2343.

Erhardt, W., Pruess, A. 2001. Organic contaminants in sewage sludge for agricultural use. Report prepared by UMEG Center for Environmental Measurements Environment Inventories and product safety, Germany for the European Commission, Joint Research Centre, Institute for Environment and Sustainability, Soil and Waste Unit. (Access date 26 June, 2011)

Eriksson, E. Christenssen, N., Schmidth, J.E., Ledin, A. 2008. Potential priority pollutants in sewage sludge. Desalination 226, 371–388.

EU RAR 2001. European Union Risk Assessment report, 4,4'-methylenedianiline, Volume 9. European Commission (EC), Publication Nr. EUR 19727, Eds. B.G.Hansen, S.J. Munn, S.Pakalin, C.J.A. Heidorn, R. Allnou, S. Scheer, G. pellegrini, S. Vergo, M. Luotamo, J. De Bruijin, F. Berthault, H. Loonen, K, Vormann, A. Naughton, V. Anfossi, L. Praderio., Luxembourgh, 125.

Fang, H.H.P, Liang, D., Zhang, T. 2007. Aerobic degradation of diethylphthalate by Sphingomonas sp. Bioresour. Technol. 98, 717–720.

la Farré, M., García, M-J, Castillo, M., Riu, J., Barceló, D. 2001. Identification of surfactant degradation products as toxic organics compounds present in sewage sludge. J. Environ. Monit. 3, 232–237.

Favoino, E. 2005. Biodegradable polymers and the optimization of models for source separation and composting of municipal solid waste. In Handbook of biodegradable polymers. Ed. C. Bastioli, Rapra Technology Limited, UK, 485–506.

Finkenstadt, V.L., Tisserat, B. 2010. Poly(lactic acid) and Osage Orange wood fiber composites for agricultural mulch films. Ind. Crops Prod. 31, 316–320.

Di Franco, C.R., Cyras, V.P., Busalmen, J.P., Ruseckaite, R.A., Vazquez, A., 2004. Degradation of polycaprolactone/starch blends and composites with sisal fibre. Polym. Degrad. Stab. 86, 95–103.

Fritz, J. 1999. Ecotoxicity of biogenic materials during and after their biodegradation, The University for Agriculture, Vienna, Austria. Ph.D Thesis, 145.

Fritz, J. 2005. Ecotoxicological aspects in the biodegradation process of polymers. In Handbook of biodegradable polymers. Ed. C. Bastioli, Rapra Technology Limited, UK, 103–144.

Fritz, J., Link, U., Braun, R. 2001. Environmental impacts of biobased/biodegradable packaging. Starch 53, 105–109.

Fritz, J., Sandhofer, M., Stacher, C., Braun, R. 2003. Strategies for detecting ecotoxicological effects of biodegradable polymers in agricultural applications. Macromol. Symp. 197, 397–409.

Fromme, H., Kuchler, T., Otto,T., Pilz, K., Muller,J., Wenzel, A. 2002. Occurrence of phthalates and bisphenol A and F in the environment. Water. Res. 36, 1429–1438.

Fuentes, A., Lloréns, M., Sáez, J., Aguilar, M.I., Ortuño, J.F., Meseguer, V.F. 2004. Phytotoxity and heavy metals speciation of stabilized sewage sludges. J Hazard Mater. A108, 161–169.

Fuentes, A., Lloréns, M., Saéz, J., Aguilar, M. I., Pérez-Marín, A.B., Ortuño, J.F., Meseguer, V.F. 2006. Ecotoxity, phytotoxity and extractability of

heavy metals from different stabilised sewage sludges. Environ. Pollut. 143, 355–360.

Gawlik, B.M., Bidoglio, G. 2006. Background values in European soils and sewage sludge. PART I Leschber R. Evaluation of the relevance of micro-pollutants in sewage sludge, PART III Gawlik BM, Bidoglio G. Conclusions, comments and recommendations. EUR 22265 EN –DG Joint Research Centre, Institute for the Environment and Sustainability. (Access date 19 Dec, 2006)

de Groot, J.H. de Vrijer, R. Wildeboer, B.S. Spaans C.S. and Pennings A.J. 1997. New biomedical polyurethane ureas with high tear strengths. J. Polym. Bull. 38, 211–218.

Gross, R.A., Kalra, B. 2002. Biodegradable polymers for the environment. Science 297, 803–807.

Hakkarainen, M. 2002. Aliphatic Polyesters: Abiotic and Biotic Degradation and Degradation Products. Adv. Polym Sci. 157, 113–138.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M., Ikonomou, M.G. 2003. Polybrominated diphenyl ether flame retardants in the North American environment. Environ. Int. 29, 771–779.

Hale, R.C., La Guardia, M.J., Harvey, E., Gaylor, M.O., Mainor, T.M. 2006. Brominated flame retardant concentrations and trends in abiotic media. Chemosphere 64, 181–186.

Hamdi, H., Manusadžianas, L., Aoyama, I., Jedidi, N. 2006. Effects of anthracene, pyrene and benzo[a]pyrene spiking and sewage sludge compost amendment on soil ecotoxicity during a bioremediation process. Chemosphere 65, 1153–1162.

Harrison, E.Z., Oakes, S.R., Hysell, M., Hay, A. 2006. Organic chemicals in sewage sludge. Sci. Total Environ. 367, 481–497.

Hernandez-Raquet, G., Soef, A., Delgene`s, N, Balaguer, P. 2007. Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. Water Res. 41, 2643–2651.

Hiltunen, K., Seppälä, J., Itävaara, M., Härkönen, M. 1997. The biodegradation of lactic acid based poly(ester-urethanes). J. Environ. Polym. Degrad. 5, 167–173.

Horn, O., Nalli, S., Cooper, D., Nicell, J. 2004. Plasticizer metabolites in the environment. Water Res. 38, 3693–3698.

ISO 6341. Water quality – Determination of the inhibition of the mobility of Daphnia magna Straus (*Cladocera*, *Crustacea*) – Acute toxicity test. International Organization for Standardization 1996.

ISO 11261. Soil quality – Determination of total nitrogen – Modified Kjeldahl method. International Organization for Standardization 1995.

ISO 11263. Soil quality – Determination of phosphorus – Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution. International Organization for Standardization 1994.

ISO 11267. Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Organization for Standardization 1999.

ISO 11268. Soil quality – Effects of pollutants on earthworms. International Organization for Standardization.

ISO 11269-2. Soil quality – Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the emergence and growth of higher plants. International Organization for Standardization 2005.

ISO 11348. Water quality – Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test). International Organization for Standardization.

ISO 14852. Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium – Method by analysis of evolved carbon dioxide. International Organization for Standardization 1999.

ISO 15685. Soil quality – Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation. International Organization for Standardization 2004.

ISO 16387. Soil Quality: Effects of pollutants on *Enchytraeidae* – determination of effects on reproduction and survival (*Echytraeus albidus*). International Organization for Standardization 2004.

ISO 17088. Specifications for compostable plastics. International Organization for Standardization 2008.

ISO 21338. Water Quality – Kinetic determination of the effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri** (kinetic luminescent bacteria test). International Organization for Standardization 2010.

Itävaara, M. Vikman, M. 1996. An overview of methods for biodegradability testing of biopolymers and packaging materials. J. Environ. Polym. Degradat. 4, 29–36.

Itävaara, M., Venelampi, O., Samsøe-Petersen, L., Lystad, H., Bjarnadottir, H., Öberg, L. 1998. Assessment of compost maturity and ecotoxicity Technical Report 404, NORDTEST-PROJECT 1363–97, 84.

Itävaara, M., Karjomaa, S., Selin, J-F. 2002a. Biodegradation of polylactide in aerobic and anaerobic thermophilic conditions. Chemosphere 46, 879–883.

Itävaara, M., Venelampi, O., Vikman, M., Kapanen, A. 2002b. Compost maturity – problems associated with testing. In Microbiology of Composting Eds. H. Insam, N. Riddech, S. Klammer. Springler-Verlag Berlin Heidelberg. pp. 373–382.

Itävaara, M., Vikman, M., Kapanen A., Venelampi O., Vuorinen A. 2006. Kompostin kypsyystestit. Menetelmäohjeet. VTT Tiedotteita 2351, p. 43.

Itävaara M., Vikman M, Maunuksela L, Vuorinen A. 2010. Maturity tests for composts – verification of a test scheme for assessing maturity. Compost Sci. Util. 18, 174 – 183.

Jayasekara, R., Sheridan, S., Lourbakos, E., Beh, H., Christie, G.B.Y., Jenkins, M., Halley, P.B., McGlashan, S., Lonergan, G.T. 2003. Biodegradation and ecotoxicity evaluation of a bionolle and starch blend and its degradation products in compost. Int. Biodeterior. Biodegradation 51, 77–81.

Jensen, J. 2004. Ecotoxicological effect assessment and risk characterization of selected contaminants in sewage sludge. PhD Thesis, The Danish University of Pharmaceutical Sciences Department of Analytical Chemistry.

Jensen, J., Jepsen, S-E. 2005. The production, use and quality of sewage sludge in Denmark. Waste Manag. 25, 239–247.

Jianlong, W., Xuan, Z. 2004. Biodegradation of phthalic acid esters (PAEs) in soil bioaugmented with acclimated activated sludge. Process Biochem. 39, 1837–1841.

JIS K 6953. Determination of the ultimate aerobic biodegradability of plastic. Japanese Industrial Standards.

Johnson, K.E., Pometto III, A.L., Somasundaram, L., Coats, J. 1993. Microtox assay to determine the toxicity of degradation products from degradable plastics. J. Environ. Polym. Degradat. 1, 111–116.

Jobling, S., Reynolds, T., White, R., Parker, M.G., Sumpter, J.P. 1995. A variety of environmentally persistent chemicals, including some phthalate plasticisers, are weakly estrogenic. Environ. Health Perspect. 103, 582–587.

Jonsson, S., Baun, A. 2003. Toxicity of mono- and diesters of *o*-phthalic esters to a crustacean, a green alga, and a bacterium. Environ. Toxicol. Chem. 22, 3037–3043.

Joutti, A. Ahtiainen, J. 1994. Saastuneen maaperän biokemiallisia ja biologisia tutkimusmenetelmiä. *Vesi- ja Ympäristöhallituksen monistesarja*, 520, 52.

Juvonen, R., Martikainen, E., Schultz, E., Joutti, A., Ahtinen, J., Lehtokari, M. 2000. A battery of toxicity tests as indicators of decontamination in composting oily waste. Ecotoxicol. Environ. Safe. 47, 156–166.

Jätelaki 1072/1993. Waste Act (in Finnish).

Kale, G., Auras, R., Singh, S.P. 2007a. Comparison of the Degradability of Poly(lactide) Packages in Composting and Ambient Exposure Conditions. Packaging Technol. Sci. 20, 49–70.

Kale, G., Kijchavengkul, T., Auras,R., Rubino, M., Selke, S. E., Singh S. P. 2007b. Compostability of Bioplastic Packaging Materials: An Overview. Macromol. Biosci. 7, 255–277.

Kapanen, A., Itävaara, M. 2001. Ecotoxicity tests for compost applications. Ecotoxicol. Environ. Safe. 49, 1–16.

Kapanen, A., Venelampi, O., Vikman, M., Itävaara, M. 2002. Testing the ecotoxicity of biodegradable plastics. In KTBL-Schrift 414: Biodegradable materials and natural fibre composites. Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt, Germany, 107–111.

Kapanen, A., Priha, O., Maunuksela, L., Itävaara, M. 2009. Biotestien kehittäminen lannoitevalmisteiden valvontaan; lietteiden hyötykäytön edistäminen (Bioliete) 4543/501/2006. Tutkimusraportti 2007–2008. VTT-R-02284-09, 51.

Kim, M-N., Lee, A-R., Yoon, J-S., Chin, I-J. 2000. Biodegradation of poly(3-hydroxybutyrate), Sky-Green[®] and Mater-Bi[®] by fungi isolated from soils. Eur. Polym. J. 36, 1677–1685.

Klee, N., Gustavsson , L., Kosmehl ,T., Engwall, M., Erdinger, L., Braunbeck, T., Hollert, H. 2004. Changes in Toxicity and Genotoxicity of Industrial Sewage Sludge Samples Containing Nitro- and Amino-aromatic Compounds Following Treatment in Bioreactors with Different Oxygen Regimes. Environ. Sci. Pollut. Res. 11, 313–320.

Kroes, R. 1995. Toxicity testing and human health. In Risk Assessment of Chemicals: An Introduction, Eds. C.J van Leeuwen and J.L.M. Hermen, 147–174. Kluwer Academic Publishers.

Kunioka, M., Ninomiya, F., Funabash, M. 2006. Biodegradation of poly(lactic acid) powders proposed as the reference test materials for the international standard of biodegradation evaluation methods. Polym. Degradat. Stab. 91, 1919–1928.

Kyrikou, I., Briassoulis, D. 2007. Biodegradation of Agricultural Plastic Films: A Critical Review J. Polym. Environ. 15(2), 125–150.

Landis, G.W., Yu, M-H. 1995. Introduction to Environmental Toxicology: Impacts of chemicals upon ecological systems, p. 328. Lewis Publischers, CRC Press, Inc.

Lannoitevalmistelaki 539/2006. Act on Fertilizers (in Finnish).

Lappalainen, J. 2001. Improved use and new applications of luminescent bacteria. Turku University, A1, 264. Doctoral Thesis, 54.

Laturnus, F, von Arnold K, Grøn C. 2007. Organic Contaminants from Sewage Sludge Applied to Agricultural Soils. Env. Sci. Pollut. Res. 14, Special Issue 1, 53–60.

Leusch, F.D.L., Chapman, H.F., van den Heuvel, M.R., Tan, B.L.L. Gooneratne, S.R., Tremblay, L.A. 2006. Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand. Ecotoxicol. Environ. Saf. 65, 403–411.

Liang, D.-W., Zhang, T. Fang, H.H.P, He, J. 2008. Phthalates biodegradation in the environment. Appl. Microbiol. Biotechnol. 80, 183–198.

Lopez, M.J., Vargas-García, M.C., Suárez-Estrella, F., Guisado, G., Moreno, J. 2010. Flash microbial toxicity test as monitoring parameter at composting: comparison of ecotoxicity level for different substrates. Proceedings in 14th Ramiran International Conference, Lisboa, Portugal.

Luckachan, G.E., Pillai, C.K.S. 2011. Biodegradable Polymers- A Review on Recent Trends and Emerging Perspectives. J. Polym. Environ. 19, 637–676.

MacNaughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A., Chang, Y-J., White, D.C. 1999. Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microb. 65, 3566–3574.

Mantis, I., Voutsa, D., Samara, C. 2005. Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods. Ecotoxicol. Environ. Saf. 62, 397–407.

Marttinen, S., Hänninen, K., Rintala, J. 2004. Removal of DEHP in composting and aeration of sewage sludge: Chemosphere 54, 262–272.

Moeller, J., Reeh, U. 2003. Degradation of DEHP, PAHs and LAS in source separated MSW and sewages sludge during composting. Compost Sci. Util. 11, 370–378.

Mudgal, S., Lyons, L, Bain, J., Dias, D., Faninger, T. Johansson, L., Dolley, P., Shields, L., Bowyer, C. 2011. Plastic Waste in the Environment – Revised Final Report for European Commission DG Environment. Downloaded from http://www.ec.europa.eu/environment/waste/studies/pdf/plastics.pdf (Acces date 5 Dec, 2011).

Müller, R.-J. 2005a. Biodegradability of Polymers: Regulations and Methods for Testing. In Biopolymers 10. Ed. A. Steinbuchel, 365–392.

Müller, R.-J. 2005b. Biodegradation behaviour of polymers in liquid environments. In Handbook of Biodegradable Polymers. Ed. C. Bastioli, Rapra Technology Limited, UK, 33–55.

Murk, A., Legler, J., van Lipzig, M., Meerman J., Belfroid, A., Spenkelink, A., van der Burg, B., Rijs, G., Vethaak, D. 2002. Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environ. Toxicol. Chem. 21, 16–23.

Natal-da-Luz, T., Tidona, S., Jesus, B., Morais, P.V., Sousa, J.P. 2009. The use of sewage sludge as soil amendment. The need for an ecotoxicological evaluation. J Soils Sediments 9, 246–260.

Norton, J. M., Javier J. Alzerreca, J. J., Suwa, Y., Klotz, M.G. 2002. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Arch. Microbiol. 177(2), 139–149.

Ødegaard, H., Paulsrud, B and Karlsson, I. 2002. Wastewater sludge as a resource: sludge disposal strategies and corresponding treatment technologies aimed at sustainable handling of wastewater sludge. Water Sci. Technol. 46(10), 295–303.

OECD 201. OECD guideline for testing of chemicals. "Alga, Growth Inhibition Test".Organisation for Economic Cooperation and Development 2011.

OECD 202. OECD guideline for testing of chemicals. "Daphnia sp., Acute Immobilisation Test and Reproduction Test".Organisation for Economic Cooperation and Development 2004.

OECD 207. OECD guideline for testing of chemicals. "Earthworm, Acute Toxicity Tests".Organisation for Economic Cooperation and Development 1984.

OECD 208. OECD guidelines for the testing of chemicals. Terrestrial plant test: seedling emergence and seedling growth test. Organisation for Economic Cooperation and Development 2006.

OECD/SIDS 1994 OECD Screening Information Data Set (SIDS) for High Production Volume Chemicals, Hexamethylenediamine, 91.

OECD/SIDS 2004. OECD Screening Information Data Set (SIDS) for High Production Volume Chemicals, Hexamethylene diisocyanate, 73.

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz I., Kusk, K. O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look K.J.W., Tyler, C.R. 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Phil. Trans. R. Soc. B 2009 364, 2047–2062.

Oleszczuk, P. 2007. Changes of polycyclic aromatic hydrocarbons during composting of sewage sludges with chosen physico-chemical properties and PAHs content, Chemosphere 67, 582–591.

Oleszczuk, P. 2008. Phytotoxicity of municipal sewage sludge composts related to physic-chemical properties, PAHs and heavy metals. Ecotoxicol. Environ. Saf. 69, 496–505.

Oleszczuk, P. 2010. Toxicity of light soil fertilized by sewage sludge or compost in relation to PAH content. Water Air Soil Pollut. 210, 347–356.

ÖNORM S 2023. Untersuchungsmethoden und Güteüberwachung von Komposten. Austrian Standards Institute 1993.

Pagga, U. 1997. Testing biodegradability with standard methods. Chemosphere 35, 2953–2972.

Pagga, U. 1998. Biodegradability and compostability of polymeric materials in the context of the European packaging regulation. Polym. Degrad. Stab. 59, 371–376.

Palojärvi, A., Alakukku, L., Martikainen, E., Niemi, M., Vanhala, P., Jørgensen, K., Esala, M. 2002. Luonnonmukaisen ja tavanomaisen viljelyn vaikutukset maaperään. Maa- ja elintarviketalous 2, 88.

Park, G.S., Chung, C.S., Lee, S.H., Hong, G-H., Kim, S-H., Park, S.Y., Yoon, S. J., Lee, S. M. 2005. Ecotoxicological evaluation of sewage sludge using bioluminescent marine bacteria and rotifer. Ocean Sci. J. 40, 91–100.

Pérez, S., Farré, M., García, M.J., Barceló, D. 2001. Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contribution to its toxicity in the ToxAlert® 100 bioassay. Chemosphere 45, 705–712.

Pessala, P. 2008. Tracking and identifying wastewater toxicants and assessing their biodegradation products. PhD Thesis, University of Helsinki, 54.

Phillips, C.J., Harris, D., Dollhopf, S.L., Gross, K.L., Prosser, J.I., Paul, E.A. 2000. Effects of agronomic treatment on structure and function of ammonia-oxidizing communities. Appl. Environ. Microbiol. 66, 5410–5418.

Poulsen, T.G., Bester, K. 2010. Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. Environ. Sci. Technol. 44, 5086–5091.

Priha, O., Kapanen, A., Maunuksela L. 2009. Tutkimustietoa lietekompostien hyötykäytöstä. Uusiouutiset 20(3–4), 20–21.

Ramírez, W.A., Domene, X., Ortiz, O., Alcañiz, J.M. 2008. Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresour. Technol. 99, 7168–7175.

Ratto, J., Stenhouose, P.J., Auerbach, M., Mitchell, J., Farrell, R. 1999. Processing performance and biodegradability of a thermoplastic aliphatic polyester/starch system. Polymer, 40 , 6777–6788.

Renoux, A.Y., Tyagi, R.D., Samson, R. 2001. Assessment of toxicity reduction after metal removal in bioleached sewage sludge. Water Res. 35, 1415–1424.

Rudnik, E. 2008. Compostable polymer materials. Elsevier Ltd, 211.

Rudnik, E., Milanov, N., Matuschek, G., Kettrup, A. 2007. Ecotoxicity of biocomposites based on renewable feedstock – Preliminary studies. Chemosphere 70, 337–340.

Rutkowska, M., Krasowska K., Heimowska A., Kowalczuk M. 2001. Biodegradation of the blends of Atactic poly[(R,S)-3-hydroxybutanoic acid] in natural environments. In "Biorelated Polymers: Sustainable Polymer Science and Technology", Eds. E. Chiellini, H., Gil, G. Braunegg, J. Buchert, P. Gatenholm, M. van der Zee., Kluwer Academic/Plenum Publishers, 313–320.

Rychter, P. Kawalec, M., Sobota, M., Kurcok, P., Kowalczuk, M. 2010. Study of Aliphatic-Aromatic Copolyester Degradation in Sandy Soil and Its Ecotoxicological Impact. Biomacromolecules 11, 839–847

Saarma, K., Tarkka, M.T., Itävaara, M., Fagerstedt, K.V. 2003. Heat shock protein synthesis is induced by diethyl phthalate but not by di(2-ethylhexyl) phthalate in radish (*Raphanus sativus*), J. Plant Physiol. 160, 1001–1010.

Schettini, E., Vox, G., De Lucia B. 2007. Effects of the radiometric properties of innovative biodegradable mulching materials on snapdragon cultivation. Sci. Hortic. 112, 456–461.

Schowanek, D., Carr, R., David, H., Douben, P., Hall, J., Kirchmann, H., Patria, L., Sequi, P., Smith, S., Webb, S. 2004. A risk-based methodolgy for deriving quality standards for organic contaminants in sewage sludge for use in agriculture – Conceptual Framework. Regul. Toxicol. Pharmacol. 40, 227–251.

Seppälä, J.V., Helminen, A.O., Korhonen, H. 2004. Degradable polyesters through chain linking for packaging and biomedical applications. Macromol. Biosci. 4, 208–217.

Selivanovskaya, S.Yu., Latypova, V.Z. 2003. The use of bioassays for evaluating the toxicity of sewage sludge and sewage sludge-amended soil. J Soils Sediments 3, 85–92.

Shen, J. and Bartha, R. 1996. Priming effect of substrate addition in soil-based biodegradation tests. Appl. Environ. Microbiol. 62, 1428–1430.

Shogren, R.L. 2000. Biodegradable mulches from re-newable resources. J. Sustain. Agr. 16, 33–47.

Siotto, M., Tosin, M., Degli Innocenti, F., Mezzanotte, V. 2011. Mineralization of monomeric components of biodegradable plastics in preconditioned and enriched sandy loam soil under laboratory conditions. Water Air Soil Pollut. 221, 245–254.

Smith, S.R. 1996. Agricultural Recycling of Sewage Sludge and the Environment, Wallingford, UK, CAB International.

Smith, S.R. 2000. Are controls on organic contaminants necessary to protect the environment when sewage sludge is used in agriculture? Prog. Environ. Sci. 2, 129–146.

Song, J.H., Murphy, R.J., Narayan, R., Davies, G.B.H. 2009. Biodegradable and compostable alternatives to conventional plastics. Philos. Trans. R. Soc. B 364, 2127–2139.

Stamatelatou, K., Pakou, C., Lyberator, G. 2011. Occurrence, toxicity, and biodegradation of selected emerging priority pollutants in municipal sewage sludge. In Comprehensive Biotechnology. Ed. M-Y. Murray, 2nd edition, Vol. 6, 473–484.

Staples, C.A., Adams, W.J., Parkerton, T.F., Gorsuch, J.W. 1997a. Review, aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16, 875–891.

Staples, C.A., Peterson, D.R., Parkerton, T.F., Adams, W.J. 1997b. The environmental fate of phthalate esters: a literature review. Chemosphere 35, 667–749.

Staples, C.A., Parkerton, T.F., Peterson, D.R. 2000. A risk assessment of selected phthalate esters in North American and Western European surface waters. Chemosphere 40, 885–891.

Stephen, J.R., Chang, Y-J., Gan, Y-D., Peacock, A., Pfiffner, S.M., Barcelona, M.J., White, D.C., Macnaughton, S.J. 1999a. Microbial characterisation of a JP-4 fuel contaminated site using a combined lipid biomarker/ PCR-DGGE based approach. Environ. Microbiol. 1, 231–243.

Stephen, J.R., Chang, Y-J., Macnaughton, S.J., Whitaker, S.L., Hicks, C,L., Leung, K.T., Flemming, C.A., White, D.C. 1999b. Fate of a mixture of five metal resistant bacteria introduced into pristine and heavy-metal-contaminated soil microcosms assessed by DGGE. Environ. Toxicol. Chem. 18, 1118–1123.

Suemori, A., Kurane, R., Tomizuka, N. 1993. Purification and properties of phthalate oxygenase from Rhodococcus erythropolis S-1. Biosci. Biotechnol. Biochem. 57, 1482–1486.

Suzuki, G., Takigami, H., Kushi, Y., Sakai, S. 2004. Evaluation of mixture effects in a crude extract of compost using the CALUX bioassay and HPLC fractionation. Environ. Int. 30, 1055–66.

Suzuki, G., Takigami, H., Kushi, Y., Sakai, S. 2006. Time-course changes of mixture effects on AhR binding-dependent luciferase activity in a crude extract from a compost sample. Toxicol. Lett. 161, 174–87.

Terveydensuojelulaki 763/1994. Health protection Act (in Finnish).

Tosin, M., Degli-Innocenti, F., Bastioli, C. 1998. Detection of a Toxic Product Released by a Polyurethane-Containing Film Using a Composting Test Method Based on a Mineral Bed. J. Polym. Environ. 6, 79–90.

Urbanczyk, H., Ast, J.C., Higgins, M.J., Carson, J., Dunlap, P.V. 2007. Reclassification of *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. Int. J. Syst. Evol. Microbiol. 57, 2823–2829.

Valtioneuvoston päätös puhdistamolietteen käytöstä maanviljelyksessä 282/1994 (in Finnish).

Venelampi, O., Weber, A., Rönkkö, T., Itävaara, M. 2003. The biodegradation and disintegration of paper products in the composting environment. Compost Sci. Util. 11, 200–209.

Vikman, M., Itävaara, M., Poutanen, K. 1995. Measurement of the biodegradation of starch-based materials by enzymatic methods and composting. J. Environ. Polym. Degradat. 3, 23–29.

Vikman, M., Karjomaa, S., Kapanen, A., Wallenius, K., Itävaara, M. 2002. The influence of lignin content and temperature on the biodegradation of lignocellulose in composting conditions. Appl. Microbiol. Biotech. 59 (4–5): 591–598.

Vikman, M., Kapanen, A., Itävaara, M. 2006. Orgaaniset haitta-aineet jätevesilietteissä. Vesitalous 3: 7–10.

Wang, Y., Ruan, C., Sun, J., Zhang, M., Wu, Y., Peng, K. 2011. Degradation studies on segmented polyurethanes prepared with poly (D, L-lactic acid) diol, hexamethylene diisocyanate and different chain extenders. Polym. Degradat. Stab. 96, 1687–1694.

WD 2000. Working document on sludge 3rd draft, 27th April, Brussels. (Access date 8 Sept, 2005)

WD 2010. Working document on sludge and biowaste. 21st September, Brussels. (Access date 11 Jan, 2011)

De Wilde, B. 2005. International and national norms on biodegradability and certification procedures. In Handbook of biodegradable polymers. Ed. C. Bastioli, Rapra Technology Limited, UK, 145–181.

De Wilde, B., Boelens, J. 1998. Prerequisities for biodegradable plastic materials for acceptance in real life composting plants and technical aspects. Polym. Degrad. Stab. 59, 7–12.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere, 46, 583–624.

Witt, U., Einig, T., Yamamoto, M., Kleeberg, I., Deckwer, W-D., Müller, R-J. 2001. Biodegradation of aliphatic-aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. Chemosphere 44, 289–299.

Witt, U., Einig, T., Yamamoto, M., Kleeberg, I., Deckwer, W-D., Müller, R-J. 2001. Biodegradation of aliphatic-aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. Chemosphere 44, 289–299.

Yang, H-S., Yoon, J-S., Kim, M-N. 2005. Dependence of biodegradability of plastics in compost on the shape of specimens. Polym. Degradat. Stab. 87, 131–135

Ying, G-G. 2005. Fate, behaviour and effects of surfactants and their degradation products in the environment. Environ. Int. 32, 417–431.

Ympäristönsuojelulaki 86/2000. The Finnish Environmental Protection Act (in Finnish).

van der Zee, M. 1997. Structure-biodegradability relationship of polymeric materials. Dissertation University of Twente, Enschede, The Netherlands, 213.

van der Zee, M. 2005. Biodegradation of polymers – Mechanisms and evaluation methods. In Handbook of Biodegradable Polymers. Ed. C. Bastioli, Rap-Rapra Technology Limited, UK, 1–31.

Zhao, J-H., Wang, X-Q., Zeng, J., Yang, G., Shi, F-H., Yan, Q. 2005. Biodegradation of Poly(Butylene Succinate) in Compost. J. Appl. Polym. Sci. 97, 2273–2278.

Zou, E., Fingerman, M., 1997. Effects of estrogenic xenobiotics on molting of the water flea, Daphnia Magna. Ecotox. Environ. Saf. 38, 281–285.

Paper IV is not included in the PDF version.
Please order the printed version to get the complete publication
(http://www.vtt.fi/publications/index.jsp).

PAPER I

Detection of toxicity released by a biodegradable plastics after composting in activated vermiculite

In: Polymer Degradation and Stability 73, pp. 101–106

Detection of toxicity released by biodegradable plastics after composting in activated vermiculite

Francesco Degli-Innocenti[a],*, Gaetano Bellia[a], Maurizio Tosin[a], Anu Kapanen[b], Merja Itävaara[b]

[a]*Novamont S.p.A., via Fauser 8, I-28100 Novara, Italy*
[b]*VTT Biotechnology, PO Box 1500, 02044 VTT, Finland*

Abstract

The composting test method based on activated vermiculite is a comprehensive system for the assessment of the environmental impact of biodegradable plastics. It allows, in a single test, (i) the measurement of the mineralization of the polymer under study; (ii) the retrieval of the final polymeric residues and (iii) determination of the biomass (to make a final mass balance); (iv) detection of breakdown products of the original polymer. In this study it is shown that the vermiculite test method is also suitable to perform ecotoxicological studies. The Flash test is a method based on kinetic measurement of bioluminescence by *Vibrio fischeri,* and was applied to evaluate the toxicity of compost samples and vermiculite samples after the biodegradation of a polyurethane (PU) based plastic material. Toxicity was detected in vermiculite samples contaminated by 4,4′ diamino diphenyl methane (MDA), a toxic breakdown product released by the PU moiety, as shown by HPLC. On the other hand, neither toxicity nor the presence of MDA was detected in mature compost. A recovery experiment previously performed had shown a 10% MDA recovery yield from mature compost. The possibility of testing the ecotoxicity of extracts obtained from mineral matrix after biodegradation makes the vermiculite test system particularly interesting for the overall assessment of the environmental impact of biodegradable plastics. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Biodegradation; Composting; 4,4′ Diamino diphenyl methane; Ecotoxicity; Flash test; Polyurethane; Vermiculite; *Vibrio fischeri*

1. Introduction

The environmental fate of the biodegradable materials is to be directly or indirectly applied and finally integrated into the soil. Plastic films used for agricultural applications (i.e. mulching) are directly integrated into the soil, while compostable materials must pass through a biological treatment (i.e. the composting process) before entering in the environment. In both cases, fertile soil used for agriculture is the final environment. Therefore, it is of primary importance to assure that the biodegradable/compostable materials do not accumulate in the soil and do not release molecules with a toxic activity against plant and animal life during degradation. In the last years a great effort has been spent by the European Standardisation Committee

(CEN) to set up test methods and criteria concerning the compostability of man-made materials. The test scheme and the procedure to assess the compostability are described in the recently approved European Norm EN13432, which represents an important reference in all Europe. The biodegradability is assessed by measuring the CO_2 evolution under controlled composting conditions, using the standard method ISO 14855. The disintegration is determined by a pilot scale composting test (ISO/FDIS 16929). With regard to the evaluation of ecotoxicity, the norm requires a plant growth test on the final compost using two higher plants. This was one of the few test methods for specifically assessing the ecotoxicity in mature compost available at the time the European norm was written. The experts involved in the standardisation were aware of the scarcity of experience in this field.

In order to evaluate the ecotoxicity risk of the biodegradable plastics it is important not only to have suitable and sensitive test methods but also to define the

* Corresponding author. Tel.: +39-0321-699607; fax: +39-0321-699601.
E-mail address: fdi@materbi.com (F. Degli-Innocenti).

timing for the assessment. A plastic material can be safe before biodegradation, but may turn toxic during degradation.

According to EN13432, the ecotoxicity test should be performed on compost samples three months old, obtained in a pilot scale composting process (equivalent to the ISO/FDIS 16929). In order to strengthen the potential toxic exposure, the plastic material is added at a very high, unrealistic concentration (10%) to the initial bio-waste and composted for three months. 1% of the material can be added as pieces of film or sheets, to evaluate the disintegration after screening the final compost with a 2 mm sieve. If the test material is compostable, more than 90% of the initial specimens will disappear during the test. At this stage the compost is tested for possible negative effects on plants. However, it is not known what is the biodegradation level of the residual disintegrated test material at that moment, when the compost is subjected to the ecotoxicological testing. The disintegration of a polymer can be a consequence of splitting of a limited number of chemical bonds. If biodegradation is not yet completed, possible toxic molecules might be missed out simply because they are not yet released after 3 months composting. However, if the biodegradation is completed later in the soil (after compost application) the toxic molecules could be eventually released in the soil. Therefore, it seems important to couple the ecotoxicological testing with the measurement of mineralization in a integrated test system. A "comprehensive" laboratory scale composting test method should, at the same time, allow: to measure biodegradation as CO_2 evolution; to retrieve possible final residues of the original polymer (to allow a cross-verification of the CO_2 results); to detect possible low molecular weight molecules released by the test material into the solid matrix; to measure biomass; to perform ecotoxicological tests. To pursue this objective the test method for measuring mineralization of plastics under composting conditions (ISO 14855) has been modified by using activated vermiculite as a solid matrix instead of mature compost [1–3]. Vermiculite is a clay mineral applied for building purposes and it is known to be particularly suitable as a microbial carrier, allowing survival and full activity of microbes [4]. The advantage of using vermiculite instead of mature compost lays in the fact that, at the end of the test, vermiculite can be easily treated with suitable solvents to obtain clear solutions, suitable for further chemical analysis. On the other hand, the extraction from mature compost is a long, tedious procedure and the extracts obtained are generally not suitable for further chemical analysis because of their very complex organic composition [2].

In the present work, samples of vermiculite and mature compost have been analysed with a new test method: the Flash test. This method has been previously applied to evaluate the toxic effects of solid and coloured samples

such as soil and compost [5–7]. The method is based on the kinetic measurement of bioluminescence by *Vibrio fischeri*, a heterotrophic bacterium capable of light production as a part of its metabolism. In the Flash test each sample can be used as a reference of its own. In a typical kinetic profile of a non-toxic sample, the peak value of the luminescence is attained rapidly after dispensing the *Vibrio fischeri* suspension onto the sample and the luminescence level stays fairly constant during the 30 s exposure time. If the sample is toxic for the test organism, the light intensity might be reduced even before the peak maximum is reached [8].

2. Materials and methods

The plastic material 2030/489 is an experimental product composed of starch, polycaprolactone, 18% (w/w) polyurethane (Estane 54351) and a minor amount of plasticizers. Estane 54351 (BF Goodrich, USA) is a thermoplastic polycaprolactone-type polyurethane (PU). The mineralization of the material 2030/489 and cellulose was previously tested in vermiculite and in mature compost [2]. At the end of the experiment, the three replicates of each series (see Table 1) were pooled and samples of the different fermentation matrices (vermiculite + 2030/489; vermiculite blank; compost + 2030/489; compost blank: see Table 1) were extracted and analysed by HPLC. MDA was only detected in the vermiculite supplemented with 2030/489. The MDA concentration was 87.8 µg/g vermiculite (dry weight). In mature compost supplemented with 2030/489 no MDA was detected.

The pH of each sample was determined in water suspensions obtained by mixing compost and water in a 1:5 ratio (Table 1). Dry weight was determined after a 105°C treatment for 24 h. Compost and vermiculite samples were stored at +4°C until analysis.

Acute toxicity studies were carried out with a modified bioluminescent Flash-test [5]. The luminescence measurement is based on the BioTox™ kit utilizing the bioluminescent micro-organism *Vibrio fischeri* as a test organism. Kinetics measurement was performed with 1251 Luminometer (Bio-Orbit, Turku, Finland) at 20°C. Luminescence was measured throughout the whole

Table 1
List of tested samples

Samples	pH	Dry weight (%)
Compost blank	7.9	46.8
Compost supplemented with cellulose	8.1	47.7
Compost supplemented with 2030/489	8.1	46.9
Vermiculite blank	8.4	23.8
Vermiculite supplemented with cellulose	8.3	24.3
Vermiculite supplemented with 2030/489	8.5	25.1

exposure time (30 s). The peak value of luminescence was obtained within the first 5 s and it was followed by a reduction in the case of toxicity of the sample. On the other hand, no bioluminescence decrease was recorded in the absence of toxicity. The results were presented as percentages of inhibition in light production. Inhibition percentage was calculated as the ratio of the maximum light production (0–5 s) against the light production after 30 s exposure time. 2% NaCl solution was used as the test control in all experiments performed.

2.1. Pre-treatments of the samples

Three different pre-treatments were adopted for each sample.

1. 1 g (fresh weight) of the solid sample was mixed with 4 ml of the sample diluent (2% NaCl). The suspension was mixed for 1 min, left undisturbed for 5 min and mixed again for 1 min. Then it was neutralised in the case of pH below 6 or above 8. Finally the suspension was cooled down to 15°C and analysed without filtration.

2. The suspension, obtained using the same procedure as the first treatment, was filtered with a Whatman 4 filter paper after the pH correction, in order to remove compost or vermiculite particles.

3. The extraction step was prolonged overnight maintaining the sample in agitation (120 rpm) at 4°C. The suspension was not filtered.

A 500 μl aliquot of bacterial suspension then was added to 500 μl of the suspension, obtained as described before.

The luminescence was measured kinetically for the whole exposure time. Luminescence inhibition of MDA (4,4′ diamino diphenyl methane obtained by Fluka) was also determined. 0.41 g of MDA was diluted with 25 ml of ethanol. Because ethanol was shown to cause light reduction until dilution 1:40, MDA was studied in concentrations of 0.8–205 mg/l. The result was expressed as EC50 value (the concentration causing a 50% light reduction).

3. Results and discussion

Ecotoxicity of a plastic material, called 2030/489 containing a thermoplastic polycaprolactone-type polyurethane (PU) was studied. The 2030/489 is used as a model compound because the PU is based on the aromatic amine 4,4′ diphenyl methane diisocyanate (MDI). A concern in using MDI-based polyurethane in biodegradable materials is that during biodegradation the toxic breakdown product 4,4′ diamino diphenyl methane (MDA), might be released [9] (Fig. 1). The biodegradation levels of polyurethane starch blend 2030/489 in a vermiculite based test, were in good agreement with

the results obtained in the traditional controlled composting test, reaching the value of 89.7% in vermiculite and 88.2% in compost after 56 days [2]. At the end of the experiment, extracts of the composting matrices (see Table 1; abbreviations used onwards: vermiculite blank; vermiculite + 2030/489; compost blank; compost + 2030/489) were analysed by HPLC. MDA was only detected in vermiculite + 2030/489 (87.8 μg/g of dry vermiculite). In compost + 2030/489 no MDA was detected [2]. Considering the detection limit of our HPLC system and the test conditions, this means a MDA concentration $\leqslant$ 20 μg/g.

A dose–response curve for the Flash test was established using a stock solution of MDA. Concentrations tested were in the range 0.8–205 mg/l (Fig. 2). The EC_{50} value for MDA in the Flash test was 38 mg/l. Also at the lowest tested concentration (0.8 mg/l) MDA affected the light production of the test organism, causing a 7% inhibition. MDA has been found to be toxic in other toxicity tests based on single organisms, such as *Pseudomonas* and *Daphnia*, at concentrations of 15 and 0.25 mg/l, respectively [10]. Extracts of the different composting matrices (Table 1) were then subjected to the Flash test. The results are shown in Fig. 3.

3.1. Compost supplemented with 2030/489

The extracts of Compost blank, compost + cellulose and compost + 2030/489, prepared with the procedure 1 (see Materials and methods) showed no notable inhibitory effect when tested with the Flash test. Therefore, the effect of the compost + 2030/489 was not different from the background activity. An unexpected result was obtained by testing the compost extracts after filtration, according to procedure 2 (see Materials and methods). A slight background luminescence inhibition was measured in all the filtered compost samples (Fig. 3, white bars). Also in this case no difference between compost + 2030/489 and compost blank was evidenced. This result was in agreement with the HPLC analysis, unable
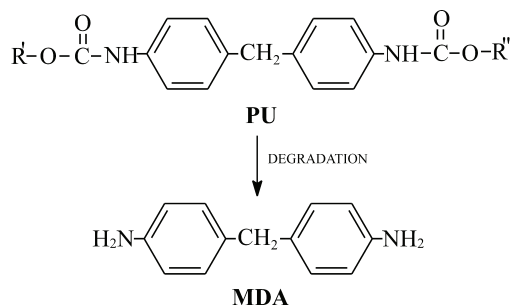

Fig. 1. Schematic representation of the production of 4,4′ diamino diphenyl methane (MDA) from an aromatic polyurethane (PU).
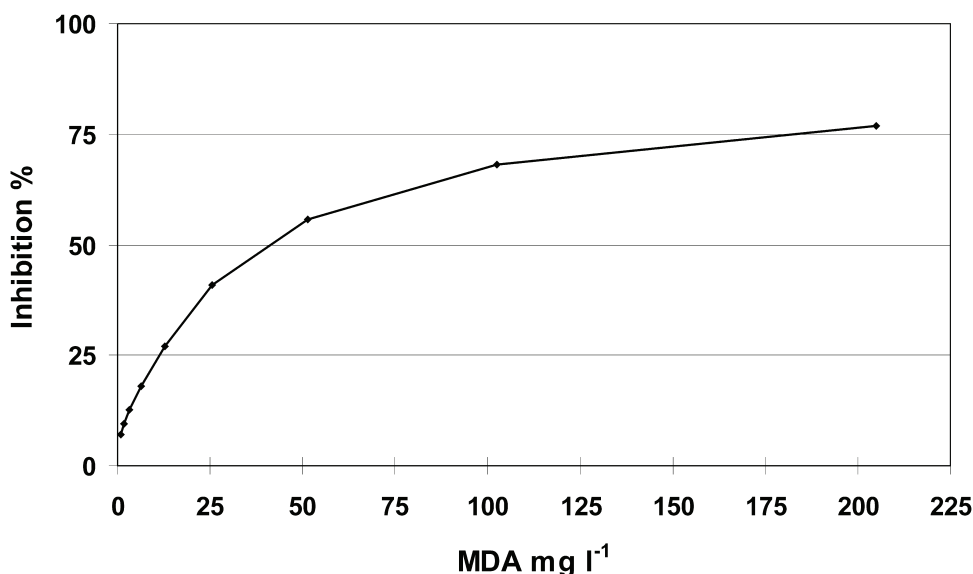
Fig. 2. Inhibition caused by MDA in the range from 0.8 to 205 mg/l in the Flash luminescent bacteria test.

to detect any MDA present in the extracts of compost + 2030/489.

3.2. Vermiculite supplemented with 2030/489

A clear toxic effect was found in vermiculite + 2030/489. The absolute luminescence inhibition was 14%. The vermiculite blank (and also vermiculite + cellulose) caused a 5% light stimulation. Therefore, the normalized inhibition effect over the background, taken equal to zero, is 19% (the sum of 14 and 5%; see Fig. 3, black bars). A luminescence inhibition was recorded also testing the vermiculite + 2030/489 after filtration (13%). The normalized inhibition value is lower (9%), because the blank shows also a inhibition effect.

The MDA concentration in the vermiculite + 2030/489 was 87.8 μg/g of dry vermiculite [2]. The suspension used in Flash test was obtained by suspending 1 g of fresh vermiculite (water content = 74.9%) in 4 ml of NaCl solution. The expected final concentration of MDA was 22 μg/4.749 ml. This suspension was diluted 1:1 before testing, therefore, the final concentration was 2.32 mg/l. This concentration (estimated through HPLC) and the resulting inhibition effect were in agreement with the dose–response curve determined with MDA (Fig. 2).

The filtration of the extracts caused an unexpected result, the increase of the luminescence inhibition of the samples. A release of toxic compounds during the filtration step can be excluded, because the NaCl solution, also filtered, did not show a remarkable increase of the inhibition. A possible explanation is that the presence of

solid particles (both compost and vermiculite) covered some background toxicity. The solid particles, may contain substances such as nutrients which stimulated the light production, and counteracted the decrease in light production due to toxicity present in the solution. This, however, did not happen with the vermiculite supplemented with the 2030/489. This suggests that filtration could trap some MDA together with the stimulatory factors.

3.3. Overnight extractions

The pre-treatment method based on the overnight extraction confirmed the toxicity of vermiculite + 2030/489 obtained with the short time extractions (Fig. 4). The modest background toxicity of the compost samples detected in the short time extraction was converted into a light stimulation, evidenced in all samples, both compost and vermiculite. This suggests that, during the longer extraction, more stimulating factor has been released. Only the vermiculite + 2030/489 still showed a clear inhibition (11%). The difference between vermiculite + 2030/489 and the vermiculite blank was 17% (the sum of the 11% inhibitory effect plus the 6% stimulatory effect of the blank), in agreement with the value found in the short time extraction. The time of extraction did not affect the inhibition level in the vermiculite + 2030/489: the same inhibition level was obtained both with the shorter (Fig. 3) and the longer extraction time (Fig. 4).

In the present work it is shown that toxicity is detectable in the vermiculite fermentation bed. The inhibition
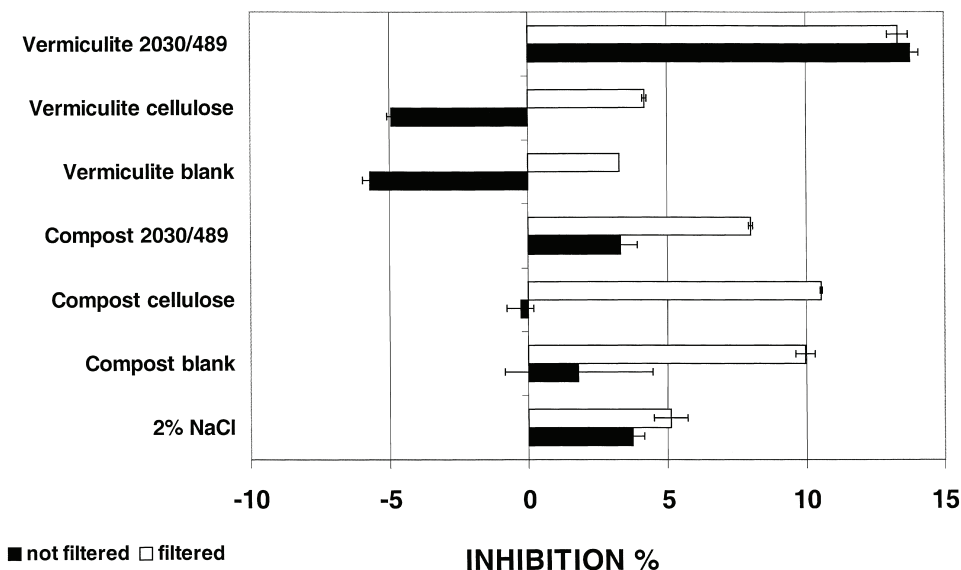
Fig. 3. Inhibition in light production of *Vibrio fischeri* in the Flash test caused by extracts of vermiculite and compost samples, with (black bars) and without filtration (white bars).
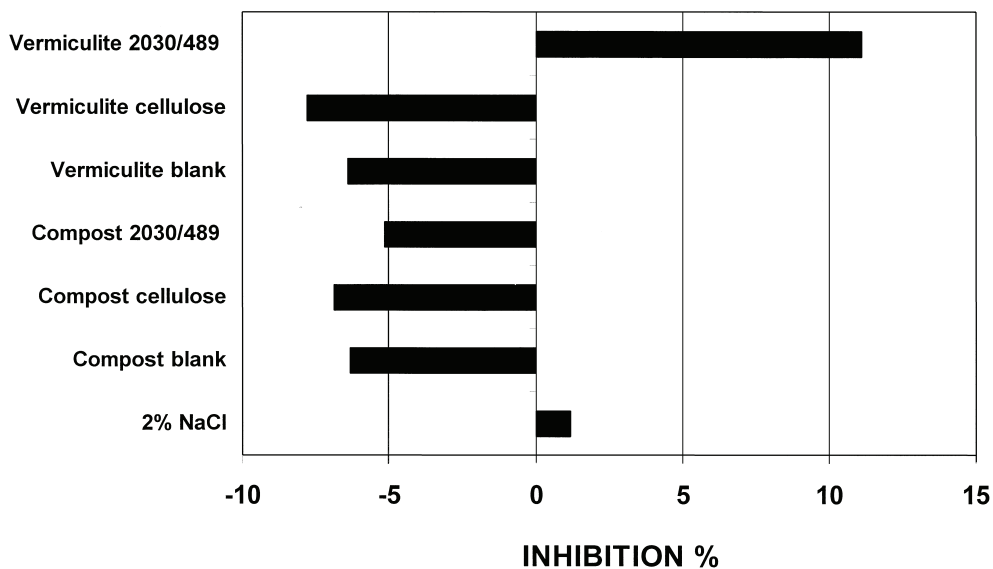

Fig. 4. Inhibition in light production of *Vibrio fischeri* in the Flash test caused by vermiculite and compost samples after an over night extraction.

effect is very well related with the measured concentration of MDA as detected by HPLC, taking into account the dose–response curve of MDA.

Flash test has been proven to give comparable results with the conventional toxicity tests and it was proposed as a range finder test for more expensive and slower toxicity tests [7]. Also in this case, the Flash test resulted in being a suitable and fast method to establish a toxic effect caused by MDA released during the biodegradation process.

It remains to be understood why the MDA was detectable (both by HPLC and as a toxic effect by the Flash test) only in extracts from vermiculite and not in extracts from mature compost. There are two possible explanations: either MDA is more degradable in compost than in vermiculite or MDA is not extractable from compost. It has been shown [1] that the recovery yield of MDA from compost is very low (10%) while the recovery from vermiculite is good (90%). MDA could be adsorbed by compost particles and make itself no more extractable with water. If this were the case (but more experiments are needed to confirm it) then the vermiculite test system would be preferable for ecotoxicological assessments.

4. Conclusion

The test method based on activated vermiculite is a comprehensive test system for the assessment of the biodegradability. It is possible to follow the CO_2 evolution and determine the biodegradation of solid materials with results which are substantially identical to those found in mature compost. It is also possible to treat vermiculite after test termination with suitable solvents to easily recover potential polymeric residues [1,2]. The residues can then be analysed to confirm their identity and to understand the degradation mechanism. In this way both the substrate of the reaction (test material) and the products (the CO_2 and low molecular weight molecules) are taken into account in a final balance.

In the present work the Flash test has been applied to verify the ecotoxicity of extracts from vermiculite where a PU-based test material had released some MDA during degradation [2]. The results of this study suggest that the Flash test in combination with the vermiculite biodegradation test can be reliably used for ecotoxicological analysis of biodegradable plastics.

References

[1] Tosin M, Degli-Innocenti F, Bastioli C. Detection of a toxic by-product released by a polyurethane-containing film using a composting test method based on a mineral bed. J Environ Polymer Degrad 1998;6:79.

[2] Bellia G, Tosin M, Floridi G, Degli-Innocenti F. Activated vermiculite, a solid bed for testing biodegradability under composting conditions. Polym Degrad Stab 1999;66:65.

[3] Bellia G, Tosin M, Degli-Innocenti F. The test method of composting in vermiculite is unaffected by the priming effect. Polym Degrad Stab 2000;69:113.

[4] Pesenti-Barili B, Ferdani E, Mosti M, Degli-Innocenti F. Survival of Agrobacterium radiobacter K84 on various carriers for crown gall control. Applied Environ Microb 1991;57:2047.

[5] Lappalainen J, Juvonen R, Vaajasaari K, Karp M. A new flash method for measuring the toxicity of solid and colored samples. Chemosphere 1999;38(5):1069.

[6] Juvonen R, Martikainen E, Schultz E, Joutti A, Ahtiainen J, Lehtokari M. A battery of toxicity tests as indicators of decontamination in composting oily waste. Ecotoxicology and Environmental Safety 2000;47:156.

[7] Põllumaa L, Kahru A, Eisenträger A, Reiman R, Maloveryan, Rätsep A. Toxicological investigation of soils with the solid-phase Flash assay: comparison with other ecotoxicological tests. ATLA 2000;28:461.

[8] Lappalainen J, Juvonen R, Vaajasaari K and Karp M. A new flash assay for measuring the toxicity of solid and colores samples. In: Persoone G, Janssen C, De Coen W, editors. New microbiotests for routine toxicity screening and biomonitoring. Kluwer Academics/Plenum Publishers, New York 2000.

[9] Szycher M, Poirier V, Dempsey DJ. Development of an aliphatic biomedical-grade polyurethane elastomer. Elastomers and Plastics 1983;15:81.

[10] Nikunen E, Leinonen R., Kemiläinen B, Kultamaa A. Environmental properties of chemicals, vol. I. Environment Guide 71, Finninsh Environment Institute, Edita Ltd., Helsinki 2000.

PAPER II

Biodegradation of lactic acid based polymers in controlled composting conditions and evaluation of the ecotoxicological impact

In: Biomacromolecules 3(3), pp. 445–455.

Biodegradation of Lactic Acid Based Polymers under Controlled Composting Conditions and Evaluation of the Ecotoxicological Impact

Jukka Tuominen,[†] Janne Kylmä,[†] Anu Kapanen,[‡] Olli Venelampi,[‡] Merja Itävaara,[‡] and Jukka Seppälä*,[†]

Department of Chemical Technology, Polymer Technology, Helsinki University of Technology, P.O. Box 6100, FIN-02150 HUT, Finland, and VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland

The biodegradability of lactic acid based polymers was studied under controlled composting conditions (CEN prEN 14046), and the quality of the compost was evaluated. Poly(lactic acids), poly(ester-urethanes), and poly(ester-amide) were synthesized and the effects of different structure units were investigated. The ecotoxicological impact of compost samples was evaluated by biotests, i.e., by the Flash test, measuring the inhibition of light production of *Vibrio fischeri*, and by plant growth tests with cress, radish, and barley. All the polymers biodegraded to over 90% of the positive control in 6 months, which is the limit set by the CEN standard. Toxicity was detected in poly(ester-urethane) samples where chain linking of lactic acid oligomers had been carried out with 1,6-hexamethylene diisocyanate (HMDI). Both the Flash test and the plant growth tests indicated equal response to initial HMDI concentration in the polymer. All other polymers, including poly(ester-urethane) chain linked with 1,4-butane diisocyanate, showed no toxicological effect.

Introduction

Biodegradable polymers offer a viable alternative to commodity plastics in a number of bulk applications where recycling is impractical or uneconomical. Among present waste management methods, composting, which is traditionally used for the disposal of food and garden waste, is also highly suitable for the disposal of other biodegradable materials together with soiled or food contaminated paper.[1] The use of biodegradable polymers in the agricultural sector as well as in the packaging field or in other disposable articles places special requirements on the material. Ideally, such materials should be part of the natural life cycle of biomass, i.e., the raw materials should be renewable and should biodegrade in compost to harmless, natural products. In addition, polymers should have good physicomechanical properties and processability for targeted applications, and production technology should be cost effective. Lactic acid based polyesters (PLA) are well-known biodegradable thermoplastic polymers and would appear to meet these strict requirements. The raw material, lactic acid, is nontoxic, naturally occurring, and derived from natural renewable sources (e.g., corn) by microbial fermentation of biomass. PLA has excellent physical properties, which mimic conventional thermoplastics, and it is truly a biodegradable polymer, which decomposes rapidly and completely in a typical compost environment to yield carbon dioxide, water, and biomass.[2–5] Lactic acid oligomers with less than 10 monomer units are also claimed to possess plant growth stimulation activity.[6]

A ring-opening polymerization of lactide, i.e., cyclic diesters of lactic acid, is usually applied in the preparation of high molecular weight PLA.[7,8] Alternatively, the chain extending of lactic acid condensation polymers is feasible. Chain extenders, such as diisocyanates, bis(2-oxazolines), bis(epoxides), and bis(ketene acetals), are difunctional low molecular weight monomers that can increase the molecular weight of polymers in a fast reaction, with only low concentrations of chain extender agent required and no need for separate purification steps. The chain extenders also introduce new functional groups and flexibility to manufacture copolymers, which in turn affect physical and mechanical properties as well as the biodegradability of the resulting polymers.[9–14]

Heterochain polymers, particularly those containing oxygen and/or nitrogen atoms in the main chain, are generally susceptible to hydrolysis. For hydrolysis to occur, the polymer must contain hydrolytically unstable bonds, such as ester, amide, or urethane, and show some degree of hydrophilicity. In addition, the hydrolysis rate of the polymer is affected by properties such as molecular weight, glass transition temperature, and crystallinity and also hydrolysis conditions such as pH, temperature, and the presence of enzymes and microorganisms.[15] The hydrolysis of PLA polymers has been widely studied both in vivo and in vitro. Hydrolytic degradation of poly(DL- and L-lactic acids) proceeds by random hydrolytic chain scission of the ester linkages, eventually producing the monomeric lactic acid.[16] The mechanism by which PLA polymers degrade depends on the biological environment to which they are exposed. In mammalian bodies, PLA is initially degraded by hydrolysis and the oligomers that form are metabolized or mineral-

---

* To whom correspondence should be addressed.
† Helsinki University of Technology.
‡ VTT Biotechnology.

ized by cells and enzymes. Abiotic hydrolysis is known to participate at the initial stage of microbial biodegradation of PLA in nature. However, the degradation rate has been shown to increase in the compost environment in the presence of an active microbial community compared to the abiotic hydrolysis.[17,18]

Polyurethanes based on polyesters are hydrolytically vulnerable polymers, whose hydrolytic stability and degradation rate are dependent on the chemical structure, such as the amount of methylene groups present within the polyester moiety and the nature of the diisocyanate used in synthesis.[19] Lactic acid based poly(ester-urethanes) have been shown to be susceptible to hydrolysis above ambient temperature. As shown in our previous study, their degradation rate is dependent on molecular weight, temperature, length of ester block, and amount of different stereostructures in the polyester chain.[20] The ester group in polyurethanes has been found to be 1 order of magnitude more susceptible to hydrolysis than the urethane group.[21] Thus, the degradation of poly(ester-urethanes) is mainly a consequence of the degradation of polyester or polyol rather than urethane bonds. However, it has also been shown that some microorganisms are capable of biodegrading urethane bonds of poly(ester-urethanes), forming corresponding amides as degradation products, and that bacteria may play a significant role in the biodegradation and biodeterioration of poly(ester-urethanes).[22–24] In addition, lactic acid based poly(ester-urethanes) have been shown by the headspace test method to be completely biodegradable under thermophilic conditions.[25] However, after the polymer has been shown to be biodegradable, it is important to ensure that the degradation products are harmless and that the mature compost is not toxic for plant growth. This is to ensure that the accumulation of harmful substances into the environment will be avoided.

Recently a standard test for biodegradation and compostability of packaging material was harmonized by the European Standardization Committee (CEN). This standard ("Requirements for packaging recoverable through composting and biodegradation", EN 13432, 2000)[26] requires the characterization of materials, the biodegradability of each organic constituent in the product, disintegration studies on packaging, and evaluation of the quality of the compost product. A controlled composting test (prEN 140466, 2000)[27] that simulates the thermophilic composting conditions is used to determine the biodegradability of the packaging materials.[28] All components that represent more than 1% of the weight of the packaging material need to be tested for biodegradability. Biodegradation of the material and its components has to be at least 90% of the positive control used in the test. A positive control, for example cellulose, is a material that is known to be well biodegraded under the test conditions.

The quality of compost is related to its agronomic and commercial value as an organic solid conditioner.[29] Quality requirements for the compost product cover both analytical and biological criteria. Analytical criteria include volumetric weight, total dry solids, volatile solids, salt content, and the content of inorganic nutrients such as total nitrogen, phosphorus, magnesium, or calcium and ammonium nitrogen. For compost to be safe to use, there need to be limits on the content of pathogens. Until now, the harmfulness of compost products has mainly been estimated on the basis of their chemical composition. Limit values have been set only for heavy metals, and there are no limits on the ecotoxicity of chemical compounds in compost. Because it is difficult to assess the impact on the environment purely on the basis of the concentration of chemical constituents, biotests are needed as well.[30] The need for biotests is also clearly mentioned in the international standards.
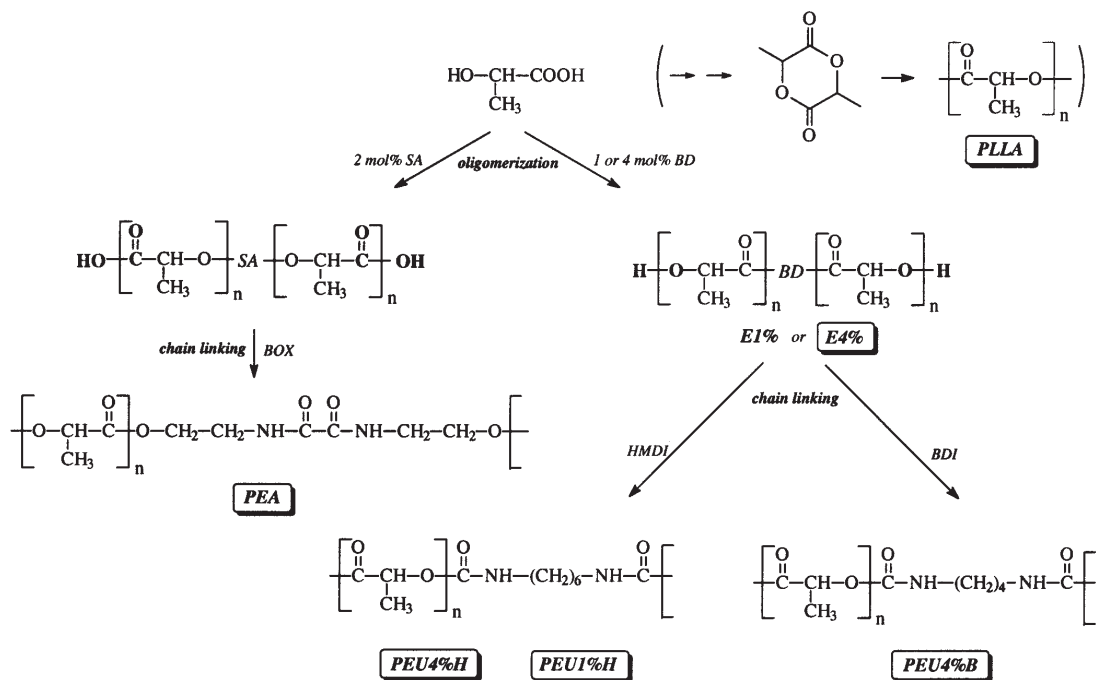
Biotests can be used to assess the ecotoxicological quality of chemicals or of environmental samples such as water, soil, and compost. Biotests are methods that employ enzymes, microbes, soil fauna, or plants to provide data to evaluate the fate and effect of any pollutants in the receiving ecosystem. Biotests are already available to evaluate the ecotoxicological profile of the product in composting of biowaste, biodegradable packaging, paper products, and contaminated soils.[30] The usefulness of biotests for study of the compost product after the composting of biodegradable plastics has recently been assessed.[31,32] It was recommended that the ecotoxicity evaluation should be coupled with measurement of the mineralization to ensure that the biodegradation of the polymer was complete. If the biodegradation is still on going, some of the toxic substances may still be bound to the polymer and not bioavailable for the test organism. The recently approved norm (EN 13432) for compostability includes the requirement for plant growth assay using two higher plants as a compost quality assessment. This was one of the few available methods for testing toxicity in compost at the time the standard was set. Other tests for ecotoxicity of compost are thus not mentioned. There are no standard methods for evaluating compost toxicity, which means that further studies and more experience are needed to develop a reliable test scheme for the evaluation of potential compost toxicity and establishment of limit values.

Our work on the synthesis and characterization of biodegradable lactic acid polymers has led us to be interested in a controlled compost test for testing their biodegradation and in ways of assessing the environmental quality of the mass derived from the composting. A series of polyesters, poly-(ester-urethanes), and poly(ester-amide) (see Scheme 1) were synthesized to investigate the effect of different structure units on the biodegradation. The ecotoxicological impact of the degradation intermediates was evaluated by biotests, i.e., by Flash, which is a microbial test, and plant growth tests.

Experimental Section

Polymerization. The prepolymers for poly(ester-urethane) and poly(ester-amide) synthesis were condensation polymerized in a rotation evaporator. L-Lactic acid (88% L-lactic acid in water, 99% optically pure; ADM) was purified by distillation under vacuum. In condensation polymerizations, 1 or 4 mol % 1,4-butanediol (BD, used as received, Acros Organics) was added to produce hydroxyl-terminated prepolymers (E1% and E4%, respectively) and 2 mol % succinic

Scheme 1

HO–CH–COOH
CH₃

2 mol% SA   oligomerization   1 or 4 mol% BD

PLLA

E1%   or   E4%

chain linking   BOX

chain linking

HMDI   BDI

PEA

PEU4%H   PEU1%H   PEU4%B

anhydride (SA, used as received, Fluka) was added to produce carboxyl-terminated prepolymer. Stannous octoate (tin(II) ethylhexanoate, 0.01 mol %, used as received, Aldrich) was used as a catalyst. The flask was purged with nitrogen and placed in an oil bath. The reaction mixture was polymerized at 200 °C for 24 h, with a continuous nitrogen stream fed under the surface of the melt, at a reduced pressure of 20 mbar. The rotation speed was approximately 100 rpm. The prepolymers obtained were used without further purification.

Chain-linking polymerization of the prepolymers was carried out in a 150 cm³ DIT (Design Integrated Technology) batch reactor equipped with nitrogen inlet and outlet tubes for nitrogen atmosphere, using 1,6-hexamethylene diisocyanate (used as received, Fluka), 1,4-butane diisocyanate (used as received, Aldrich), or 2,2′-bis(2-oxazoline) (BOX, used as received, Tokyo Kasei) as a chain extender. The polymerizations were carried out at 60 rpm and 180−200 °C. Typically, 150 g of the dried prepolymer powder was charged into the preheated reactor. After 1 min the prepolymer was completely molten, and the chain extender was added. The course of the reactions was followed by means of torque-vs-time curves measured during the polymerization. All polymers were ground before the compost test.

Other Materials. Polylactide, PLLA (POLLAIT, Fortum Oil and Gas, Finland), was used as reference polymer, and chaffed Whatman Chromatography paper (3 mm Chr) was used as positive control for the biodegradation test.

Polymer Characterization. Molecular weights were determined by room-temperature size exclusion chromatography, SEC (Waters System Interface module, Waters 510

HPLC Pump, Waters 410 differential refractometer, Waters 700 Satellite Wisp, and four PL gel columns, 10^4 Å, 10^5 Å, 10^3 Å, and 100 Å, connected in series). Chloroform (Riedel-de Haën) was used as solvent and eluent. The samples were filtered through a 0.5 μm Millex SR filter. The injected volume was 200 μL, and the flow rate was 1 mL min^{-1}. Monodisperse polystyrene standards were used for primary calibration, which means that the Mark−Houwink constants were not used.

Thermal properties were determined with a Mettler Toledo Star DSC821 differential scanning calorimeter (DSC), in a temperature range of 0−180 °C and with a heating and cooling rate of 10 °C min^{-1}. Glass transition temperatures were recorded during the second heating scan to ensure that thermal histories were the same.

The carbon content of the samples was determined with a carbon analyzer (Carlo-Erba NA 1500, Carlo Erba Insruments, Italy).

Biodegradation. Biodegradation of the prepolymer E4% and the polymers PEU1%H, PEU4%H, PEU4%B, PEA, and PLLA (see Table 1 for definitions) was studied in a controlled composting test (prEN 14046, 2000). In this test, CO_2 evolution is followed until the 90% biodegradation requirement relative to the positive control is achieved. Two separate runs were performed. In the first test run, samples were E4%, PEU1%H, PEU4%H, PEA, and PLLA. The first test was carried out during 112 days, except for PLLA where the test continued for 230 days. In the second run, the biodegradation of PEU4%B was studied for 70 days. Whatman Chromatography paper was used as a positive control in both runs.

Milled (0.1−0.6 mm particles) polymers were mixed with stable postcompost on a 1:6 dry weight basis. Composting was performed as earlier described.[28] The mixtures were incubated in 5-L Schott bottles at 58 °C (± 2 °C) in a water bath. Three replicates of each sample and a background control (compost without sample) were aerated throughout the experiment. CO_2 evaluation was followed at 2-h intervals with an infrared CO_2 analyzer (Normocap 200, Datex, Finland).

Biodegradation percentage (D_t) was calculated as

$$D_t = \frac{(CO_2)_t - (CO_2)_b}{ThCO_2} \times 100$$

where $(CO_2)_t$ is the accumulated amount of carbon dioxide released by each compost vessel, $(CO_2)_b$ is the accumulated amount of carbon dioxide released by the background controls, and $ThCO_2$ is the theoretical amount of carbon dioxide of the test material in the test vessel.

Toxicity of Polymer Components. The standardized luminescent bacteria test (ISO 11348-3, 1998) and the Flash test were used for testing the toxicity of reagents. The tested chemicals were lactic acid, 1,4-butanediol, stannous octoate, 1,6-hexamethylene diisocyanate, 1,6-hexamethylenediamine, 1,4-butane diisocyanate, 1,4-butane diamine, lactide, succinic anhydride, succinic acid, and 2,2-bis(2-oxazoline). The tested concentrations of the chemicals are presented in Table 2. The measurement in the standard test was performed according to the standard protocol, and the luminescence was measured with the 1253 Luminometer (Bio-Orbit, Turku, Finland). For the Flash test, samples were diluted with 2% NaCl. The test protocol is described in detail below.

The Flash Test. The Flash test was applied to evaluate the toxicity of the compost samples derived from the biodegradation test. The Flash test[33] is a method based on kinetic measurement of the bioluminescence of *Vibrio fischeri*, a heterotrophic bacterium capable of light production as a part of its metabolism. Fresh weight of 1 g of solid sample was mixed with 4 mL of the sample diluent (2% NaCl). The suspension was mixed for 1 min, left undisturbed for 5 min, and mixed again for 1 min. Where the pH was below 6 or above 8, the sample was neutralized. Finally the suspension was cooled to 15 °C. A 500 μL aliquot of bacterial suspension was then added to 500 μL of the suspension, obtained as described above. The BioTox Kit was utilized to obtain the bioluminescent microorganism *Vibrio fischeri* as test organism. Kinetic measurement was performed with the 1251 Luminometer (Bio-Orbit, Turku, Finland) at 20 °C. Luminescence was measured throughout the exposure time (30 s). The peak value of luminescence was obtained within the first 5 s, and this was followed by a reduction in the case of toxicity of the sample. No decrease in bioluminescence was recorded in the absence of toxicity. The results are presented as percentages of inhibition in light production. Inhibition percentage was calculated as the ratio of the maximum light production (0−5 s) to the light production after 30 s of exposure time. NaCl solution (2%) was used as the test control in all experiments.

Plant Growth Test. The plant growth test was performed with cress (*Lepidium sativum*), radish (*Raphanus sativus*), and barley (*Hordeum vulgare*), and adapting the OECD 208 Terrestrial plants, Growth test[34] for compost toxicity studies. The medium for the plant growth test was a 1:1 (v/v) mixture of compost derived from the composting test and commercial potting medium (Kekkilän ruukutusmulta, Kekkilä, Finland). Cress, radish, and barley seeds (100, 40, and 50, respectively) were sown in the medium described. Plants were grown under controlled conditions for 14 days (16 h light/8 h dark, 20 °C/15 °C, relative humidity 55%). After a 2 week growing period, the emerged seedlings were counted and the dry weight of the plants above the soil was determined. Dry weight of the plants was determined after drying at 75 °C until constant weight was achieved.

The pH and conductivity of the growth medium were determined before the plant growth test to ensure the suitability of the medium for plant growth. pH was determined in a water solution of the growth medium in volume ratio 1:5. Conductivity was measured in a solution containing 40 mL of medium and 150 mL of water.

HPLC Analysis. The identification of 1,6-hexamethylenediamine (HMDA) in the compost after the controlled compost test was carried out by HPLC (Agilent Technologies 1100 series) with a variable-wavelength UV detector adjusted to 250 nm. An Agilent Eclipse XDB-C8 reversed phase column (5 μL, 4.6 × 150 mm) with quaternary pump and a column compartment thermostated at 25 °C was used. The injected volume was 5 μL and the flow rate 1 mL min^{-1}, with methanol (Merck)/Milli-Q water (Millipore) (58:42 v/v) used as mobile phase.

Compost samples and a native compost sample (native compost extracted with water) were prepared by extracting 100 g of compost with 500 mL of distilled water for 24 h and then centrifuging and filtering the solution. Two control samples were prepared by adding 0.214 g of HMDA (Fluka, +99%) to the native compost slurry and, after extraction, HPLC measurement was carried out at once and after 1 month. A third control sample was prepared by adding HMDA directly to the native compost extraction solution. HPLC samples (from these solutions) and standards were prepared by adapting the method described by Redmond and Tseng.[37] The standards were prepared from 0.01−1 mL samples of 0.5 μmol/mL HMDA−water solutions to which were added 1 mL of sodium hydroxide (Merck, +99%) (2 mol/L) and 0.5 μL of benzoyl chloride (Merck-Schuchardt, +99%). After mixing for a short time, the benzoylation reaction was allowed to proceed for 30 min to ensure the complete acylation. Saturated sodium chloride (2 mL) was added to the solution, which was then extracted with 2 mL of diethyl ether (Merck, +99.5%). The diethyl ether layer was separated and evaporated in a stream of argon. The residue was dissolved in 1 mL of methanol. Other HPLC samples were prepared by the same procedure except that the HMDA−water solution was replaced by 1 mL of compost sample.

Results

Polymer Synthesis. Table 1 summarizes the polymer codes, compositions, and properties of the synthesized lactic

Table 1. Characteristics of the Lactic Acid Polymers

polymer	prepolymer composition (in feed, mol %)			chain extending reaction		carbon content	SEC		DSC		
	LA	BD	SA	extender	(wt %)	Carlo-Erba (wt %)	M_w (g/mol)	MWD	T_g (°C)	T_m (°C)	%[a]
E1%	99	1				50.2	14 000	1.8	45		
E4%	96	4				50.3	5 500	1.5	25		
PEU1%H	99	1		HMDI	2.0	50.0	97 000	3.2	48		
PEU4%H	96	4		HMDI	8.3	51.1	71 000	1.7	44		
PEU4%B	96	4		BDI	7.2	50.8	180 000	3.0	45		
PEA	98		2	BOX	3.7	50.7	200 000	3.0	52		
PLLA						50.2	305 000	1.7	61	176	50

[a] 93.6 J/g was used as the melting enthalpy for 100% crystalline poly(L-lactide).[47]

acid polymers and other materials used in the study. Lactic acid polymers were polymerized by polycondensation followed by chain linking. The detailed synthesis and characterization of these materials have recently been described.[10,12,35] The synthetic routes and structures of the polymers are depicted in Scheme 1. The self-condensation polymerization of lactic acid results in a low molecular weight oligomer, characterized by an equimolar concentration of hydroxyl and carboxyl groups. The chain extenders used in this study will preferentially react with either the hydroxyl or carboxyl group, and thus, to increase the molecular weight by the chain-linking technique, the prepolymers should be hydroxyl- or carboxyl-terminated. These modifications were carried out in the condensation polymerization by synthesizing poly(lactic acid) in the presence of difunctional hydroxyl or carboxyl compound.

For the synthesis of poly(ester-urethanes) (PEU), two different hydroxyl-terminated prepolymers (designated here as prepolymer E1% and E4%) were prepared by using 1 or 4 mol % 1,4-butanediol (BD). The use of different amounts of BD affected the amount of prepolymer chains and thereby the molecular weight, as shown in the SEC results presented in Table 1. In the corresponding poly(ester-urethanes) chain linked with 1,6-hexamethylene diisocyanate (HMDI), the two different esterblock lengths required the use of two different amounts of HMDI. Thus, we were able to monitor not only the effect of the length of the esterblock on biodegradation but also the effect of a 4-fold amount of HMDI on the degradation products. To examine the effect of another diisocyanate, 1,4-butane diisocyanate (BDI) was applied as chain-linking agent. These polyurethanes are referred to as PEU1%H, PEU4%H, and PEU4%B, where the percentage refers to the concentration of butanediol and the abbreviations H and B identify the diisocyanate as HMDI and BDI, respectively.

Poly(ester-amide) (PEA) was polymerized from carboxyl-terminated lactic acid prepolymer with 2,2′-bis(2-oxazoline) as chain extender. The prepolymer was condensation polymerized from lactic acid and 2 mol % succinic anhydride. Poly(L-lactide) (PLLA), which is polymerized by ring-opening polymerization of lactide, i.e., cyclic dimer of lactic acid, was used as a reference consisting only of lactic acid structure units. Also, pure hydroxyl-terminated prepolymer (E4%) was employed in biodegradation and biotests. Stannous octoate has been used as a catalyst in all polymerizations.

All synthesized polyester prepolymers and chain-linked poly(ester-urethanes) and poly(ester-amides) were amorphous, because racemization during the condensation polymerization causes changes in optical activity of lactic acid units in the esterblocks (measured by ^{13}C NMR).[35] Thus, the synthesized polymers showed no sign of crystallinity. On the contrary, the reference polymer PLLA is a semicrystalline material due to the different polymerization method. Differences in structure units of these polymers caused variation in the glass transition temperature (Table 1). The organic carbon content of the polymers was determined with a carbon analyzer (Carlo-Erba NA 1500) after milling, to calculate the biodegradability percentage.

Biodegradation in Compost Environment. Biodegradation of the polymers was studied in a controlled composting test. The biodegradation of the polymer was calculated by subtracting the amount of CO_2 evolved from the background compost from the amount of CO_2 evolved from the compost containing the sample. The difference in the CO_2 evolution was expressed as percent of the theoretical maximum amount of CO_2 released from the sample. The results are shown in Figure 1.

Two separate composting tests were performed, where the positive control (Whatman Chromatography paper) was biodegraded in test run I by 95% (±0.7%) under controlled conditions in 112 days and in test run II by 94% (±4%) in 70 days. If the polymer biodegrades to 90% of the positive control, it meets the requirements set for the standard biodegradation test for packaging materials. The biodegradation percentage of the prepolymer E4% was 85% (±3%) during 112 days, which was equivalent to 90% biodegradation relative to the biodegradation of the positive control. As expected, the biodegradation of the prepolymer began with a shorter lag-period than that of the actual polymer samples. Biodegradation percentages of the tested polymers were 87% (±4%) for PEU1%H, 95% (±6%) for PEU4%H, and 100% (±9%) for PEA in 112 days; 91% (±0.2%) for PEU4%B in 70 days; and 92% (±17%) for PLLA in 202 days. The biodegradation of PLLA at 150 days was 56% (±5%) and the deviation of the three replicates increased when the test was prolonged.

Biotest Evaluation of the Components. Chemicals tested for toxicity were lactic acid, 1,4-butanediol, stannous octoate, 1,6-hexamethylene diisocyanate, 1,4-butane diisocyanate, 1,6-hexamethylenediamine, 1,4-butane diamine, lactide, succinic anhydride, succinic acid, and 2,2-bis(2-oxazoline). The
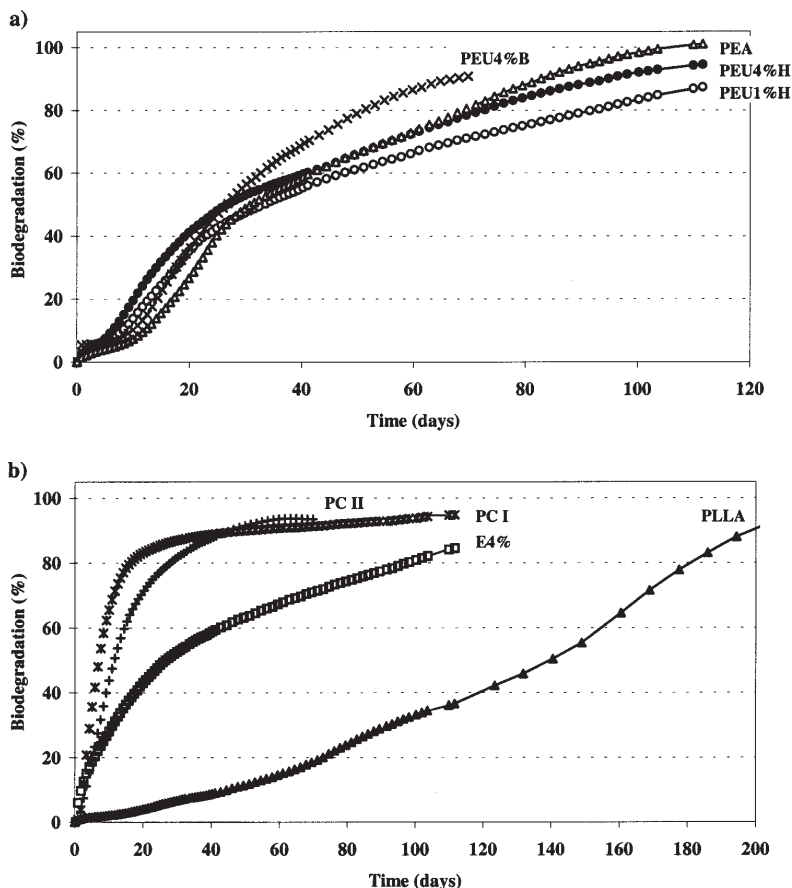
Figure 1. Biodegradation in controlled composting test: (a) PEU1%H, PEU4%H, PEU4%B, and PEA; (b) positive controls (PC) in test runs I and II, E4%, and PLLA.

**Table 2.** Toxicity of the Reagents Measured by Flash Test and by Standardized Luminescent Bacteria Test

	concentrations tested		Flash		standard		
components	Flash	standard	$EC50_{30s}$	$EC20_{30s}{}^b$	$EC50_{5min}{}^c$	$EC50_{15min}{}^c$	$EC50_{30min}{}^c$
lactic acid (g/L)	18−180	5−18	65	50	10.5	11.5	10.8
1,4-butanediol (g/L)	4−203	0.8−81	70	11	2.9	10.8	15.2
stannous octoate (g/L)	0.05−5	0.01−5	0.6	0.1	0.2	0.2	0.2
1,6-hexamethylene diisocyanate (μg/L)	0.01−0.5	0.02−0.1	0.1	0.05	0.05	0.02	0.02
1,4-butane diisocyanate (μg/L)	0.01−0.5	0.0005−0.5	0.2	0.02	0.09	0.03	0.02
1,6-hexamethylenediamine (g/L)	17−100	17−100	46	33	38	37	36
1,4-butane diamine (g/L)	17−85	1.5−30	20	11	5.3	3.3	3.1
lactide (g/L)	31−63	3−10	64	42	5.5	5.6	5.5
succinic acid anhydride (g/L)	5−50	5−83	43	20	16	21	26
succinic acid (g/L)	13−50	13−50	+++[a]	+++[a]	31	35	39
2,2′-bis(2-oxazoline) (g/L)	2−50	2−50	51	36	13	15	15

[a] Increase in light production. [b] With 30 s contact time. [c] With 5, 15, and 30 min contact time.

luminescent bacteria test with *Vibrio fischeri* was used to evaluate the range of concentrations of specific components of polymers that would have an effect on light production of the test organism. Tested concentrations and EC20 and EC50 values are presented in Table 2. EC20 is an effective concentration that reduces light production by 20%, while EC50 reduces it to one-half. EC50 values are, on average,

higher with the Flash test than with the standard test owing to the shorter contact time.

The most toxic of the tested chemicals were 1,6-hexamethylene diisocyanate and 1,4-butane diisocyanate. The $EC50_{30min}$ value was 0.02 μg/L for both HMDI and BDI in standard test and in Flash test EC50 values were 0.1 and 0.2 μg/L, respectively. EC50 values for the other chemicals
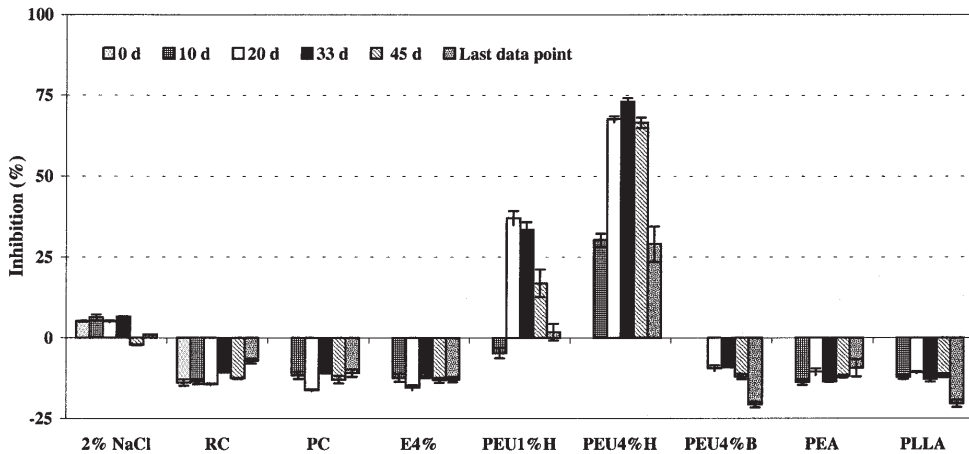
Figure 2. Inhibition in light production of *V. fischeri* in the Flash test: 2% NaCl = test control; RC = reference compost; PC = positive control. Last data point for RC, PC, E4%, PEU1%H, PEU4%H, and PEA was 112 days, for PLLA 202 days, and for PEU4%B 70 days.

were in g/L range. Stannous octoate gave EC50 values of 0.2 g/L in the standard test and 0.6 g/L in the Flash test. The EC values of all the other chemicals were so high that corresponding concentrations could not reasonably be expected after the standard composting process.

Evaluation of Toxicity of Compost Samples by the Flash Test. The Flash test, based on kinetic measurement of bioluminescence of *Vibrio fischeri*, was applied to evaluate the formation of potentially toxic metabolites in the compost matrix during the biodegradation study. The Flash test measurements were carried out for compost samples after 10, 20, 33, and 45 days of composting and at the end of the composting test, as shown in Figure 2.

Two of the composted polymers, PEU1%H and PEU4%H, gave toxic response in the test. Toxicity, reported as inhibition in the light production of *V. fischeri*, was highest (73%) with PEU4%H after 33 days of composting. At that point, over 50% of the polymer had already been mineralized to CO_2. The toxic effect was not pronounced, through evident, at the beginning of the composting experiment. As the biodegradation proceeded, more degradation products were released into the compost matrix raising the acute toxicity level. However, the toxicity at no point slowed the biodegradation rate. The toxicity dropped to 30% inhibition after 112 days when the biodegradation was 95%. Polymer PEU1%H gave a toxic response between 20 and 45 days of composting (biodegradation at 35−60%), when the inhibition decreased from 37% to 17%. No toxicity was observed in samples originating from PEU1%H at the end of the composting experiment. The amount of light generated in the 30 s test was increased in all the other compost samples, and those samples can be declared to be nontoxic to *V. fischeri*.

Toxicity Evaluation by the Plant Growth Test. The pH and conductivity of the growth medium were determined to ensure that the medium was suitable for plant growth and that any negative effects would be derived from the composted sample and not the medium. The optimal pH range for most plants is about 5.5.[36] Extremely high or low pH

may change the solubility of nutrients and affect their availability. In this study the pH of the medium varied from 7.0 to 7.8, which was quite high for plant growth. Despite this, the growth of the control plants in these test conditions was good and it can be assumed that high pH did not have an adverse effect on growth. Conductivity of the compost medium derived from the background samples was between 800 and 1000 μS/cm and that of medium derived from samples composted with polymers was about 600−900 μS/cm. Conductivity was higher in the background samples than in the samples containing polymers, so biodegradation of the polymers during the composting did not increase the conductivity of the tested growth medium.

To assess the effect of biodegradation intermediates on the quality of the compost product, we examined the growth of cress, radish, and barley. The growth medium consisted of a 1:1 (v/v) mixture of compost derived from the composting test and commercial potting medium. After a 2-week growth period, plants were harvested; germination and visual growth were observed and gravimetric measurements were made. The compost samples containing the different polymers, and which went through the controlled composting test, did not have an adverse effect on seedling emergence of any of the tested plant species. However, the compost sample containing PEU4%H caused 33, 24, and 12% inhibition in the plant growth test with cress, radish, and barley, respectively (Figure 3). Cress was the most sensitive and barley the most resistant of the three plant species; therefore PEU1%H had a minor negative effect and only on cress growth. With all the other polymers—E4%, PEU4%B, PEA, and PLLA—plant growth was excellent.

PEU4%H and PEU1%H had a severe effect on plant metabolism, resulting in chlorosis and damage to the shoot tips. As seen in Figure 4 for PEU4%H, the shoot tips of barley were curled and the edges of the leaves of cress and radish were damaged. The effect of PEU4%H was stronger than the similar effect of PEU1%H, which contains fewer 1,6-hexamethylene diisocyanate units. PEU4%B, a similar polymer to PEU4%H but with a different diisocyanate (1,4-
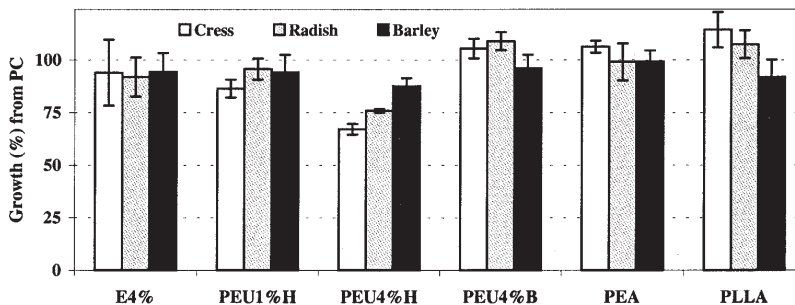
Figure 3. Growth of cress, radish, and barley measured as percent of dry weight against the positive control (PC).
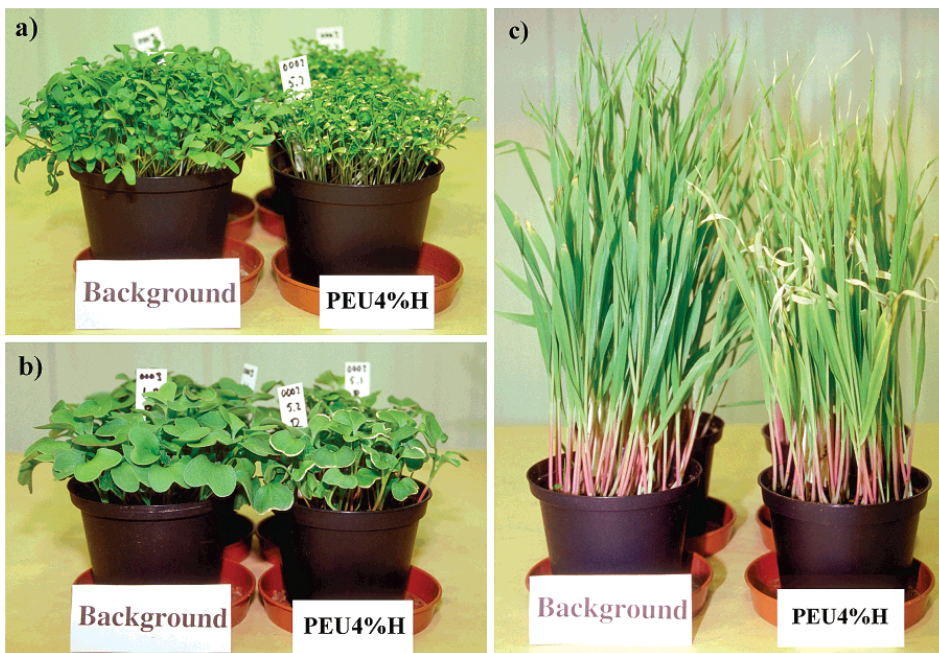

Figure 4. Growth of (a) cress, (b) radish, and (c) barley in PEU4%H/compost medium and in background compost medium.

butane diisocyanate) used as extender, did not cause any visible damage or have a growth inhibitory effect on the plants (Figure 5). Likewise, PEA and the control samples of E4% and PLLA did not cause any visible changes.

HPLC Measurements. 1,6-Hexamethylenediamine (HMDA), which is assumed to be a degradation product of PEU1%H and PEU4%H, was identified in compost samples after the controlled composting test by applying water extraction, Scotten-Baumann benzoylation for the derivatization of HMDA, and HPLC determination of benzoylated HMDA.[37] Standards were prepared for the identification of the peak and retention time of benzoylated HMDA (5.10– 5.12 min). As expected, the native compost sample did not give any signals for benzoylated HMDA. The amounts of HMDA added to control samples were the same as the theoretical amounts if all HMDI hydrolyzed to HMDA (about 0.37 mg/mL of H_2O (3.2 mol/mL)). When HPLC measurement was carried out immediately after HMDA addition to

the extraction solution, a large peak was observed corresponding to the benzoylated HMDA. The size of the peak was only one-fifth of this when HMDA was in contact with the native compost slurry during the whole period of water extraction (24 h). More surprising was that when this sample was measured after a 1-month storage, only a very small peak was observed (size only about 1/2000 that of the first control sample). In the actual PEU4%H compost sample, only traces of HMDA were evident, although a distinct peak of HMDA could be seen (about 1/5000 of the theoretical amount). In the PEU1%H compost sample, where the amount of HMDI units was one-fourth the amount in PEU4%H, no peak was detected.

Discussion

Biodegradation of different lactic acid based polyesters, poly(ester-urethanes), and poly(ester-amide) under compost-

Figure 5. Growth of barley in PEU4%B/compost medium and in background compost medium.

ing conditions was studied. In addition, the ecotoxicity of polymers and their degradation products was examined to determine the effects of the type and amount of chain extender and the length of the esterblock. All the polymers biodegraded, i.e., produced 90% of the theoretical CO_2, during 6 months, which is the time set in the CEN standard for biodegradability of packaging materials. The difference between the degradation of two polymers (PEU1%H and PEU4%H) with different lengths of the poly(lactic acid) chain and different amounts of 1,6-hexamethylene diisocyanate was about 8%. The biodegradation of PEU4%H was slightly faster throughout the whole composting experiment, but at the end the difference between the samples was diminished. This is in agreement with our previous biodegradability study, which was carried out using the headspace test.[25] To determine the effect of different chain extenders on degradation, we tested 1,4-butane diisocyanate as well. Even though the biodegradation of PEU4%H and PEU4%B was tested in separate composting experiments, it can be concluded that PEU4%B biodegraded faster than PEU4%H. The lag time was slightly longer, but after that the degradation rate was faster. This faster biodegradation might have been due to the activating effect of a degradation product of BDI, because the chain linking agent is the only chemical difference between these polymers. The difference in lag times of tested polymers was attributed to their T_g values. In poly(ester-amide), the diisocyanate is replaced with another chain extender, 2,2′-bis(2-oxazoline), and oxamide groups are introduced to the polymer structure. Moreover, 1,4-butanediol is substituted with succinic acid in the ester

chain. Indeed, it was found that the degradation behavior of PEA was similar to that of PEU1%H and PEU4%H. PEA began to biodegrade with longer lag periods than that of PEUs, but it reached an even higher level of biodegradation. The extensive biodegradation (100%) of PEA might be explained by an error in the measurement arising during the long test period, i.e., diminishing CO_2 levels, or the sample might have activated the degradation of the compost matrix itself, a phenomenon known as the priming effect.[38] PLLA reached the required biodegradation level in the controlled composting test in the standard test environment but at slower rate than the other polymers. The slower biodegradation of PLLA is attributed to the higher T_g (61 °C) and the semicrystalline nature of the polymer (50% crystallinity). Since the degradation of PLLA is sensitive to temperature especially around the T_g, the constant 58 °C temperature, as specified in the standard, probably retarded degradation.[25] PLLA has been found to degrade faster in bench-scale composting conditions where the temperature rises higher than 58 °C.[5]

Polyurethanes, and especially the degradation products derived from the diisocyanates, have been the subject of intensive discussion, because most of the diisocyanates used in conventional urethane chemistry may yield toxic substances or fragments upon degradation. It is generally accepted that, in the degradation of polyurethanes, diisocyanate units hydrolyze to the corresponding amines. In particular, the aromatic amines produced in the degradation of aromatic diisocyanates (e.g. diphenylmethane diisocyanate) are considered toxic.[39,40] To examine the effect of different diisocyanates, HMDI and BDI were applied as chain extenders. Use of BDI is of special interest because, upon degradation, it yields 1,4-butane diamine, also known as putrescine, which is present in mammalian cells.[41] We observed a clear toxic effect in poly(ester-urethane) samples where chain-linking was carried out with HMDI. This was seen both in the Flash test and in the plant growth experiments measured as dry weight and visual growth of cress, radish, and barley. Since the other polymers tested did not show any ecotoxicological effect, not even PEU4%B where lactic acid prepolymers are chain-linked with another diisocyanate, BDI, the toxicity is evidently due to the breakdown product of HMDI units.

The HMDI concentration in the PEU1%H and PEU4%H polymer samples was significant in the detection of toxicity, although both samples showed an inhibitory effect. Initially, PEU4%H contained approximately 8 wt % and PEU1%H 2 wt % of HMDI. In the Flash test the inhibition in the light production of PEU4%H was 2-fold or even more relative to PEU1%H. Also in plant growth experiments, the toxicity effect was more distinct with the higher concentration of HMDI. We note, nevertheless, that no effect on dry weight of radish or barley was observed with the lower amount of HMDI. In our experience, there are differences in sensitivity among the test plant species. The most sensitive of our plant species was cress, which showed minor damage in the shoot tips with PEU1%H even when other plants were not affected.

Although HMDI was evidently indirectly responsible for the toxicity, the form in which the toxicity was expressed

was not self-evident. Polyester-based polyurethanes contain many ester bonds that are vulnerable to hydrolysis. Thus, microbial degradation of ester-based polyurethanes is thought to be mainly a consequence of the hydrolysis of ester bonds, at least at the beginning of degradation in compost environment.[42] The formation of small molecules in the hydrolysis allows microbiological degradation to begin. In the ester-based polyurethanes, the degradation of the diurethane molecules has been found to be highly ineffective relative to the degradation of the poly(ϵ-caprolactone) chain, and thus it has been thought possible that complete degradation of the polyester would yield undegradable diurethane residue.[43] However, in our controlled composting experiment the toxicity in the Flash test was already evident in the 10-day sample of PEU4%H (in the 20-day sample for PEU1%H). At that point, only about 20% of the polymer had been mineralized to CO_2. Thus, it is likely that urethane bonds in fact tend to degrade (or hydrolyze) at a surprisingly early stage. However, the toxic compound cannot be the supposed hydrolysis product of the HMDI unit, HMDA, because the concentration at which HMDA could be detected in the Flash test, as shown in Table 2, was at least 15-fold ($EC20_{30s}$) the highest possible amount in the compost. The toxicity results for the pure chemicals show the threshold values for each compound in the Flash or standardized luminescent bacteria test, i.e., the possible toxicity levels in compost that can be detected with these biotests. This approach does not, however, take into account the combined effects of substances in the compost sample. EC values of all tested chemicals except HMDI were so high that such concentrations could not reasonably be expected to appear after the composting process. The highest possible amount of HMDA that could originate in the hydrolysis of PEU is 2.14 g/kg of compost, calculated from the amount of HMDI in feed, which is still far below the toxicity level measured in the Flash or the standardized luminescent bacteria test. Also HMDI and stannous octoate reagents cannot be the cause of the toxicity for *Vibrio fischeri*. After polymerization, no residual HMDI in the polymer is detected by FTIR, which means that the concentration of HMDI is under the resolution of a spectrometer.[10] In addition, the long contact with the compost water at 58 °C ensures that no free HMDI is present. The maximum possible amount of stannous octoate catalyst in the compost was 0.019 g/kg of compost, which is only one-tenth of the toxic level. Also, the amount of stannous octoate was the same in all the other polymers, and these did not exhibit an ecotoxicological effect.

During the compost test the toxicity value of PEU4%H started to decrease from its highest value of 73% after 33 days of composting to 30% inhibition (112 days). Evidently the degradation product or its derivative undergoes further chemical or biochemical reactions during composting, causing the toxicity to decrease. Studying the toxicity together with the mineralization, i.e., as a function of biodegradation, as we did in this experiment, allows the detection of toxic effects when the maximum amount of degradation products is in the compost medium. As observed in the case of PEU1%H, even though the toxicity was evident during the biodegradation study, the Flash test showed only minor inhibition in light production of *Vibrio fischeri* at the end of the test. Thus, if no samples had been taken during the biodegradation, the toxic response in the Flash test might have been missed.

Owing to the highly complex organic composition of compost medium, the extraction and analytical detection of residuals or toxic intermediates at low concentration tend to be more complicated than in a synthetic aqueous environment.[31,44] Despite this, HMDA was identified in the compost from the controlled compost test by HPLC. The chromatograms showed that only traces of the HMDA were left in the compost after the controlled compost test with PEU4%H. Similarly only traces were seen in the control samples where a known amount of HMDA was allowed to be in contact for a period of time with native compost slurry. HPLC, like the Flash test above, confirms that urethane bonds degrade first to HMDA, which then, chemically or biochemically, further converts to a more toxic compound, whose concentration decreases during composting. HPLC analysis, together with the biotests, showed that the toxic compound in the compost was not HMDA but the toxicity was due to the derivatives or degradation products of HMDA.

With the test scheme we applied, it is possible to detect the release of toxic components from polymers during the biodegradation. Both the Flash test and plant growth experiments revealed the toxic effect and its relation to HMDI concentration in HMDI linked polymers. The Flash test is fast and a valuable toxicity test for pre-evaluation of the quality of compost, and it has been found to work well in composting studies with polymers[31] as well as oily wastes and sludges.[45,46] Toxicity tests with plants should always include a test protocol when studying compost toxicity, as mentioned in the standard.

The concentrations of polymers and of the degradation products formed during our composting study were much higher than could be expected in a real composting process. The amount of polymer in the biodegradation experiment was about 16% of the dry weight of the compost, whereas the amount of biodegradable polymers in future biowaste is assumed to be a maximum 1 wt %.[44] In other words, the high concentration of polymer in the present composts represents a worst-case scenario. Thus, where toxicity was not detected, the polymer can be considered safe, without negative effect on compost quality.

Conclusions

Complete biodegradation of all tested lactic acid polymers was confirmed. Our biotests clearly showed that poly(ester-urethane) where lactic acid prepolymers were chain linked with 1,4-butane diisocyanate did not exhibit any ecotoxicological effect, and neither did poly(ester-amide) or poly(lactide). However, the results strongly suggest that 1,6-hexamethylene diisocyanate, which is frequently used in urethane chemistry and as a connecting agent in different polymers, should not be used as a structure unit in biodegradable polymers because of the environmental risk. In materials designed for mass volume applications it is important to pay attention not only to the biodegradation of

the material but also to the composting process. This study has clearly shown the advantages of biotests in the examination of compost quality. Although there was no evidence of hazardous amounts of toxic chemicals present in any PEU samples, the biotests showed evidence of toxicity in PEU1%H and PEU4%H. Clearly it is essential to understand the effect of degradation products of polymers in composting when using compost for plant production.

References and Notes

(1) Bastioli, C. *Macromol. Symp.* **1998**, *135*, 193−204.

(2) Vert, M.; Schwarch, G.; Coudane, J. *J. Macromol. Sci., Pure Appl. Chem.* **1995**, *A32*, 787−796.

(3) Hartmann M. H. In *Biopolymers from renewable resources*; Kaplan, D. L., Ed.; Springer: Germany 1998; pp 367−411.

(4) Jacobsen, S.; Degée, Ph.; Fritz, H. G.; Dubois, Ph.; Jérôme, R. *Polym. Eng. Sci.* **1999**, *39*, 1311−1319.

(5) Itävaara, M.; Karjomaa, S.; Selin, J.-F. *Chemosphere* **2002**, *46*, 879−885.

(6) Barrows T. H. In *High Performance Biomaterials*; Szycher, M., Ed.; Technomic Publishing: Basel 1991; pp 243−258.

(7) Leenslag, J. W.; Pennings, A. J. *Macromol. Chem.* **1987**, *188*, 1809−1814.

(8) Kricheldorf, H. R.; Kreiser-Saunders: I.; Boettcer, C. *Polymer* **1995**, *36*, 1253−1259.

(9) Seppälä, J. V.; Selin, J.-F.; Su, T. Fin. Pat. 92592, 1994.

(10) Hiltunen, K.; Seppälä, J. V.; Härkönen, M. *J. Appl. Polym. Sci.* **1997**, *63*, 1091−1100.

(11) Loontjens, T.; Pauwels, K.; Derks, F.; Neilen, M.; Sham, C. K.; Serné, M. *J. Appl. Polym. Sci.* **1997**, *65*, 1813−1819.

(12) Tuominen, J.; Seppälä, J. *Macromolecules* **2000**, *33*, 3530−3535.

(13) Bonsignore, P. V. US5470944, 1995.

(14) Heller, J.; Barr, J.; Ng, S. Y.; Shen, H.-R.; Achwach-Abdellaoui, K.; Emmahl, S.; Rothen-Weinhold, A.; Gurny R. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 121−128.

(15) Lenz, R. W. In *Biodegradable Plastics and Polymers*; Doi, Y., Fukuda, K., Eds.; Elsevier: Amsterdam 1994; pp 11−23.

(16) Pitt, C. G. In *Biodegradable Polymers and Plastics*; Vert, M., Feijen, J., Albertsson, A., Scott, G., Chiellini, E., Eds.; The Royal Society of Chemistry: Cambridge, 1992; pp 7−17.

(17) Hakkarainen, M.; Karlsson, S.; Albertsson, A.-C. *J. Appl. Polym. Sci.* **2000**, *76*, 288−239.

(18) Hakkarainen, M.; Karlsson, S.; Albertsson, A.-C. *Polymer* **1999**, *41*, 2331−2338.

(19) Rehman, I.; Andrews, E. H.; Smith, R. *J. Mater. Sci. Mater. Med.* **1996**, *7*, 17−20.

(20) Hiltunen, K.; Tuominen, J.; Seppälä, J. *Polym. Int.* **1998**, *47*, 186−192.

(21) Pegoretti, A.; Penati, A.; Kolarík, J. *J. Therm. Anal.* **1994**, *41*, 1441−1452.

(22) Owen, S.; Masaoka, M.; Kawamura, R.; Sakota, N. *J. Macromol. Sci., Pure Appl. Chem.* **1995**, *A32*, 843−850.

(23) Tosin, M.; Degli-Innocenti, F.; Bastioli, C. *J. Environ. Polym. Degrad.* **1998**, *6*, 79−90.

(24) Kay, M. J.; Morton, L. H. G.; Prince, E. L. *Int. Biodeter.* **1991**, *27*, 205−222.

(25) Hiltunen, K.; Seppälä, J.; Itävaara, M.; Härkönen, M. *J. Environ. Polym. Degrad.* **1997**, *5*, 167−173.

(26) EN 13432, European Committee for Standardization, Packaging−Reqirements for packaging recoverable through composting and biodegradation−Test scheme and evaluation criteria for the final acceptance of packaging, 2000.

(27) prEN 14046, European Committee for Standardization, Packaging−Evaluation of the ultimate aerobic biodegradability and disintegration of packaging materials under controlled composting conditions−Method by analysis of released carbon dioxide, 2000.

(28) Venelampi, O.; Weber, A.; Rönkkö, T.; Itävaara, M. Submitted for publication in *Com. Sci. Util.*

(29) Degli-Innocenti, F.; Bastioli, C. *J. Environ. Polym. Degrad.* **1997**, *5*, 183−189.

(30) Kapanen A.; Itävaara, M. *Ecotoxicol. Environ. Saf.* **2001**, *49*, 1−16.

(31) Degli-Innocenti, F.; Bellia, G.; Tosin, M.; Kapanen, A.; Itävaara, M. *Polym. Degrad. Stab.* **2001**, *73*, 101−106.

(32) Fritz, J. Ökotoxizität biogener Werkstoffe während und nach ihrem biologischen Abbau. Dissertation, Interuniversitären Forschunginstituts für Agrarbiotechnologie; IFA-Tulln: Austria, 1999.

(33) Lappalainen, J.; Juvonen, R.; Vaajasaari, K.; Karp, M. *Chemosphere* **1999**, *38*, 1069−1083.

(34) OECD 208, Terrestrial plant growth test. OECD Guidelines for testing of chemicals, 1984.

(35) Hiltunen, K.; Härkönen, M.; Seppälä, J. V.; Väänänen, T. *Macromolecules* **1996**, *29*, 8677−8682.

(36) Marschner, H. *Mineral nutrition of higher plants*, 2nd ed.; Academic Press: UK, 1995.

(37) Redmond, J. W.; Tseng, A. *J. Chromatogr.* **1979**, *170*, 479−481.

(38) Shen J.; Bartha, R. *Appl. Environ. Microbiol.* **1996**, *62*, 1428−1430.

(39) Storey, R. F.; Wiggins, F. S.; Mauriz, K. A.; Puckett, A. D. *Polym. Compos.* **1993**, *14*, 17−25.

(40) Hirt, T. D.; Neuenschwander, P.; Suter, U. W. *Macromol. Chem. Phys.* **1996**, *197*, 4253−4268.

(41) de Groot, J. H.; de Vrijer, R.; Wildeboer, B. S.; Spaans, S. C.; Pennings, A. J. *Polym. Bull.* **1997**, *38*, 211−218.

(42) Nakajima-Kampe, T.; Shigeno-Akutsu, Y.; Nomura, N.; Onuma, F.; Nakahara T. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 134−140.

(43) David, C.; Dupret, I.; Lefèvre, C. *Macromol. Symp.* **1999**, *144*, 141−151.

(44) Witt, U.; Einig, T.; Yamamoto, M.; Kleeberg, I.; Deckwer, W.-D.; Müller, R. J. *Chemosphere* **2001**, *44*, 289−299.

(45) Juvonen, R.; Martikainen, E.; Schultz, E.; Joutti, A.; Ahtiainen, J.; Lehtokari, M. *Ecotoxicol. Environ. Saf.* **2000**, *47*, 156−166.

(46) Rantala, P.-R.; Vaajasaari, K.; Juvonen, R.; Schultz, E.; Joutti, A.; Mäkelä-Kurtto, R. *Water Sci. Technol.* **1999**, *49*, 187−194.

(47) Fischer, E. W.; Sterzel, H. J.; Wegner, G. *Kolloid Z. Z. Polym.* **1973**, *251*, 980−990.

BM0101522

Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community

In: Chemosphere, 67(11), pp. 2201–2209.

Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community

A. Kapanen [a], J.R. Stephen [b,c], J. Brüggemann [b,d], A. Kiviranta [a],
D.C. White [b,✠], M. Itävaara [a,*]

[a] *VTT, P.O. Box 1000, Tietotie 2, 02044 VTT, Finland*
[b] *Center for Biomarker Analysis, University of Tennessee, Knoxville, 37932-257, USA*
[c] *Australian Genome Research Facility, PMB1 University of Adelaide, South Australia 5064, Australia*
[d] *Molecular Plant Breeding Cooperative Research Centre, University of Adelaide, Australia*

Abstract

There is a great need to understand the environmental impacts of organic pollutants on soil health. Phthalates are widely used in consumables and can be found extensively. We studied the toxicity of diethyl phthalate (DEP), spiked in a compost plant growth substrate, by means of the acute toxicity Flash test and on the basis of the germination and plant growth of radish seedlings. The response of the microbial community to DEP in the growth substrate was studied by PCR-DGGE (denaturing gradient gel electrophoresis). In the acute toxicity test, DEP was found to be less toxic as a pure compound than when mixed with the compost mixture. This suggests the synergistic effect of unknown toxic compounds or the release of compounds due to DEP addition. The same DEP concentration level in compost substrate induced toxic response in both plant test and microbial community analysis. The diversity of the major microbial community was reduced from a broad community to only 10 major species at toxic concentrations of DEP. Several of the identified microbial species are known to be able to degrade phthalates, which means that the suppression of other microbial species might be due to the substrate availability and toxicity. The major species identified included *Sphingomonas* sp., *Pseudomonas* sp., *Actinomycetes* sp.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Diethyl phthalate; Phytotoxicity; Compost; Microbial community structure; Biotest

1. Introduction

Phthalate esters are a group of synthetic chemicals, which are used to plasticize polymers such as polyvinyl chloride (PVC), polyvinyl acetates, cellulosics and polyurethanes. These polymers are widely used in food wraps, plastic tubing, furniture, toys, shower curtains, cosmetics, etc. (Williams et al., 1995; Staples et al., 1997a,b). Dimethyl and diethyl phthalate esters are typically used in cellulose ester-based plastics, such as cellulose acetate and butyrate. As they are not chemically bound to the polymer material, phthalates readily migrate from the plastics to the environment. The release of phthalate esters into the environment during manufacture, use, and disposal has been extensively reviewed (e.g Wams, 1987; Cadogan et al., 1993). Concern has recently been shown about the occurrence and behaviour of phthalate esters in landfill leachates (Bauer and Herrmann, 1998; Jonsson et al., 2003).

The environmental fate and bioaccumulation of phthalate esters have been intensively studied (Staples et al.,

---
* Corresponding author. Tel.: +358 207225172; fax: +358 207227071.
E-mail addresses: Anu.Kapanen@vtt.fi (A. Kapanen), john.stephen@agrf.org.au (J.R. Stephen), Julia.Brueggemann@adelaide.edu.au (J. Brüggemann), Arto.Kiviranta@vtt.fi (A. Kiviranta), Dwhite1@utk.edu (D.C. White), Merja.Itavaara@vtt.fi (M. Itävaara).
✠ Deceased.

1997b, 2000). Their low solubility in water is considered to reduce aquatic toxicity, because the bioavailability of phthalates in the environment is considerably lower than the total concentration (Gledhill et al., 1980; Staples et al., 1997a). On the other hand, Staples et al. (1997a) reported that the lower molecular weight phthalate esters are both acutely and chronically toxic at concentrations below their solubility level, and that toxicity increases with increasing alkyl chain length up to four carbon atoms. Several investigators have established that phthalates are teratogenic at high dosages (Lokke and Rasmussen, 1983). Some of the phthalates are suspected to be carcinogenic, and there are indications that low molecular weight phthalates are liver carcinogenic (FDA, 1982; Zhang and Reardon, 1990). The biological effects and environmental fate of phthalates have been reviewed by Thomas et al. (1978) and Staples et al. (1997a,b), who suggested that maximum residue levels would be found at intermediate trophic levels rather than at the top of the food chain. Therefore, phthalates undergo biotransformation in mammalian system.

Great concern about the effects of these compounds in nature has arisen as a result of their endocrine-disrupting properties (Jobling et al., 1995; Zou and Fingerman, 1997). Most of the studies on the endocrine-disrupting effects of chemicals in nature are based on their effects on the reproduction of fish and on changes in their genital structures. A comprehensive reviews of the effect of environmental xenoestrogens on human male reproductive health have been published by Toppari et al. (1996) and Vidaeff and Sever (2005).

Biodegradation of phthalates has been reported in marine and terrestrial environments (Saeger and Tucker, 1976; Engelhardt and Wallnöfer, 1978; Taylor et al., 1981), and in municipal sewage waters (Wang et al., 1998). Several Gram positive and Gram negative bacteria, as well as actinomycetes, can degrade phthalate esters in aerobic and anaerobic conditions. Although some individual microbes are able to completely mineralize phthalate esters, a mixed microbial community, which is typically the case in the environment, appears to have a more efficient phthalate-degrading ability (Aftring et al., 1981; Kurane, 1986). The biodegradable properties of phthalate esters vary depending on the structure of the compound. An increase in molecular weight and alkyl chain length of the molecule decreases the biodegradability. In nature, environmental conditions such as temperature, nutrient composition and the presence of degrader organisms, affect the degradation rate and mechanism (Zhang and Reardon, 1990; Staples et al., 1997b).

Compared to other xenobiotics, relatively little information is available about the impact of phthalates on individual microorganisms, as well as on microbial population dynamics in general (Cartwright et al., 2000b). However, recent investigations have suggested that ecotoxicological studies may also benefit from the determination of changes in the affected microbial communities (Stephen et al., 1999a,b; MacNaughton et al., 1999).

Diethyl phthalate (DEP), the phthalate that we focus on this study, has been found to have diverse acute and chronic toxic effects on several species at different trophic levels, as well as endocrine-disrupting properties (Colborn et al., 1993; Staples et al., 1997b; Zou and Fingerman, 1997). Even though the fate of DEP in aquatic environments has been comprehensively investigated, its effects in soil or compost have not been so well described. There are few recent studies on the degradation pathways and impacts of DEP in soil (Cartwright et al., 2000a,b; Jianlong and Xuan, 2004). While DEP biodegrades relatively rapidly in soil, its membrane-disrupting properties are a potential environmental risk. At concentrations higher than 1 mg g^{-1}, DEP has a significant effect on microbial communities and subsequently potential consequences for environmental processes.

Chemicals in the environment and their effect on soil health have led to increasing interest in monitoring acute and chronic toxicity and mutagenicity in soil (Maxam et al., 2000; White and Claxton, 2004; Fernández et al., 2006). ISO Soil Quality 190 is the major standardization body involved in the development of assays for soil monitoring purposes. However, there is a great need to understand the behaviour of multispecies communities, especially the impacts of toxic chemicals on the degrader communities and the growth of plants. In addition, concern has recently been raised about the health effects and tentative transportation of chemicals from soil to plants. Composted municipal sewage sludges are widely used as fertilizer or as growth media for plants in order to improve the chemical and physical properties of the soil. However, sludges are known to contain variable amounts of inorganic and organic contaminants, including phthalates (Abad et al., 2005; Harrison et al., 2006). Among the phthalic acid esters, DEHP has been widely detected in sludge targeted for composting and agricultural use. DEP is formed as a result of the degradation of DEHP during sludge treatment, and it has been detected in composted sludges (Amir et al., 2005).

The present study was performed in order to determine whether a kinetic luminescent bacteria test, the FLASH test (Lappalainen et al., 1999) for measuring acute toxicity, can be used as an indicator of DEP contamination in compost. We have earlier utilized this method to study the toxicity of compost due to the toxic compounds released during the biodegradation of biodegradable plastics (Degli-Innocenti et al., 2001; Kapanen and Itävaara, 2001; Tuominen et al., 2002). In addition, the FLASH test has been used as an indicator of compost immaturity (Itävaara et al., 2002b).

The responses of plants (OECD 208 Terrestrial Plant Growth test) and microbial communities to DEP in the growth substrate were evaluated in order to gain a better understanding of the processes taking place in plant growth substrate contaminated with phthalates. Changes in microbial communities were analysed by PCR-DGGE (Denaturing Gradient Gel Electrophoresis).

2. Materials and methods

2.1. Compost medium

Fruit and vegetable waste mixed with a bark/peat mixture was composted in controlled composting conditions in insulated 220 l composter bins as earlier described (Itävaara et al., 2002a). After 12 weeks, the compost was mixed (1:2, w:w) with a commercial growth medium (Kekkilän ruukutusmulta, Kekkilä Ltd., Finland) comprising a peat/sand mixture. Diethylene phthalate (SIGMA P5787) was added at doses of 0.01 g, 0.1 g, 1 g, 10 g and 100 g kg^{-1} (fw) to a mixture of air-dried compost (200 g) and commercial growth medium (400 g) and mixed thoroughly by hand. Deionised water was added to give a total fresh weight of 1 kg. The compost medium was used in the plant growth test. Part of the medium was frozen at $-20\,°C$ for later determination of acute toxicity in the solid phase luminescent bacteria test and the DEP concentration.

The pH of the prepared compost medium, measured in a compost:water mixture (volume ratio 1:6) was from 7.4 to 7.6 in the control sample and in the samples containing 0.01–10 g DEP kg^{-1}. In the compost medium containing 100 g DEP kg^{-1}, the pH decreased to 5.9. The dry matter content (105 °C for 24 h) of the compost medium was 42%.

2.2. Plant growth and acute toxicity studies

Forty radish (*Raphanus sativus*; Köpenhavns Torve) seeds were sown in the compost medium described above. Four replicate pots were used at each concentration of DEP. The plants were grown in a phytotron (Weiss Umwelttechnik GMBH, Germany), exposed to light for 16 h (1700 lux) and dark for 8 h at a temperature of 20 °C and 15 °C, respectively at 55% relative humidity. Germination of the seeds was checked daily and the number of germinated seeds counted. The fresh weight of the seedlings was determined after two weeks, and the dry weight of the plant seedlings after freeze-drying. The compost medium was sieved through a 5 mm sieve and frozen at $-20\,°C$ until further analysis.

Acute toxicity studies were carried out using a modified bioluminescent bacteria test, the Flash Assay. The measurement was based on the BioTox^{TM} Kit test utilizing bioluminescent *Vibrio fischeri*. Kinetic measurement was carried out on a 1251 Luminometer (Bio-Orbit, Turku, Finland) according to Lappalainen et al. (1999). Luminescence was measured throughout the whole exposure period (30 s), and the peak luminescence value was obtained at $I(0)$ and after exposure time $I(t)$. Results were expressed as inhibition percentage.

$$\text{Inhibition } \% = \left(1 - \frac{I(t)}{I(0)}\right) \times 100$$

$I(0)$ = the maximum value of luminescence during the first 5 s after addition of the *V. fischeri* suspension.
$I(t)$ = the value of luminescence after 30 s exposure.

The acute toxicity of compost medium to *V. fischeri* was tested before and after the plant growth test. Twenty and 40 g (dry weight) of all the samples were suspended in 1 l of 2% NaCl, and the pH was adjusted to 7. The EC50 (effective concentration) and the EC10 values for pure DEP were determined with the Flash Assay and the standard luminescent bacteria test with *V. fischeri* (EN ISO 11348-3, 1998). The dilution series for toxicity analysis with DEP was made with methanol, and the effect of methanol in the sample was taken into account. The DEP concentrations for the Flash and standard tests were 0.35–11.2 and 0.23–1.9 g l^{-1}, respectively. EC50 represents the concentration of the tested substance when the amount of light produced by *V. fischeri* is reduced to one half, and the EC10 concentration when the light reduction is 10%.

2.3. Diethyl phthalate analysis

The amount of diethyl phthalate in the compost medium was determined before and after the plant growth test. The DEP concentration was determined by high performance liquid chromatography (HPLC-DAD) in order to determine the amounts of DEP biodegraded or volatilized. The samples (1–7 g) were extracted with acetonitrile (ACN, 3×10–50 ml). The combined ACN extracts were analysed thereafter by HPLC, and quantitation of DEP was performed using the external standard method. The limit of quantitation was 1 mg kg^{-1}, and the uncertainty of the measurement ±30%. The chromatography conditions were as follows. HPLC column: Hypersil ODS (5 μm), length 100 mm, diameter 2.1 mm; eluant: gradient eluation from 20% H$_2$O/80% ACN to 100% ACN/5 min; eluation rate 0.4 ml/min; oven temperature $+40\,°C$; the DAD wavelengths: 205 nm and 227 nm.

3. Effects of diethyl phthalate on microbial community structure

3.1. Nucleic acid extraction, PCR and DGGE

Samples for PCR and DGGE were taken after the plant growth test. Nucleic acid extractions were carried out in duplicate for native compost and each concentration of diethyl phthalate/compost mixture as described by Stephen et al. (1999a), the only modification being that 0.25 g of compost, rather than 0.5 g, was used. PCR was conducted as described in Muyzer et al. (1993): initial denaturation of DNA at 94 °C for 3 min and amplification in 35 cycles in 94 °C (1 min), 55 °C (60 s), 68 °C (45 s), in 25 μl reactions with 1.25 units of Expand LT polymerase (Boehringer Mannheim, Indianapolis, IN, USA) and 0.3 ng template DNA using a Robocycler thermocycling unit (Stratagene, LaJolla, CA, USA). Amplified 16 S rDNA gene fragments were inspected by agarose gel electrophoresis (1.2% agarose, $0.5 \times$ TBE (0.04 M Tris base plus 0.02% M acetic acid plus 1.0 M EDTA, pH 7.5, 5 μl ethidium bromide)) prior to DGGE analysis. Ethidium bromide stained bands were

quantified by UV fluorescence using an AlphaImager 2000 and manufacturer supplied software (Alpha-Innotech, San Leandro, CA, USA). The DGGE analyses were as described in Muyzer et al. (1993), except that the gels consisted of a 10–65% gradient of denaturant (100% denaturant = 7 M urea, 40% (vol/vol) formamide; Biorad) using a D-Code system (Biorad) at a constant temperature of 60 °C. Strong DNA bands in the polyacrylamide gel were excised, and the DNA re-amplified as described in Kowalchuk et al. (1997) prior to sequence analysis.

3.2. DNA sequence analysis and phylogenetic inference

Re-amplified rRNA -gene fragments were purified by means of GeneClean Spin columns (BIO-101, Vista, CA, USA) and quantified by fluorometry (Hoefer DyNA-Quant 200[TM] Fluorometer and Hoechst H33258 dye binding assay; Pharmacia Biotech. Inc, Piscataway, NJ, USA). Double-strand sequencing was carried out on an Applied Biosystems automated sequencer (model 373) with "Prism[TM]" dye terminators. All the clones were sequenced using the primer 519r (Lane et al., 1985), *E. coli* numbering (Brosius et al., 1981), and the sequences were edited using "Seqpup Version 0.6." (Gilbert, 1996). Reference sequences were recovered from the RDP release 7.0 of July 1998 (Cole et al., 2003). Supplemental sequences were retrieved from GenBank *via* the National Institute for Biotechnology Information (NCIB) Internet node using the Entrez facility (Schuler et al., 1996). Crude alignments of recovered sequences were performed using the ALIGN facility of the RDP followed by manual alignment within Seqpup V. 0.6. Ambiguous bases were deleted from the phylogenetic analysis by means of the Genetic Data Environment 2.2 "mask" function operated within ARB (Strunk and Ludwig, 1998). Phylogenetic algorithms (DNA-DIST, NEIGHBOR and SEQBOOT) also operated within the ARB software environment.

3.3. Nucleic acid accession numbers

Sequences recovered from DGGE bands were submitted to GenBank under the accession numbers AF187050–AF187056.

4. Results

DEP was toxic for radish in the plant growth test at the three highest concentrations studied. Plant growth, determined as seedling dry weight, was 25%, 92% and 100% inhibited by the compost medium with DEP concentrations of 1, 10 and 100 g kg^{-1}, respectively (Fig. 1a). Germination of the seeds was a less sensitive indicator, and gave a clear response at only the two highest phthalate concentrations (Fig. 1b). Germination was inhibited totally at the highest DEP concentration. Inhibition of germination by 10 g kg^{-1} DEP was 72%, while most of the seedlings did not show good growth.
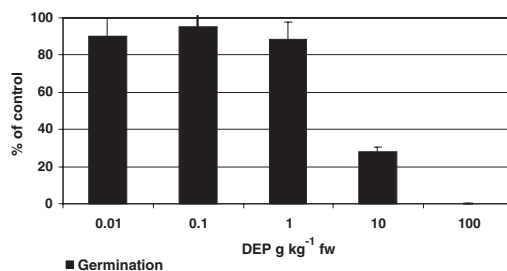

Fig. 1a. Germination expressed as % of the control in the radish (*Raphanus sativus*) germination test at different levels of DEP contamination.
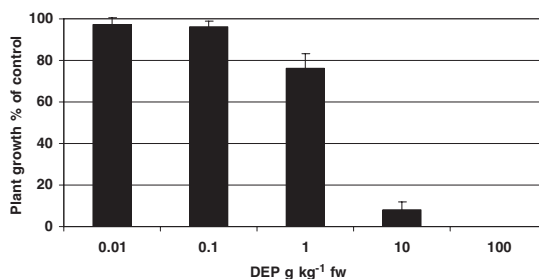

Fig. 1b. Plant growth expressed as % of the control with radish (*Raphanus sativus*) at different levels of DEP contamination.

The concentration of DEP in the compost medium decreased during the 14-day plant growth test (Table 1). After the plant growth test, the concentration in the medium that originally contained 100 g kg^{-1} of DEP was only 64 g kg^{-1}. Less than 1% of the DEP was left in the medium that originally contained 10 g kg^{-1} of DEP. Furthermore, no detectable amounts of DEP were present after the 14 day-plant growth test in the media that originally contained 1–0.01 g DEP kg^{-1} compost substrate.

Before planting the seeds, the toxicity of the DEP-spiked compost medium was evaluated with the Flash luminescent bacteria test. This test, which measures acute toxicity, showed a clear toxic response at the two highest diethyl

Table 1
DEP concentrations (g kg^{-1} fw) in the compost medium before and after the plant growth test

Theoretical DEP concentration g kg^{-1} fw[a] (dw[b])	DEP g kg^{-1} fw[a] before the plant growth test	DEP g kg^{-1} fw[a] after the plant growth test
Control (0)	0.0085	<0.001
0.01 (0.024)	0.011	<0.001
0.1 (0.24)	0.080	<0.001
1 (2.4)	0.780	<0.001
10 (24)	8.500	0.014
100 (240)	100.000	64.000

[a] fw = fresh weight.
[b] dw = dry weight.

phthalate concentrations (Fig. 2a). Inhibition of the light production of *V. fischeri* with 10 and 100 g kg^{-1} fw DEP in the compost media with dilutions of 40–20 g l^{-1} was 27–51% and 71–75%, respectively. In contrast, the smaller concentrations of diethyl phthalate in the media caused an increase in bioluminescence by 10–20%. After the two-week plant growth assay, the toxicity of the compost media was again studied. In this case, only the highest concentration of DEP gave a toxic response and around 70% inhibition was detected (Fig. 2b). All the other samples increased the amount of light produced by up to 17%.

The toxicity of the 100 g DEP kg^{-1} compost medium did not decrease during the two-week plant growth period, even though the other samples were no longer toxic at the end of the experiment due to the biodegradation or volatilization of DEP.

In order to study the EC50 and EC10 values of DEP toxicity in compost, a dilution series was analyzed by the *V. fischeri* test. The DEP concentrations in the test solutions derived from the compost media are presented in Table 2. DEP concentrations of between 9.4 and 0.47 g l^{-1} in the compost media gave a toxic response in the flash test. The EC10$_{30s}$ value for DEP measured as a

Table 2
Concentration of DEP (g kg^{-1} dw) in the compost media dilutions (40 and 20 g dw l^{-1}) for the Flash test

DEP g kg^{-1} fw in compost medium	DEP g kg^{-1} dw in dilution 40 g dw l^{-1}		DEP g kg^{-1} dw in dilution 20 g dw l^{-1}	
	B	A	B	A
100	9.4	8.2	4.7	4.1
10	0.94	0.002	0.47	0.001
1	0.094	<0.0001	0.047	<0.0001
0.1	0.0094	<0.0001	0.0047	<0.0001
0	0	0	0	<0.0001

Samples were taken before (B) and after (A) the plant growth test.

Fig. 3. PCR-DGGE analysis of 16S rDNA fragments recovered from compost. Labels indicate the bands that were excised and successfully subjected to sequence and phylogenetic analysis.
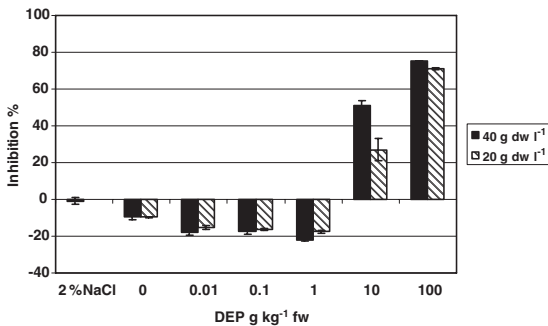

Fig. 2a. Inhibition % in the Flash test with freshly DEP-spiked (0, 0.01, 0.1, 1, 10 and 100 g kg^{-1}) compost medium using sample concentrations of 40 and 20 g dw l^{-1}. The test control was 2% NaCl.
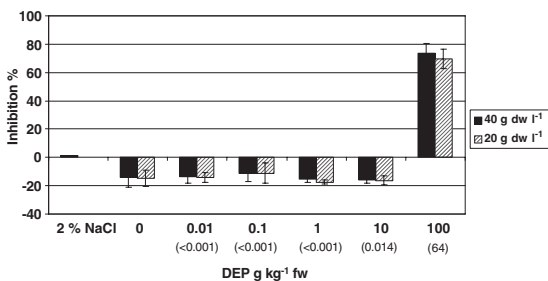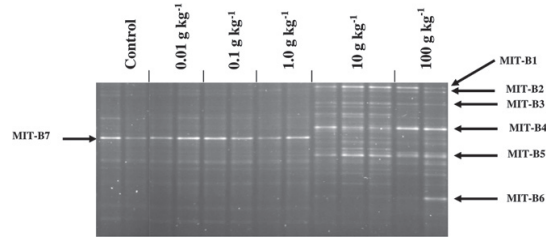

Fig. 2b. Inhibition % in the Flash test with the compost medium at the end of the 14-d plant growth test with DEP-spiked substrates. The test control was 2% NaCl. The concentrations of DEP in the growth media are expressed as nominal (without parentheses) and as measured (in parenthesis) at the end of the plant growth test.

pure chemical with the Flash assay was 0.92 g l^{-1} (standard deviation 0.25), and the EC50$_{30s}$ value was 9.40 g l^{-1} (standard deviation 1.28). The standard luminescent bacteria test with a longer contact time gave a stronger inhibition effect for DEP, while EC50$_{30min}$ was 0.50 g l^{-1} (0.02) and EC20$_{30min}$ 0.16 g l^{-1} (0.004).

Microbial community analysis by PCR-DGGE revealed a DEP-induced change in the community structure at a concentration of 10 and 100 g kg^{-1} compost medium (Fig. 3). The community structure of the DEP/compost mixtures below these levels was not discernibly different from the control compost (Fig. 3). PCR-DGGE analysis of the control and low-level DEP composts revealed only a single strong band in a highly complex background of minor PCR products. Sequence analysis of this band showed that the source organism was a member of the δ-proteobacteria (Fig. 4). At toxic concentrations, as defined by plant growth and germination, this band was no longer visible. The community profiles of compost containing 10 and 100 g kg^{-1} DEP were dominated by *ca.* 10 major bands, six of which generated legible sequences. Comparison of these sequences to those of cultured organisms suggested association with the genera *Sphingomonas*, *Pseudomonas* and the *Actinomycetes* (Fig. 4).

5. Discussion

Phthalates are compounds which are included in the priority list to be monitored in sludge targeted for agriculture
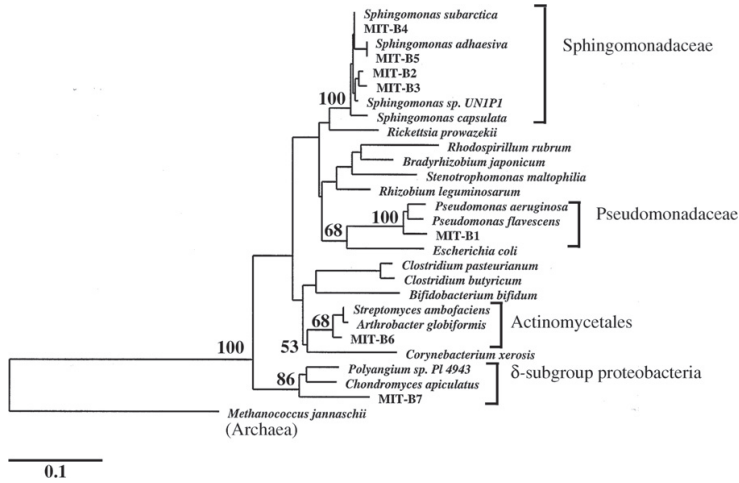
Fig. 4. Neighbour-joining dendrogram with Jukes and Cantor correction of sequences recovered from DGGE bands with reference sequences derived from the Ribosomal Database Project (Maidak et al., 1999). Numbers represent Bootstrap support on 100 replicates for branches immediately to their right and, for simplicity, are shown only for nodes of relevance to the novel sequences described. All analyses were carried out within the ARB sequence management system for the Linux operating system (Strunk and Ludwig, 1998). Scale bar represents a 10% estimated change.

in the 3rd draft of the "Working document of sludge" (EU, 2000; Andersen, 2001). Diethyl phthalate is known to be biodegradable and, in this study, was shown to decrease to nontoxic levels in the plant growth substrate during two weeks' cultivation of the test plants.

Cartwright et al. (2000a) reported that DEP added to soil at concentrations of $10 \, mg \, g^{-1}$ or below was up to 90% biodegraded in 59 days, and Jianlong and Xuan (2004) showed DEP degradation in sludge-amended soils with a half life of 3.7 days. Compared to these values, the degradation in our compost medium was more effective. On the other hand, translocation and absorption of the chemical by the plants has to be taken in consideration. Rapid biodegradation of DEHP (diethylhexyl phthalate) in municipal solid waste and sewage sludge composting has also been reported by Moeller and Reeh (2003).

The concentration of DEP in the compost environment that induced toxic effect in the Flash test and plant assay was in the range of $1–10 \, g \, kg^{-1}$. Lower concentrations stimulated the light production of V. fischeri. This phenomenon has earlier been reported by Degli-Innocenti et al. (2001). Aquatic toxicity studies with microorganisms, algae, invertebrates and fish show that EC/LC50 values for acute toxicity for DEP vary from about $10–130 \, mg \, l^{-1}$, and for chronic toxicity from 3.65 to $25 \, mg \, l^{-1}$ (Staples et al., 1997b). There is a lack of information about the toxic levels of DEP in soil and compost ecosystems. In the acute toxicity test (Flash test) used in this study, EC50 for DEP was $9.4 \, g \, l^{-1}$, and in the standard luminescent bacteria test it was $500 \, mg \, l^{-1}$. This seems to be a relatively high value compared to other reported EC50 values (Colborn et al., 1993; Staples et al., 1997a,b; Zou and Fingerman, 1997).

Due to the low solubility of DEP, acute toxicity measured with luminescent bacteria is probably not a very sensitive method for determining the toxicity of DEP, especially.

It was assumed that the amount of organic matter in the compost would have reduced the level of toxicity induced by DEP, but our results did not support this. Surprisingly, in the compost matrix, $0.47 \, g \, l^{-1}$ DEP caused 25% inhibition in the Flash test, even though inhibition with the same concentration but with the pure chemical (without compost) was less than 10% ($EC10_{30s}$ $0.9 \, g \, l^{-1}$). This may be due to changes in the compost matrix induced by DEP, and the conversion of certain compounds in the compost into more bioavailable forms, resulting in toxicity to the test organism. According to Cartwright et al. (2000b), DEP is more toxic in soils containing low amounts of organic matter than in soils with high organic matter. In our study, however, DEP in with the presence of the organic matter in the compost did induce higher toxic levels than DEP in pure water. Therefore, the effect of DEP in an organic matter rich environment should be investigated in more detail.

There is very little information about the physiological responses of plants to DEPs and about their bioaccumulation in plants. In a related study the toxic response of radish seedlings was studied at the protein level. Toxic concentrations of DEP lead to prominent changes in protein synthesis and the formation of several stress related proteins, such as heat shock proteins (HSPs), when the small aseptically cultivated seedlings were exposed to $222 \, mg \, l^{-1}$ DEP in liquid medium. Novel proteins that might be specific for DEP induction were also detected (Saarma et al., 2003). In the present study, toxic effects were induced in the plant assay and Flash test at higher

concentrations in the range of 1–10 g kg^{-1}, probably due to the presence of a solid matrix and lower bioavailability or lower sensitivity of the used methods.

The indigenous microbial communities present in plant growth media are important not only for the degradation and mineralization of organic compounds in the medium and the release of nutrients for the plants, but also because they represent an important suppressive microbial community that prevents the growth of pathogens. In the present study, the microbial community composition in the compost medium changed at the same toxic concentration that was found to be toxic in the single-species toxicity tests. This phenomenon is probably due either to the suppression of sensitive populations by the high concentration of DEP, or to the emergence of a resistant degrader population.

The *Actinomycetes* group, which emerged at higher DEP concentrations, is known to include proficient DEP-degrading strains (Suemori et al., 1993; Chauret et al., 1995). We have not found any report of DEP-degrading *Sphingomonas* sp. but, considering the broad-substrate utilization of this genus and its strong implication in the bioremediation of organic pollutants (White et al., 1996; Kästner et al., 1998; Barkay et al., 1999; Hamann et al., 1999 and references cited therein), its occurrence as a major phthalate-degrader *in situ* is not surprising. However, we did not find any members of the genus *Burkholderia*, for which phthalate degradation has been well described under laboratory conditions (Chang and Zylstra, 1999). The response of the indigenous bacterial community to the addition of DEP was observed at the same level of contamination as was required to elicit an effect in both the single-species phytotoxicity and microbial-toxicity tests described above. This response may have been due to a number of factors, principally to the growth of specific organisms on DEP and to inhibition of the growth of other organisms by DEP. Therefore the response should not be considered as a measure of the toxicity of DEP to the *in situ* microbial community *per se*. Instead, the coincidence of the two ecotoxicological measurements with a clear alteration in the *in situ* bacterial community structure supports the argument that microbial community typing is an effective alternative method for monitoring pollutants and their remediation (Almeida et al., 1998; White et al., 1998).

Cartwright et al. (2000b) reported that pseudomonas were more sensitive to higher concentrations of DEP (10–100 mg g^{-1}) than the total culturable bacteria. Lower concentrations stimulated pseudomonas momentarily, but DEP was shown to be inhibitory in both water and soil at concentrations above 10 mg l^{-1}. One of the major microbial groups in the samples containing 10–100 g DEP kg^{-1} in our test setup was found to belong to the *Pseudomonas* genera (*sensu stricto*). Concentrations above 1 g kg^{-1} had a significant effect on the microbial community and environmental processes. What are the fundamental agricultural and environmental effects of these changes in the microbial population that have been detected in all contaminated soils? The important questions to be addressed

in the future are the effect of organic pollutants on soil health and on the balance of pathogenic and biocontrol microorganisms in agricultural soils.

The recycling of organic matter back to soil is required to maintain the fertility of soils. However, sludge and compost may contain high amounts of organic pollutants such as phthalates, and this poses a potential threat for the contamination of agricultural soils. In order to meet the challenges set by EU legislation for the recycling of sludge and biowaste compost, more information is needed about their bioaccumulation due to continuous application on fields. There is also a need for screening tests (i.e. fast biotests) to evaluate the potential adverse effects of the compost used for soil amelioration, and inexpensive methods for determining the amount of organic pollutants in the environment.

6. Conclusions

This study demonstrated that in compost plant growth substrate, microbes and plants responded to the same toxic concentration of DEP. In the acute toxicity test the presence of organic matter in the compost did not, contrary to expectations, reduce the toxicity. DEP was found to be less toxic as a pure chemical than when mixed with the mature compost. The microbial community response exhibited suppression of the most heterotrophic microorganisms at toxic concentrations of DEP. The diversity of the microbial community was reduced to 10 major species at concentrations above 1 g kg^{-1} DEP. In addition to the sensitivity of some microbial species to DEP, the reason for the change in the microbial community was probably due to the increase in the degrader population resulting from abundant substrate availability. The major species identified were *Sphingomonas* sp., *Pseudomonas* sp., and *Actinomyces* sp.

Acknowledgements

References

Abad, E., Martínez, K., Planas, C., Palacios, O., Caixach, J., Rivera, J., 2005. Priority organic pollutant assessment of sludges for agricultural purposes. Chemosphere 61, 1358–1369.

Aftring, R.P., Chalker, B.E., Taylor, B.F., 1981. Degradation of phthalic acids by denitrifying mixed cultures of bacteria. Appl. Environ. Microb. 41, 1177–1183.

Almeida, J.S., Leung, K., Macnaughton, S.J., Flemming, C., Wimpee, M., Davis, G., White, D.C., 1998. Mapping changes in soil microbial community composition signalling bioremediation. Bioremediat. J. 1, 255–264.

Amir, S., Hafidi, M., Merlina, G., Hamdi, H., Jouraiphy, A., El Gharous, M., Revel, J.C., 2005. Fate of phthalic acid esters during composting

of both lagooning and activated sludge. Process Biochem. 40, 2184–2190.

Andersen, A., 2001. Disposal and recycling routes for sewage sludge – Part 3 – Scientific and technical sub-component report, 23 October 2001. European commission DG Environment – B/2.

Barkay, T., Navon-Venezia, S., Ron, E.Z., Rosenberg, E., 1999. Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. Appl. Environ. Microb. 65, 2697–2702.

Bauer, M.J., Herrmann, R., 1998. Dissolved organic carbon as the main carrier of phthalic acid esters in municipal landfill leachates. Waste Manage. Res. 16, 446–454.

Brosius, J., Dull, T.L., Sleeter, D.D., Noller, H.F., 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. 148, 107–127.

Cadogan, D.F., Papez, M., Poppe, A.C., Scheubel, J., 1993. An assessment of the release, occurrence and possible effect of plasticizers in the environment. Prog. Rubber Plast. Technol. 10, 1–19.

Cartwright, C.D., Owen, S.A., Thompson, I.P., Burns, R.G., 2000a. Biodegradation of diethyl phthalate in soil by a novel pathway. FEMS Microbiol. Lett. 186, 27–34.

Cartwright, C.D., Thompson, I.P., Burns, R.G., 2000b. Degradation and impact of phthalate plasticizers on soil microbial communities. Environ. Toxicol. Chem. 19, 1253–1261.

Chang, H.K., Zylstra, G.J., 1999. Role of quinolinate phosphoribosyl transferase in degradation of phthalate by *Burkholderia cepacia* DBO1. J. Bacteriol. 181, 3069–3075.

Chauret, C., Mayfield, C.I., Inniss, W.E., 1995. Biotransformation of di-*N*-butyl phthalate by a psychrotrophic *Pseudomonas fluorescens* (Bgw) isolated from subsurface environment. Can. J. Microbiol. 41, 54–63.

Colborn, T., vom Saal, F., Sotto, A.M., 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Persp. 101, 378–384.

Cole, J.R., Chai, B., Marsh, T.L., Farris, R.J., Wang, Q., Kulam, S.A., Chandra, S., McGarrell, D.M., Schmidt, T.M., Garrity, G.M., Tiedje, J.M., 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucl. Acids Res. 31, 442–443.

Degli-Innocenti, F., Bellia, G., Tosin, M., Kapanen, A., Itävaara, M., 2001. Detection of toxicity released by biodegradable plastics in activated vermiculite. Polym. Degrad. Stabil. 73, 101–106.

Engelhardt, G., Wallnöfer, P.R., 1978. Metabolism of di- and mono-*n*-butyl phthalate by soil bacteria. Appl. Environ. Microb. 35, 243–246.

EN ISO 11348-3, 1998. Water Quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* – Part 3: Method using freeze-dried bacteria.

EU, 2000. Working Document on Sludge 3rd Draft. Unpublished, 19 p.

FDA, US Department of Health Education and Welfare, Food and Drug Administration, 1982. Notice, Food Chem News 24, 32.

Fernández, M.-D., Vega, M.M., Tarazona, J.V., 2006. Risk-based ecological soil quality criteria for the characterization of contaminated soils. Combination of chemical and biological tools. Sci. Total Environ. 366, 466–484.

Gilbert, D.G., 1996. SeqApp sequence alignment editor. Bloomington, Ind.

Gledhill, W.E., Kaley, R.G., Adams, W.J., Hicks, O., Michael, P.R., Saeger, V.W., LeBlanc, G.A., 1980. An environmental safety assessment of butyl benzyl phthalate. Environ. Sci. Technol. 14, 301–305.

Hamann, C., Hegemann, J., Hildebrandt, A., 1999. Detection of polycyclic aromatic hydrocarbon degradation genes in different soil bacteria by polymerase chain reaction and DNA hybridization. FEMS Microbiol. Lett. 1731, 255–263.

Harrison, E.Z., Oakes, S.R., Hysell, M., Hay, A., 2006. Organic chemicals in sewage sludge. Sci. Total Environ. 367, 481–497.

Itävaara, M., Karjomaa, S., Selin, J., 2002a. Biodegradation of polylactide in aerobic and anaerobic thermophilic conditions. Chemosphere 46, 879–885.

Itävaara, M., Venelampi, O., Vikman, M., Kapanen, A., 2002b. Compost maturity – problems associated with testing. In: Insam, H., Riddech, N., Klammer, S. (Eds.), Microbiology of Composting. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 373–382.

Jianlong, W., Xuan, Z., 2004. Biodegradation of phthalic acid esters (PAEs) in soil bioaugmented with acclimated activated sludge. Process Biochem. 39, 1837–1841.

Jobling, S., Reynolds, T., White, R., Parker, M.G., Sumpter, J.P., 1995. A variety of environmentally persistent chemicals, including some phthalate plasticisers, are weakly estrogenic. Environ. Health Persp. 103, 582–587.

Jonsson, S., Ejlertsson, J., Ledin, A., Meriowsky, I., Svensson, B.H., 2003. Mono- and diesters from o-phtalic acid in leachates from different European Landfills. Water Res. 37, 609–617.

Kapanen, A., Itävaara, M., 2001. Review – Ecotoxicity tests for compost applications. Ecotox. Environ. Safe. 49, 1–16.

Kästner, M., Breuer-Jammali, M., Mahro, B., 1998. Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Appl. Environ. Microb. 64, 359–362.

Kowalchuk, G.A., Gerards, S., Woldendorp, J.W., 1997. Detection and characterization of fungal infections of *Ammophila arenaria* (marram grass) roots by denaturing gradient gel electrophoresis of specifically amplified 18s rDNA. Appl. Environ. Microb. 63, 3858–3865.

Kurane, R., 1986. Microbial degradation of phthalate ester-assimilating bacteria. Agr. Biol. Chem. 41, 1031–1038.

Lane, D.J., Pace, B., Olsen, G.J., Stahl, D.A., Sogin, M.L., Pace, N.R., 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA 82, 6955–6959.

Lappalainen, J., Juvonen, R., Vaajasaari, K., Karp, M., 1999. A new flash method for measuring the toxicity of solid and coloured samples. Chemosphere 38, 1069–1083.

Lokke, H., Rasmussen, L., 1983. Phytotoxicological effects of di-(2-ethyl hexyl)-phthalate and di-*n*-butyl-phthalate on higher plants in laboratory and field experiments. Environ. Pollut. A32, 179–199.

MacNaughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A., Chang, Y.-J., White, D.C., 1999. Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microb. 65, 3566–3574.

Maidak, B.L., Cole, J.R., Parker Jr., C.T., Garrity, G.M., Larsen, N., Li, B., Lilburn, T.G., McCaughey, M.J., Olsen, G.J., Overbeek, R., Pramanik, S., Schmidt, T.M., Tiedje, J.M., Woese, C.R., 1999. A new version of the RDP. Nucl. Acids Res. 27, 171–173.

Maxam, G., Rila, J.P., Dott, W., Eisentraeger, A., 2000. Use of bioassays for assessment of water-extractable ecotoxic potential of soils. Ecotox. Environ. Safe. 45, 240–246.

Moeller, J., Reeh, U., 2003. Degradation of DEHP, PAHs and LAS in source separated MSW and sewage sludge during composting. Compost Sci. Util. 11, 370–378.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microb. 59, 695–700.

Saarma, K., Tarkka, M.T., Itävaara, M., Fagerstedt, K.V., 2003. Heat shock protein synthesis is induced by diethyl phthalate but not by di(2-ethylhexyl)phthalate in radish (*Raphanus sativus*). J. Plant Physiol. 160, 1001–1010.

Saeger, V.W., Tucker, E.S., 1976. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microb. 31, 29–34.

Schuler, G.D., Epstein, J.A., Ohkawa, H., Kans, J.A., 1996. Entrez: molecular biology database and retrieval system. Method. Enzymol. 266, 141–162 <http://www.ncbi.nlm.nih.gov/Entrez> (last accessed 9.15.1999).

Staples, C.A., Adams, W.J., Parkerton, T.F., Gorsuch, J.W., 1997a. Review, aquatic toxicity of eighteen phthalate esters. Environ. Toxicol. Chem. 16, 875–891.

Staples, C.A., Peterson, D.R., Parkerton, T.F., Adams, W.J., 1997b. The environmental fate of phthalate esters: a literature review. Chemosphere 35, 667–749.

Staples, C.A., Parkerton, T.F., Peterson, D.R., 2000. A risk assessment of selected phthalate esters in North American and Western European surface waters. Chemosphere 40, 885–891.

Stephen, J.R., Chang, Y.-J., Gan, Y.-D., Peacock, A., Pfiffner, S.M., Barcelona, M.J., White, D.C., Macnaughton, S.J., 1999a. Microbial characterisation of a JP-4 fuel contaminated site using a combined lipid biomarker/ PCR-DGGE based approach. Environ. Microbiol. 1, 231–243.

Stephen, J.R., Chang, Y.-J., Macnaughton, S.J., Whitaker, S.L., Hicks, C.L., Leung, K.T., Flemming, C.A., White, D.C., 1999b. Fate of a mixture of five metal resistant bacteria introduced into pristine and heavy-metal-contaminated soil microcosms assessed by DGGE. Environ. Toxicol. Chem. 18, 1118–1123.

Strunk, O., Ludwig, W., 1998. ARB: a software environment for sequence data. <http://www.mikro.biologie.tu-muenchen.de>. Munich: Department of Microbiology, Technische Universität München, Germany.

Suemori, A., Kurane, R., Tomizuka, N., 1993. Purification and properties of phthalate oxygenase from *Rhodococcus erythropolis* S-1. Biosci. Biotech. Bioch. 57, 1482–1486.

Taylor, B.F., Curry, R.W., Corcoran, E.F., 1981. Potential for biodegradation of phthalic acid esters in marine regions. Appl. Environ. Microb. 42, 590–595.

Thomas, J.A., Thomas, D.D., Wallin, R.F., Garvin, P.J., Martis, L., 1978. A review of biological effects of di(2-ethylhexyl) phthalate. Toxicol. Appl. Pharm. 45, 1–27.

Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr., L.J., Jegou, B., Jensen, T., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J., Meyer, O., Müller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., Skakkebæk, N.E., 1996. Male reproductive health and environmental xenoestrogens. Environ. Health. Persp. 104, 741–803.

Tuominen, J., Kylmä, J., Kapanen, A., Venelampi, O., Itävaara, M., Seppälä, J., 2002. Biodegradation of lactic acid based polymers in controlled composting conditions and evaluation of the ecotoxicological impact. Biomacromolecules 3, 445–455.

Vidaeff, A.C., Sever, L.E., 2005. In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. Reprod. Toxicol. 20, 5–20.

Wams, T.J., 1987. Diethylhexylphthalate as an environmental contaminant – a review. Sci. Total Environ. 66, 1–16.

Wang, J., Liu, P., Shi, H., Qian, Y., 1998. Kinetics of biodegradation of di-*n*-butyl phthalate in continuous culture system. Chemosphere 37, 257–264.

White, P.A., Claxton, L.D., 2004. Mutagens in contaminated soil: a review. Mutat. Res. – Rev. Mutat. 567, 227–345.

White, D.C., Sutton, S.D., Ringelberg, D.B., 1996. The genus *Sphingomonas*: physiology and ecology. Curr. Opin. Biotech. 7, 301–306.

White, D.C., Flemming, C.A., Leung, K.T., Macnaughton, S.J., 1998. In situ microbial ecology for quantitative appraisal, monitoring, and risk assessment of pollution remediation in soils, the subsurface, the rhizosphere and in biofilms. J. Microbiol. Meth. 32, 93–105.

Williams, M.D., Adams, W.J., Parkerton, T.F., Biddinger, G.R., Robillards, K.A., 1995. Sediment sorbtion coefficient measurements for four phthalate esters:experimental results and model theory. Environ. Toxicol. Chem. 14, 1477–1486.

Zhang, G., Reardon, K.F., 1990. Parametric study of diethyl phthalate biodegradation. Biotechnol. Lett. 12, 699–704.

Zou, E., Fingerman, M., 1997. Effects of estrogenic xenobiotics on molting of the water flea, *Daphnia Magna*. Ecotox. Environ. Safe. 38, 281–285.

PAPER V

Biotests for environmental quality assessment of composted sewage sludge

Biotests for environmental quality assessment of composted sewage sludge

Kapanen, Anu[a], Vikman, Minna[a*], Rajasärkkä, Johanna[b], Virta, Marko[b], and Itävaara, Merja[a]

[a] VTT, P.O.Box 1000, FI-02044 VTT, Finland

[b] University of Helsinki, Food and Environmental Sciences, P.O.Box 66, FIN-00014 Helsinki, Finland

Corresponding author[*]

Minna Vikman

VTT Technical Research Centre of Finland

P.O.Box 1000, Tietotie 2, FI-02044 VTT, Finland

Telephone +358 20 722 7155

Fax +358 20 722 7071

Minna.Vikman@vtt.fi

Abstract

The quality of sewage sludge and sludge-based products, such as composts and growth media, is affected by the contamination of sewage sludge with, potentially, hundreds of different substances including organic pollutants. Therefore, it is difficult to achieve the reliable environmental quality assessment of sewage sludge-based products solely based on chemical analysis. In the present work, we demonstrate the use of the kinetic luminescent bacteria test to evaluate acute toxicity and the Vitotox[TM] test to monitor genotoxicity. In addition, endocrine-disrupting and dioxin-like activity was studied using yeast-cell-based assays. The relative contribution of industrial waste water treated at the Waste Water Treatment Plants (WWTP) was shown to affect the amount of organic contaminants detected in sewage sludge; it led to elevated concentrations of polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) leading to raised concern regarding the potential agricultural use of the sewage sludge. The effect of elevated amounts of organic contaminants in sewage sludge could also be identified with biotests able to demonstrate higher acute toxicity, genotoxicity, and potential for endocrine-disruptive properties. Composting in a pilot-scale efficiently reduced the amounts of linear alkylbenzensulphonates (LAS), nonylphenols and nonylphenolethoxylates (NPE/NP) and PAH with relative removal efficiencies of 84%, 61% and 56%. In addition, decrease in acute toxicity, genotoxicity and endocrorine-disrupting and dioxin-like activity during compost processing could be detected with applied biotests. However, the biotests did have limitations in accessing the ecotoxicity of test media rich with organic matter, such as sewage sludge and compost, and effects of sample characteristics on biotest organisms must be acknowledged. The compost matrix itself, however, which contained a high amount of nutrients, bark, and peat, reduced the sensitivity of the genotoxicity tests and yeast bioreporter assays. The maturity of the compost may affect the response of the test organism, therefore, it is recommended to always determine the stage of maturity when the ecotoxicity of compost is evaluated.

Key words
Sewage sludge, compost, organic contaminant, biodegradation, ecotoxicity, bioreporter

1 Introduction

A total of ten million tons (dry matter) of sewage sludge is produced in Europe every year, and of this, an annual amount of 160 000 tons is produced in Finland (Stamatelatou et al. 2011; EC, 2008; Laturnus, 2007). According to the Sewage Sludge Directive 86/278/EEC, the safe use of municipal sewage sludge is an essential objective of European legislation. The European Commission is reviewing the Sewage Sludge Directive and a working document draft on sludge and biowaste is available (WD, 2010). The Working Document on Sludge and Biowaste (WD, 2010 and 2000) sets the frame for monitoring the quality of sewage sludge in Europe. It is not legally binding but provides a basis for discussions. In addition, the disposal and recycling of sewage sludge in Europe, as well as the content of heavy metals and organic pollutants in sewage sludge, have been discussed in several reports (Amlinger et al., 2004; Andersen, 2001; Brändli et al., 2004; Erhardt and Pruess, 2001; Gawlik and Bidoglio, 2006; EC, 2008).

Sewage sludge and products derived from sewage sludge such as soil conditioners contain beneficial nutrients and organic matter, but they also contain heavy metals, pathogens and organic contaminants. Of European countries German, Denmark, Sweden, France and Austria have set national limit values for organic contaminants in sewage sludge. At EU-level the limit values have not been defined yet but are under discussion (Table 1). Major organic contaminants found from municipal sewage sludge include adsorbable organic halogens (AOX), linear alkylbenzensulphonates (LAS), nonylphenols and nonylphenolethoxylates (NP and NPE), di-ethylhexylphthalate (DEHP), polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) listed in Working document on sludge 3rd draft (Anderssen, 2001; Müller, 2003; Gawlik and Bidoglio, 2006; Amlinger, 2004; Abad et al., 2005; Schowanek, 2004; Marttinen et al., 2003; Jensen and Jepsen, 2005; Samsøe-Peteren, 2003; Stewens et al. 2001; Koch et al., 2001). Polybrominateddiphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA), and hexabromocyclododekane (HBCD), known as bromated flame retardants (BFR), are one of the emerging contaminant groups in sewage sludge (Cincinelli et al. 2012; Hale et al., 2006).

The disposal and handling of ever-increasing amounts of sewage sludge challenge the established treatment technologies targeted at preventing harmful effects on human health, agricultural land,

and the environment. Composting is one of the most efficient organic waste treatment technologies to reduce the amount of biodegradable organic waste and potentially biodegradable organic contaminants present in sewage sludge. The amount of LAS, NP, NPE, DEHP, and some PAHs, has been successfully reduced by composting (Stamatelatou et al. 2011; Amir et al., 2005; Ramirez et al., 2008; Marttinen et al., 2004; Oleszczuk 2007; Cheng et al. 2008; Paulsen and Bester, 2010). On the contrary, PCB and PCDD/F persist throughout the composting process (Abad et al., 2005).

The quality of sewage sludge, and composted sewage sludge, cannot be evaluated based only on chemical analysis, while it has been shown to carry more than 500 organic chemicals (Harrison et al., 2006; Eriksson et al., 2008). The synergistic effects of several harmful compounds can only be evaluated with biotests which measure biological responses of living organisms. The most commonly used application of biotests to evaluate the toxicity of sewage sludge, composted sewage sludge, or soil amended with either sewage sludge or compost, seems to be tests based on the determination of the inhibitory effect of samples on the light emission of the *Vibrio fischeri* (Alvarenga et al. 2007; Lopez et al. 2010; Mantis et al., 2005; Fuentes et al. 2006). The kinetic luminescent bacteria test has been found to be well suited for determining the acute toxicity of coloured samples e.g. compost and composted sludge (Kapanen and Itävaara 2001; Kapanen et al. 2007; Kapanen et al. 2009). Other biotests applied in ecotoxicity assessment of sewage sludge and sewage sludge amended soils include *Daphnia magna* mortality, earthworms, springtails, phytotoxicity tests, and ostracod mortality and growth inhibition (Domene et al. 2011; Natal-da-Luz et al. 2009; Oleszczuk 2008; Oleszczuk 2010; Ramirez et al. 2008; Hamdi et al., 2006). Not much attention, however, has been paid to the compatibility of the test method with the sewage sludge or compost samples.

Sewage sludge may also contain genotoxic chemicals such asbenzo[a]pyrene, fluoranthene, fenantrane, and chrysene, however information pertaining to the biotests used for the detection of the genotoxicity of sewage sludge is scarce. Ames tests, and SOS-Chromotests, have been applied for genotoxicity assessment of sewage-sludge (Pérez et al. 2003; Renoux et al 2001). Furthermore, the genotoxic potential of sewage sludge amended soil has been studied with plants by Amin et al. (2011). In addition to the evaluation of acute toxicity or genotoxicity, the endocrine disruption potential (Svenson and Allard 2004; Leush et al., 2006; Murk 2002; Engwall et al., 1999; Hernandez-Raquet et al., 2007) and the presence of dioxin-like compounds (Suzuki et al. 2004; Suzuki et al. 2006) in sewage and sewage sludge have also been studied with biotests. When Murk et al. (2002) used three different assays, ER-Calux, YES, and ER-binding, to predict the oestrogenic

4

potencies in wastewater and sludge, ER-binding assay was the most sensitive of the applied tests. In the present work we used recombinant receptor transcription assays that use yeast cells with response element-regulated reporter genes are commonly used in the first stage of *in-vitro* screening of chemicals. Yeast tests can be useful in the first stage of screening, as they are easy and inexpensive to perform compared with tests using mammalian cells (OECD 2001), which require laborious and costly cell culturing. Furthermore, yeast cells are more resistant to environmental contaminants than mammalian cells, which is an important advantage when measuring complex environmental samples.

There remains a great deal of concern regarding the amount of different toxic chemicals present in sewage sludge; thus, the expanding monitoring needs for an increase in the amount of known chemicals are well acknowledged (Eriksson et al., 2008; Gawlik and Bidoglio, 2006). This has brought about an increasing demand for more affordable, reliable, and fast methods for monitoring the environmental fate of sewage sludge. Biotesting methods could be more widely used to complement the currently established chemical analyses employed in sewage sludge quality evaluation. However, there is a lack of information about the suitability of biotesting in environmental fate assessment of sewage sludge and composted sewage sludge.

In this study, we conducted a pilot composting experiment to study the efficiency of composting in reducing the load of organic contaminants and ecotoxicity of sewage sludge targeted for agricultural or landscaping applications. The aim of this study was to evaluate the acute toxicity, genotoxicity, endocrine disruptive, and dioxin-like activity in sewage sludge and composted sewage sludge by using biotests. We focused on evaluating the potential and limitations of biotests in evaluation of ecotoxicity of sewage sludge and composted sewage sludge. Applied biotests included a kinetic luminescent bacteria test with *Vibrio fischeri* measuring acute toxicity (ISO 21338), the Vitotox® test kit for genotoxicity evaluation, and a panel of yeast cell bioreporters for monitoring endocrine-disruptive activity and dioxin-like activity. A panel of bioluminescent (using the*luc*gene from Photinuspyralis) yeast strains was based on the human Oestrogen Receptor (ER) (Leskinen et al. 2005), Androgen Receptor (AR) (Michelini et al. 2005), or Aryl hydrocarbon Receptor (AhR) (Leskinen et al. 2008). Strains with ER and AR measure oestrogenic and androgenic activity, respectively. The AhR strain reacts to many polyhalogenated persistent organic pollutants (POP), such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and diphenyl ethers. As far as we know, there is no previous experience in using this yeast bioreporter in studying the environmental fate of sewage sludge and compost samples.

2 Materials and Methods

2.1 Municipal sewage sludge

Anaerobically digested municipal sewage sludge samples were collected after dewatering from two different wastewater treatment plants (WWTP), A and B. The WWTPs varied in size, yearly sludge production volume, and share of industrial wastewaters (Table 2). Six sewage sludge subsamples of 0.5 L were mixed thoroughly and divided for ecotoxicity and chemical analysis. Sewage sludge sample of about 100 L was collected from WWTP A for pilot composting. Samples of 200 ml were sent for chemical analysis to Lantmännen Analycen Ltd (Tampere, Finland) immediately after the sampling, and samples for ecotoxicity analysis were stored at -20 °C.

2.2 Pilot composting

In order to study the efficiency of composting as a remediation technology and biotests as a tool to study the quality of the sewage sludge compost, we performed a pilot scale composting study in 200 L composter bins (Biolan Ltd, Finland) with continuous aeration, on-line temperature, and CO_2 monitoring according to Itävaara et al. (2002a). Sewage sludge from WWTP A was mixed with bark and peat at a volume ratio of 1:2 and turned weekly. A 5 L sample was taken after mixing the compost thoroughly after 26, 55, 87, and 124 days of composting. The sample was sieved through a 10 mm mesh, divided into three parts for maturity, ecotoxicity, and chemical analysis, and stored at -20 °C until analysed. The maturity was tested immediately after the samplings without freezing the samples. The maturation of the compost was monitored by measuring the following chemical and physical, as well as the specific, maturity parameters.

The maturity tests applied were: CO_2 production (mg CO_2-C g^{-1} VS d^{-1}), nitrate-N/ammonium-N ratio, and germination index. The battery of maturity tests used in this study is presented in detail by Itävaara et al. (2006 and 2010). When CO_2 evolution is greater than 3 mg CO2-C g^{-1} VS d^{-1}, compost is not considered stabile. The nitrate-N/ammonia-N ratio describes the mineralization phase of organic nitrogen, and a ratio higher than 1 represents mature compost. The phytotoxicity of the compost samples was determined by germinating 50 seeds (*Lepidium sativum* L.) in a fresh compost sample in glass Petri dishes (ø 7-10 cm) for 28 hours in the dark at room temperature. The number of germinated seeds was counted, and the root length measured and compared with the control (Saadi et al. 2007; Saharinen and Vuorinen, 1998). In the control, cress seeds were

6

germinated on germination paper (Munktell, 1701, ø 88 mm). When the germination index was below 80%, the sample was considered phytotoxic. The germination index (GI) was calculated as described by Itävaara et al. (2010). In addition, dry weight (%), pH, conductivity (mSm^{-1}), and organic matter (%) were determined according to European standards EN 13040, EN 13037, EN 13038 and EN 13039, respectively.

2.3 Field scale compost samples

Field-scale compost samples were collected from two composting plants (1 and 2) treating municipal sewage sludge in Finland. Samples were selected to represent different stages of maturity to gain better understanding on the quality of full scale composts in relation to the composting phase. The test scheme for assessing the stage of maturity as earlier has been described by Itävaara et al. (2010) was used. Altogether, three compost samples were collected, one from the plant 1 (compost 1), and two from plant 2 (compost 2a and compost 2b). The age of the sampled compost pile in plant 1 was between one and two years, and the age of the compost samples collected from plant 2 were 3 months and 1 year. Samples were collected as composite sample. Five 10 L samples were taken from compost piles, mixed thoroughly, sieved with a 10 mm mesh, and then divided for chemical, ecotoxicity, and maturity analysis. Samples for maturity analysis were stored at +4 °C and samples for biotests and chemical analysis at -20 °C.

2.4 Chemical analysis

The organic contaminants listed in Table 1 were analysed from sewage sludge, pilot composting, and field-scale compost samples by Lantmännen Analycen Oy (Tampere, Finland) according to the following accredited methods: EPA-PAH 16, DEHP and NP/NPE by Gas Chromatography (GC) - Mass Spectrometer, PCB-7 by GC-Electron Capture Detector (ECD), LAS by High-Pressure Liquid Chromatography (HPLC) with UV Detector, and PCDD/F by GC- High Resolution Mass Spectrometry (HRMS).

2.5 Biotests

The acute toxicity, genotoxicity and endocrine disrupter and dioxin-like activity of sewage sludge from the two large-scale municipal wastewater treatment plants A and B, both with an anaerobic digestion step, but different amounts of treated industrial wastewater, was studied with biotests. Biotests were also applied to study the impact of the composting process on the quality of the sewage sludge targeted for soil improvement.

2.5.1 Acute toxicity by the kinetic luminescent bacteria test

Acute toxicity of sewage sludge and compost samples was measured according to ISO 21338, Water quality – Kinetic determination of the inhibitory effects of sediment and other solids and colour containing samples on the light emission of *Vibrio fischeri* (Kinetic luminescent bacteria test). The organism *Vibrio fischeri* was treated according to the BioTox[TM] Kit instructions (Aboatox, Turku, Finland). The luminescence was measured kinetically with a 1251 Luminometer (Bio-Orbit, Turku, Finland). In addition to the ISO 21338 standard protocol, two additional protocols for sewage sludge were applied using DMSO (dimethylsulfoxide, Sigma-Aldrich, Germany) and hexane (Rathburn Chemicals Ltd, Scotland) as solvents. Ten grams of sludge and compost samples were extracted with 20 ml of 100% DMSO for 2 hours at room temperature (RT) at 200 rpm. Hexane extraction was performed for conducting the tests with yeast bioreporter; the extraction protocol is described in yeast cell bioreporter chapter (2.5.3). DMSO and hexane extractions were conducted to increase the bioavailability of organic contaminants to the test organisms *Vibrio fischeri*.

The highest concentration of the sewage sludge and compost samples studied for ecotoxicity was 100 g l^{-1} and pH of the samples was adjusted to between pH 6-8. For sewage sludge samples the results are presented as EC50 values after 30 second and 30 minutes contact time. EC50$_{30min}$ values for reference chemicals 3,5-dichlorophenol, nonylphenol, and phenanthrene, were 2.4 mg L^{-1}, 2 mg L^{-1}, and 0.5 mg L^{-1}, respectively. Compost concentrations studied in kinetic luminescent bacteria were 100 g L^{-1} with samples treated according to the standard procedure (ISO 21338) or 3 g L^{-1} with an additional DMSO treatment step. EC50 values were not determined for the compost samples. The results were presented as percentages of inhibition in light production of *Vibrio fischeri*.

2.5.2 Genotoxicity by Vitotox[TM]

For the genotoxicity assay, 2 g samples were extracted with 4 ml of 100% DMSO at RT for 1 hour at 150 rpm. The genotoxic potential of the sewage sludge and compost was studied with the Vitotox[TM]10Kit (Thermo Electron Corp, Vantaa, Finland) based on the SOS response in the genetically modified luminescent *Salmonella typhimurium* TA104 *rec*N2-4 strain induced by the genotoxic compounds (van der Lelie et al., 1997). Luminescence was measured by the Fluoroscan Ascent FL fluorometer (Labsystems, Finland). The test was performed according to Verschaeve et al. (1999), introducing a *S. typhimurium* TA 104 *pr*1 strain to the test, with constantly expressed *lux* genes and according to the manufacturer's instructions for the Vitotox[TM]10 Kit. As an exception, four times the recommended amount of S9 was used with samples containing high levels of organic

matter. The results were expressed as a geno/cyto ratio of > 1.5 for genotoxic samples. The sample was considered cytotoxic if the S/N (signal/noise) ratio was below 0.8.

2.5.3 Yeast cell bioreporter test

The yeast strains employed here express human nuclear receptors that control the expression of a luciferase reporter gene. Oestrogenic activity was tested using the yeast strains containing the human estrogen receptors alpha (ER α) or beta (ER β), androgenic activity with the yeast strain containing the human androgen receptor (AR), and dioxin-like activity with the yeast strain expressing the human aryl hydrocarbon receptor (AhR). To test the possible increase or decrease in light emission due to the inhibitory effects of the toxic substances, or stimulating effects caused by nutrients in the samples, a control yeast strain that constitutively expressed luciferase was used. All the applied *Saccharomyces cerevisiae* strains have been described before by Leskinen et al. (2005 and 2008). Hexane and hexane + sulphuric acid extractions of sewage sludge and compost samples were tested with yeast cell bioreporters to detect endocrine-disrupting and dioxin-like activity. A sulphuric acid extraction step was needed to detect the dioxin-like compounds. In the hexane (Rathburn Chemicals Ltd, Scotland) extraction, 4 g dw of sludge or compost was mixed with 25 ml of acetone:hexane (1:9) (volume) for 1 hour at 200 rpm and centrifuged for 10 minutes (1000 x g). Hexane extraction was replicated and the replicates combined. The hexane extract was divided into two separate samples of which 2/3 was evaporated until dry under nitrogen and 1/3 was evaporated to about 4 ml. Thereafter, 3 ml of sulphuric acid (Merck, USA) was added, mixed at 170 rpm for 30 minutes and separated at +4 °C. The extract was centrifuged (800 x g) for 10 minutes, and the hexane phase was collected. The hexane extraction was repeated. The samples were evaporated, diluted to DMSO and stored at -20 °C. The concentration of the final extract was 13 g L^{-1}. The test scheme is presented in Figure 1.

Yeast was grown overnight at 30 °C with vigorous shaking in a synthetic minimal (SD) medium (6.7g/910 ml Difco yeast nitrogen base without amino acids, BD, USA) supplemented with D-glucose (Amresco, Ohio, USA) and the required amino acids (Sigma-Aldrich Co, St Louis, USA). The culture was diluted to an optical density (OD_{600}) of 0.4 and then grown at 30 °C until the OD_{600} reached 0.6-0.7. Aliquots of 90 µl were then pipetted onto a 96-well plate and 10 µl of the sample (diluted in 10% Dimethylsulfoxide) was added. The plate was shaken for 20 s and then incubated at 30 °C for 2.5 h or,in the case of the dioxin-strain, 3.5 h. After the incubation, the plate was shaken for another 20 s and 100 µl of 1 mM D-luciferin (BioThema (Sweden, in 0.1 M Na-citrate buffer pH 5) was added to the wells containing the induced cultures. The plate was briefly shaken and then

9

immediately measured with a Victor3 1420 multilabel counter (Perkin–Elmer Wallac, Turku, Finland) in the luminescence mode using a one-second counting time.

The data was analysed as described by Leskinen et al. (2005). In brief, the fold induction (FI) was determined by comparing the relative light units (RLU) measured by the luminometer with the RLU of the background luminescence in wells containing only yeast and solvent. The FI was corrected with the correction factor (CF), which was obtained by comparing the light emission of the control yeast strain incubated with the sample and with only solvent. If the correction factor was over 2 (light emission of the solvent+yeast was more than two-fold compared with the sample+yeast), the sample was considered to be too toxic to obtain reliable results, and the experiment was repeated with a more diluted sample. The minimum response considered positive, i.e., the limit of detection (LOD), was determined as described by Hakkila et al. (2004). The corrected fold inductions were used to calculate the corresponding equivalent values (eqv) by using standard curves measured with the same experimental procedure using dilutions of 17 beta-estradiol (Sigma-Aldrich Co, St Louis, USA), 5alpha-dihydrotestosterone (donated by Hormos Medical Oy, Turku, Finland) orbenzo[a]pyrene (Sigma-Aldrich Co, St Louis, USA), for oestrogen receptors (ERα or ERβ), the androgen receptor (AR) and the aryl hydrocarbon receptor (AhR), respectively.

3   Results

3.1   Organic contaminants in sewage sludge before composting

Concentrations of the studied organic contaminants present in the municipal sewage sludge samples from the two different wastewater treatment plants (A and B) are presented in Table 1. AOX and LAS concentrations were very similar within the two sewage sludge samples studied. NP was responsible for approximately 90% of the overall NPE load in sewage sludge A and B; its concentration was 13.3 mg kg^{-1} dw in sample A and 7.8 mg kg^{-1} dw in sample B. Sample B contained a very high amount of DEHP, 110 mg kg^{-1} dw (versus 57 kg^{-1} dw for sample A), however, the amount of PAH was higher in sample A (5.8 mg kg^{-1}), compared to sample B (0.3 mg kg^{-1}) (PAHs were calculated according to WD 2000). The most significant PAHs in this sample were phenanthrene (1.2 mg kg^{-1}dw), fluoranthene (1.6 mg kg^{-1}dw), and pyrene (1.2 mg kg^{-1}dw). The amount of benzo[a]pyrene was lower 0.36 mg kg^{-1}dw. If all of the 16 analysed PAHs are calculated, the PAH content was as high as 8.0 mg kg^{-1}dw, in sludge A, which also carried the

higher content of PCB (1.4 mg kg^{-1}dw). In addition, amount of PCDD/Fs in sewage sludge A was ten times higher than in sample B.

3.2 Sewage sludge composting

Pilot scale composting was performed with anaerobically treated sewage sludge A. At the commencement of composting, the temperature inside the composter bin increased quickly to over +60 °C and then decreased to room temperature after two weeks. The maturation characteristics of pilot composting, as well as three full scale sewage sludge compost samples, are presented in Table 3. After 26 days of composting, the CO_2 production of 3.8 mg CO_2-C g^{-1} VS d^{-1} indicated that the composted sewage sludge A was still immature. Increased compost stability was shown after 124 days of composting, the CO_2 production had decreased to 0.6 mg CO_2-C g^{-1} VS d^{-1}. The relatively high conductivity, the nitrate-N/ammonium-N ratio below one, and the low germination index, suggest that composting should still be continued to reach full maturity, however. The degradation of LAS, NPE, NP, DEHP, PAH, and PCB was followed during the pilot composting process and the amount, as well as the degradation percentage, of these organic contaminants was measured after 26 and 124 days of composting (Table 1). The concentration of each compound at the beginning of the test was calculated considering the dilution of the sewage sludge after mixing it with bark and peat. The degradation of LAS, NPE, and NP was very efficient at the beginning of pilot composting. After just 26 days of composting, LAS has degraded by 83%, NPE 69%, and NP 66%. The degradation of PAH was slower, but at the end of pilot composting only 16% of the original amount of PAHs remained in the compost. The amount of DEHP at the end of pilot composting was 11 mg kg^{-1}dw and its degradation efficiency in pilot composting was only 56%. In addition, the amount of PCB detected during pilot composting decreased from 0.6 to 0.2 mg kg^{-1}dw.

Full scale compost 1 could be classified as mature (Table 3). After composting for more than one year, the CO_2 production in compost 1 was 0.7 CO_2-C g^{-1} VS d^{-1}, the nitrate-N-ammonium-N ratio was over 1, and the germination index was above 80%. According to the maturity parameters, CO_2 above 4 CO_2-C g^{-1} VS d^{-1} and a low nitrate-ammonium ratio of 0.02, full scale compost 2a was classified as raw. Compost sample 2b, also taken from the same compost pile but after one year of composting, showed matured properties in CO_2 production, but the nitrate-N-ammonium-N ratio was still below 1 and the conductivity was high. Compost 2b was classified as nearly mature. In addition to the maturation phase determination, the amount of organic contaminants in industrial-scale composts was analysed (Table 1). The overall level of organic contaminants in compost 1 was

lower than in samples collected from compost 2. Composts 2a and 2b clearly contained higher amounts of surfactants LAS and NP than compost 1. The DEHP content was at its highest in raw compost, compost 2a, at 38 kg^{-1}dw. Overall, the PAH content was relatively low, in compost 1 and compost 2b it was below the detection limit, and in compost 2a it was 1.2 mg kg^{-1}dw. There were also detectable concentrations of PCB (between 0.02 and 0.06 mg kg^{-1}dw) and PCDD/F (average 3.4 ng ITEQ kg^{-1}dw).

3.3 Toxicity evaluation by biotests

3.3.1 Kinetic luminescent bacteria test

According to the kinetic luminescent bacteria test, sewage sludge A was more acutely toxic than sewage sludge B (Table 4). The $EC50_{30s}$ in A and B were higher than 100 g L^{-1}. However, after a longer exposure time of 30 minutes, $EC50_{30min}$ value in A was 9 g L^{-1}, and in B between 50 and 100 g L^{-1}. With the additional DMSO extraction step, sewage sludge A proved toxic to the test organism even after the shorter exposure time of 30 s, with an $EC50_{30s}$ of 2.7 g L^{-1}. To avoid the toxic effect of DMSO on the test organisms *Vibrio fischeri,* the maximum sample concentration of the DMSO treated sewage sludge or compost samples could be 3 mg L^{-1}. With sewage sludge B, a concentration of 3 mg L^{-1} induced a 56% inhibition of light production after a 30s exposure time, and 85% after 30 minutes. The hexane extract was the most toxic of the tested extracts with both sludge samples. For sewage sludge A, the exposure time did not affect the EC50 value of the hexane extract, which was 1.7 g L^{-1}. In addition, the hexane extract of sewage sludge B was less toxic than sewage sludge A and demonstrated an $EC50_{30s}$ of 3.7 g L^{-1} and $EC50_{30min}$ of 2.9 g L^{-1}.

In pilot composting, acute toxicity had already disappeared after 26 days of composting (Figure 2). After 30 minutes of exposure time to compost, the light production of *Vibrio fischeri* was increased by 10% for 26 days composting, 14% for 55 days, 47% for 87 days, and 412% for 124 days. The inhibition of light production (30 minutes) in the DMSO treated samples decreased from 96% to 31% after 55 days of composting (Figure 6). In the sample taken at the end of the pilot composting, however, the DMSO extract inhibited light production by 94% after 30 minutes of exposure time.

Mature field-scale compost sample 1 did not cause any inhibition in the kinetic luminescent bacteria test (Figure 2a and 2b). Compost samples 2a and 2b were both toxic to the luminescent bacteria. Compost 2a induced inhibition in light production by 7% after 30 seconds, and 55% after 30

minutes exposure times (Figure 2a). DMSO treatment increased the toxicity of compost samples, especially after a shorter exposure time of 30 s. Compost sample 2b from the same composting plant, but sampled after a longer composting time than 2a, was more acutely toxic than compost 2a. After 30 minutes of exposure, light production was inhibited 82% and an additional DMSO treatment step even increased the inhibition to 99 %.

3.3.2 Genotoxicity by Vitotox

The genotoxicity of sewage sludges A and B, and composted sewage sludge samples, from the pilot composting test were studied with the Vitotox[TM] 10 Kit. Sewage sludge A was found to be genotoxic in the tested concentrations of 0.008- 1 g L^{-1}, that yields a geno/cyto ratio between 3.3 and 6.1 (Table 5). Sewage sludge B was not genotoxic in the applied test and the geno/cyto ratio was below 1.5. Composting reduced genotoxicity of sewage sludge A but a genotoxic response was still detectable after 124 days of composting (Table 5). In this more mature compost, the genotoxic concentrations were above 1.5 g L^{-1}.

3.3.3 Endocrine disrupters and dioxin-like activity by yeast cell bioreporter

Sludge samples A and B, tested with yeast cell bioreporter, both had relatively high oestrogenic activity. Sludge A had more than double the ER α activity (20 ng g^{-1}) compared with sludge B (8.5 ng g^{-1}), however, there was no difference between the ER β activity in these samples (Table 5). The androgenic activity in both of the studied sewage sludge samples was also considerably higher. In sewage sludge A, DHT eqv was 94 and in B 185 ng g^{-1}dw. The oestrogenic and androgenic activities were mostly eliminated during the composting process since no activity was detected from any of the compost samples tested (Table 5). The sludge samples contained large amounts of the AhR receptor ligands (Table 5). Dioxin-like activity was 15 BaPeqv µg g^{-1}dw in sludge A and 18 BaPeqv µg g^{-1}dw in sludge B. Again, the vast majority of the AhR binding activity was removed during composting, although a small fraction of the activity appeared to resist degradation. Due to the toxicity of the bark/peat mixture, the extracts for the yeast-based assay had to be diluted 200-300-fold prior to the tests. The bark/peat mixture alone was too toxic to be measured reliably.

4 Discussion

The source of wastewater, and the variation in industrial wastewater load to the municipal waste water treatment plant, influenced the contaminant concentrations and the ecotoxicity of the sewage sludges. A similar conclusion was drawn by Natal-da-Luz et al. (2009) when they studied the ecotoxicity of industrial and municipal sewage sludges amended with soil. The influence of higher amounts of treated industrial wastewater was mainly seen in our study as the elevated concentration of PAHs, PCB, and PCDD/F in the sewage sludge. The overall level of the organic pollutants in studied sewage sludges was in line with previously reported concentrations of organic pollutants in Finnish sewage sludges (Vikman et al. 2006). In Finland, the average amount of PAH in the sewage sludge is 4.6 mg kg^{-1}dw (Vikman et al. 2006), which is lower than the proposed limit value of 6 mg kg^{-1}dw in WD (2010). In this study, the limit value for PAHs or for benzo[a]pyrene proposed in WD (2010) was not exceeded. Similarly, Abad (2005) reported that the 6 mg kg^{-1} dw PAH concentration was exceeded only in 3 % of the studied sewage sludges. However, sewage sludges with high PAH concentrations, far over the limit value, have, however, also been reported (Pauslrud et al., 2000; Stevens et al., 2003). Even though the amount of PCB in sewage sludge has been decreasing and is generally very low (Gawlik and Bidoglio, 2006), we observed a high amount of PCB (1.4 mg kg^{-1}dw) in sewage sludge A. In our study, the amount of DEHP in sample B was slightly higher than the average amount of DEHP reported in sewage sludge that is below 70 mg kg^{-1}dw in Finland, Denmark, and Sweden (Marttinen et al. 2003; Jensen and Jepsen, 2005; Samsøe-Petersen, 2003). In contrast, very high DEHP concentrations have been detected in Spain, where DEHP concentrations over 3000 mg kg^{-1}dw have been measured (Abad et al. 2005).

Sewage sludge A was found to be more acutely toxic than sewage sludge B. In addition, sewage sludge A showed potential for being genotoxic. Oestrogenic activity, androgenic activity, and dioxin-like activity was detected in both studied sewage sludge samples. Surprisingly, sewage sludge B showed higher androgenic activity. The traditional luminescent bacteria test (ISO 11348) has previously been used in the assessment of sewage sludge toxicity (la Farré et al., 2001; Perez et al., 2001; Mantis et al., 2005; Fuentes et al., 2006), however, with this test it is difficult to take to account for how the colour of the sample affects the result. The advantage of the kinetic luminescent bacteria test used in our study is that the test is especially designed for measuring the toxicity of colourful samples as shown in our earlier studies (Kapanen et al., 2007; Kapanen et al., 2009). Some cases such as la Farré et al. (2001) and Perez et al. (2001) were able to correlate observed toxicity with the presence of PAH, PCB, NP, or NP carboxylates by using traditional

14

luminescent bacteria test. Even if we did not use the approach of fractionation to study the correlation of toxicity with specific organic pollutants, we could demonstrate that DMSO treatment increased the bioavailability and resulted in elevated toxic responses in the kinetic luminescent bacteria test with both studies' sewage sludge samples. Extraction with hexane further increased the toxic response while also affecting other factors, for example, PAHs and other more water insoluble substances could be detected. It is noteworthy that the acute toxicity test with luminescent bacteria, as such, does not give a complete picture of the toxicity of PAHs, since PAHs are not soluble in water or bioavailable to the test organism (Perez, 2001). Presence of heavy metals in sewage sludge also contributes to the level of toxicity. However, in Finland the overall level of heavy metals is reported to be relatively low (Rantanen et al. 2008). In this study the effects of heavy metals is not discussed.

The sensitivity of the Vitotox test, measuring genotoxicity, is based on a low nutrient level in the media of the test organism during the exposure time. The Vitotox test was therefore not easily applicable to nutrient-rich sewage sludge samples and sensitivity of the test might be compromised. However, we discovered that sewage sludge A showed genotoxic potential in the Vitotox test. Genotoxic substances benzo[a]pyrene and phenantrene, present in sewage sludge A, could have had an impact on the detected response while their genotoxicity is reported to be revealed by the Vitotox test (Westerink et al., 2009). Sewage sludge B, which contained a lower organic contaminant load, was not genotoxic. Klee et al. (2004) also observed genotoxicity and mutagenicity in industrial sewage sludge derived from wastewater treatment plant receiving influents containing pharmaceutical substances, chemical intermediates, and explosives, by a battery of *in vitro* biotests.

Many chemicals, such as NP, bisphenol-A, dibutylphtalate (DBP) and DEHP found in sewage sludge have endocrine-disrupting properties (Soares et al. 2008; Harrison, et al., 2006; Fromme et al., 2002). When we detected the endocrine disrupting potential using yeast-cell bioreporter, sewage sludge B clearly caused a more substantial activation of ER β (versus ER α) in comparison to sewage sludge A, although the ligand range of ER α and ER β has been shown to be quite similar (Kuiper et al. 1997). Some plant-derived nonsteroidal compounds such as genistein and coumestrol were shown to have a significantly higher affinity for ER ß however, which raises the question of the presence of those (or structurally similar) compounds in sample B. Compared to our study, both lower and higher oestrogenic activities in sewage sludge have been reported by Murk et al. (2002) using ER-binding-, YES -, and ER-CALUX assays. In their study, a yeast oestrogen screen showed oestrogenic potencies below 3.55 ng g^{-1}dw. We also used yeast bioreporter for the detection of the

dioxin-like activity in sewage sludge. Observed dioxin –like activities in sewage sludges A and B were less than 20 µg g^{-1}dw. Similar and higher results were reported by Engwall et al. (1999) using EROD induction in cultured chicken embryo liver to recognize the dioxin-type compounds in sludge: bio-TEQ in the studied Swedish sludge extracts ranged from 6 to 109 pg g^{-1}dw.

When sewage sludge A was composted, the acute toxicity, genotoxicity, and endocrine disruption potential were reduced. Sorption of organic contaminants on organic matter in compost may reduce bioavailability and thus decreases toxicity of organic contaminants. On the other hand, during composting degradation of biodegradable organic contaminants is efficient. We demonstrated that pilot scale composting of sewage sludge A reduced the amount of LAS, NPE, DEHP, and PAH efficiently. Similar results on reduction of organic contaminants by composting sewage sludge have been reported by Poulsen and Bester (2010), Cheng et al. (2008), Oleszczuk (2007), Jensen and Jepsen (2005), and Moeller and Reeh (2003). There is evidence that microbes are able to biodegrade most of the surfactants, such as LAS and NP, after being released to the environment (Ying, 2005). In our pilot composting experiment, the degradation of LAS was very efficient (92%) and NPE also degraded by more than 60% during 124 days of composting. Similarly, Amundsen et al. (2001) reported that composting decreased concentrations of LAS by 75% and NPE by 50%. Furthermore, Moeller and Reeh (2003) found that the amount of LAS decreased from 150 mg kg-1 to below 5 mg kg^{-1}dw after 18 days of composting. Correspondingly, at the end of our pilot composting, the LAS concentration had decreased from 647 mg kg^{-1}dw, to below 50 mg kg^{-1}dw. In addition to the pilot-scale composting experiment, slightly higher LAS concentrations were found in mature industrial-scale composts 1 (50 mg kg^{-1}dw) and 2b (310 mg kg^{-1}dw). The NP level was also higher in the 2b compost sample than in the pilot-scale experiment. PAHs and DEHP have a high affinity to organic matter but still degrade during the composting process (Jensen and Jepsen, 2005). Moeller and Reeh (2003) reported that the PAH concentration was already 70% lower after 25 days of composting. Accordingly, the degradation of PAHs was very efficient in our study and 84% of PAHs was degraded during pilot composting. In addition to the biodegradability of the compound, degradation is dependent on the testing conditions as well as the duration of the composting: with a longer composting period higher degradation rates can be achieved. For example, the degradation rate of DEHP is affected by the type of the sludge, the DEHP concentration, and the temperature during composting (Amir et al. 2005). Moeller and Reeh (2003) observed a 69% decrease in DEHP at a composting temperature of 55 °C, and more efficient degradation, a 91% reduction in DEHP, was detected at 65 °C during a 25-days composting experiment. In our experiment, with a natural composting temperature gradient, with the highest

16

temperature of 65 °C during 124 days of composting, degradation of DEHP in pilot composting was only 56%. Similar degradation levels, from 34% to 64%, for phthalates in the composting process have been reported by Amundsen et al. (2001) and Marttinen et al. (2004). Poulsen and Bester (2010) described a 93% removal of DEHP during full scale composting. Abad, et al. (2005) studied a total of 30 compost samples between 2001 and 2003, and the median for the studied DEHP concentration in composted sewage sludge was 14.5 mg kg-1 dw, a concentration very similar to that in our study. Surprisingly, both the PAH and DEHP concentrations were similar or even lower in mature industrial-scale compost than at the end of our pilot-scale composting. Of the substances studied, PCB binds tightly to organic matter and is very persistent in the environment. PCDD/Fs are also very persistent, enriched into the sludge, and do not degrade during the composting process (Amlinger, 2004). Abad et al. (2005) reported PCDD/F concentrations in composted sludge from 7.7 to 78.3 ng kg I-TEQ (Median 14.9). In the samples derived from industrial composting plants 1 and 2, the PCDD/F concentrations were lower, namely 3.2 (plant 1) and 3.6 (plant 2) ng kg I-TEQ.

The acute toxicity, genotoxicity, and endocrine disruption potential were reduced substantially already after 26 days of composting. The pilot compost (A) sample was not acutely toxic in the kinetic luminescent bacteria test: on the contrary, when sewage sludge composted for 124 days was tested, it activated light production strongly. This type of response to mature compost, by luminescent bacteria, has been reported previously by Itävaara et al. (2002b). Lopez et al. (2010) also reported that after composting of sewage sludge, no toxicity could be detected with the kinetic luminescent bacteria test when water was used as an extractant. In the DMSO-extracted samples, we observed that the decrease in acute toxicity was slower, but after 55 days the inhibition in light production after 30 minutes exposure time was reduced to 31%. It is not clear what induces the sudden increase in toxicity after 124 days in the DMSO extracted compost sample. Perhaps some degradation products, humic substances, or increased bioavailability of remaining contaminants extractable with DMSO are responsible for this effect. The acute toxicity of the samples collected from industrial-scale composting plants ranged from non-toxic to toxic. Mature compost 1 was not acutely toxic, while both the raw compost 2a and more mature compost 2b were acutely toxic in the luminescent bacteria test, which has previously been found to give a toxic response when immature compost is tested (Itävaara et al. 2002b). When compost samples are tested, the stage of maturity of the compost sample should therefore always be addressed.

The Vitotox tests performed here have also confirmed that the genotoxicity of sewage sludge could be reduced with composting. After 124 days of composting, the concentration required to induce a

genotoxic response in the Vitotox test was over 1 g L^{-1}. To our knowledge, the Vitotox test has not been applied previously in the genotoxicity assessment of compost samples. However, Klee et al. (2004) used the comet assay to illustrate that aerobic treatment reduced the genotoxicity of sludge, whereas anaerobic treatment was not so effective in removing the genotoxicity.

It was especially interesting to study the endocrine-disruptive activity of the sewage sludge during composting, given that similar data on this type of measurement are scarce. When Motoyama et al. (2006) screened 21 compost samples for oestrogenic and androgenic compounds with a yeast two-hybrid assay, they detected oestrogenic activity in over a half of the studied samples and androgenic activity was detected in two of the composts. Even if we could detect the ER α, ER β, AR, and AhR activities with the yeast cell bioreporter in the starting material of pilot composting, sewage sludge A, the activity of the studied endocrine disrupters in composting was already under the detection limit after 26 days of composting. In addition, the dioxin-like activity disappeared during composting; thus, these endocrine reporters were overall very sensitive to composting treatment by this measure. These results are in line with a previous study by Hernandez-Raquet et al. (2007), who applied an oestrogen-responsive reporter cell line (MELN bioassay) for detecting the reduction in oestrogenic activity of NP-spiked sewage sludge after anaerobic and aerobic treatment. In their study, anaerobic treatment did not affect the oestrogenic activity but only 10% of the original activity was left after aerobic treatment of sewage sludge. Therefore, the yeast bioreporter that we have tested here gave valuable results on the fate of endocrine disruptive substances in compost. We did face some limitations regarding the applicability of this biotest, however. To our surprise, the bark/peat mixture used in the composting process was toxic to the yeast *S. cerevisiae*, which forced us to dilute the compost samples significantly. This probably diluted the part of the endocrine disrupter activity that may hypothetically have remained present in the samples, but below to the limit of detection. This is illustrated in the biotesting of the compost samples, since the natural sample itself, with no additives of industrial toxic chemicals, can complicate ecotoxicity testing.

5 Conclusions

Due to mixed contamination of sewage sludge with potentially hundreds of different substances, environmental quality assessment of sewage sludge or composted sewage sludge is challenging and cannot be achieved with chemical analysis alone. Ecotoxicity assessment provides valuable information on the environmental fate of these materials. However, the suitability of applied

biotests in the assessment of sewage sludge and compost materials should be addressed. We observed that the amount of industrial wastewaters treated at the WWTP, led to elevated concentrations of PAHs, PCB, and PCDD/F in the sewage sludge produced. Furthermore, this was detected as acute toxicity, genotoxicity, and elevated endocrine disrupter and dioxin-like activities. Composting reduced efficiently of the amount of LAS, NPE, PAHs, and DEHP in sewage sludge resulting in decrease of acute toxicity, genotoxicity, and endocrine-disrupter potential. Biotests proved to be a good tool when the quality of the sewage sludge or composted sewage sludge, and the potential risks of their use as fertilizer in agriculture or landscaping applications, was evaluated. It is challenging, however, to try to take into account how the natural characteristics of the tested material, such as sewage sludge and compost, affect the test organism. For example, the maturity of the compost samples affected the responses of the biotests, and it is recommended that the stage of maturity is determined when the ecotoxicity of compost is evaluated. Of the tests applied, the kinetic luminescent bacteria test was well suited for screening of the acute toxicity of sewage sludge and composted sewage sludge, if the stage of the compost maturity was acknowledged. Not all of the applied tests were ideal for testing the toxicity of sewage sludge and compost materials, however. Several challenges are posed in the application of this genotoxicity assay and yeast bioreporter, including the high amount of nutrients and bark/peat present in compost samples, which can affect specific measurements in these tests.

6 Acknowledgements

7 References

1. Abad, E., Martínez, K., Planas, C., Placios, O., Caixach, J. Rivera, J., 2005. Priority organic pollutant assessment of sludges for agricultural purposes. Chemosphere 61, 1358-1369.

2. Alvarenga, P., Palma, P., Gonçalves A.P., Fernandes R.M., Cunha-Queda A.C., Duarte E., Vallini G. 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33, 505–513.

3. Amin, A.W. 2011. Evaluation of the Genotoxicity of Residual Repeated Applications of Sewage Sludge on M2 Meiocytes of Zea Plants. Res. J. Environ. Toxicol. 5: 235-250.

4. Amir S., Hafidi M., Merlina G., Hamdi H., Jouraiphy A., El Gharous M., Revel J.C., 2005. Fate of phthalic acid esters during composting of both lagooning and activated sludge. Process Biochem. 40, 2184-2190.

5. Amlinger, F., Pollack, M., Favoino, E., 2004. Heavy metals and organic compounds from waste used as organic fertilizers Ref.Nr.: Tend/AML/2001/07/20, NV.A.2./ETU/2001/0024, Summary Report.(access date 26.06.11)

6. Amundsen, C.E., Eggen, T., Lystad, H., 2001. Stabilitet og nedbrytning av organiske forurensninger i avløpsslam ved storskala kompostering – Resultater fra rankekompostering ved Lindum Ressurs og Gjenvinning. TemaNord, 588.

7. Andersen, A., 2001. Disposal and recycling routes for sewage sludge – Part 3 – Scientific and technical sub-component report, 23 October 2001. European commission DG Environment – B/2. (access date 23.09.10)

8. Brändli, R., Kupper, T., Bucheli, T., Mayer, J., Stadelmann F.X., Tarradellas, J., 2004. Occurrence and relevance of organic pollutants in compost, digestate and organic residues – Literature review. Final report, p.193. (access date 06.03.06)

9. Cheng, H-S, Kumar, M., Lin, J-G. 2008. Degradation kinetics of di-(2-ethylhexyl) phthalate (DEHP) and organic matter in sewage sludge during composting. J. Hazard. Mater. 154, 55-62.

10. Cincinelli, A., Martellini, T., Misuri, L., Lanciotti, E., Sweetmen, A., Laschi, S., Palchetti, I. 2012. PBDEs in Italian sewage sludge and environmental risk of using sewage sludge for land applications. Environ. Poll. 161, 229-243.

11. Domene, X., Solà, L., Ramírez, W., Alcañiz, J. M., Andrés, P. 2011. Soil bioassays as tools for sludge compost quality assessment. Waste Manag. 31, 12-522.

12. EC, 2008. Environmental, economic and social impacts of the use of sewage sludge on land. Milieu LTD, WRc and RPA for the European commission, DG Environment under the study contract DG ENV.G.4/ETU/2008/0076r. (access date 10.06.10)

13. Engwall, M., Brunström, B., Näf, C. Hjelm, K., 1999. Levels of dioxin-like compounds in sewage sludge determined with a bioassay based on erod induction in chicken embryo liver cultures. Chemosphere, 38, 2327-2343.

14. Erhardt, W.,Pruess, A., 2001. Organic contaminants in sewage sludge for agricultural use. Report prepared by UMEG Center for Environmental Measurements Environment Inventories and product safety, Germany for the European Commission, Joint Research

Centre, Institute for Environment and Sustainability, Soil and Waste Unit. (access date 26.06.11)

15. Eriksson, E. Christenssen, N., Schmidth, J.E., Ledin, A., 2008. Potential priority pollutants in sewage sludge. Desalination 226, 371-388.

16. Farré, M., Barceló, D., 2003. Toxicity of wastewater and sewage sludge by biosensors, bioassays and chemical analysis. Trends in Analyt. Chem. 22, 299-310.

17. la Farré, M., García, M-J, Castillo, M., Riu, J., Barceló, D., 2001. Identification of surfactant degradation products as toxic organics compounds present in sewage sludge. J. Environ. Monit. 3, 232-237.

18. Fromme H, Küchler T, Otto T, Pilz K, Müller J, Wenzel A., 2002. Occurrence of phthalates and bisphenol A and F in the environment. Water Res. 36, 1429-1438.

19. Fuentes, A., Lloréns, M., Saéz, J., Aguilar, M. I., Pérez-Marín, A.B., Ortuño, J.F., Meseguer, V.F. 2006. Ecotoxicity, phytotoxicity and extractability of heavy metals from different stabilised sewage sludges. Environ. Pollut. 143, 355-360.

20. Gawlik BM, Bidoglio G., 2006. Background values in European soils and sewage sludge. PART I Leschber R. Evaluation of the relevance of micro-pollutants in sewage sludge, PART III Gawlik BM, Bidoglio G. Conclusions, comments and recommendations. EUR 22265 EN –DG Joint Research Centre, Institute for the Environment and Sustainability. (access date 19.12.06)

21. Hakkila, K., Green, T., Leskinen, P, Ivask, A., Marks, R.T., Virta, M., 2004. Detection of bioavailable heavy metals from Eilatox -Oregon samples by utilization of whole-cell luminescent bacterial sensors and fiber-optic biosensors. J. Appl. Toxicol. 24, 333-342.

22. Hale, R.C., La Guardia, M.J., Harvey, E., Gaylor, M.O., Mainor, T.M., 2006. Brominated flame retardant concentrations and trends in abiotic media. Chemosphere 64, 181-186.

23. Harrison, E.Z., Oakes, S.R., Hysell, M., Hay, A., 2006. Organic chemicals in sewage sludge. Sci. Total Environ. 367, 481-497.

24. Hamdi H., Manusadžianas L., Aoyama, I., Jedidi, N., 2006. Effects of anthracene, pyrene and benzo[a]pyrene spiking and sewage sludge compost amendment on soil ecotoxicity during a bioremediation process. Chemosphere 65, 1153-1162.

25. Hernandez-Raquet G., Soef A, Delgene`s N, Balaguer P., 2007. Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. Water Res. 41, 2643-2651.

26. Itävaara, M., Karjomaa, S., Selin, J-F., 2002a. Biodegradation of polylactide in aerobic and anaerobic thermophilic conditions. Chemosphere 46, 879-883.

27. Itävaara M, Venelampi O, Vikman M, Kapanen A., 2002b. Compost maturity – problems associated with testing, in: Insam, H., Riddech, N., Klammer, S., (Eds.), Microbiology of Composting, Springer-Verlag, Berlin, Heidelberg, pp. 373-382.

28. Itävaara M, Vikman M, Kapanen A, Venelampi O, Vuorinen A., 2006. Compost maturity – Method book (in Finnish). VTT – Research notes 2351 p. 38.

29. Itävaara M, Vikman M, Maunuksela L, Vuorinen A., 2010. Maturity tests for composts - verification of a test scheme for assessing maturity. Compost Sci. Util. 18, 174 – 183.

30. Jensen, J., Jepsen, S-E., 2005. The production, use and quality of sewage sludge in Denmark. Waste Manag. 25, 239-247.

31. Kapanen, A., Itävaara, M. 2001. Ecotoxicity tests for compost applications. Ecotoxicol. Environ. Safe. 49, 1–16.

32. Kapanen, A., Stephen, J.R., Brüggeman, J., Kiviranta, A., White, D.C., Itävaara, M. 2007. Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community". Chemosphere, 67 (11): 2201-2209.

33. Kapanen, A., Priha, O., Maunuksela, L., Itävaara, M. 2009. Biotestien kehittäminen lannoitevalmisteiden valvontaan; lietteiden hyötykäytön edistäminen (Bioliete) 4543/501/2006. Tutkimusraportti 2007-2008. VTT-R-02284-09 p. 51.

34. Klee, N., Gustavsson , L., Kosmehl ,T., Engwall, M., Erdinger, L., Braunbeck, T., Hollert, H. 2004. Changes in toxicity and genotoxicity of industrial sewage sludge samples containing nitro- and amino-aromatic compounds following treatment in bioreactors with different oxygen regimes. Environ. Sci. Pollut. Res. 11, 313 – 320.

35. Koch, M. Knoth, W., Rotard W., 2001. Source identification of PCDD/Fs in a sewage treatment plant of a German village. Chemosphere, 43: 737-741.

36. Kuiper, G.J.M., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J-Å. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors a and b. Endocrinology 138, 863-870.

37. Laturnus F, von Arnold K, Grøn C, 2007. Organic contaminants from sewage sludge applied to agricultural soils. Env. Sci. Pollut. Res. 14, Special Issue 1, 53–60.

38. Leskinen, P., Michelini, E., Picard, D., Karp, M.,Virta, M., 2005. Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices. Chemosphere 61, 259-266.

39. Leskinen, P., Hilscherova, K., Sidlova, T., Pessala, P., Salo, S., Verta, M., Kiviranta, H. Virta, M. 2008. Detecting AhR ligands in sediments using bioluminescent reporter yeast. Biosens.Bioelectron.23, 1850-1855.

40. Leusch, F.D.L., Chapman, H.F., van den Heuvel, M. R. Tan, B.T.T., Gooneratne, S.R., Tremblay, L.A., 2006. Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand. Ecotoxicol. Environ. Saf. 65, 403-411.

41. Lopez, M.J., Vargas-García, M.C., Suárez-Estrella, F., Guisado, G., Moreno, J. 2010. Flash microbial toxicity test as monitoring parameter at composting: comparison of ecotoxicity level for different substrates. Proceedings in 14th Ramiran International Conference, Lisboa, Portugal.

42. Mantis, I, Voutsa, D., Samara, C., 2005. Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods. Ecotoxicol. Environ.Saf. 62, 397-407.

43. Marttinen, S.K., Kettunen, R.H., Rintala, J.A., 2003. Occurrence and removal of organic pollutants in sewages and landfill leachates. Sci. Total Environ. 301, 1-12.

44. Marttinen, S., Hänninen, K., Rintala, J., 2004. Removal of DEHP in composting and aeration of sewage sludge: Chemosphere 54, 262-272.

45. Michelini, E., Leskinen, P., Virta, M., Karp, M., Roda, A., 2005. A new recombinant cell-based bioluminescent assay for sensitive androgen-like compound detection. Biosens. Bioelectron. 20, 2261-2267.

46. Moeller J, Reeh U., 2003. Degradation of DEHP, PAHs and LAS in source separated MSW and sewages sludge during composting. Compost Sci. Util. 11, 370-378.

47. Müller, G., 2003. Sense or no-sense of the sum parameters for water soluble ″adsorbable halogens" (AOX) and "absorbed organic halogens"(AOX-S18) for the assessment of organohalogens in sludges and sediments. Chemosphere 52, 371-379.

48. Murk A, Legler J, van Lipzig M, Meerman J, Belfroid A, Spenkelink A, van der Burg B, Rijs G, Vethaak D., 2002. Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environ. Toxicol. Chem.21, 16-23.

49. Myamoto, M., Toyokazu, K., Nomiya, K., Nakagawa, S., Matsuo, H., Shinohara, R. 2006. The assessment of the estrogen and androgen in the compost produced from the sewage sludge and solid waste of livestock. J. Environ. Chem. 16, 615-626.

50. Natal-da-luz, T., Tidona, S., Van Gestel, C.A.M. Morais, P.V., Sousa , J.P. 2009. The use of Collembola avoidance tests to characterize sewage sludges as soil amendments. Chemosphere 77, 1526-1533.

51. OECD, (2001) Detailed review paper. Appraisal of test methods for sex hormone disrupting chemicals. Series on testing and assessment no 21. Organisation for economic Cooperation and Development, Paris.

52. Oleszczuk, P. 2007. Changes of polycyclic aromatic hydrocarbons during composting of sewage sludges with chosen physico-chemical properties and PAHs content, Chemosphere 67, 582–591.

53. Oleszczuk, P., 2010. Toxicity of light soil fertilized by sewage sludge or compost in relation to PAH content. Water Air Soil Pollut. 210, 347-356.

54. Poulsen, T.G., Berster, K. 2010. Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. Environ. Sci. Technol. 44, 5086-5091.

55. Paulsrud, B.A, Wien, A., Nedland, K.T., 2000. A survey of toxic organics in Norwegian sewage sludge, compost and manure. Report. Aquatem, Norwegian Water technology Centre AS, Rodeløkka, Oslo, Norway. (access date 13.03.06)

56. Pérez, S. Farré, M., García, M.J., Barceló, D., 2001. Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contribution to its toxicity in the ToxAlert® 100 bioassay. Chemosphere 45, 705-712.

57. Pérez, S., Reifferscheid, G., Eichhorn, P., Barceló, D.2003. Assessment of the mutagenic potency of sewage sludges contaminated with polycyclic aromatic hydrocarbons by an Ames fluctuation assay. Environ. Toxicol. Chem. 22, 2576–2584.

58. Ramírez, W.A., Domene, X., Ortiz, O., Alcañiz, J.M., 2008. Toxic effects of digested, composted and thermally-dried sewage sludge on three plants. Bioresource Technol. 99, 7168-7175.

59. Rantanen P. Valve M Kangas A. 2008. Disposal of sludge – preliminary survey (In Finnish) Suomen Ympäristökeskuksen raportteja I, p. 80.

60. Renoux, A.Y., Tyagi, R.D., Samson, R. 2001. Assessment of toxicity reduction after metal removal in bioleached sewage sludge. Water Res. 35, 1415-1424.

61. Saharinen, M., Vuorinen, A., 1998. Aerobisesti prosessoidun orgaanisen jätteen laatu: Analyysimenetelmiä (in Finnish). Matemaattis-luonnotieteellisen tiedekunnan raporttisarja nro 38. Joensuun Yliopisto, 50 s.

62. Saadi, I., Laor, Y., Raviv, M. and Medina, S., 2007. Land spreading of olive mill wastewater: Effects on soil microbial activity and potential phytotoxicity. Chemosphere 66, 75-83.

63. Samsøe-Petersen, L. 2003. Organic contaminants in sewage sludge. Review of studies regarding occurrence and risks in relation to the application of sewage sludge to agricultural soils. Swedish Environmental Protection Agency 5217, pp. 66.

64. Schowanek, D., Carr, R., David, H., Douben, P., Hall, J.,Kirchmann, H., Patria, L., Sequi, P., Smith, S., Webb, S., 2004. A risk-based methodology for deriving quality standards for organic contaminants in sewage sludge for use in agriculture - Conceptual Framework. Regul. Toxicol.Pharmacol. 40, 227-251.

65. Soares, A., Guieysse, B, Jefferson, B., Cartmell, E. and Lester, J.N. 2008. Nonylphenol in the environment; A critical review on occurrence, fate, toxicity and treatment in waste water. Environ. Int. 34, 1033-1049.

66. Stamatelatou, K., Pakou, C., Lyberator, G. 2011. Occurrence, toxicity, and biodegradation of selected emerging priority pollutants in municipal sewage sludge. In Comprehensive Biotechnology Eds. M-Y Murray, 473-484.

67. Stevens J.L, Northcott G.L, Stern G.A., Tomy G.T.,Jones, K.C., 2003. PAHs PCBs, PCNs, organochlorinepesticides, synthetic musks, and polychlorinated n-alkanes in UK-sewage sludge: Survey results and implications. Environ. Sci. Technol. 37, 462-467.

68. Stevens, J., Green, N.J., Jones, K.C., 2001. Survey of PCDD/Fs and non-ortho PCBs in UK sewage sludges. Chemosphere 44, 1455-62.

69. Suzuki, G., Takigami, H., Kushi, Y., Sakai, S., 2004. Evaluation of mixture effects in a crude extract of compost using the CALUX bioassay and HPLC fractionation. Environ. Int. 30, 1055-66.

70. Suzuki, G., Takigami, H., Kushi, Y., Sakai, S., 2006. Time-course changes of mixture effects on AhR binding-dependent luciferase activity in a crude extract from a compost sample. Toxicol.Lett. 161, 174-87.

71. Svenson, A., Allard, A.S., 2004. Occurrence and some properties of the androgenic activity in municipal sewage effluents. J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng. 39, 693-701.

72. van der Lelie, D., Regniers, L., Borremans, B., Provoost, A.,Verschaeve, L., 1997. The VITOTOXR test, an SOS bioluminescence Salmonella typhimurium test to measure genotoxicity kinetics. Mutat. Res. 389, 279-290.

73. WD, 2000. Working document on sludge 3rd draft, 27th April, Brussels. (access date 08.09.05)

74. WD, 2010. Working document on sludge and biowaste. 21st September, Brussels. (access date 11.01.11)

75. Verschaeve, L., Van Gompel, J., Thilemans, L.,Regniers, L., Vanparys, P., van der Lelie, D., 1999. The VITOTOX (R) genotoxicity and toxicity test for the rapid screening of chemicals. Environ. Mol. Mutagen. 33, 240-248.

76. Westerink W.M.A., Stevensona J.C.R., Lauwersb, A., Griffioenb, G., Horbacha G. J.,. Schoonena W.G.E.J. 2009. Evaluation of the VitotoxTM and RadarScreen assays for the rapid assessment of genotoxicity in the early research phase of drug development. Mutat. Res. 676, 113-130.

77. Vikman, M., Kapanen, A., Itävaara, M. 2006. Orgaaniset haitta-aineet jätevesilietteissä. Vesitalous 3: 7-10.
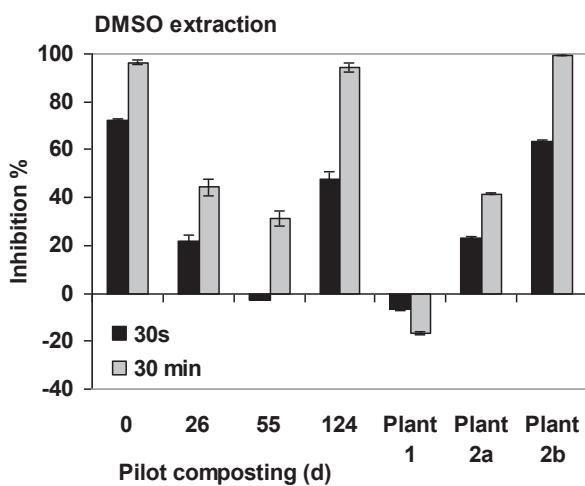
78. de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere, 46, 583-624.

79. Ying, G-G., 2005. Fate, behaviour and effects of surfactants and their degradation products in the environment. Environ. Int. 32, 417-431.
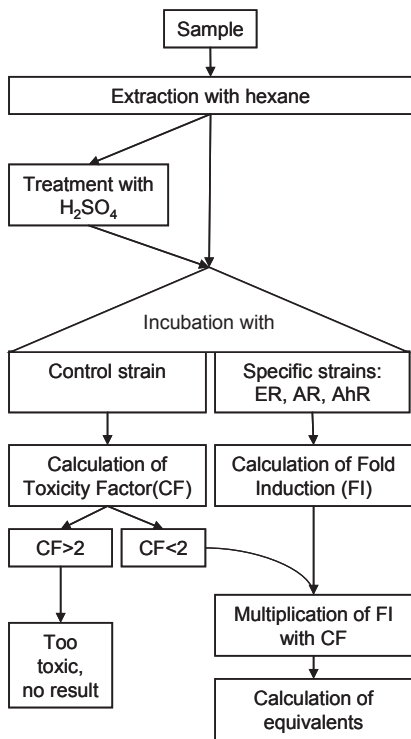
Figure 1. Test scheme for the yeast cell biosensor (CF=correction factor).
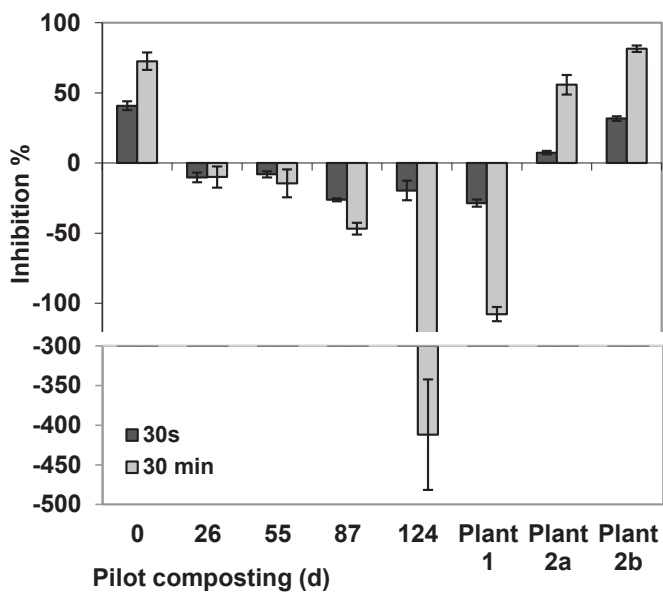
a)

b)

Figure 2. a) Toxicity measured as inhibition (%) after 30s and 30 min exposure time in light production in a kinetic luminescent bacteria test (ISO 21338) of composted sewage sludge from pilot composting test and field-scale composts 1 (mature), 2a (raw) and 2b (in maturation). Studied concentrations were 100 g L-1 in test conducted according to the ISO 21338 and b) 3.3 g L-1in sample extracted with DMSO.

Table 1. Amount of organic contaminants in sewage sludge samples A and B, in compost samples from pilot composting with sewage sludge A and in compost samples from two field scale composting plants (1 and 2). Degradation % of organic contaminants after 26 days and 124 days of composting. Suggested limit values for organic contaminants in the EU in sewage sludge used as soil improvers (WD 2000 and 2010).

mg kg^{-1}dw (Degradation %)	A	B	Pilot composting		Field scale composting			WD 2000	WD 2010
			26 d	124 d	1	2a	2b		
AOX	260	170	nd	nd	75	80	nd	500	-
LAS	1500	1300	110 (83 %)	<50 (92 %)	50	240	310	2600	-
DEPH	57	110	20 (20 %)	11 (56 %)	2	38	16	100	-
NPE	15.2	8.9	2.0 (69 %)	2.6 (61 %)	<0.6	26.0	47	50	-
of which NP	13.3	7.8	2.0 (66%)	2.2 (62%)	0.2	23	46		
PAH-16[a]	8.0	0.5	2.3	0.6	< 0.5	1.2	<0.3	6	6
PAH-9[b]	5.8	0.3	1.8 (28%)	0.4 (84%)	< 0.3	0.9	<0.3		(2)[e]
PCB[c]	1.4	0.04	0.7 (-17%)	0.2 (67%)	0.02	0.06	0.06	0.8	-
PCDD/F [d](ng I-TEQ kg^{-1}dw)	3.8	0.3	nd	nd	3.2	3.6	nd	100	-

[a]EPA-PAH-16: Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, bentzo[a]antracene, chrysene/trifenyyli?, benzo[bjk]fluoranthene, benzo[a]pyrene, indeno[1,2-cd]pyrene, dibenzo[ah]antracene, benzo[ghi]perylene
[b]PAH-9: calculated according to WD (2000)
[c]PCB-7; PCB 28,PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180
[d]PCDD/F;2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8, 9-HxCDD; 1,2,3,4,6,7,8-HpCDD; 1,2,3,4,6,7,8,9-OCDD, 2,3,7,8-TCDF; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 1,2,3,4,6,7,8.HpCDF; 1,2,3,4,7,8,9-HpCDF; 1,2,3,4,6,7,8,9-OCDF
[e]orbenzo[a]pyrene

Table 2. Descriptions of the wastewater treatment plants involved in the study.

Sample	Amount of sludge m^3/a (2002)	Share of industrial wastewaters (2002)	Anaerobic digestion	TS% (2002)
A	57 690	17%	Yes	31
B	18 830	8%	Yes	30

Table 3. Maturation characteristic of field-scale and pilot-scale composts.

	Compost 1	Compost 2a	Compost 2b	Pilot composting (A)	
Age	1-2 years	3 months	1 year	26 days	124 days
Dry weight %	37	36	32	35	35
VS %	58	67	61	76	69
pH	6.1	6.5	6.3	6.8	4.2
Conductivity (mSm^{-1})	18	84	104	75	114
CO_2 production mg CO_2-C g^{-1} VS d^{-1}	0.7	4.1	1.9	3.8	0.6
NO_3-N/NH_4-N	1.1	0.02	0.1	-	0.4
Germination index	127	92	nd	32	40
Maturity	Mature	Raw	In maturation	Raw	In maturation

Table 4. Sewage sludge ecotoxicity evaluated by luminescent bacteria test (ISO 21338).

Method	Sewage sludge A			Sewage sludge B		
Acute toxicity by luminescent bacteria	ISO 21338	+ DMSO extraction	+ hexane extraction	ISO 21338	+ DMSO extraction	+ hexane extraction
$EC50_{30s}$ g L^{-1}	> 100	2.7	1.7	> 100	$(56\%)^b$	3.7
$EC50_{30min}$ g L^{-1}	9	2.3	1.7	$50>^a<100$	$(85\%)^b$	2.9

[a]The EC50 value lies between 50 and 100 g L^{-1}

[b]Inhibition percentages with tested maximum concentration 3 mg L^{-1}

Table 5. Genotoxicity and amount of endocrine disrupters in sewage sludge and pilot compost samples after 26 and 124 days of composting.

Method			Sewage sludge A	Sewage sludge B	Pilot compost 26 days	Pilot compost 124 days
Genotoxicity by Vitotox						
[c] g L^{-1} (+S9)			0.008-1	not genotoxic	0.08-0.3	1.25-5
Geno/ cyto ratio (+S9)			3.3-6.1	< 1.5	1.54-2.12	1.39-2.12
Yeast cell assay						
17- β-estradioleqv	ER	α	20±0.4	9±0.6	<0.01	<0.01
(ngg^{-1}dw)	ER β		21±6.5	23±3.2	<0.07	<0.06
DHT eqv (ngg^{-1}dw)	AR		92±15	185±15	<0.3	<0.1
BaPeqv (µg g^{-1} dw)	AhR		38±11	75±1.1	<LOD[a]	22±1.5
+ sulphuric acid extraction	AhR		15±0.4	18±0.7	<LOD[a]	<LOD[1]

[a]<LOD = below limit of detection. The LODs for ER α, ER β, AR, and AhR were 2.3 ng g^{-1}, 14 ng g^{-1}, 60 ng g^{-1} and 12 µg g^{-1} of the respective equivalents.

Title	**Ecotoxicity assessment of biodegradable plastics and sewage sludge in compost and in soil**
Author(s)	Anu Kapanen
Abstract	Biodegradable plastics, either natural or synthetic polymers, can be made from renewable or petrochemical raw materials. The most common applications for biodegradable plastics are packaging materials and waste collection bags. The one thing in common for all biodegradable items is that at the end of their life cycle they should degrade into harmless end products, during a specified time frame. Depending on the target application, the degradation may take place in soil, in water, in an anaerobic digestion plant or in compost. In addition to its use as a waste treatment process for biodegradable plastics, composting can be used in the removal of organic contaminants from sewage sludge. In this study the biodegradation properties of bioplastics targeted for agricultural and compost applications were investigated. In addition, the potential of biotests were evaluated in ecotoxicity assessment of biodegradable plastics and their components during the biodegradation process in vermiculite, compost and soil. During the biodegradation of chain-linked lactic acid polymers and polyurethane-based plastic material in controlled composting conditions the release of toxic degradation products could be demonstrated by biotests. Clear toxic responses in the luminescent bacteria test and/or plant growth test (OECD 208) were observed. The fate of an endocrine disrupting plasticizer, diethyl phthalate (DEP) was also studied in a controlled composting test, in pilot composting scale and in plant growth media. A high concentration of DEP induced changes in the microbial community, gave a clear response in the biotest and its degradation was inhibited. However, in pilot scale composting toxicity was not detected and the degradation of DEP was efficient. The studied starch-based biodegradable mulching films showed good product performance, good crop quality and high yield in protected strawberry cultivation. Furthermore, no negative effects on the soil environment, Enchytraeidae reproduction (ISO 16387) or amoA gene diversity were detected. If sewage sludge is used as a soil conditioner, many harmful substances can potentially end up in the environment. In our study, composting reduced efficiently the amount of organic contaminants such as DEHP, PAH, LAS, and NPs in sewage sludge. In addition, composting resulted in reduction in acute toxicity, genotoxicity and endocrine-disruption potential of the sewage sludge. The use of biotests is recommended as an indicator of potential risk when sewage sludge-based products are used in agricultural or landscaping applications. Potentials and also limitations were recognized in the performances of different biotests when studying the ecotoxicity of biodegradable materials during biodegradation processes in compost and in soil environment. Soil, compost and sludge as testing environments do set limitations for the use of biotests. Colour, amounts of nutrients, additional carbon sources, presence of bark and peat, compost immaturity, and high microbial activity are some of the factors limiting the use of biotests or complicate interpretation of the results.
ISBN, ISSN	
Date	November 2012
Language	English, Finnish abstract
Pages	92 p. + app. 82 p.
Keywords	Biodegradable plastic, sewage sludge, biotest, ecotoxicity, acute toxicity, phytotoxicity, bioreporter, organic contaminant, soil, compost, biodegradation
Publisher	

Nimeke	**Biohajoavien muovien sekä jätevesilietteen ympäristömyrkyllisyyden arviointi kompostissa ja maaperässä**
Tekijä(t)	Anu Kapanen
Tiivistelmä	Biohajoavat muovit voivat koostua luonnonpolymeeristä tai synteettisistä polymeereistä, joita valmistetaan joko uudistuvista tai uusiutumattomista raaka-aineista kuten petrokemikaaleista. Biohajoavia muoveja voidaan käyttää raaka-aineena hyvin erilaisissa sovelluksissa. Yleisimpiä sovelluksia ovat pakkausmateriaalit ja jätepussit. Biohajoavien tuotteiden tulee käyttöikänsä jälkeen hajota haitattomiksi lopputuotteiksi. Biohajoamisen tulisi tapahtua tietyn ajan sisällä käyttöympäristön tai käytetyn jätteenkäsittelyteknologian asettamien vaatimusten mukaisesti. Sovelluskohteen ja käyttötarkoituksen mukaan hajoaminen voi tapahtua joko maassa, vedessä, anaerobiprosessissa tai kompostissa. Useat biohajoavista muoveista on suunniteltu täyttämään kompostoituville materiaaleille asetetut vaatimukset. Kompostointia hyödynnetään yleisesti myös jätevesilietteiden käsittelyssä. Kompostoinnilla on mahdollista alentaa lietteen sisältämien orgaanisten haitta-aineiden pitoisuuksia ja näin parantaa lietteen hyötykäyttömahdollisuuksia. Tässä työssä tutkitut kompostoituvat ja maatalouskäyttöön tarkoitetut biohajoavat muovit biohajosivat tavoitteiden mukaisesti suhteessa käyttötarkoituksen asettamiin vaatimuksiin. Materiaalien biohajoavuusominaisuuksien lisäksi tarkasteltiin myös biotestien mahdollisuuksia biohajoavien muovien ja niiden komponenttien ympäristömyrkyllisyyden arvioinnissa biohajoavuusprosessin aikana sekä kompostissa, maaperässä että vermikuliitissa. Tutkimuksessa osoitettiin, että maitohappopohjaisten polymeerien ja polyuretaanipohjaisten biomuovien biohajoamisen aikana ympäristöön voi vapautua ympäristölle haitallisia yhdisteitä. Haittavaikutukset voitiin osoittaa sekä kineettisellä valobakteeritestillä että kasvitestillä (OECD 208). Tämän lisäksi tutkittiin muovin pehmittimenä käytetyn ja hormonin kaltaisesti vaikuttavan dietyyliftalaatin (DEP) käyttäytymistä laboratorio- ja pilot-mittakaavan kompostiprosessissa sekä kompostista valmistetussa kasvualustassa. Korkeat DEP-pitoisuudet aiheuttivat muutoksia kasvualustan mikrobidiversiteetissä ja mikrobien toiminnassa sekä vaikuttivat kasvien kasvuun. Pilot-mittakaavan kompostointiolosuhteissa DEP hajosi tehokkaasti, eikä haitta-vaikutuksia havaittu biotesteillä. Tutkitut tärkkelyspohjaiset biohajoavat katekalvot toimivat hyvin kenttäolosuhteissa, materiaali oli kestävää eikä alentanut tuotetun mansikkasadon laatua tai määrää. Käytön jälkeen peltomaahan kynnetyn katekalvon hajoaminen kesti noin vuoden. Katekalvon hajoamisella ei havaittu olevan vaikutusta maaperän laatuun, kun maan laatua tarkasteltiin Enchytraeidae-jälkeläisten määrän (ISO 16387) ja amoA-geenin diversiteetin avulla. Biotesteillä osoitettiin myös että kompostointi vähensi lietteen akuuttia myrkyllisyyttä, genotoksisuutta sekä hormonin kaltaisesti vaikuttavien yhdisteiden määrää lietteessä. Tämän tutkimuksen perusteella biotestien käyttöä voidaan suositella maatalouskäyttöön tai viherrakentamiseen tarkoitettujen jätevesilietteiden laadun tarkkailussa. Biohajoavat materiaalit sekä komposti ja maaperä testiympäristöinä asettavat biotesteille suuria haasteita. Vaikka biotesteillä voitiin tässä tutkimuksessa osoittaa biohajoavuuden aikana tapahtuvia muutoksia näytteiden haitallisuudessa, testiympäristö asetti biotestien käytölle myös rajoituksia. Tutkittavien näytteiden väri, orgaaninen hiili, kuorike, turve, kompostin kypsyysaste ja korkea mikrobiaktiivisuus ovat niitä tekijöitä jotka vaikuttavat biotestien toimintaan ja tekevät biotestien tulosten tulkinnasta haastavaa.
ISBN, ISSN	ISBN 978-951-38-7465-0 (nid.) ISSN 2242-119X (nid.) ISBN 978-951-38-7466-7 (URL: http://www.vtt.fi/publications/index.jsp) ISSN 2242-1203 (URL: http://www.vtt.fi/publications/index.jsp)
Julkaisuaika	Marraskuu 2012
Kieli	Englanti, suomenkielinen tiivistelmä
Sivumäärä	92 s. + liitt. 82 s.
Avainsanat	Biodegradable plastic, sewage sludge, biotest, ecotoxicity, acute toxicity, phytotoxicity, bioreporter, organic contaminant, soil, compost, biodegradation
Julkaisija	VTT PL 1000, 02044 VTT, Puh. 020 722 111

VTT Technical Research Centre of Finland is a globally networked multitechnological contract research organization. VTT provides high-end technology solutions, research and innovation services. We enhance our customers' competitiveness, thereby creating prerequisites for society's sustainable development, employment, and wellbeing.

Turnover: EUR 300 million
Personnel: 3,200

VTT publications

VTT employees publish their research results in Finnish and foreign scientific journals, trade periodicals and publication series, in books, in conference papers, in patents and in VTT's own publication series. The VTT publication series are VTT Visions, VTT Science, VTT Technology and VTT Research Highlights. About 100 high-quality scientific and professional publications are released in these series each year. All the publications are released in electronic format and most of them also in print.

VTT Visions
This series contains future visions and foresights on technological, societal and business topics that VTT considers important. It is aimed primarily at decision-makers and experts in companies and in public administration.

VTT Science
This series showcases VTT's scientific expertise and features doctoral dissertations and other peer-reviewed publications. It is aimed primarily at researchers and the scientific community.

VTT Technology
This series features the outcomes of public research projects, technology and market reviews, literature reviews, manuals and papers from conferences organised by VTT. It is aimed at professionals, developers and practical users.

VTT Research Highlights
This series presents summaries of recent research results, solutions and impacts in selected VTT research areas. Its target group consists of customers, decision-makers and collaborators.

Ecotoxicity assessment of biodegradable plastics and sewage sludge in compost and in soil

Biodegradable plastics, either natural or synthetic polymers, can be made from renewable or petrochemical raw materials. The most common applications for biodegradable plastics are packaging materials and waste collection bags. The one thing in common for all biodegradable items is that at the end of their life cycle they should degrade into harmless end products, during a specified time frame. Depending on the target application, the degradation may take place in soil, in water, in an anaerobic digestion plant or in compost. In addition to its use as a waste treatment process for biodegradable plastics, composting can be used in the removal of organic contaminants from sewage sludge. Due to mixed contamination present in soil, in compost, or in sewage sludge the environmental impact of biodegradable materials is difficult to assess based only on concentrations of chemical constituents. Therefore, biotests are needed for detecting potential risks derived from the use of biodegradable materials in environmental applications.

Potentials and also limitations were recognized in the performances of different biotests when studying the ecotoxicity of biodegradable materials during biodegradation processes in compost and in soil environment. With biotests it was possible to identify potential hazardous polymer components or degradation products that might be released to the environment during the degradation. In addition, the biotest could be used to monitor the detoxification of sewage sludge during the composting process. However, soil, compost and sludge as testing environments do set limitations for the use of biotests. Colour, amounts of nutrients, additional carbon sources, presence of bark and peat, compost immaturity, and high microbial activity are some of the factors limiting the use of biotests or complicate interpretation of the results.

VTT