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Biodegradation of aliphatic–aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates

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Abstract

The biological degradation behaviour of the aliphatic–aromatic copolyester Ecoflex® was investigated with regard to the degree of degradation and the intermediates formed during the degradation process. The individual thermophilic strain *Thermomonospora fusca*, isolated from compost material, was used for the degradation experiments in a defined synthetic medium at 55°C. After 22 days of degradation more than 99.9% of the polymer had depolymerized and with regard to the degradation of the diacid and diol components of Ecoflex® only the monomers of the copolyesters (1,4-butanediol, terephthalate and adipate) could be detected by gas chromatography/mass spectroscopy (GC–MS) measurements in the medium. In interrupted degradation experiments predominantly the monoesters of adipic acid and terephthalic acid with 1,4-butanediol were observed in addition to the monomers. In toxicological tests with *Daphnia magna* and *Photobacterium phosphoreum* no significant toxicological effect was observed, neither for the monomeric intermediates nor for the oligomeric intermediates. From a risk assessment it can be concluded that there is no indication for an environmental risk when aliphatic–aromatic copolyesters of the Ecoflex-type are introduced into composting processes.

Keywords: Aliphatic–aromatic copolyesters; Actinomycetes; Ecotoxicology; Biodegradation

1. Introduction

As a result of increasing problems with domestic waste, particularly with plastics, a new class of polymers especially designed to be biodegradable, has been undergoing development for more than 10 years. Now several of such materials have entered the market and current forecasts deal with a future medium term potential of 200 000–380 000 t/a for western Europe (Verrottungsbeschleuniger für Polyolefin-Folien, 1994; BASF Press release, 1998).

Generally, biodegradability is exclusively a function of the polymer structure and does not depend on the origin of the raw materials – whether petrochemically based or from renewable resources (Witt et al., 1999). At present, synthetic biodegradable plastics provide a number of advantages compared to those made from naturally produced macromolecules (e.g. starch, cellulose, poly(hydroxyalkanoates)). Better use properties,

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processability comparable to conventional plastics, constant material quality and in many cases a significantly lower price point in favour of synthetic materials, at least as components in biodegradable plastic compositions.

Beside the natural polyesters (e.g. PHB), many other synthetic aliphatic polyesters are susceptible to microbial attack. Recently, it was shown that synthetic copolyesters containing aromatic constituents are also degraded by microorganisms. The degradation decreases as the amount of aromatic components increases (Witt et al., 1995, 1997). In 1998, an aliphatic–aromatic copolyester was commercialised by BASF (Germany) under the trade name Ecoflex®. This copolyester overcomes the disadvantages of many aliphatic materials and combines useful material properties with biodegradability.

This product is certified according to German standard DIN V 54900 (1998a,b,c) with regard to its biodegradability and compostability. In particular, the fate of aromatic sequences during biodegradation has been thoroughly investigated with regard to the environmental safety of the material (Witt et al., 1996a). In other investigations the metabolization of the copolyester was proven in a compost environment (Witt et al., 1999) but the results have to be discussed within the experimental limits originating from the use of a complex compost matrix as degradation environment. However, a number of thermophilic actinomycetes have recently been isolated from compost and taxonomically identified. These depolymerize the aliphatic–aromatic copolyesters very rapidly, also in defined synthetic media, but do not metabolize significant amounts of the low-molecular-weight substances formed (Kleeberg et al., 1998).

The work presented here investigates the absolute extent of biodegradation and potential ecotoxicological effects from degradation intermediates of aliphatic–aromatic copolyesters in a compost system. The tests were performed with the copolyester Ecoflex® which, as a commercial product, was optimized for a practical application. Using an individual strain of the thermophilic actinomycete *Thermomonospora fusca* in a defined medium increases the accuracy of the analytical procedures for determining the degradation intermediates by gel permeation chromatography (GPC) and gas chromatography/mass spectroscopy (GC–MS) and permits toxicological tests to be run with the artificially enriched degradation intermediates. Thus, the results are expected to be much more accurate than those obtained for example from common standard tests.

2. Materials and methods

2.1. Chemicals

Chemicals were used from the following suppliers: 1,4-butanediol, adipic acid, N-methylpyrrolidone: BASF AG, Germany; buthylbenzene, tetrabuthylorthotitanate (TBOT): Fluka, Germany; dimethyl terephthalate (DMT): Hoechst AG, Germany; N-methylene-*n*-trisilyl-trifluoro-acetamide (MSTFA): Macherey-Nagel, Germany; 1,4-dioxane, peptone, terephthalic acid dichloride, triethylamine, tryptic soy broth (usage in vitro): Merck, Germany; hexafluoroisopropanoic acid (Riedel de Häen, Germany). 1,4-butanediol, adipic acid and DMT were industrial grade. All chemicals except these were reagent grade.

2.2. Polymer

The aliphatic–aromatic copolyester Ecoflex® was provided by BASF AG (Germany). Polymer granules were milled with liquid nitrogen and fractionated by sieving according to the particle size. The particle size fraction of 100–250 μm was used in this study.

The composition of the polymer was determined by ^{13}C-NMR measurement (terephthalic acid: 22.2 mol%, adipic acid: 27.8 mol% and butanediol: 50 mol%), with regard to the content of the diacids and the diol. For the degradation experiments the powder (15 g) was sterilized by washing in 10% H_2O_2 (200 ml) for 2 h, rinsed with deionized, sterile water (5000 ml) and dried under reduced pressure at room temperature.

2.3. Aromatic model oligomers

The aromatic oligomers do not contain any modular components mentioned in Fig. 1.

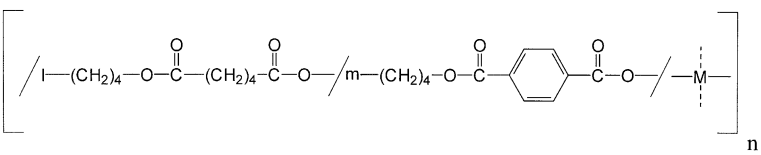

Fig. 1. Chemical structure of the aliphatic–aromatic copolyester Ecoflex® (M = modular components, e.g. monomers with a branching or chain extension effect).

2.3.1. OH–OH-terminated oligomer mixture

The aromatic oligomer mixture with OH-terminal group was synthesized by bulk condensation. DMT (5 mol/971 g), 1,4-butanediol (10 mol/901 g) and tetrabutyl orthotitanate (1.8 g) were heated in a 2 l round bottom flask equipped with a metal stirrer and a cooler at 160–190°C for 17 h. The reaction mixture was cooled, and the product was washed thoroughly with acetone and dried at 70°C under reduced pressure for 16 h.

2.3.2. COOH–COOH-terminated oligomer mixture

Terephthalic acid dichloride (160 mmol/32.48 g) was dissolved in 100 ml of N-methylpyrrolidone (NMP), and triethylamine (320 mmol/32.32 g) was added as a neutralizing agent. Then 1,4-butanediol (100 mmol/9.0 g) was added. A precipitation of the products was observed, due to the low solubility of the products in the solvent. The mixture was then poured into 2 l of water, stirred well and filtered. The product was washed with sufficient water and acetone and dried under reduced pressure at 50°C for 16 h.

2.3.3. OH–OH-terminated monomer and trimer

These aromatic model oligomers were synthesized according to the method of Hässlin et al. (1980).

2.4. Microorganisms

The organism used for the degradation experiment was the actinomycete *T. fusca* (DSM 43793), obtained from the German collection of microorganisms and cell cultures (DSMZ, Braunschweig, Germany).

The mixed inoculum from compost was prepared as follows: 10 g of mature compost from green waste (Watenbüttel compost plant, Germany) was suspended in 90 ml of deionized water for 30 min. The suspension was filtered through a folded paper filter (Schleicher and Schüll 595-1/2). The first 5 ml of filtrate were rejected. The filtrate was used as inoculum.

2.5. Degradation test

Liquid cultures were grown in 300-ml Erlenmeyer flasks containing 80 ml of an "optimized mineral salt medium" (DIN V 54900 Teil 2, 1998b) supplemented with peptone from soymeal (0.5% wt/vol, papain-digested, Merck 1.07212). Sterile Ecoflex® powder (350 ± 5 mg/80 ml) was added aseptically to the cooled sterile medium before inoculation. The cultures were inoculated (1% vol/vol) with a mycelium suspension of *T. fusca.* This mycelium suspension was harvested from a 30 ml preculture grown overnight in Tryptic Soy Broth (Merck 1.05459) by washing and resuspending the biomass once with the same volume of 80 mmol phosphate buffer (pH 7.0). All cultures were incubated at 55°C on a rotary shaker at 120 rpm. Experiments were run in triplicate. The activity of the actinomycetes was checked in a flask containing Ecoflex® films (14 films, 2.5 mm diameter, 50 μm thickness, 25 ± 3 mg film weight).

2.6. Respirometer test

For respirometric tests mixed populations of compost were cultured in aerated 1000 ml flasks containing 500 ml of "optimized mineral salt medium" (DIN V 54900 Teil 2, 1998b). The diacids and diol related monomers of Ecoflex® – 1,4-butanediol, Na terephthalate, Na adipate – were added (0.3% wt/vol) to the medium before sterilization. The medium was inoculated (1% vol/vol) with a suspension made from compost as described before. The cultures were incubated over a period of 22 days at 22°C with gentle stirring (magnetic stirrer) and CO_2-evolution was monitored by infrared measurements (Respirotec, München, Germany).

As a control, the medium (optimized mineral salt medium) without any supplements was inoculated as the other cultures. The experiments were run in triplicate.

2.7. Sample preparation

The culture solutions were centrifuged at 12 000 rpm for 120 min and the pellets were dried at 40°C under reduced pressure for 24 h. The samples were used for the GPC measurement. The clear supernatants were used for the GC measurement.

2.8. GC/GC–MS analysis

2.8.1. Determination of 1,4-butanediol

For determination of 1,4-butanediol 100 mg sample were dissolved in 2 ml of dioxane. After addition of butylbenzene solution in dioxane as internal standard the sample solution was derivatized with 3 ml MSTFA for 4 h at 80°C. The degree of recovery was checked by spiking the sample with an appropriate amount of 1,4-butanediol in dioxane. The content of the derivatized sample solution was determined under the following GC conditions. The GC apparatus (HP 5890 series II with FID and automatic sample injector HP 7673, Hewlett-Packard) was equipped with a DB-1 column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm, J&W Scientific). As carrier gas helium (column head pressure 1.0 bar, split 20 ml/min, septum purge 4 ml/min) and as combustion gas hydrogen and synthetic air (as recommended by the instrument supplier) was used. The injection volume was 1 μl. The temperature of the injector was adjusted to 250°C and those of the FID detector to 320°C. A temperature profile (80°C, 3 min isothermal; 80°C → 140°C, 5°C/min; 140°C → 300°C, 20°C/min; 300°C, 5 min isothermal) was applied for the column oven.

2.8.2. Determination of adipic acid, terephthalic acid and esters thereof with 1,4-butanediol

25 g of the aqueous samples were lyophilized for 4 days. The residue was extracted with 5 ml of dioxane and derivatized with 2 ml of MSTFA for 4 h at 80°C. The degree of recovery was checked by spiking the sample with appropriate amounts of adipic acid and terephthalic acid and terephthalic diester with 1,4-butanediol in dioxane. The contents of the esters of adipic acid and 1,4-butanediol identified by GC–MS (see below) were estimated based on the comparison of the corresponding peak areas. The content of the derivatized sample solution was determined under the following GC-conditions. The GC apparatus (HP 5890 series II with FID and automatic sample injector HP 7673, Hewlett-Packard) was equipped with a DB-1 column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm, J&W Scientific). As carrier gas helium (column head pressure 1.0 bar, split 50 ml/min, septum purge 4 ml/min) and as combustion gas hydrogen and synthetic air (as recommended by the instrument supplier) was used. The injection volume was 1 μl. The temperature of the injector was adjusted to 250°C and those of the FID detector to 320°C. A temperature profile (80°C, 3 min isothermal; 80°C → 325°C, 5°C/min; 325°C, 30 min isothermal) was applied for the column oven.

2.8.3. Identification of unknown components by GC–MS

The unknown degradation products in the samples were identified by GC–MS using a Finnigan 9610 GC with a Finnigan 4500 mass spectrometer (ionization: electron impact (EI) and chemical ionization (CI), scan rate: 1 scan/s, ionization (EI): 70 eV, ionization gas (CI): ammonia, mass range 25–600 amu, ion source temperature: 100°C, transfer line temperature: 300°C). The chromatographic conditions were similar to those used for determination of compounds in the lyophilized samples. Peaks related to the biological matrix (e.g. amino acids, carbohydrates) may be coextracted with residual traces of water.

2.9. GPC measurements

15 mg of each sample were dissolved in 10 ml of hexafluoroisopropanoic acid (HFIP). The insoluble part (biomass) was filtered off and the filtrate was used for the GPC-measurement. The GPC measurements were performed in a conventional GPC apparatus (pump: Kontron instrument 420, detector: GAT LGD 503, Gamma Analysentechnik; elution solvent: HFIP + 0.05% wt/vol trifluoro acid Ka-salt, column temperature: room temperature, flow: 0.5 ml/min, injection: 125 μl of 0.15% wt/vol solution, columns: Shodex HFIP-800P $\phi = 8$ mm × 5 cm/Shodex HFIP 803 $\phi = 8$ mm × 30 cm/Shodex HFIP 803 $\phi = 8$ mm × 30 cm, silica columns).

2.10. Solubility of oligomers

For the analysis of the residual polymer and oligomers, the polymer and oligomers must be able to be extracted with hexafluoroisopropanol (HFIP). For this reason the solubility especially of the aromatic model oligomers in HFIP was checked. Different amounts of oligomers (1, 2 mg, etc.) were added to 10 ml of HIFP and it was visually observed if the material dissolved after intense mixing.

From the solubilities of the oligomers (Copolyester, OH-terminated aromatic oligomer mixture and OH-terminated aromatic trimer: >1000 mg/10 ml HFIP; COOH-terminated aromatic oligomer mixture: 7 mg/10 ml HFIP; COONa-terminated aromatic oligomer mixture: 2 mg/10 ml HFIP) it can be concluded that, if they are present in a sample, they would be detectable by the GPC measurement after extraction.

2.11. Toxicity tests

2.11.1. Ecotoxicity test with Daphnia

The test was performed according to DIN 38412 part 30 (1989). The pH of the test solutions was adjusted to 7.0 ± 0.2 with sodium hydroxide solution. The test solutions with different dilution rate were prepared using the water, which was used for growing *Daphnia*. *Daphnia* can be damaged by the test substances. The loss of the ability to swim was measured. In a beaker 10 *Daphnia* were incubated with test solution at 20°C for 24 h and the number of the *Daphnia*, which could still swim was counted. If at least 9 *Daphnia* can swim for the first time in a sample diluted x times, the G_D ("Giftigkeit" = toxicity for *Daphnia*) value of x is stated.

2.11.2. Ecotoxicity test with Photobacterium phosphoreum

The test with luminescent bacteria was run according to DIN 38412 part 34 (1989). The pH of the test solutions was adjusted to pH 7.0 ± 0.2 with sodium hydroxide solution. Sodium chloride was added to the neutralised solution to get a concentration of 2.0% wt/vol (20 g NaCl/l). The test solutions with different dilution rates were prepared using 2.0% wt/vol NaCl solution and the test solutions were then incubated at 15°C with the luminescent organism *Photobacterium phosphoreum* (10 μl of 10^8 cells/ml-bacteria suspension) for 30 min. The decrease of the photoemission compared to the initial time ($t = 0$) was measured. If the decrease of photoemission was less than 20% for the first time compared to the initial time for a sample diluted x times, the G_L ("Giftigkeit" = toxicity of luminescent bacteria) value of x is stated.

3. Results

3.1. Analysis of degradation products and intermediates

A degradation test with the actinomycete strain *T. fusca* DSM 43793 in the synthetic medium was run for 21 days using Ecoflex® powder. In the control experiment, a Ecoflex® film (50 μm thickness) was visually decomposed after approximately 4 days under comparable test conditions. Thus, an almost complete depolymerization of the polyester powder could be expected.

In the degradation experiment initially 2133 mg Ecoflex® was added to the medium. At the end of the test (21 days) 127 mg of solid substance, consisting of potential residual polymer-derived substance and biomass was isolated by centrifuging, corresponding to a depolymerization of the solid polyester of more than 94%. In the polymer blank test, a small mass loss was observed, probably due to migration of some low-molecular-weight material. The amount of microorganisms grown in the inoculum blank test indicates that a significant part of the insoluble fraction of the degradation experiment consists of biomass (see Table 1).

To obtain more quantitative information about potential polymer-derived residues in the solid fraction, a GPC analysis was performed. Fig. 2 shows the GPC chromatogram of the HFIP extract of the residues in comparison to the initial polyester and an aromatic model oligomer (synthesized by condensation of 1,4 butanediol and terephthalic acid).

Neither residual polymer nor an accumulation of aromatic oligomers could be observed. Concentrations of 0.1 mg polymer/10 ml HFIP could still be detected by the GPC experiments. From this minimum detection level and the absence of any polymer or oligomer peak it can be estimated (15 mg of the sample was suspended in 10 ml HFIP) that a maximum of 0.84 mg of the total amount of residuals can originate from the polymer. Thus, in total a mass loss of more than 99.9% for Ecoflex® can be calculated from this experiment.

The composition of the soluble intermediates were further analysed by GC measurements. In the medium mainly the diol and diacids related monomers (1,4-butanediol, terephthalate and adipate) and no significant amounts of soluble diol and diacids related oligomers of

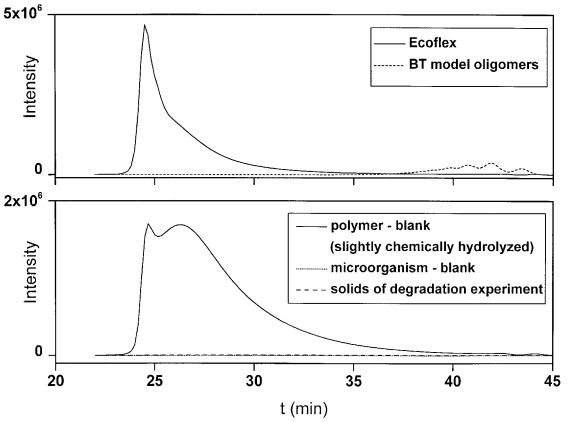

Fig. 2. GPC analysis of the residual non water-soluble material after degradation of Ecoflex® powder with *T. Fusca* for 21 days at 55°C in shaking flasks. In the lower diagram the chromatograms of the extract of the solid residuals of the degradations experiment, the polymer blank test (medium not inoculated) and the microorganism blank (inoculated medium without polymer) are shown. The upper diagram gives comparison of the chromatograms of native Ecoflex® and a synthetic oligomer mixture (from terephthalic acid and 1,4-butanediol) as reference for possible aromatic residues after microbial degradation.

Ecoflex®, neither aromatic nor aliphatic, could be detected as soluble components by GC after 21 days of microbial depolymerization. (see Fig. 3). In addition small amounts of intermediates related to the modular components of Ecoflex® (see Fig. 1) were detected and identified, too.

It can be estimated from the total area of the minor matrix contaminations observed in the chromatogram that the content of these dissolved species is more than 100 times lower than the concentration of diol and diacids related monomers in Ecoflex®. This indicates that more than 99% of the initial polymer has been depolymerized into monomers.

The monomeric intermediates of Ecoflex® artificially enriched during the polyester depolymerization by *T. fusca* are rapidly degraded when a mixed microbial population is present. This is demonstrated by an experiment, in which after degradation with an individual culture of the actinomycete for 7 days an eluate of

Table 1
Solid matter before and after degradation of Ecoflex® powder with *T. fusca* for 21 days in a mineral salt medium at 55°C in shaking flasks

	Initial polymer mass (mg)	Insoluble fraction (polymer + biomass) after incubation (mg)	Minimum solubilization of the polymer (%)
Polymer + medium	1144	1071	–
Inoculum + medium	–	65	–
Polymer + inoculum + medium	2133	127	94

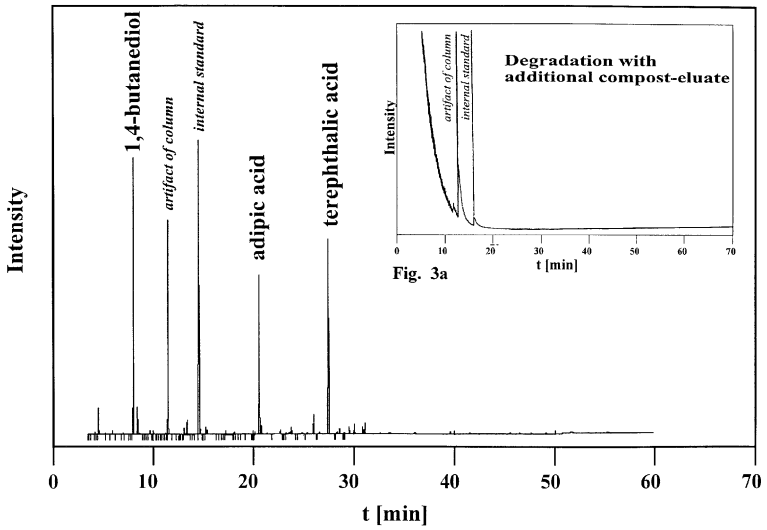

Fig. 3. GC-chromatogram of water-soluble degradation intermediates after 21 days incubation of Ecoflex® with *T. fusca* at 55°C. The small diagram: (a) 14 days incubation with *T. fusca* and afterwards incubation with a mixed culture from compost for 7 days.

compost material was added and then the test continued for further 2 weeks. In the chromatogram (see Fig. 3(a)) of the water soluble products all peaks of Ecoflex® related intermediates have totally disappeared.

These results were confirmed by a test, in which 1,4-butanediol, Na adipate and Na terephthalate, dissolved in a synthetic MSV/0.5% pepton medium (Kleeberg, 1999), were inoculated with a mixed microbial population from compost. The degradation was then monitored via CO_2 evolution (see Fig. 4). After lag-phases of some days, all substances were rapidly mineralized by the mixed microbial population.

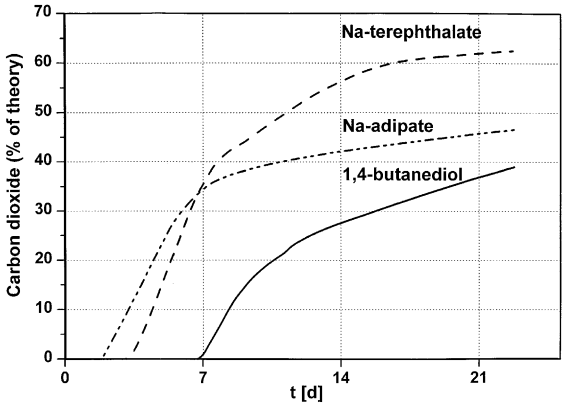

Fig. 4. Degradation of diol and diacids related monomers of Ecoflex® – 1,4-butanediol, terephthalate and adipate with a mixed microbial population from compost at ambient temperature in a synthetic MSV/0.5% pepton medium (Kleeberg, 1999).

To check if any oligomeric intermediates are detectable, one degradation experiment was stopped after 7 days of incubation with *T. fusca*. (No polymer was visually detectable in the degradation medium.) Fig. 5 shows the GC of the water-soluble components of this experiment.

The chromatograms expanded in *y*-dimension to show small peaks too. In addition to the diol and diacids related monomers of Ecoflex®, aliphatic oligomers can also be observed. The main intermediates here are the monoester (BA) of adipic acid (A) and butanediol (B) and the BAB diester. Small amounts of ABAB oligomer can also be detected.

The concentration ratios for these species were estimated by comparing the peak areas of the signals identified by GC–MS. No signals of aromatic esters were observed at all. From the signals of the present esters, it can be estimated that all other species (e.g. aromatic esters or mixed aliphatic–aromatic esters) are of a lower concentration than BABA and therefore present in concentrations of less than 0.4% of the monomers.

The relative concentration of the oligomers in Fig. 5 can be estimated by comparing the corresponding peak areas with the total peak area of the monomers. According to this calculation, the oligomer AB has a concentration of 8% of monomer peak area, BAB 4% of monomer peak area and ABAB only 0.4% of monomer peak area. Thus, all other oligomers not detectable in the chromatogram must exhibit concentrations lower than 0.4% of the monomer peak area.

A number of other substances identified by GC–MS are present in the medium in minor amounts. Apart from small amounts of modular components related

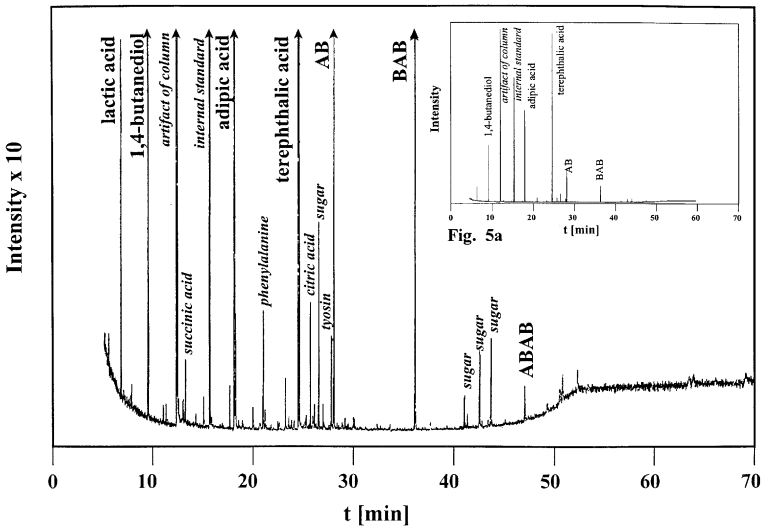

Fig. 5. GC-chromatogram of water-soluble degradation intermediates after 7 days incubation with *T. fusca* (10-fold magnification). The small diagram shows the initial scaling of the diagram.

intermediates of Ecoflex® all others turned out to be naturally occurring structures. Sugars and amino acids, for instance, are components of the medium, and succinic acid is probably an oxidation product of 1,4-butanediol.

In another experiment, Ecoflex® was used in a five-fold concentration (21.9 g polyester/l medium) in a degradation experiment. The degradation by the actinomycetes stopped due to a pH shift of the medium caused by the release of acids while solid polymer was still present. In this case, an aromatic oligomer (monoester of butanediol and terephthalic acid/BT) was identified in addition to the aliphatic AB oligomer (see Fig. 6).

The results indicate that during the degradation process with the actinomycete strain, aliphatic and aromatic oligomers are released into the medium, which then rapidly hydrolysed into the monomeric components. These readily can be metabolized when a mixed culture is present. The concentration ratios were estimated as described above. In this experiment, the peak of the AB-oligomer has an area of 1.7% of the monomer peak areas, the BT-oligomer exhibits a much

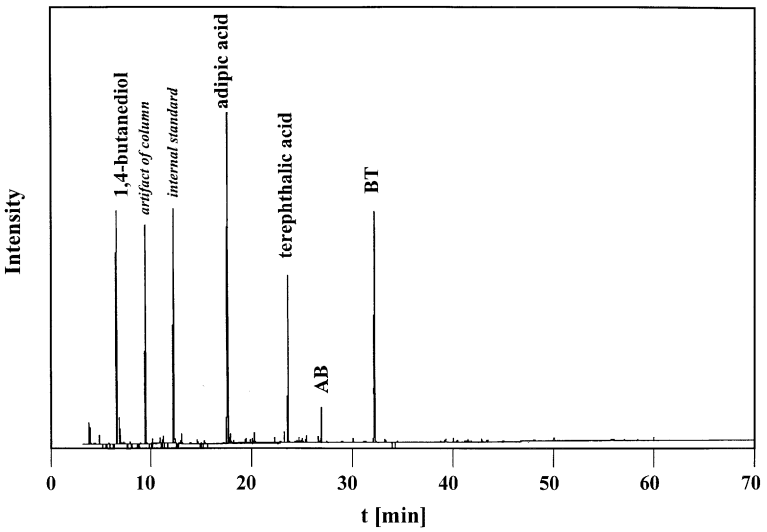

Fig. 6. GC-chromatogram of water-soluble degradation intermediates after 21 days incubation with *T. fusca*. The polymer mass was five-fold of that used in Fig. 3. The degradation was interrupted due to a pH-shift.

higher concentration of 24% of monomer peak areas. Also, here it can be estimated that the concentration of the esters, which are not observable in the chromatogram, must be lower than 1.7% of the sum of the monomers.

3.2. Ecotoxicity

For the environmentally safe application of such degradable plastics, it is important to prove that intermediates, even those degradable, do not have any ecotoxicological effect. To verify this for the aliphatic–aromatic copolyester Ecoflex®, the acute toxicity of the aqueous fraction of two tests was examined using two toxicity tests with *Daphnia* and *P. phosphoreum*. Due to evaporation of the medium during the degradation test and a potentially small metabolization of the intermediates by the actinomycetes the concentrations of the intermediates were directly calculated from the DOC values determined taking into account the carbon content of the medium at the end of incubation with the microorganisms. Table 2 summarizes the results from these toxicity tests. The solutions containing the test samples are in both tests comparable in the toxicological effect to the control samples (medium + polymer and medium + actinomyces).

In test (a) the polyester was totally depolymerized (no oligomers could be detected) by the actinomycetes while in test (b) the degradation was incomplete (residual polymer present). Here aliphatic oligomers as well as aromatic oligomers were present in addition to aliphatic and aromatic monomers.

The toxicity of the solutions, which contain the degradation products is comparable to that of the control solutions which contain only medium. The toxic effect of the medium and test solutions in the test to *Daphnia* can be attributed to the high conductivity caused by the different minerals, e.g. adipate and terephthalate because *Daphnia magna* are fresh-water organisms.

3.3. Risk assessment

The concentration of the monomers and oligomers formed during the laboratory tests described is much higher than can be expected in the real environment.

Estimations of the future amount of biodegradable plastics in the biowaste assume a maximum concentration of 1% wt/wt in Germany (DIN V 54900 Teil 3, 1998a,b,c). Assuming a biowaste mass of 10^7 t/a (Witt et al., 1994) which will be composted within the next years, this would correspond to 100 000 t/a of biodegradable plastics. On the production side the market potential of any biodegradable plastic products is considered to be in the range of about 200 000–380 000 t/a for the whole of western Europe.

For an assessment of the potential concentration of Ecoflex® intermediates in the soil, the following assumptions are made:

Table 2
Data of the ecotoxicity tests with media containing intermediates from the degradation of Ecoflex® with *T. fusca*

	Control	Control	Test a	Test b
	Medium + polymer (21 days)	Medium + actinomycetes (21 days)	Complete depolymerization[a] (21 days)	Incomplete depolymerization[b] (21 days)
Detected water-soluble intermediates	–	–	A, B, T, [M][c]	A, B, T, [M] BA, BT[c]
Ecotoxicity test with *Daphnia* G_D[d]	8	6	8	8
Ecotoxicity test with *P. phospherum* G_L[e]	64	128	64	128
DOC[f] (mg/l)	2546	1756	6508	8697
Degradation intermediates[g] (mg/l)	–	–	7665	11 195
Conductivity (μS/cm)	8800	9600	13 600	11 900
pH value before adjustment	6.8	7.1	6.2	5.1

[a] Polymer was completely depolymerized.
[b] Polymer was only partially depolymerized due to using the five-fold concentration of the polyester and subsequently stopping the degradation by a pH-shift of the medium caused by the release of free acids (see Fig. 6). Aliphatic and aromatic oligomers are still present in the aqueous medium.
[c] A = adipic acid, B = 1,4 butanediol, T = terephthalic acid, M = modular components related intermediates of Ecoflex®.
[d] $G_D = 8$ means that test substance diluted 8 times (1 part test solution + 7 parts water) is for the first time not toxic to *Daphnia*.
[e] $G_L = 64$ means that the test solution diluted 64 times (1 part test solution + 63 parts water) is for the first time not toxic to *P. phosphoreum*.
[f] DOC – dissolved organic carbon.
[g] The concentration of degradation intermediates was calculated by $(DOC_{solution} - DOC_{blank})/0.62$. The figure 0.62 documents the carbon content of the polymer.

1. the amount of the copolyester in the biowaste is 1% wt/wt (no other products than Ecoflex® are considered),
2. the weight reduction of the biowaste during the composting process is 50%,
3. the copolyester is depolymerized but no metabolization of intermediates takes place (even readily biodegradable intermediates will be accumulated),
4. the amount of compost applied to 1 hectare of agriculturally used area is 30 tons (a maximum application of this amount is recommended by the German biowaste directive within 3 years (Bioabfallverordnung, 1998), and
5. the compost will be mixed with the soil to a depth of 30 cm (specific weight of the soil is 1500 kg/m^3).

From these data it is possible to calculate a maximum concentration of substances in the soil originating from the copolyester of approximately 130 ppm directly after the application of the compost. For these conservative assumptions, any concentration of intermediates in the soil would be lower than the determined toxicity levels by one order of magnitude. A toxicity effect compared to the medium in the test with *Daphnia magna* was observed at a dilution factor of 8 (concentration of degradation products was calculated from DOC measurement to be 11 195 mg/l in the undiluted solution) corresponding to a monomer and oligomer concentration of 1390 ppm. Probably the toxic effect can be attributed to the higher conductivity of the solution containing the degradation products.

In summary, it can be stated that under practical realistic conditions no toxic effects can be expected from composting this copolyester.

4. Discussion

In investigations applying mixed microbial cultures it was previously demonstrated that aliphatic–aromatic copolyesters comparable to Ecoflex® can be classified as totally degradable within the detection limits of the test methods applied (e.g. ASTM D 6002-96, 1996; DIN V 54900, 1998a,b,c). However, in respirometric tests with aqueous synthetic media, the detection limit of the extent of degradation was demonstrated to reach a maximum of approximately ±10% even when the calculation of a carbon balance is incorporated (Urstadt et al., 1995). Applying a matrix of mature compost instead of an aqueous medium in a so called "controlled composting test" (ASTM D 5338-92, 1992; Pagga et al., 1995; DIN V 54900 Teil 2, 1998a,b,c) usually increases the degradation rate of the copolyesters, but the accuracy in determining the absolute degradation level of the polymers is limited in this case by the significant background CO_2-evolution from the compost. Due to the complex "medium" of compost, additionally the analytical detection of residual or toxic intermediates at a low concentration level tends to be more complicated than in a synthetic aqueous environment. Thus, it cannot surely be excluded from such tests that some small amounts (in the range of some percent) of the polymers remain undegraded.

Particularly the fate and the environmental effect of aromatic oligomeric intermediates in aliphatic–aromatic copolyesters was the subject of an intensive discussion, because aromatic homopolyesters such as poly(ethylene terephthalate) (PET) or poly(butylene terephthalate) (PBT) are resistant to microbial attack. Aromatic ester sequences of different length are present in various amounts in such statistical copolymers, depending on the composition of the material. Degradation tests with specific model oligomers synthesized from 1,4-butanediol (B) and terephthalic acid (T) applying mixed microbial populations showed that BTB esters and also BTBTB sequences are rapidly degraded by microorganisms, while longer aromatic oligomers turned out to be much more resistant (Witt et al., 1996a). However, GPC measurements of residual materials after a degradation experiment with a copolyester on an agar plate using a pre-adapted inoculum showed much lower concentrations of longer aromatic sequences than could be calculated from the polymerization statistics (Witt et al., 1996b). This observation indicates that longer oligomers are also inherently biodegradable.

With the test strategy presented in this paper, it is possible to gain more detailed information about the fate and environmental impact of the aromatic constituents in aliphatic–aromatic copolyesters. The test organism used here belongs to the group of actinomycetes, which has been shown to be ubiquitous and to play an important role during the thermophilic phase of a composting process (McCarthy and Williams, 1992). Thus, it is likely that the degradation processes observed in the laboratory tests with the selected actinomycete can also occur in a real compost environment. The test microorganism *T. fusca* DSM 43793 has a high potential in depolymerizing Ecoflex® but is not able to readily metabolize the monomers and oligomers formed. This results in an artificially high enrichment of the degradation intermediates which, in combination with the use of defined media, enables these substances to be analysed by GC and GPC measurements very precisely and ecotoxicological tests to be run without the problems involved when eluates from real compost materials are used.

From our results, it can be definitely concluded that also the aromatic sequences in aliphatic–aromatic copolyesters like Ecoflex® are subject to degradation under the test conditions, simulating a composting process. Ecoflex® contains approximately 9% wt/wt of aromatic sequences with a length $\geqslant 3$ repeating units (calculated from polymerization statistics). From the GPC measurements a minimum solubilization of Ecoflex® of at least 99.9% was observed in the tests. This indicates that

during the depolymerization of the polymer chains either the aromatic sequences built water-soluble aromatic oligomers (e.g. if acid terminated) or are cleaved into smaller parts by the microbial enzymes. In the aqueous medium the monoesters BT and AT and minor amounts of the soluble aliphatic oligomers BAB and ABAB (from butanediol and adipic acid or terephthalic acid) could be detected as intermediates beside the monomers.

These data definitely prove that the longer aromatic sequences within the Ecoflex® copolyester are also enzymatically cleaved and do not remain as insoluble or soluble residuals. The short soluble oligomeric intermediates, however, are hydrolysed within 3 weeks into monomers, which are easily metabolized, when a mixed microbial population from compost is present.

In conclusion, a complete degradation of Ecoflex® under conditions present in a composting system can be concluded from these experiments, taking into account conservative lower detection limits of the analytical methods. There is no evidence that an accumulation of aromatic components (soluble and insoluble) occurs and the intermediates released during the degradation exhibit no acute ecotoxicological effect up to concentrations, which will never be present in a real compost system. The monomers themselves, formed as main depolymerization products, were intensively investigated with regard to their toxicological impact (BUA-Stoffbericht 68, 1991; BUA Stoffbericht 69, 1991; BASF-AG, Ökologische Stoffdaten-1,4-Butandiol, 1994), and exhibit no critical toxic behaviour.

From these results, it can be forecast that aliphatic–aromatic copolyesters comparable to Ecoflex® can be fully degraded in an environmentally safe manner in a composting process.

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