Aromatic components in copolyesters: Model structures help to understand biodegradability

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SUMMARY: Copolyesters build of aliphatic and aromatic components have been shown to be degraded by microorganisms in a certain range of composition.
While aliphatic polyesters of diol and dicarbonic acid often are hydrolyzed e.g. by lipases, pure aromatic polyesters like polyethylene terephthalate are not biodegradable. To understand the degradation mechanism of complex aliphatic/aromatic copolymers, we performed degradation experiments with different enzymatic systems and also especially screened highly active microorganisms. Several polymers and monomeric esters representing distinct structure elements within the polymer-chain have been synthesized. These model structures were investigated in terms to understand the correlation of the stereo selectivity of the tested enzymes and the biodegradability of the polymer structure.

Introduction

Recently, polymers, which can be degraded by microorganisms are discussed to be a serious contribution in solving parts of the increasing waste problems. A number of biodegradable polyester materials are already available on the market[1)], many of them based on polyesters.
For industrial application pure aliphatic materials do not always provide optimal properties for use. Polycaprolactone, for example, which is applied in various biodegradable blends (mainly with starch) has only a melting point of 60°C. This excludes the corresponding products from applications at elevated temperatures, for instance cups for hot drinks.
Therefore, some years ago we looked for a way to improve the properties of biodegradable aliphatic polyesters and we started to synthesize copolyesters of aliphatic and aromatic monomers[2)]. In this paper, we present some experiments which help to understand the complex degradation mechanism of these aliphatic/aromatic copolyesters and the relationship between polymer structure and biodegradability, using also model substances.

Experimental

Materials

Adipic acid, toluene-4-sulfonic acid (monohydrate) and phenol were purchased from Merck

 CCC 1022-1360/98/$ 10.00

(Darmstadt, Germany), 1,4-butylene glycol, trifluoroacetic anhydride and 1-butanol from Riedel-de Haen (Seelze, Germany), phenyl benzoate from Aldrich (Steinheim, Germany).

Synthesis

All polyesters were synthesized by bulk polycondensation as described elsewhere[2].

The aromatic model oligomer was prepared by bulk polycondensation by applying a twofold surplus of diol[3].

The model esters dibutyladipate and 1,4-bis-benzoyloxy-butan were synthesized by bulk polycondensation with toluene-4-sulfonic acid as catalyst. Phenyl adipate was prepared according to Bourne et al.[4]. Dibutyladipate was purified by distillation (107°C, 1hPa), 1,4-bis-benzoyloxy-butan and phenyl adipate were extracted with chloroform and washed with 1M Na_2CO_3.

Measurements

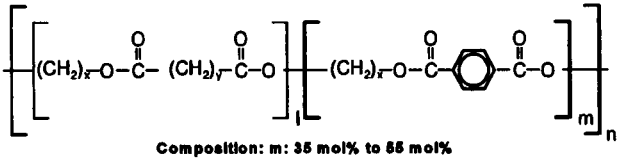
Enzym assay: A 10ml flask equiped with a pH microelectrode (U 402 M6 S7; Ingold Meßtechnik GmbH, Steinbach/Ts., Germany) was used as reaction vessel. Registration of the pH value was performed by an automatic titration system (TTT 80 Titrator, PHM 82 pH-Meter, ABU 80 Autoburette; Radiometer, Copenhagen, Denmark). 5.4 ml of a solution of 0.9% sodium chloride in deionized water was equilibrated to 30°C and stirred magnetically. Nitrogen, washed with 5M KOH, was used as inert gas. 40 mg of the model ester sample and 600 µl of a solution (4 $mg \cdot ml^{-1}$ in sodium chloride solution) of lipase from *Rhizomucor miehie* (Fluka analysis no. 43578/1 686) were placed in the flask and the pH value was kept at pH 7.00 by continuous addition of 0.05M NaOH. Monitoring of sodium hydroxid addition was done by a computer system. In the blank tests, enzyme was omitted from the reaction mixture.

Agar plate tests: The degradation tests were carried out at 55°C with BTA-films (2.5 cm diameter, 100 µm thickness) on mineralsalt agar plates. Films were inoculated with suspensions of (a) a mixed culture grown (pre-adapted) on BTA and (b) actinospores of a thermophilic actinomycete[5], both originated from mature compost made of green waste. Weight loss of polymer films was monitored over a period of time.

Results and discussion

Biodegradation of aliphatic/aromatic copolyesters

For synthesis of the copolyesters different diols, two different aliphatic dicarboxylic acids and terephthalic acid as aromatic component were used (Fig. 1). Some of these educts are cheap bulk chemicals, and some of the monomers are also available from renewable resources.



Composition: m: 35 mol% to 65 mol%

Diols			Dicarboxylic acids		
1,2 Ethanediol	(X=2)	=> E	Adipic acid	(Y=4)	=> A
1,3 Propanediol	(X=3)	=> P	Sebacic acid	(Y=8)	=> S
1,4 Butanediol	(X=4)	=> B	Terephthalic acid		=> T

Available from renewable resources

Fig. 1: Monomer components used for synthesis of the aliphatic/aromatic copolyesters

Best results with regard to the use properties were obtained with copolyesters from 1,4-butanediol, adipic acid and terephthalic acid, abbreviated as BTA-copolyesters (UPAC nomenclature for BTA: Poly(tetramethylene-terephthalate-co-tetramethylene-hexanedioate))[2]. Within certain limits of composition, which range approximately from 30 to 50 mol% of terephthalic acid refered to the total amounts of acids, materials with improved properties compared to the pure aliphatic components were obtained, which are still biodegradable in terms of weight loss measurements [Fig. 2].

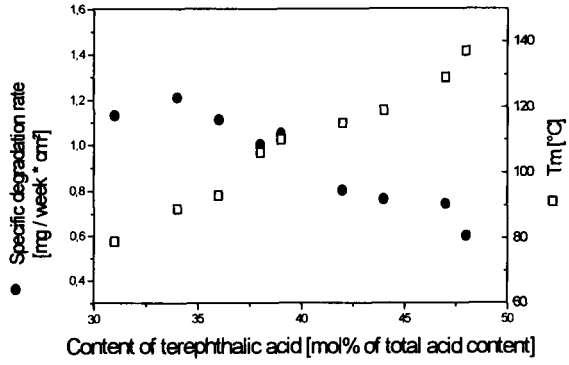


Fig. 2: Erosion rates (●) and melting points (□) of BTA-copolyesters depending on the amount of terephthalic acid in the acid components.

The data of Fig. 2 have been obtained by using mixed cultures in a simple agar plate test. Additionally, we screened for BTA degrading microorganisms with high depolymerisation potential. With the help of such microorganisms, improved degradation tests can be performed under defined laboratory conditions. Highest degradation rates were obtained with an aerobic grown thermophilic actinomycete which was isolated from compost. These actinomycetes degrade films of the BTA-copolyester approximately tenfold faster than it is observed in compost material or even with pre-adapted mixed cultures from compost [Fig. 3]. Tests can now be performed in a much faster time scale and patterns of intermediates can be analysed in the synthetic media used.

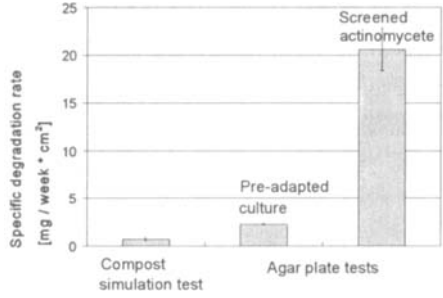


Fig. 3: Degradation rates of BTA-copolyesters films with different laboratory biodegradability test systems. Degradation rates were calculated from data of the linear area of degradation curves.

Biodegradation of aromatic model oligomer

The degradation data in Fig. 3 are based on weight loss measurements. Defining biodegradation of polymers as a complete metabolization of the material by microorganisms[6], weight loss of a sample is not sufficient to prove real biodegradability. The metabolization usually is characterized by measuring microbial metabolic products like carbon dioxide. Improved methods use carbon balances for the evaluation of biodegradability[7]. However, the aliphatic/aromatic copolyesters are only poorly degradable in synthetic aqueous media and carbon balances can not really be calculated in complex matrices like soil or compost.

Thus, due to the analytical limitations of the standard biodegradation test methods, the existence of not degradable residues in a range of approximate less than 10% of the initial polymer mass can not be excluded by sure.

Even in random type copolyesters as the BTA material[8], aromatic blocks of a length of some repeating units can be built in an amount of several mass percent during primary degradation of the polymer. Taking into account that aromatic polyesters like polyethylene terephthalate are resistant against microbial attack the question arises, what happens with residues consisting of some aromatic repeating units? To answer this question we synthesized aromatic model oligoester of 1,4-butanediol and terephthalic acid by applying a surplus of alcohol in the polycondensation reaction[2]. These oligomers represent potential aromatic intermediates from a primary degradation of the aliphatic/aromatic copolyesters. The materials were characterized by size exclusion chromatography (SEC) and mass spectroscopy measurements. Average molar masses of the oligomers ranged from approx. 600 g/mol to 2000 g/mol (SEC measurement referred to polystyrene standards). Distinct degrees of polymerization up to 6 can be detected. It was expected that the degradability of the oligoester decreases with increasing chain length. In Fig. 4 the size exclusion chromatograms of the oligoesters are shown before and after biodegradation experiments in different environments. The dashed line represents the oligomer material after storage under sterile conditions in water.

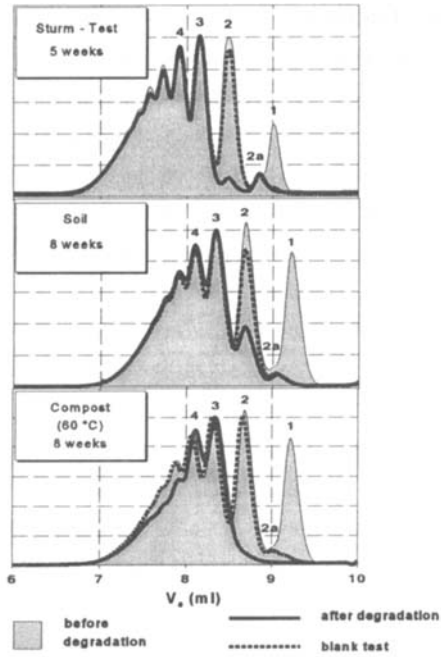


Fig. 4: Size exclusion chromatograms of an oligomer mixture (oligoesters from terephthalic acid and 1,4-butanediol) before and after incubation in different environments.

Surprisingly, only the monomer and the dimer of the oligomeric material had been degraded. Taking into account the results from the blank tests where the monomer and the dimer exhibited a certain solubility in water, it can be supposed that the degradation of the soluble oligomers takes place within the microbial cells, while probably no exoenzymes are present, being able to cleave the aromatic ester bonds of the insoluble material. This would imply that depending on the composition, from every copolyester a certain amount of aromatic blocks would remain undegraded. However, in the composting test at 60°C also longer oligomers have been shown to disappear, probably due to a simple chemical hydrolysis. This would mean, that the aliphatic/aromatic copolymers finally will degrade totally in the environment.

Enzymatic degradation of model esters

From the degradation experiments with the aromatic oligomer the question arose, why aromatic

oligoesters are so resistant against microbial attack. For this investigation we choose enzymatic degradation experiments with especially synthesized monomeric model esters, representing distinct structural elements in a virtual polymer chain. Using these low molecular substances, the influence of other, polymer related parameters like molar mass or crystallinity could be separated from the substrate specificity of the enzymes used. For the experiments a commercially available lipase from *Rhizomucor miehie* was used, which has been shown to degrade a number of different aliphatic polyesters[9]. On the right side of Fig. 5, the degradation results of some model esters are shown.

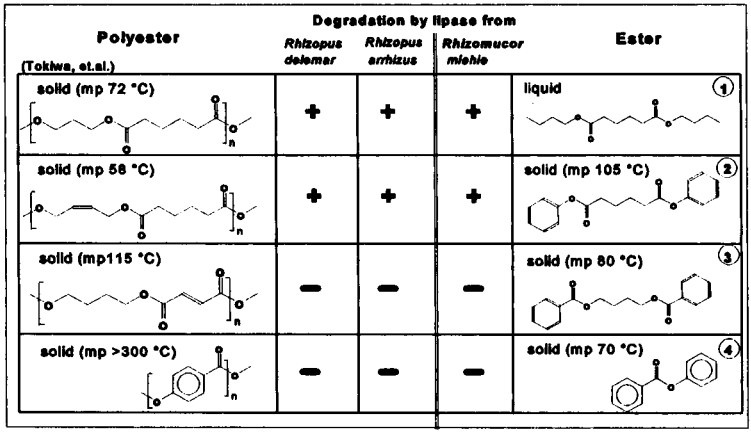


Fig. 5: Degradability of different ester structures by lipases

The pure aliphatic ester dibutyladipate (substance 1 in Fig. 5) is hydrolyzed by the lipase, while 1,4-bis-benzoyloxy-butan (substance 3 in Fig. 5), which represents the aromatic component in BTA-copolymers is not attacked by the enzyme. Also phenyl benzoat is resistant to enzymatic hydrolysis. However, an ester of an aliphatic dicarboxylic acid and a phenol (substance 2 in Fig. 5) is cleaved. Apart from the orientation of the ester group, this structure is very similar to the not degradable ester from benzoic acid and 1,4-butanediol. Thus, the diacid group seems to determine the accessibility of the ester group to the active site of the enzyme.

Instead of monomers Tokiwa et al.[10] (left part of Fig. 5) synthesized polymers, which were subjected to an enzymatic degradation tests with lipases from *Rhizopus delemar* and *Rhizopus arrhizus*. Comparing the results obtained with monoesters and polyesters, some interesting correlations can be seen. All polyesters containing an aromatic structure or a double bond in the

diacid component were not enzymatically hydrolyzed, while a double bond in the dialcohol component does not exclude the enzymatic cleavage. It is likely, that the rigidity of the diacid component influences significantly biodegradation.

Furthermore, it can be concluded from this comparison, that not preferably the melting point is the controlling parameter, as Tokiwa stated in his publication, but the molecular structure. Substance 2 in Figure 5 with a melting point of 105 °C was degraded by the lipase, while Substance 3 with a much lower melting point (80 °C) kept unhydrolyzed.

Although, these results are of basic importance, it has

to be considered, that only the degradation with a very specific kind of lipases has been investigated here. Further enzymes, lipases and other hydrolyzing enzymes, have to be taken into account, when discussing the relation between biodegradation and polymer structure in general.

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