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EFFECT OF POLYMER POSITION IN NUTRIENT-SALT AGAR MEDIUM ON FUNGAL DEGRADATION OF POLYCAPROLACTONE

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Abstract—In order to investigate the effect of contact type between microorganism and polymer on the degradation of polycaprolactone (PCL), fungal degradation test was performed by placing PCL films on the top, middle, and bottom of the nutrient-salt agar medium. After 3 weeks incubation, the pH values of agar containing PCL films on the top, middle, and bottom were 5.82, 3.78, and 4.06, respectively. The weight losses of the PCL films were 5.10%, 13.89%, and 6.92%, respectively. However, the molecular weight change by degradation was not observed. It is supposed that the degradation of PCL films on the top position was caused by biophysical, biochemical and direct enzyme action, but that of PCL films on the middle or bottom position was caused by biochemical and direct enzyme action.

Key words: *Polycaprolactone, Polymer Position, Fungal Degradation*

INTRODUCTION

Essential properties of durability and resistance to degradation in synthetic polymers have become an environmental concern because of its refractory nature in a landfill site [Evans and Sikdar, 1990; Swift, 1993]. Recycling and incineration can reduce the volume of solid waste of durable plastics to some extent. Landfill and composting can be alternatives for disposable plastics if biodegradability can be built into its characteristic functionality [Evans and Sikdar, 1990; Swift, 1993; Babinchak, 1991; Taylor, 1979]. Although biodegradable plastics have been recently under intense development, relatively less efforts were made in developing suitable test methods for evaluating the biodegradability of such materials [Swift, 1993].

Currently enzymatic, bacterial, and fungal degradation, soil and marine simulation, degradation by macroorganism, and compost simulation are used for testing the biodegradability of polymer materials [Huang et al., 1979; Tokiwa et al., 1988; Jesudason et al., Benedict et al., 1983; Leonas and Gorden, 1993; Gonsalves et al., 1991; Wool and Goheen, 1989; Greizerstein et al., 1993; Gu et al., 1993; Kim et al., 1992]. Fungal test method of ASTM G21-70 (ASTM G21-70, 1989) for solid surface growth involves casting polymer films on the surface of nutrient-salt agar plates. However, it is insensitive and ineffective for assessing biodegradability of synthetic polymers properly. Rhee et al. [1990] developed a soft agar overlay method by modifying the ASTM G21-70.

Maddever and Chapman [1989] suggested that the breakdown caused by microorganisms can be three different types:

1. A biophysical effect, in which cell growth can cause mechanical damage.
2. A biochemical effect, in which substances from the microorganisms can act on the polymer.
3. Direct enzymatic action, in which enzymes from the microorganisms attack components of the plastic product, leading to splitting or oxidative breakdown.

However, none of these was proven experimentally. Therefore, the objective of this study is to investigate some preliminary experimental evidence of the degradation type and the effect of polymer position on the fungal degradation of polycaprolactone (PCL) in a nutrient-salt agar medium. In addition, the relationship between pH and weight loss was studied.

MATERIALS AND METHODS

1. Polymer and Film Preparation

PCL was purchased from Aldrich Chemical Co. (USA). The films of PCL were prepared by compression molding between polyimide films, then aged 3 weeks at room temperature to reach an equilibrium crystallinity.

2. Fungal Degradation in Agar Plate

The microorganism used in this study was *Aspergillus niger* (KCTC 1374) obtained from Genetic Engineering Research Institute, Taejon, Korea. *A. niger* was kept on potato dextrose agar plates at 4℃ and reinoculated monthly. The cultivation medium described in ASTM G21-70 was used and the medium had the following composition, in g/L : KH_2PO_4, 0.7; $MgSO_4 \cdot 7H_2O$, 0.7; NH_4NO_3, 1.0; NaCl, 0.005; $FeSO_4 \cdot 7H_2O$, 0.002; $ZnSO_4 \cdot 7H_2O$, 0.002; $MnSO_4 \cdot 7H_2O$, 0.001; agar, 15. The initial pH was 6.45. The PCL films (5×5 cm in size) were sterilized with 70% ethanol and placed on the top, middle, and bottom of nutrient-salt agar medium as shown in Fig. 1. The polymer containing plates were inoculated with 1 mL of conidia suspension (5×10^5 conidia/mL), and were incubated in a moist chamber at 30℃ for 3 weeks. In addition, two controls were used. One (control 1) was used to observe the growth of *A. niger* in plate without PCL film. The other (control 2) was used to determine the hydrolytic degradation of PCL film in plate without the *A. niger*.

After incubation, fungal degradability of PCL was measured by changes in pH, weight loss, and molecular weight. The pH

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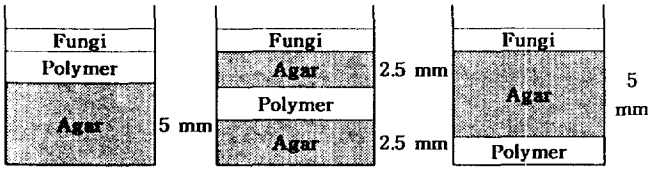

Fig. 1. Schematic diagram of polymer position in nutrient-salt agar medium.
(a) Top position, (b) Middle position, (c) Bottom position

measurements were made as follows. The agar containing fungi was placed in a 50 mL distilled water (pH 6.29) and stirred virgously for 5 hours. Then, the mixture was centrifuged at 2,500 rpm for 10 min. After centrifugation, the pH of supernatant was measured. The PCL film was washed with distilled water and dried *in vacuo* for 24 hours at 30℃, and then, weight loss and molecular weight were determined. The gold coated film was examined and photographed with scanning electron microscope (SEM; Philips, Model 525M/535M, The Netherlands).

3. GPC Analysis

Molecular weight data of PCL were obtained at 40℃ using a 150CV Waters GPC system equipped with refractive index detector and a series of 500, 10^3, 10^4, 10^5 Å μ-styragel columns. Tetrahydrofuran (THF) was used as the eluant at a flow rate 1.0 mL/min. A molecular weight calibration curve of PCL was obtained on the basis of universal calibration method with polystyrene standard samples of low polydispersities.

RESULTS AND DISCUSSION

For the fungal degradation test of PCL films, PCL films were placed on the top, middle, and bottom of nutrient-salt agar medium. The growth of *A. niger* was not observed in control 1 which did not contain PCL film [Fig. 2(a)] and control 2 which did not contain *A. niger* conidia suspension [data not shown]. While, the growth of *A. niger* was observed in agar plate containing PCL films. This indicates that soluble small molecules of PCL films produced by fungal degradation and/or hydrolysis [Pitt et al., 1981] serve as carbon sources of *A. niger*. Jun et al. [1994] reported that the enzymatic degradation products of PCL consisted of the molecules less than the hexamer of PCL. When PCL film was placed on the top position, *A. niger* was grown mainly on the edge of the PCL films [Fig. 2(b)]. While, the uniform growth was observed through the whole PCL films of middle and bottom positions [Fig. 2(c), (d)]. The most active growth was observed on the middle position among the tested. PCL film acted as diffusional resistance of the nutrient except the carbon source in the case of top position. And solidified agar acted as diffusional resistance of the biochemical and/or enzyme produced by *A. niger* and small molecules (carbon sources of *A. niger*) of PCL films

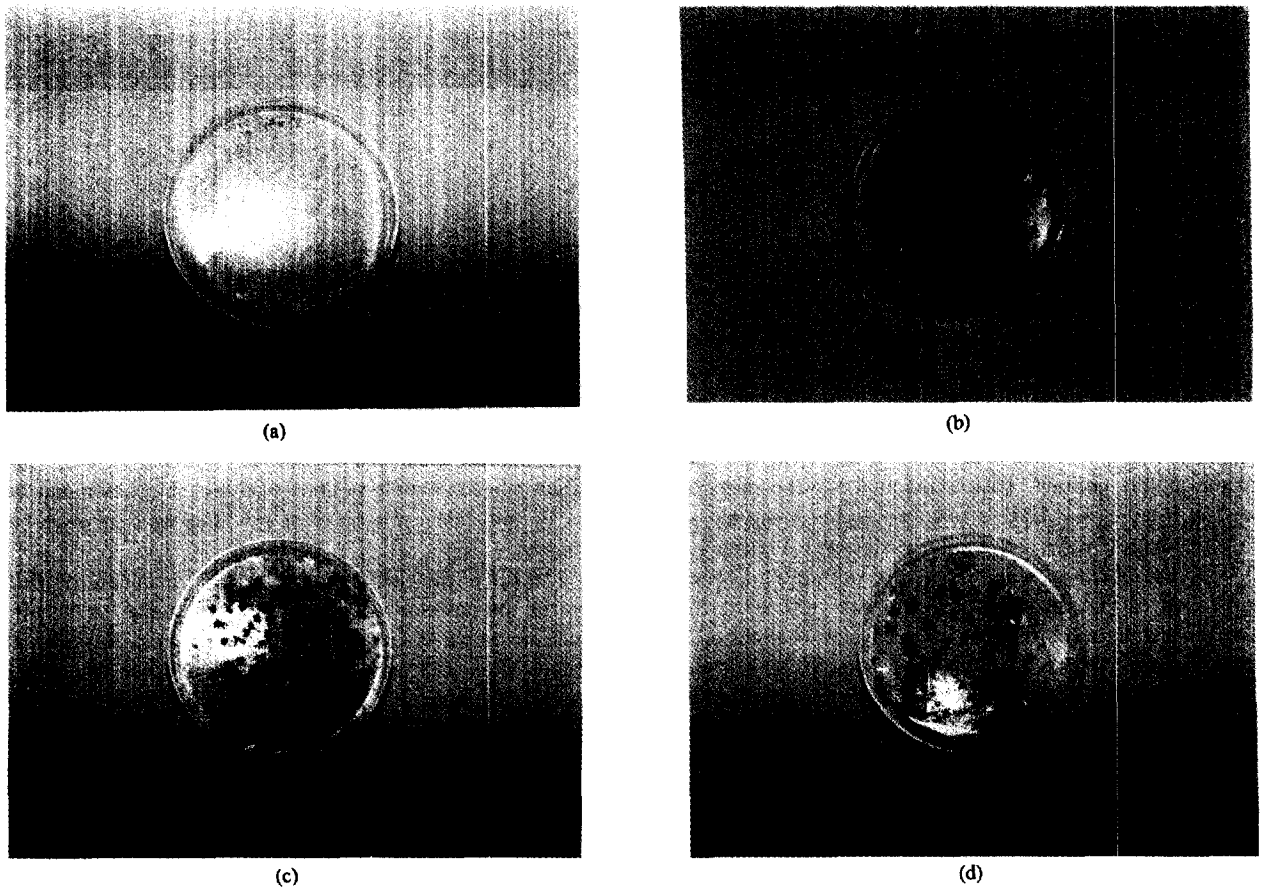

Fig. 2. Photographs of PCL film after 3 weeks incubation.
(a) Control, (b) Top position, (c) Middle position, (d) Bottom position

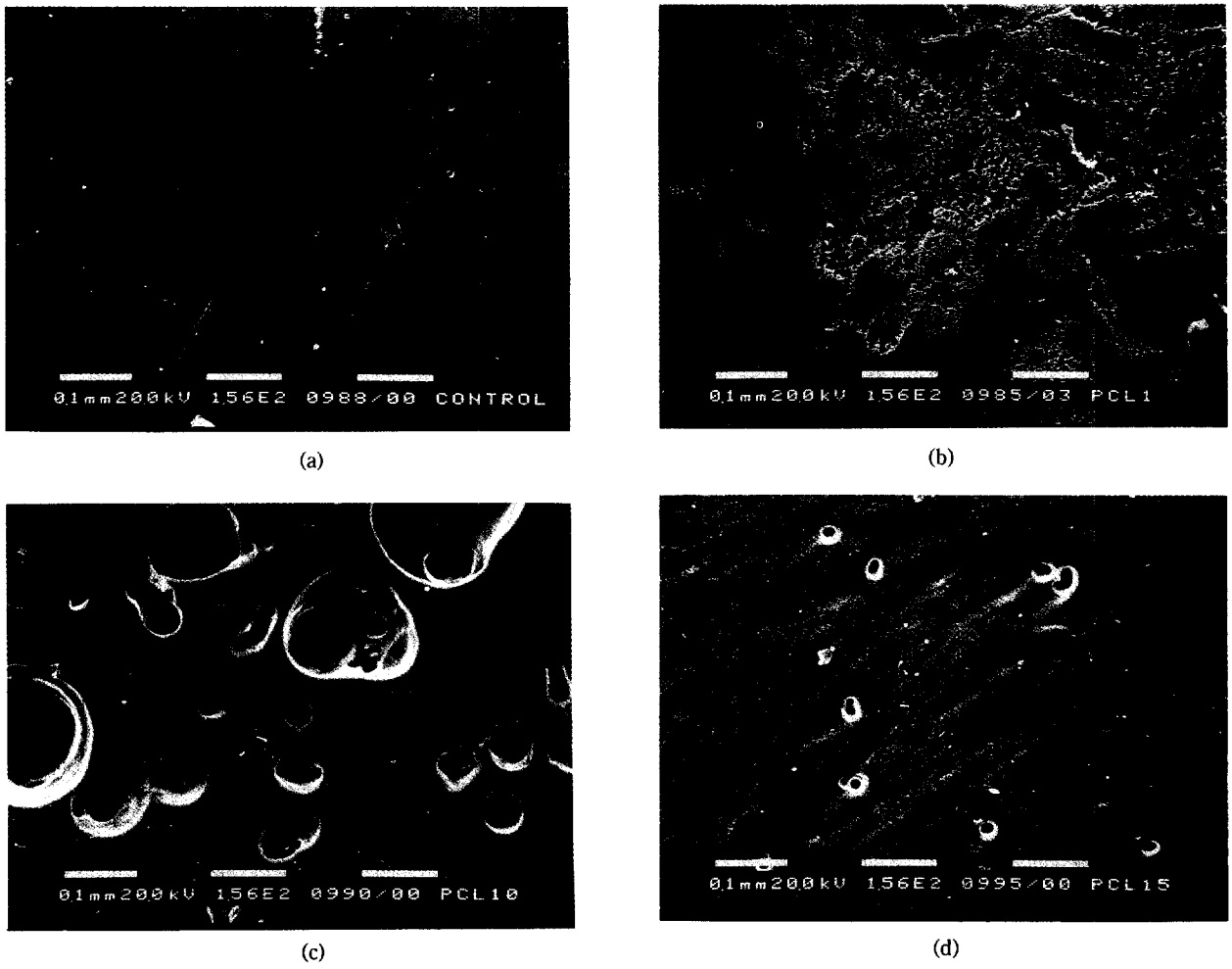

Fig. 3. SEMs (X156) of PCL film after 3 weeks incubation.
(a) Control, (b) Top position, (c) Middle position, (d) Bottom position

produced by degradation in the case of middle and bottom positions. The depth of solidified agar may influence the fungal degradation behavior, since the extracellular enzyme and/or biochemical involved in the degradation and small molecules serve as *A. niger*'s carbon sources and must pass through it. In the case of middle position, the depth of solidified agar was shorter than that in the bottom position. Therefore, most active growth was observed in the middle position. This assumption may be reasonable considering Rhee et al. [1990]'s results obtained using top agar (0.8%) containing *A. niger* conidia suspension and Augusta et al. [1993]'s results obtained using different concentrations of agar.

Degradation of PCL by fungal growth was verified by SEM as shown in Fig. 3, which clearly shows physical changes on the PCL film surface compared with control sample. As shown in Fig. 3(b), the small holes were formed along the mycelia when PCL film was placed on the top position. However, in the previous studies [Kim et al., 1992], the small holes were formed over the whole surface of PCL films when the degradation test was performed by only enzyme. Therefore, there is biophysical effect as well as direct enzymatic action when PCL film was placed on the top position. While, as shown in Fig. 3(c) and (d), the spherical holes of 0.05-0.1 mm and 0.01-0.02 mm in diameter were formed over the whole surface of the degraded PCL films placed in the middle and bottom positions, respectively. The physical changes of PCL film placed on the top position may be caused by biophysical, biochemical and direct enzymatic action. While, the physical changes of PCL films placed in middle and bottom position may be caused by biochemical and direct enzymatic action. There is no biophysical effect on fungal degradation of PCL films because PCL films didn't come in contact with fungi. The larger holes in middle position than bottom position may be caused by diffusional resistance of solidified agar as described previously.

The pH of the nutrient-salt agar that contained the PCL films on the top position was 5.82, which was similar to the pH of the control sample (5.78) without carbon source. However, the pH values were 3.78 and 4.06, which were lower than the control sample, for those that contained the PCL films in the middle and bottom, respectively (Table 1). The standard deviations were less than 7%. This indicates the pH decrease was caused by PCL degradation products which were the acid forms, and metabolic products (acidic biochemicals) of *A. niger*.

The weight losses of PCL films on the top, middle, and bottom were 5.10%, 13.89%, and 6.92%, respectively (Table 1). The standard deviations were less than 20%. As expected, the highest weight loss was obtained in the case of middle position. However,

Table 1. Fungal degradability of PCL film as measured by pH, weight loss, and molecular weight

Sample position	Control	Top[b]	Middle[b]	Bottom[b]
pH[a]	5.78	5.82	3.78	4.09
Weight loss (%)	-	5.10	13.89	6.92
M_n	20,581	19,078	19,113	20,136
M_w	30,998	29,471	29,261	30,197
M_w/M_n	1.51	1.55	1.53	1.50

[a]: pH of distilled water ; 6.29

[b]: Data are averages from hexaplicates.

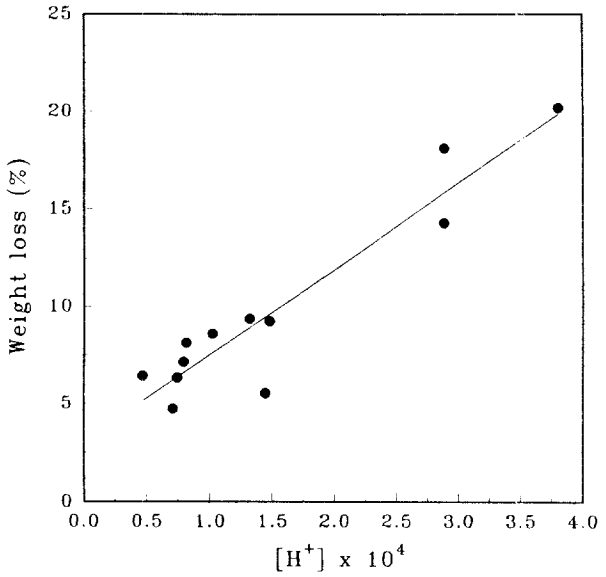

Fig. 4. Relationship between pH and weight loss.

the molecular weights and polydispersities of PCL films after incubation were almost the same as before incubation (Table 1). These results were consistent with the other's results obtained using lipases [Jun et al., 1994; Tokiwa and Suzuki, 1981]. It seems that the fungal degradation of PCL occurs on the polymer surface [Jun et al., 1994] and the enzymatic action is major degradative mechanism of fungal degradation.

As shown in Fig. 4, the weight loss was proportional to pH value (=concentration of $[H]^-$). This is consistent with the results obtained from enzymatic degradation of polyesters using *Pseudomonas* sp. lipase [Jun, 1994]. The decrease of pH accelerated the degradation of PCL films by acid hydrolysis. This relationship will able to analyze the degree of polymer degradation by pH measurement.

From the above results, we conclude the mechanism of fungal degradation of PCL films is as follows. The small molecules are first formed from the surface of the solid PCL films by simple hydrolysis, and then, the microorganism grows through the uptake of the hydrolysis products. The microorganism secretes lipase type enzymes and acidic biochemicals, and then enzymatic degradation and acid hydrolysis occur. Finally, the heavy growth of microorganism occurs.

CONCLUSIONS

The effect of polymer position on the fungal degradation of PCL films in nutrient-salt agar plates was investigated. From the photograph, SEM, weight loss and pH value, we conclude the fungal degradation rate of PCL decreased in the following order : middle>bottom>top. The microbial breakdown of the polymer was caused by a biophysical effect, a biochemical effect, and direct enzymatic action. In fungal degradation of PCL films on nutrient-salt agar medium, it is supposed that degradation of PCL films on the top position was caused by a biophysical, biochemical and direct enzyme action, but that of PCL films on the middle or bottom position was caused by mainly biochemical and direct enzyme action. In addition, the degree of polymer degradation can be measured by analyzing pH since the weight loss was proportional to pH.

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