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Effects of Various Parameters on Biodegradation of Degradable Polymers in Soil

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Abstract The effects of pH, moisture content, and the relative amount of a polymer sample on the biodegradation of degradable polymers in soil were studied using various polymer materials such as cellulose, poly-(butylene succinate-co-adipate) (SG) polycaprolactone (PCL), a blend of PCL and starch (PCL-starch), and a poly-lactic acid (PLA). As with other materials, the polymers degraded faster at a neutral pH than at either acidic or basic conditions. Moisture contents of 60 and 100% water holding capacity exhibited a similar biodegradability for various polymers, although the effects differed depending on the polymer. For synthetic polymers, biodegradation was faster at 60%, while the natural polymer (cellulose) degraded faster at 100%. Fungal hypae was observed at a 60% water holding capacity which may have affected the biodegradation of the polymers. A polymer amount of 0.25% to soil revealed the highest biodegradability among the ratios of 0.25, 0.5, and 1%. With a higher sample amount, the residual polymer could be recovered after the biodegradation test. It was confirmed that a test for general biodegradation condition can be applied to plastic biodegradation in soil.

Key words: Soil, biodegradation of polymer, biodegradation test

Biodegradable polymers have been regarded as a good alternative to solve the plastic waste problems caused by nondegradable synthetic polymers such as polyethylene and polystyrene. In the soil environment, plastics are mainly being used as a mulching film for agricultural purposes. When nondegradable plastic films such as polyethylene or polypropylene are used for this purpose, they must be collected after a certain period and treated in a solid waste treatment system. Accordingly, the collection and treatment of waste-mulching films is very troublesome and has become a major plastic waste problem. Sometimes, polyethylene fragments can remain in soil for a long time without degradation, therefore, repeated usage of polyethylene mulching films can result in an accumulation of these polyethylene fragments in the soil. Since biodegradable plastics will degrade in the soil environment after a certain period, the use of these plastics can reduce the plastic waste problem in soil.

To promote the usage of biodegradable polymers, proper evaluation methods for biodegradation are critical. Although many standard test methods have been proposed for biodegradable polymers [1-3, 5], there is no standard method for testing biodegradation in soil.

Many factors including the characteristics of the soil (pH, moisture content, sampling site, and date), test temperature, and relative sample amount are known to affect biodegradation in soil [7, 9, 11-15]. In some studies, the biodegradation test conditions used for plastic in soil are similar to those used with other materials [15]. That is, the test conditions were at pH 7.0, moisture content of 50 to 60% water holding capacity, and temperature of 20 to 30°C. Regarding the amount of the substrate in the soil, 0.5 to 1.0 mg/g soil was proposed as optimum [11]. However, plastic materials are different from the other materials with regard to their high hydrophobicity, crystalline, and solid states. As a result, longer biodegradation test periods are required compared to other materials. Since there have been few reports on biodegradation test conditions for plastics in soil [9, 13-15], it is not clear whether the above test conditions are suitable for plastic biodegradation.

This study investigates the effects of some key parameters such as soil pH, moisture content, and the relative amount of the plastic sample on the biodegradation of plastics to verify whether the test conditions mentioned above can also be applicable for plastic biodegradation.

MATERIALS AND METHODS

Polymer Samples

A synthetic aliphatic polyester, poly-(butylene succinate-co-adipate) (SG, grade 2109, SKI Corp., Korea) and

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cellulose (Sigmacell type 101, Sigma, U.S.A.), a natural polymer, were used as the main plastic samples. For the experiment related to the effect of soil moisture content on biodegradability, polycaprolactone (PCL, Tone 787, Union Carbide, U.S.A.), a blend of PCL and starch (PCL-starch, SK Corp, Korea) in which the starch content was 40% by weight, and poly-lactic acid (PLA, Kist, Korea) were also used. Except for PLA, all other polymer samples were either of film type with a 50- 100 μm thickness or powder with a size less than 250 μm. Cellophane film was used for the cellulose experiment where film was used. For PLA, a film with a thickness of about 1 mm was used.

Soil

Freshly collected sandy loam soil (moisture content, 13.9%; organic matter, 8.1%; pH 4.1) from the hill at our Institute was used in all experiments except for the biodegradation test with various soils. Big trees were located around the sampling site. Soil samples were collected at a depth of 10 centimeters below the surface. The pH of the soil was adjusted to around 7.0 by the addition of $CaCO_3$ at least 5 days prior to the test [10]. The soil moisture level was adjusted to a 60% water holding capacity except for the test on the effect of moisture content on biodegradation. $NH_4H_2PO_4$ was added to meet the initial C/N ratio between the polymer carbon and nitrogen below 40 and to provide enough phosphorus for the microorganisms.

Biodegradability Test

The tests were performed using biometer flasks (total volume of 650 ml) originally suggested by Bartha *et al.* [4]. This flask has a side arm where an alkali solution (KOH, CO_2 absorbent) absorbs the CO_2 generated by the degradation of the plastic sample in the soil. In the flasks, 300 g of soil and 3 g (1.0 weight % to soil) of the various plastic samples were added. The flasks were then incubated at a constant temperature of 30°C for a certain period. All biodegradation measurements were done in duplicate. The biodegradability was evaluated through the intermittent withdrawal and replacement of the alkali solution. After the replacement of the alkali solution, the headspace of the flask was refreshed with CO_2-free air.

Analysis

The CO_2 produced during the biodegradation test was absorbed in a 0.4 N KOH solution and precipitated with $BaCl_2$. The alkaline solution was titrated with 0.05 N HCl to determine the absorbed CO_2. The biodegradation of the polymers was defined as the percentage of the cumulative CO_2 production to the total CO_2 evolution (theoretical quantity) when all the carbon in the test material was converted to CO_2 [2]. For the recovery of residual polymer sample, solvent extraction was performed using Soxhlet. The soil samples after the biodegradation test were weighed, added into a cellulose timble, and put into the Soxhlet for extraction. Solvent (chloroform) was heated at 80°C and the extraction was proceeded for 24 h. The presence of PCL was identified using gel permeation chromatography (GPC).

RESULTS AND DISCUSSION

pH Effect on Biodegradation

The soil sampled in our Institute had a pH value of 4.1. To identify a proper pH value for a soil biodegradation test, the pH was adjusted to 4.1 (no change), 6.95, and 8.5 using $CaCO_3$. Cellophane and SG were used as the test polymers. For cellophane, the biodegradation degree was very similar at a pH of either 4.1 or 6.95 and a little lower at 8.5 (Fig. 1). The degree of biodegradation for cellophane at pHs of 4.1 and 6.95 reached 54% after 120 days, while at pH 8.5 it was slightly lower than 46%. Biodegradation started after a lag phase of a couple of days. The lag phase increased along with the pH value. At pH 8.5, the lag period was almost 20 days. That is, the adjustment of the pH caused the microorganisms to require a longer time period to adapt to a new environment than with the natural pH of 4.1. This effect was also observed with SG, a synthetic polymer (Fig. 2). The lag period increased along with the pH increase. After the lag phase, the biodegradation rate was highest at a pH of 6.95 and lowest at 4.1 for cellophane.

With SG, the biodegradation rate was very similar at a pH of either 6.95 or 8.5 and lower at 4.1. The degree of biodegradation for SG after 120 days decreased in the order of pHs 6.95, 8.5, and 4.1. Although these results reveal that the effect of pH on biodegradation is different with polymers, a neutral pH still seemed to be the most appropriate for the biodegradation test with the polymer samples tested.

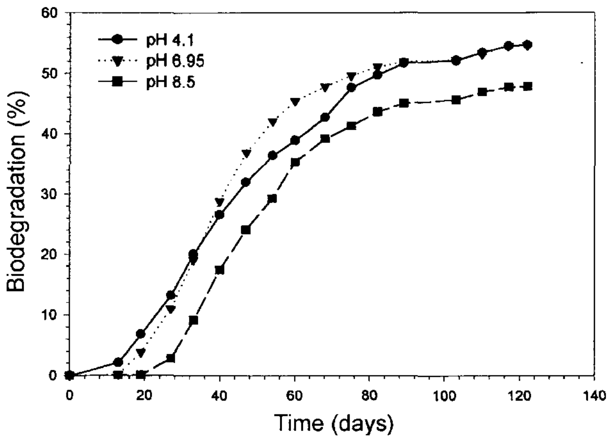

Fig. 1. Effects of soil pH on the biodegradation of cellophane in soil.

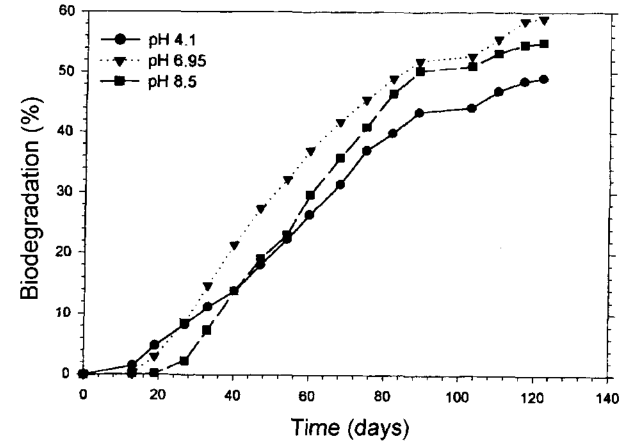

Fig. 2. Effects of soil pH on the biodegradation of SG in soil.

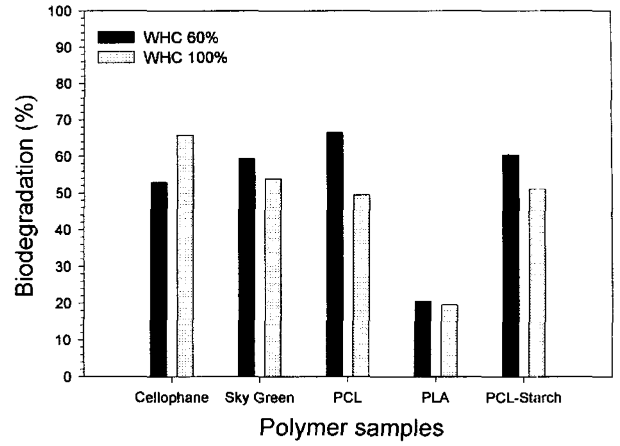

Fig. 3. Comparison of biodegradation of various polymers with two different moisture contents of 60 and 100% water holding capacity (WHC).
Biodegradation tests were conducted for 173 days.

Moisture Content Effect on Biodegradation

Moisture content is very important for the proper activity of microorganisms in soil. However, if moisture content is too high, it interferes with the transfer of oxygen from the headspace to a microorganism and, as a result, causes a local anaerobic condition which also affects the biodegradation activity of the soil microorganisms. In previous literature, the moisture content was adjusted to 50 to 60% of the water holding capacity (WHC) of the soil for a biodegradation test. Pramer and Bartha [10] found that for the aerobic biodegradation of simple or complex organic material in soil, a moisture content of 50 to 70% of the soil WHC exhibited the highest values. Later, Dibble and Bartha [8] showed that for oil sludge, the degree of biodegradation was identical at the three moisture contents of 30, 60, and 90% WHC. They postulated that the optimal moisture levels for the biodegradation of hydrophobic petroleum may be different from those for the biodegradation of hydrophilic substrates. In this study, the effect of the moisture content on the biodegradation of various polymers was compared at two moisture contents of 60 and 100% WHC. The biodegradation test was conducted for 173 days with soil collected in September.

As shown in Fig. 3, the moisture content affected the biodegradation differently depending upon the polymer. However, the degree of biodegradation was similar for all the polymers tested at both moisture contents. For the synthetic polymers, a moisture content of 60% was better than 100%, while 100% was better for the natural polymer, cellophane. Among the synthetic polymers, the difference in the degree of biodegradation was highest with PCL and lowest with PLA. When the synthetic polymer (PCL) and the natural polymer (starch) were blended together, the difference in the degree of biodegradation (9.2% for PCL-Starch) at the two moisture contents was smaller than that for the synthetic polymer alone (16.0% for PCL). These results were consistent with Yakabe's observation with a bacterial polyester PHB/HV [15]. He observed that the biodegradation rate increased with a moisture content within the range of 52 to 66%. Yet, in that study, they seemed to express the moisture content relative to the total weight rather than according to the water holding capacity.

It is possible that the moisture content affects the microbial community structure in soil. With a low moisture content, fungi may contribute more significantly to the biodegradation of polymer than with a high moisture content. It is known that fungi have advantages such as their high tolerance to pH fluctuations and to low water activities [8]. Cardenas-Gonzalez [Cardenas-Gonzalez *et al.* 1998. Correlation of biofilter media characteristics to biofilter performance. *Proceedings of 91st Annual Meeting Air and Waste Management Assoc*] also observed that fungal density increased as the moisture content decreased in a biofilter-treating VOC mixture consisting of ethylalcohol, butyraldehyde, ethyl acetate, and 1,1-diethoxybutane in air. In our study, the hyphae of fungi was observed on the soil surface with a moisture content of 60% WHC, while it was not with a 100% WHC. When soil samples around the polymers were collected and measured for the viable cell count, the number of bacteria was similar for most polymers at both moisture contents (data not shown). In contrast, the conidia count of fungi was at least 2 to 45 times higher with a 60% WHC than that with a 100% WHC (Table 1). Although the conidia count does not represent the fungal biomass, it may suggest that the growth of fungi was more active, since the conidia count was higher. Under the condition of fungal proliferation, a polymer which is degraded better by fungi than by bacteria will show a higher degree of biodegradation at a lower moisture content of 60% WHC than at 100% WHC.

Table 1. Conidia count of fungi in the soil around the plastic samples with two different moisture contents of 60 and 100 % water holding capacity (WHC).

WHC (%)	Sample (g^{-1})			
	Cellophane	PCL-starch	PCL	SG
60	8.7×10^9	9.8×10^9	9.0×10^9	1.0×10^{10}
100	3.9×10^8	2.2×10^8	3.2×10^8	5.2×10^9

Even though it was observed that fungi was more active at a moisture content of 60%, it is not certain that the polymer samples, which exhibited a higher degree of degradation with 60% WHC than with 100% WHC, are degraded better by fungi than by bacteria. Kennes observed a decrease of performance by fungal dominancy in a biofilter treating alkylbenzen vapor [6]. In the study, better performance was obtained in the biofilter with the bacteria-dominant community than with fungi.

The present results suggest that the optimal moisture content may vary depending upon the polymer. Nonetheless, as the degree of biodegradation was similar for the various polymers at both levels of moisture contents, a range of the moisture content between 60 and 100% WHC would seem to be an appropriate condition for a soil biodegradability test.

Effect of Plastic Sample Amount on Biodegradation

In this section, the proper sample amount for a soil biodegradation test was investigated. SG, a synthetic aliphatic polyester, was used as the polymer sample in both film and powder form. The polymer samples were added into soil at three different weight ratios: 0.25, 0.5, and 1% to soil. A sample amount of 1% was tested since some polymer degradation tests in soil have previously been conducted using this sample amount [13, 15]. For both types of sample, the addition of 0.25% revealed the highest degree of biodegradation after 180 days of cultivation (Fig. 4). Although the overall production of carbon dioxide increased along with the sample amount (data not shown), the degree of biodegradation decreased as the sample amount increased, because the biodegradation was defined by the ratio between the carbon converted to carbon dioxide and the total carbon present in the plastic sample.

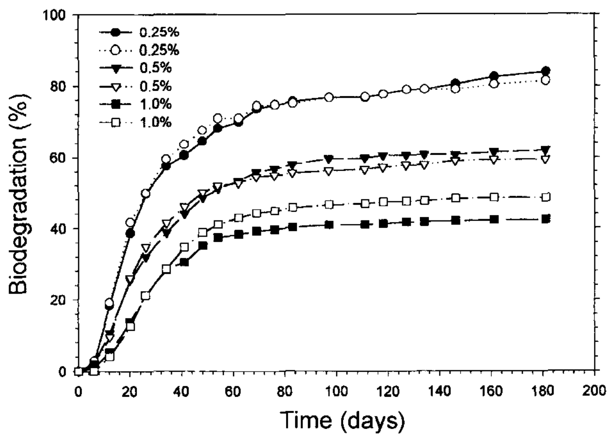

Fig. 4. Effects of the amount of a polymer sample on biodegradation in soil. Three different ratios of polymer sample (relative to soil weight) were mixed with soil. Aliphatic polyester Sky Green (SG) was used for this test. Open symbols represent the film type and close symbols represent the powder type.

Since the residual plastics were recovered by solvent extraction from the soil with sample amounts of 0.5 or 1.0% and identified as SG by gel permeation chromatography, the availability of the plastic sample was not a feasible explanation (data not shown). SG could not be recovered from the soil with a 0.25% sample. In addition, the C/N ratio was initially adjusted to around 24 by adding $NH_4H_2PO_4$ to prevent any nutrient limitation during the test. One possible explanation is that the transport limitation of nutrients may affect the growth of microorganisms in the solid state. As a result, it would seem that the polymer-degrading microorganisms could not grow fast enough to compensate for the increased sample amount in the solid state culture. Intermittent mixing (once per 1-2 weeks) was not enough to improve transfer of nutrients to the microorganisms. These results suggest that in a soil biodegradation test, the ratio of the sample amount to soil should be small enough to not be inhibited by the possible transport limitation of nutrients except for the carbon source. Considering the observation that substrate concentrations ranging between 0.5 and 1.0 mg/g of soil produced much more accurate results in a soil biodegradation test of liquid chemicals [11], the present results seems to be consistent with these observations. The powder form showed a slightly higher degradation with a sample amount of 0.25 and 0.5%, whereas a slightly lower level than film with a sample amount of 1%.

CONCLUSION

The effects of pH, moisture content, and the relative amounts of a polymer sample on the biodegradation of degradable polymers were studied to examine whether the biodegradation conditions applied to other materials could also be applied to polymer biodegradation in soil. As with other materials, polymers degraded faster at a neutral pH than at either acidic or basic conditions. Moisture contents of 60 and 100% water holding capacity exhibited similar biodegradability with the various polymers yet the effects differed according to the polymer. For synthetic polymers, biodegradation was faster at 60%, while natural polymers degraded faster at 100%. Fungal hypae was observed at 60% WHC which might affect the biodegradation of the polymers. A polymer amount of 0.25% to soil revealed the highest biodegradability among the ratios of 0.25, 0.5, and 1% tested. With a higher sample amount, the residual polymer could be recovered after the biodegradation test. Accordingly, it was confirmed that the general biodegradation test conditions in soil are also applicable for plastic biodegradation.

Acknowledgments

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REFERENCES

1. ASTM D0883-92. 1992. *Annual Book of Standards*, Vol. 08. 03.
2 ASTM D5209-92. 1992. *Annual Book of Standards*, Vol. 08. 03.
3 ASTM D5338-92. 1992. *Annual Book of Standards*, Vol. 08. 03.
4. Bartha, R. and D. Pramer. 1965. Features of a flask and method for measuring the persistence and biological effects of pesticides in soil. *Soil Science* **100:** 68- 70.
5. Charles A. Pettigrew. 1996. *ASTM Standard News*, September, pp. 32- 35.
6. Kennes, C., H. H. J. Cox, H. J. Doddema, and W. Harder. 1996. Design and performance of biofilter for the removal of alkylbenzene vapors. *J. Chem. Tech. Biotechnol.* **66:** 300-304.
7. Dibble, J. T. and R. Bartha. 1979. Effects of environmental parameters on the biodegradation of oil sludge. *Appl. Environ. Microbiol.* **37:** 729- 739.
8 Horner, K. J. and G. D. Anagnostopoulos. 1973. Combined effects of water activity, pH, and temperature on the growth and spoilage potential of fungi. *J. Appl. Bacteriol.* **36:** 427-436.
9. Kim, E. J., T. H. Park, and P. K. Shin. 1998. Measurement and acceleration of biodegradation in soil. *Kor. J. Appl. Microbiol. Biotechnol.* **26:** 465- 469.
10. Pramer, D. and R. Bartha. 1972. Preparation and processing of soil samples for biodegradation studies. *Environ. Lett.* **2:** 217- 224.
11. Sharabi, N. E.-D. and R. Bartha. 1993. Testing of some assumptions about biodegradability in soil as measured by carbon dioxide evolution. *Appl. Environ. Microbiol.* **59:** 1201- 1205.
12. Shen, J. and R. Bartha. 1996. Priming effect of substrate addition in soil-based biodegradation tests. *Appl. Environ. Microbiol.* **62:** 1428- 1430.
13. Spence, K. E., A. L. Allen, S. Wang, and J. Jane. 1996. Soil and marine biodegradation of protein-starch plastics. *ACS Symp. Ser.* **627:** 149- 158.
14. Yabannavar, A. V. and R. Bartha. 1994. Methods for assessment of biodegradability of plastic films in soil. *Appl. Environ. Microbiol.* **60:** 3608- 3614.
15. Yakabe, Y., K. Nohara, T. Hara, and Y. Fujino. 1992. Factors affecting the biodegradability of biodegradable polyester in soil. *Chemosphere* **25:** 1879- 1888.