Strategies for Detecting Ecotoxicological Effects of Biodegradable Polymers in Agricultural Applications

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Summary: Biodegradable is the long awaited and highly accepted property for materials (polymers) used in agricultural applications. Methods for determining biodegradability and material disintegration are established and already in use for routine analysis. Methods for analysing ecotoxic effects caused by biodegradable materials are neither established nor in routine use. In the past, biotests have been developed and optimised solely for investigations of single chemicals. Such tests are applicable even if they are not validated for the detection of undesired ecotoxicological effects deriving from biodegradation of polymers in soil. Since the biodegradation process does have an influence on the physical, chemical and biological status of the soil matrix, both biotests and chemical analysis are required in many cases. Theory, background data from method development and some results are presented.

Keywords: biodegradable materials; ecotoxicity; plant growth; soil quality

Introduction

The market relevance of biodegradable polymers will increase in the near future. A benefit for reduction of landfill-waste, a high public acceptance and a positive company image are the driving forces behind their propagation[1]. Farmers are highly motivated to use biodegradable polymers for agriculture because of savings of personnel time. Due to the biodegradation of agricultural tools, for example mulching foil, re-collection and disposal after use is no longer necessary. The polymer biodegrades on site.

The compatibility to crop production is of obvious interest for the farmer, but degradation residues and metabolites should not be formed and they should neither accumulate in the soil nor drift into the groundwater. Obligatory investigations at the stage of material production or product design can act as a safety net before market release of any biodegradable polymer. Those investigations could either be based on conventional chemical analysis to detect and

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identify metabolites or on ecotoxicological analysis to detect undesired effects to various soil organisms of different trophic level. The first may give full information on the chemical composition of substances without a direct answer concerning their toxicological relevance. The second provides direct information about ecotoxicity, cytotoxicity and maybe genotoxicity but does not identify the responsible substances[2].

Estimation of Ecotoxic Effects - Theoretical Approach

The probability of the formation of degradation residues and metabolites could be roughly estimated by three simple indicators:

1) In many cases microbial enzymes are responsible for the cleavage of polymer bonds[3]. The sterical accessibility could be derived from the chemical type of the bonding[4]. For some polymers a thermal hydrolysis or oxidation step is necessary to achieve inherent biodegradability. In summary, the more complex the needed prerequisites for biodegradation, the higher is the probability for the appearance of degradation residues, independent if they are toxic or not.
2) If hetero atoms (other elements than C, H and O) or aromatic monomers are part of the polymer the probability for appearance of ecotoxic degradation metabolites increases[5].
3) If heavy metals and other known toxic or harmful substances are added to a biodegradable product the probability for a release of toxic substances is obviously very high.

However, the final information about any ecotoxic effects caused by the degradation of polymers is preferably obtained by the application of biotests. The organisms used for such tests should be chosen with care. Target organisms, like higher plants are most important to gain information about the influence on the crop production. Biotests with other soil organisms, like earthworms, protozoa, soil bacteria and soil algae, are important to assure compatibility of the biodegradable polymer with the soil ecosystem. Only a stable ecosystem is capable of providing long-term soil fertility[6]. For scientific studies on inhibition mechanisms it could be of interest to apply aquatic biotests with Daphnia, freshwater algae and luminescent bacteria[2]. An overview about available standardised biotests, their

advantages and disadvantages is best obtained from basic literature[7, 8] or from standard collections. But it should be kept in mind that all those biotests were developed for testing of chemicals (for example pesticides) and may need adaptation for the analysis of biodegradable polymers.

Biodegradable polymers could be considered as organic matter that is introduced in the soil ecosystem. A direct comparison with the behaviour of organic matter of biogenic origin could be done. It is well known that harvest residues (green plants), organic fertiliser (stable manure) and other organic biodegradable substances can contribute positively to the physical structure and will therefore indirectly increase the soil fertility. Higher water holding capacity and elevated ion exchange capacity are the most often claimed causes for such improvements. Available agricultural publications do consider only natural polymers, such as starch, cellulose, ligno-cellulose (wood), proteins and fats. Several authors attributed the positive influences to plant growth, crop yield and crop quality to an increased content of organic matter (humic substances) in the soil [9, 10, 11].

Very often negative effects on plant growth were also described, caused by the presence of biodegradable substances in soil. The most prominent is the formation of toxic fermentation by-products released in the early stages of the biodegradation of organic substances[12, 13]. The prime reason for reduced plant growth is the generally increased microbial activity, which may further lead to a drop of the pH-value and to an abnormal high oxygen demand[14]. All those effects are of temporary nature and will end soon after the biodegradation is completed. Other negative impacts are explained by the mobilisation of heavy metals, which are already present in the soil. While metals, which are bound to or are included in the mineral matrix behave inert to the ecosystem, the mobile and therefore bioavailable fractions can cause serious harm to plants and animals and can accumulate in the food chain[15].

Some of the few available data are presented in the following, to demonstrate how ecotoxic effects caused by the biodegradation of polymers were detected and how they were distinguished from influences caused by microbial activation and non-toxic changes in the soil[16,17].

Materials and Methods

The biodegradable polymers and materials were: starch (J.T.Baker, No. 4010), cellulose (Fluka, No. 22181), wood in the form of saw dust (J. Rettenmaier, Lignocel BK 40-90), two biogenic materials based on the combination of starch with wood (IFA-Tulln, FASAL F129) and with sugar-beet residues (IFA-Tulln, ÖKOPUR, research lot) and a synthetic poly(ester-amide) (Bayer, BAK 1095). These materials were chosen to vary in chemical nature and to represent resources of synthetic and natural origin.

All reagents used for chemical and instrumental analysis were of analytical grade.

Soil. Commercially available garden soil was used for plant pot experiments. For the bench scale biodegradation experiment field top soil from Tulln, Austria, was used and characterised for the common physical and chemical parameters[17].

Organisms for ecotoxicity tests. Seeds of cress, rape, millet (higher plants) were purchased from a local distributor and used from one batch for the whole series of tests. Earthworms (*Eisenia foetida*) were obtained from a local pet shop. *Daphnia magna* was obtained from the High School for chemistry, Vienna. The LumisTox testkit with *Vibrio fisheri* was obtained from Dr. Lange (Art. No. LCK 486). Daphnia and earthworms were held in continuos culture at the institute and were tested for their sensitivity against standard substances before each biotest according to the requirements in OECD and DIN standards.

For all ecotoxicity tests, the procedures given in the respective DIN and OECD methods were followed. For plant tests a specific watering procedure was developed and was included to extend the standard method. All inhibition results were related to those obtained from soil samples without any additions; no synthetic references were used.

Bench scale soil degradation test. 40 kg soil were mixed with 800 g of the respective biodegradable polymer, distilled water was added to adjust a dry matter content of approximately 85 %. The mixtures were placed into solid state fermenters of 2 m height and 80 cm diameter. Periodically, air was pumped through the soil layer to ensure aerobic conditions. The experiment run at outdoor conditions without further temperature control.

Samples of approximately 4 kg were taken after 0, 14 , 28, 55, 80 and 160 days. At those times, the evaporated water was replaced and the soil was mixed[16].

Leaching of solid samples. The equivalent of 100 g soil sample dry matter was filled up to 500 g with water (for inorganic ions) or 0.01 M HCl (for organic acids). After 24 hours overhead shaking, the suspension was roughly filtered through a 0.5 mm sieve and centrifuged for 30 min at 3000 G. The supernatant was passed through a paper filter. All of the chemical and ecotoxicological analysis were performed on this extracts without any further treatment. Extracts were stored for a maximum of three weeks at -20°C if necessary.

Determination of anions and fatty acids. The inorganic anions nitrate, sulphate and phosphate and the short chain fatty acids formiate, acetate, propionate and butyrate were determined by isotachophoresis. Previously a method was developed, that allowed the simultaneous analysis of all the ions in the extracts without any further sample treatment[17].

Results and Discussion

Plant pots for breeding seedlings in the greenhouse were one of the first applications of biodegradable polymers. It was intended to set the pre-cultivated young plants together with the pots in the field. Cylindrical pots with 8 cm diameter and 8 cm height were produced from different biodegradable materials by injection molding. They were filled with commercially available garden soil and three seeds of tomatoes were added to each pot. After three weeks of incubation in a glasshouse, germination and plant biomass production were determined (Figure 1). Plants grown in readily biodegradable pots were dramatically smaller than those grown in not degradable PE-pots under the same conditions.

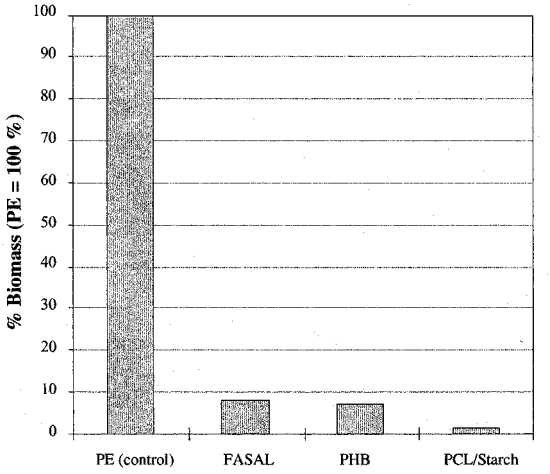

Figure 1. Biomass production (fresh weight) of tomatoes in biodegradable plant pots after three weeks of growth in the greenhouse. PE = Polyethylene, PHB = Poly(3-hydroxybutyrate), PCL = Poly(ε-caprolactone).

In the following experiment, biodegradable materials were ground to a particle size below 0.5 mm and mixed in a rate of 2% by weight with the same garden soil. The mixtures were separated for analysis of respiration and plant biotests (Figure 2). Ready biodegradable substances, like starch and ÖKOPUR did inhibit seedling germination and plant growth.

Since the observed plant incompatibility with starch, could not be explained by a chemical toxicity, additional biotests were done. The water content in the biotest trays was kept constant at different levels during germination and plant growth by restoring the evaporated water every 24 hours (Figure 3). With water contents above 100% saturation, when macro capillaries were filled at least partly, both germination and plant growth decreased significantly. The optimised water content was defined in the range between 60-100% of the water holding capacity for all subsequent biotests.

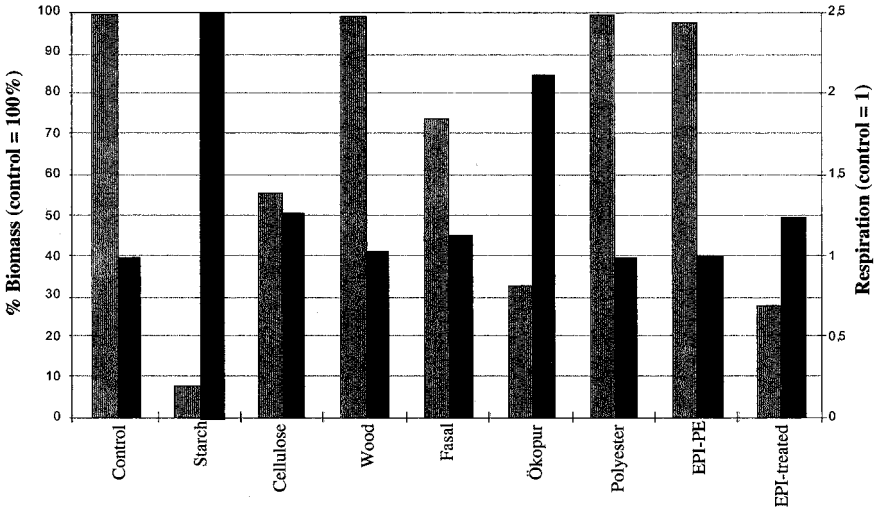

Figure 2. Biomass production (fresh weight) of cress in soil samples mixed with 2 % of biodegradable polymers and induced respiration rates in the same samples. ▩ = Plant biomass, ■ = Respiration rate relative to soil without additions.

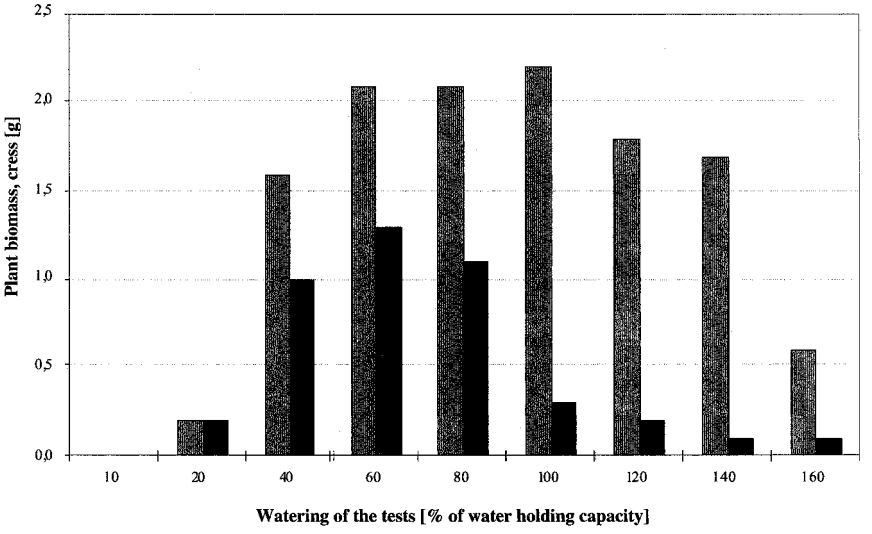

Figure 3. Biomass production (fresh weight) of cress grown in biotest trays at different watering levels. ▩ = Soil (control), ■ = Soil with 2% added starch.

In two plant biotest trays, redox-electrodes (Schott 31Rx) were mounted so that their contacts were in the centre of the layer consisting of garden soil (control) and soil mixed with 2% starch. The tests were watered at 100% saturation during the whole runtime. Data were recorded continuously (Figure 4). While the potential stood almost constant in the control, anaerobic conditions occurred during the degradation of the starch and a significantly reduced germination and plant growth was observed.

Analysis of relevant inorganic ions and organic acids were done from the bench scale soil degradation experiment. The decrease of the nitrate concentration in all experiments containing biodegradable materials is presented in figure 5. In contrast, the poly(ester-amide) did release nitrogen during its degradation. The appearance of acetate is shown in figure 6. Significant concentrations of acetate were formed only in experiments with starch and starch containing materials. Acetate does inhibit the ion transport in plant roots[18].

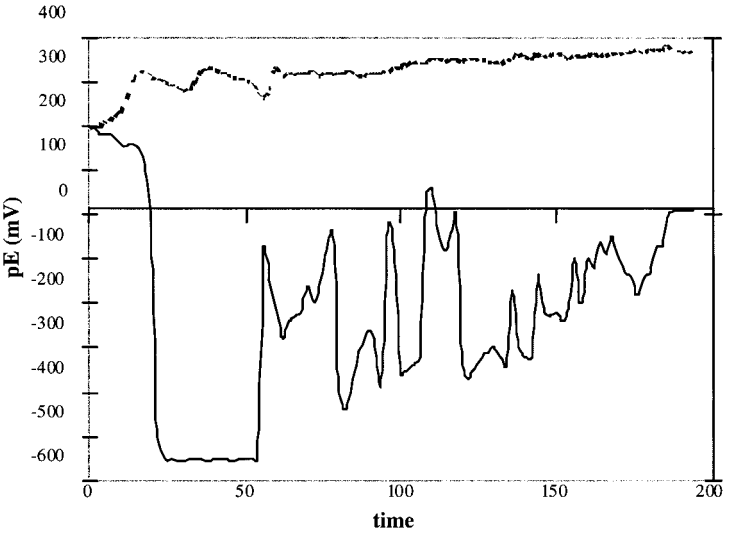

Figure 4. Influence of starch on the redox potential of plant tests at 100 % water holding capacity. Measured with a Pt-electrode positioned in the soil layer.

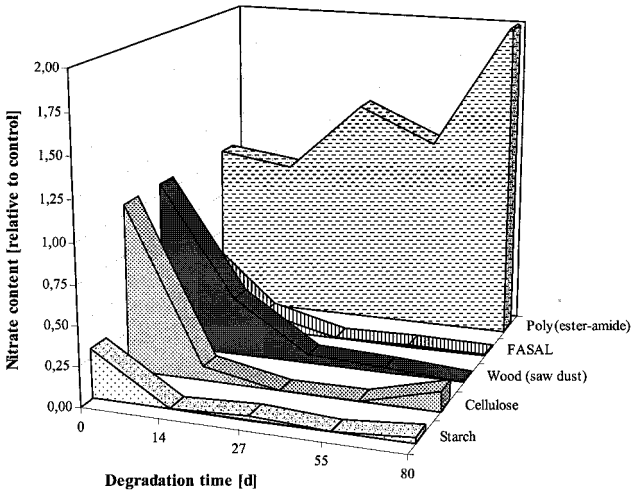

Figure 5. Variation of the nitrate concentration recorded during the bench scale biodegradation experiment. The values are referred to the control = 1. The initial concentration was 648 mg NO_3^-/kg dry matter.

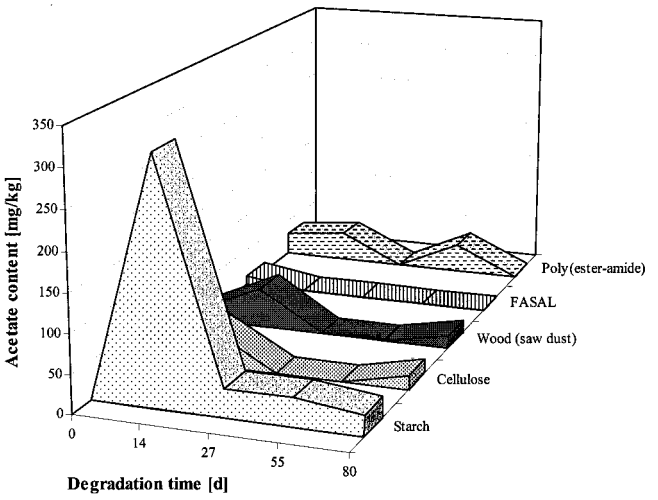

Figure 6. Variation of the acetate concentrations during the bench scale biodegradation experiment.

In parallel to chemical analysis, biotests with six different species were also performed. Starch did inhibit the growth of all three plant species at the beginning of the experiment but did not show significant inhibition to the other three species (Figure 7). After day 80, when the starch was degraded completely, no more inhibition was observed. The poly(ester-amide) did not show any significant inhibition to plants and other test species at the beginning of the experiment (Figure 8). But during the degradation of the polymer, inhibition of plant growth and minor inhibitions of Daphnia and bacteria was observed. Since neither limitation of nutrients (nitrogen) nor formation of organic acids occurred, the minor and major inhibitions of the five test species must have other reasons. No chemical analysis of specific metabolites was done. Nevertheless, it is demonstrated, that the appearance of ecotoxic effects could have different patterns over time. Earthworms were in no case inhibited as long as biodegradable polymers were present.

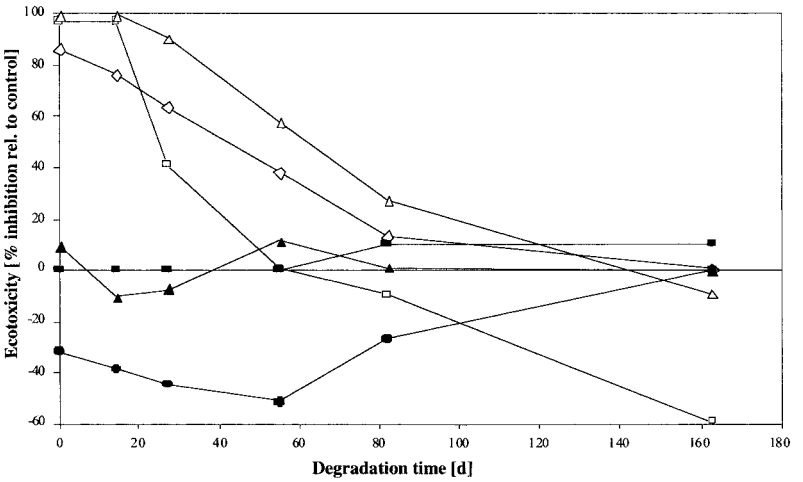

Figure 7. Ecotoxicity observed during the biodegradation of starch in the bench scale experiment. Inhibitions are plotted as positive values. ✧ = Cress, □ = Millet, △ = Rape, ● = Earthworm, ■ = Daphnia, ▲ = LumisTox.

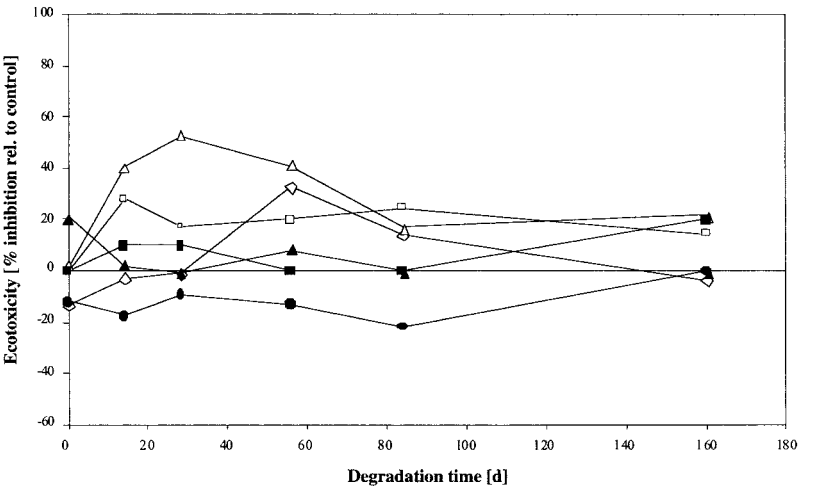

Figure 8. Ecotoxicity observed during the biodegradation of Poly(ester-amide) in the bench scale experiment. Inhibitions are plotted as positive values. ✧ = Cress, □ = Millet, △ = Rape, ● = Earthworm, ■ = Daphnia, ▲ = LumisTox.

To provide an overview including all six test substances, average values were calculated from individual biotest data at each sampling day (Figure 9). The averages are built by equal weight of each inhibition result. Such a summary does not provide new data but makes it easier to compare inhibition patterns between different samples. Starch, cellulose and wood did inhibit the plant growth significantly as long as they were not fully biodegraded but increased the plant compatibility of the soil at later time (160 days). The other three materials never reached such a positive effect during or after their biodegradation in the bench scale experiment. Most probably either ecotoxic metabolites were released or degradation residues were not compatible with plant growth.

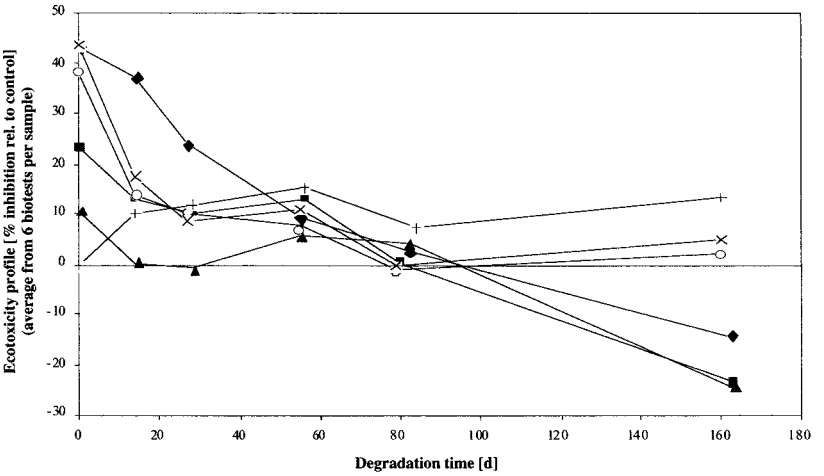

Figure 9. Average ecotoxicity (calculated from six single species biotests) observed in the bench scale experiment. Positive values are inhibitions and negative values are supported growth or biological activity. ◆ = Starch, ■ = Cellulose, ▲ = Wood, O = FASAL, x = ÖKOPUR, + = Poly(ester-amide).

Conclusions

The change of soil conditions due to microbial activities resulted in almost all cases in an incompatibility with plants and sometimes in inhibition effects against daphnia and bacteria. From single laboratory analysis, for example fresh mixtures of polymers in soil, no reliable ecotoxicity data could be expected. Only repeated measurements over a long-term degradation experiment could provide the basic data, which are needed to identify chemical ecotoxicity of metabolites and residues. Reduced plant biomass could not generally be interpreted as ecotoxicity caused by the polymer. On the other hand, as long as no biodegradation of a polymer occurs, no metabolites are formed and no effect should be expected in biotests.

Since earthworms are able to consume and digest undegraded organic matter, they do benefit from the inclusion of biodegradable polymers more than any toxic effect could harm them. Based on current knowledge, the earthworm biotest appears to be not suitable for testing

biodegradable polymers. Ecotoxicity profiles built as average of multiple biotests using different species are to prefer over biotests with only one species. Nevertheless, attention should be spent on reactions of individual test species, since each organism has specific sensitivities. The most critical detail in ecotoxicity analysis of biodegradable polymers is to differentiate between indirect effects caused by increased microbial activity and chemical toxicity of metabolites and residues. The observed changes of ion concentrations, namely appearance of short chain fatty acids and disappearance of nitrate were most probably caused by the activation of soil micro-organisms. Such biological interactions must not be mismatched with chemical ecotoxicity, even if their negative effect on plant growth is undesirable as well. For future regulations and for standardisation of biodegradable polymers, the described effects should be considered in addition to the analysis of the biodegradability.

Since nearly no ecotoxicity data are currently published, the few results presented here have to be seen as a first attempt. Continued research is necessary to validate the application of standardised biotests for samples deriving from biodegradation experiments.

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