Confirmation of Poly(1,3-dioxolan-2-one) Degrading Microorganisms in Environment

## Haruo NISHIDA\* and Yutaka TOKIWA†

Tsukuba Research Laboratory, Tokuyama Soda Co. Ltd., 40 Wadai Tsukuba, Ibaraki 300-42 
†National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, 
1-1, Higashi, Tsukuba, Ibaraki 305

Ecological biodegradability of aliphatic polycarbonates in different environments was estimated by the clear-zone method with an agar medium containing emulsified polycarbonates. It was found that poly(1,3-dioxolan-2-one) can be degraded by microorganisms in certain limited environments.

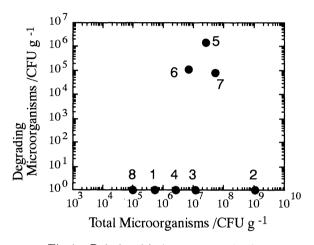
The study of the biodegradation of plastics is important for solving plastic waste problems. Many investigations concerning the biodegradation of thermoplastic resin have been mainly done with some well-known aliphatic polyesters. We recently investigated the ecological biodegradability of plastics in different environments to assess the capacity of the natural environment for accepting plastics. The populations of poly( $\beta$ -hydroxybutyrate)(PHB)- and poly( $\epsilon$ -caprolactone)(PCL)-degrading microorganisms in aerobic and anaerobic environments were estimated by the clear-zone method with an agar medium containing emulsified PHB or PCL. It was found that most cells that formed clear-zones on PHB-emulsified agar plates were able to degrade PHB.1)

In this report, aliphatic polycarbonates such as poly(1,3-dioxolan-2-one)(PEC; produced by Air Products, number-average molecular weight; Mn = 50000) and poly(4-methyl-1,3-dioxolan-2-one)(PPC; produced by Air Products, Mn = 50000) were used. Nakano<sup>4</sup>) reported that PEC was degraded enzymatically in the peritoneal cavity of rats but that PPC was not. Imai<sup>5,6</sup>) reported the enzymatic degradation of PEC by pronase. However, microbial degradation of polycarbonates has not been investigated. This is, to the authors' knowledge, the first report concerning the microbial degradation of polycarbonate.

Agar plates containing emulsified polycarbonates (1000 ppm) and yeast extract (250 ppm) (pH=7.1) were prepared on the basis of the earlier report. Soil samples were collected on 20 March 1992 from 8 locations in different environments at Tsukuba (Nos. 3-8) and its environs (Nos. 1 and 2); landfill leachate (No. 1 from Tokyo bay), sewage sludge compost (No. 2 from Tokorozawa in Saitama Prefecture), sewage sludge supernatant (No. 3), forest soil (No. 4), farm soil (No. 5), paddy soil (No. 6), roadside sand (No. 7) and pond sediment (No. 8). Each soil sample was diluted 10-10<sup>4</sup> fold with sterilized basal medium (pH 7.0). A 0.1-ml aliquot of each diluted sample was spread onto an agar medium plate containing emulsified PEC or PPC. The cultures were incubated at 30 °C for 15 d.

After 5-14 days of colony formation on some agar plates, a circular clear-zone formed around each colony containing a microorganism capable of degrading the polycarbonate. The numbers of visible colonies

and clear-zones were counted at specified times. The relationship between total colonies (total microorganisms) and clear-zones (degrading microorganisms) is shown in Figs. 1 and 2. The populations of total microorganisms and degrading microorganisms were expressed as colony- or clear-zone-forming units (CFU) per 1 g of sample. In three samples, farm soil, paddy soil and roadside sand, several kinds of colonies with clear-zones formed on the PEC-plates. The percentage of PEC-degrading microorganisms to total colonies was 0.2-5.7%. No colony with a clear-zone formed on the PPC-plates after the culture for 15 days. These results agree with those from the implantation tests.<sup>4</sup>)



Total Microorganisms /CFU g -1

Fig.1. Relationship between total microorganisms and PEC-degrading microorganisms formed on the plates containing emulsified PEC and yeast extract ( 250 ppm ); culture at 30 °C for 15d. The numbers marked represent the number of soil samples.

Fig.2. Relationship between total microorganisms and PPC-degrading microorganisms formed on the plates containing emulsified PPC and yeast extract (250 ppm); culture at 30 °C for 10d. The numbers marked represent the number of soil samples.

PHB- and PCL-degrading aerobic microorganisms are widespread over a great variety of environments. 1) However, the distribution of PEC-degrading microorganisms is limited. PPC seems to be unbiodegradable.

A total of 7 strains were isolated from colonies that formed clear-zones on the PEC-plates. Each strain was streaked onto a PEC-plate, and the clear-zone formation around the colonies was confirmed with 4-7 days culture after the inoculation.

The above results show that PEC can be degraded by microorganisms in certain limited environments.

## References

- 1) H.Nishida and Y.Tokiwa, J. Environ. Polym. Degrade., 1, 227 (1993).
- 2) H.Nishida and Y.Tokiwa, Kobunshi Ronbunshu, 50, 739(1993).
- 3) H.Nishida and Y.Tokiwa, J. Environ. Polym. Degrade., to be submitted.
- 4) T.Kawaguchi, M.Nakano, K.Juni, S.Inoue, and Y.Yoshida, Chem. Pharm. Bull., 31, 1400(1983).
- 5) Y.Imai, K.Kojima, A.Watanabe, and E.Masuhara, Jpn. J. Artif. Organs, 4, 344(1975).
- 6) Y.Imai, K.Kojima, A.Watanabe, and E.Masuhara, Jpn. J. Artif. Organs, 8, 246(1979).

(Received October 26, 1993)