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Effect of Surface Roughness on Biodegradability of Poly(3-hydroxybutyrate)

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Abstract - The effect of surface roughness on biodegradability of poly(3-hydroxybutyrate) was investigated. The PHB film prepared by cooling the molten polymer slowly (-0.5°C/min) had higher crystallinity and melting temperature than that prepared by quenching into liquid nitrogen followed by annealing at 90°C for 2 hours. However, the former sample was found to degrade faster than the latter due to presence of microscopic crack. Roughening the surface of a PHB film by hot pressing under a coarse surfaced plate accelerated the bioerosion considerably of the sample in comparison with the sample having the same thermal history but smooth surface. [Poly(3-hydroxybutyrate), biodegradability, surface roughness, the modified Sturm test].

INTRODUCTION

Plastics waste is difficult to degrade and tends to accumulate. Thus, it is considered to be one of the important pollutants in the environment. The amount of plastics waste, however, can be reduced through recycling. There have been extensive research efforts to understand the effects of multiple processing cycles on the mechanical properties and to develop blends of the recycled plastics with virgin plastics and adequate compatibilizers (Verhoogt *et al.* 1994). Also, many studies have reported that plastics waste can be reused as fuel or feedstock after decomposition and that plastics waste can be sorted according to the polymer type (Scott & Waterland 1995).

Biodegradable plastics can be used for food packaging, sanitary products, sporting goods, agricultural products or construction materials that are difficult or uneconomical to collect after use. One of the ways to enhance the properties and processibility and to lower the cost of biodegradable plastics is to blend them with naturally occurring polymers such as starch (Simmons & Thomes 1995) and cellulose (Buchanan *et al.* 1995) or with other synthetic plastics (Avella & Martuscelli 1988, 1995; Yoon *et al.* 1996). The changes in biodegradability and mechanical properties as a result of blending have been investigated.

Poly(3-hydroxybutyrate) (PHB) is a biodegradable polymer that is biosynthesized by microorganisms with mechanical properties quite similar to those of polypropylene. However, the high cost of PHB limits its applicability to some extent. Recent development in genetic engineering technology has allowed the production of PHB by the photosynthesis of plants and it had shed some light on the possibility of reducing the production cost (Williams & Peoples 1996).

PHB is degraded by depolymerases from *Aspergillus*, *Penicillium*, *Pseudomonas*, *Bacillus*, *Streptomyces* and *Alcaligenes* (Mergaert *et al.* 1993; Kim & Kang 1995). Kita *et al.* (1995) and Han *et al.* (1997) purified the depolymerases from *Alcaligenes faecalis* and *Penicillium pinophilum* respectively. The structural genes coding for PHB depolymerases of *Alcaligenes faecalis* and *Pseudomonas leignei* have been cloned and sequenced by Schirmer & Jendrossek (1994).

In this investigation biodegradability of PHB was determined by examining the specimen surface under SEM and by the modified Sturm test

according to ASTM D5209-91 (1991). The effect of the surface morphology of PHB on biodegradability was studied.

MATERIALS AND METHODS

Microbial source

Penicillium pinophilum ATCC 9644 (KCTC 2124) was supplied by Korean Collection for Type Culture. It was inoculated onto the Harrold's M40Y culture, incubated at 30°C for three days and stored at 4°C.

Sample preparation

PHB was provided by ICI Biological Product Division. The PHB powder was dissolved in chloroform at 3 wt% and poured into a Petridish after 2 hours of stirring. The solvent cast PHB film (PHBC) was obtained by evaporating the solvent at ambient condition and then by drying in a vacuum oven at 40°C.

PHBQ film was prepared by annealing the solution cast film for 3 hours in a hot press at 90°C and 250 atm that was heated for 1 min at 180°C in the hot press and then quenched in the liquid nitrogen. PHBS film was obtained by cooling the solution cast film at a rate of −1.5°C/min that was melted for 1 min at 180°C and 250 atm in the press.

Bioerosion test

PHB film (0.02 g) was added to 100 ml of the culture medium. Spore suspension of *P. pinophilum* at 1% (v/v) ($1\times10^6\sim2\times10^6$ spores/ml) was inoculated to the medium followed by incubation at 30°C in a rotary shaker (Vision, KMC-8480 SF) rotating at 180 rpm. Microorganisms were removed from the PHB film by deterging with 70% ethanol solution, and then dried at 105°C in a drying oven (Jeiotech TLC-D01) for 16 hours. Change in the surface morphology of the PHB film was observed with a SEM (JEOL JSM-T220) as a function of degradation time.

The modified Sturm test

Microbial degradation of the PHB film was evaluated by the modified Sturm test according to the ASTM D5209-91 employing the activated sludge obtained from Nanji municipal sewage treatment plant in Kyunggi-do.

Analysis

DSC analysis was carried out using a Perkin-Elmer DSC7 from room temperature to 190°C at a heating rate of 20°C/min. Spherulites were observed by the polarizing microscope (Nikon OPTIPHOT-POL). Wide-angle X-ray diffraction patterns of the films(0.5 mm thick) were obtained by the Philips X'Pert-MPD diffractometer, which utilized CuKα radiation (λ=0.1542 nm) at 27°C.

RESULTS AND DISCUSSION

PHB is a highly crystalline polymer, and its morphological properties depend on the processing conditions (Ramsay *et al.* 1993).

Fig. 1 shows the DSC thermograms of PHB films prepared by three different methods. PHBC showed a bimodal melting peak, while both PHBQ and PHBS showed a single melting peak. The melting temperature (T_m) and heat of fusion of the film (ΔH_f) decreased in the following order : PHBS>PHBQ>PHBC. It is known that the crystals formed at higher temperatures has higher melting temperature and greater crystallinity (Greco & Martuscelli 1989). In Fig. 2 it is clearly shown by the WAXS pattern that the crystallinity of PHBS was higher than that of PHBQ. Based on the WAXS intensity the crystallinity of PHBS and PHBQ was calculated to be 81.9% and 65.0%, respectively.

Fig. 3 shows the crystalline morphologies of PHBS, PHBQ and PHBC observed by the polari-

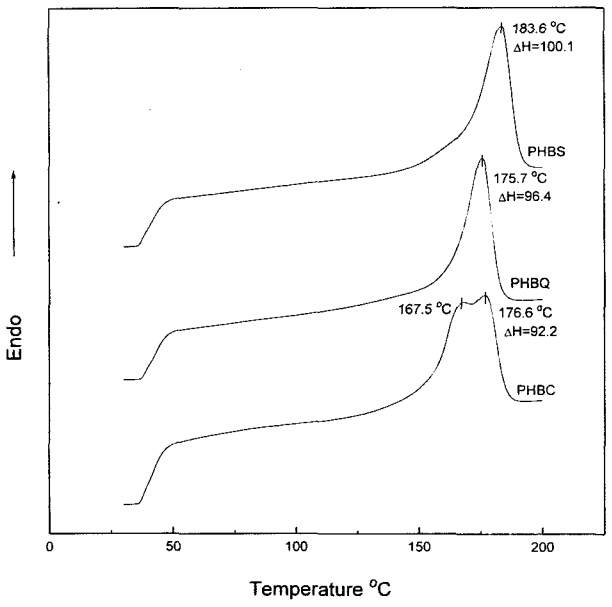

Fig. 1. DSC thermograms of PHB films.

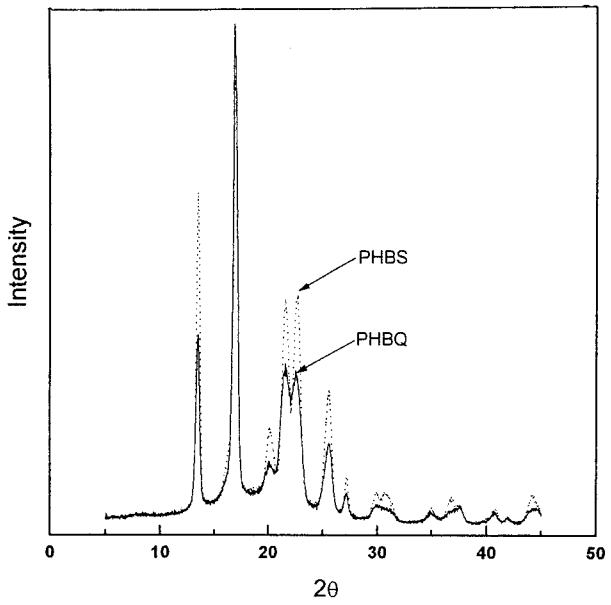

Fig. 2. X-ray scattering pattern of PHB films.

zing microscope. PHBQ and PHBC that were crystallized at a lower temperature than PHBS, shows the typical crystalline morphology containing large number of small spherulites.

SEM photos in Fig. 4 depict the biodegradation process of the PHB films by *P. pinophilum*. The surface of the PHBC film was filled with agglomerated small particles (Fig. 4. PHBC-a), and it would be the result of conglomeration of PHB particles that were precipitated while the solvent was evaporated during solvent casting process. The sizes of the particles were affected by the evaporation rate of the solvent.

The bioerosion rate of PHBC film by *P. pinophilum* was higher than that of PHBS or PHBQ film. It may be not only because the crystallinity of PHBC was the lowest but also because the surface morphology of PHBC was amenable to attachment of depolymerase of microorganism.

Since PHBS showed higher crystallinity than PHBQ, the biodegradation rate of PHBS by microorganism was expected to lower than that of PHBQ (Kumagai *et al.* 1992). However, it turned out that the bioerosion of PHBS was faster than that of PHBQ. Incidental microcracks formed during the PHBS film preparation might have facilitated the attachment of depolymerase of *P. pinophilum*. As shown in Fig. 4, the microcracks

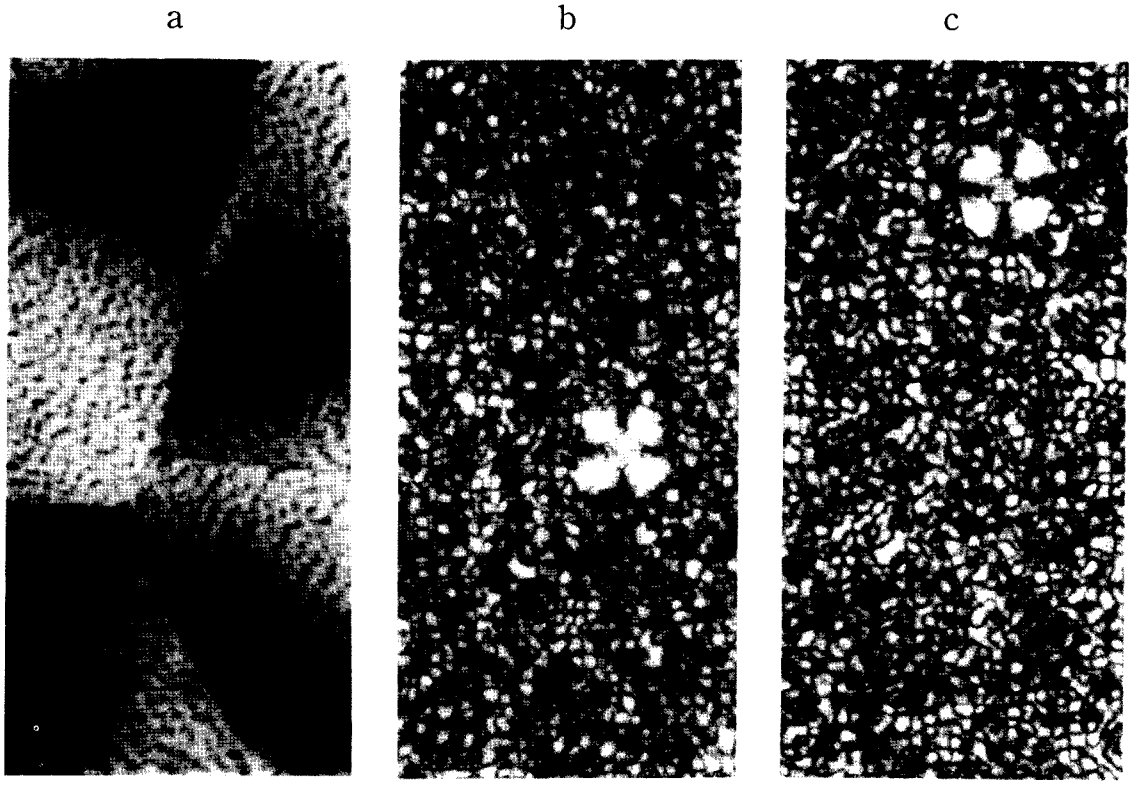

Fig. 3. Spherulites of PHB films. a: PHBS, b: PHBQ, c: PHBC.

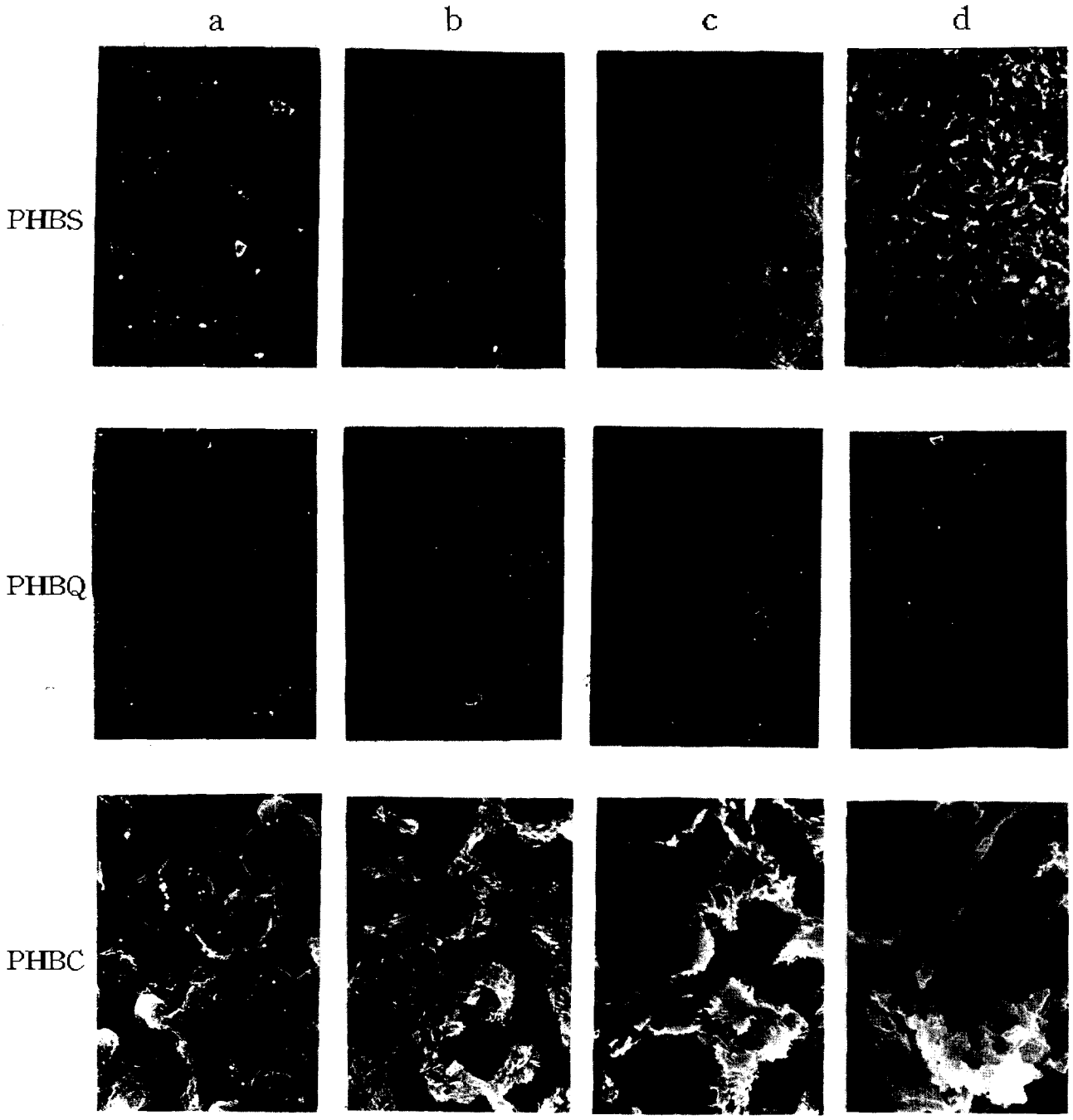

Fig. 4. Biodegradation of PHBS, PHBQ and PHBC films by *P. pinophilum*. a: control, b: after 10 days, c: after 20 days, d: after 28 days.

in the control PHBS film does not bring about a considerable change in the surface area. Therefore, the roughness of PHBS surface rather than the enlarged surface area seemed to be one of the main causes of the easier bioerosion compared with PHBQ.

The effect of surface roughness of PHB films on the degradation was investigated by SEM. Fig. 5 shows the bioeroded surface of smooth PHBQ and that of PHBQ film which was roughened by pressing the film against scratched aluminum plates. Even though the crystallinity of the

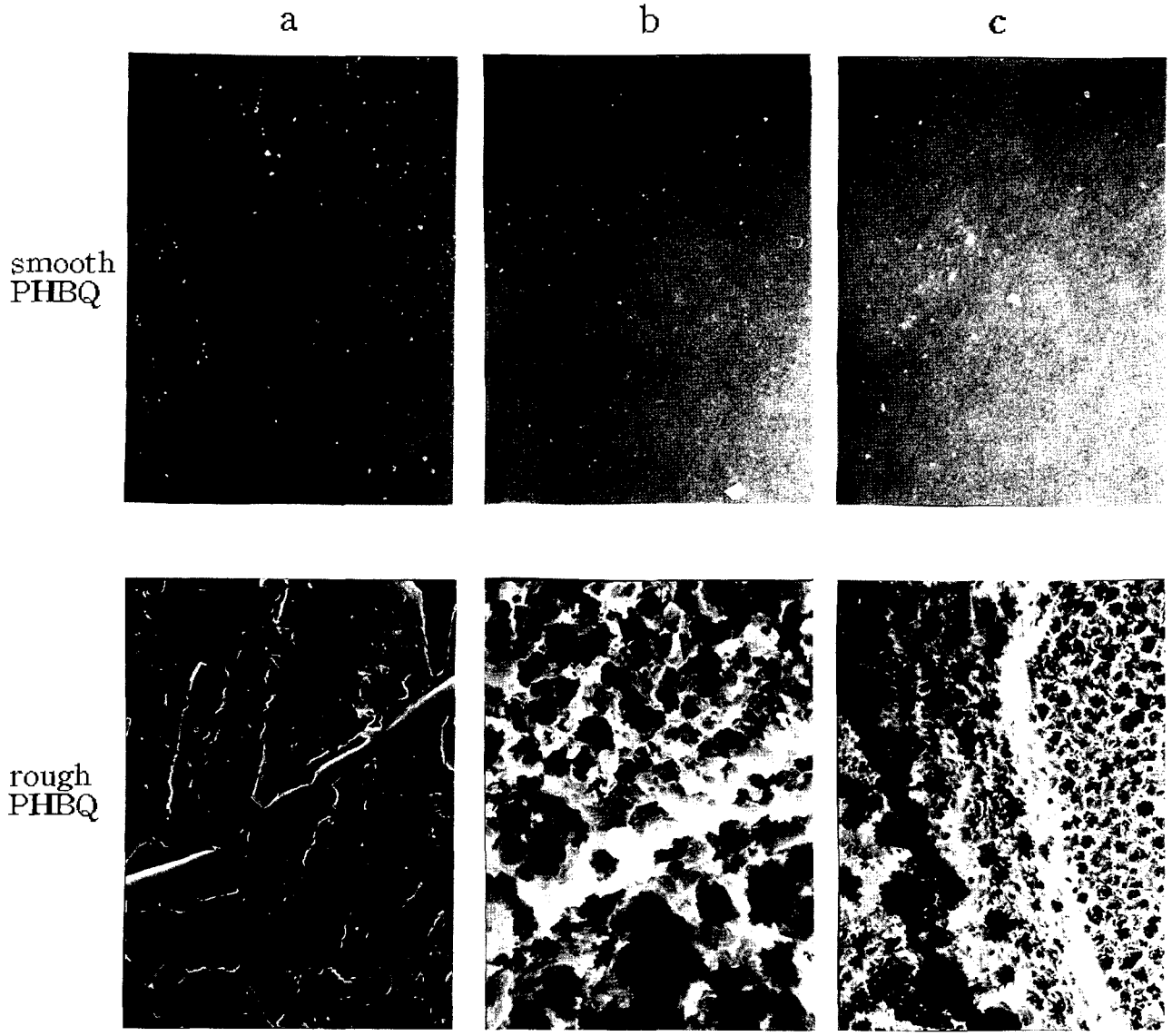

Fig. 5. Biodegradation of smooth PHBQ and rough PHBQ films by *P. pinophilum*. a: control, b: after 12 days, c: after 16 days.

smooth PHBQ and the surface roughed PHBQ films were the same, the biodegradation rate of the film with rough surface far exceeded the film with smooth surface.

The same results were obtained with the PHB films, evaluated by the modified Sturm test utilizing the activated sludge as the microbial sources (Fig. 6). Smooth PHBQ films did not show any significant change in the surface morphology up to 28 days of degradation, as shown in Fig. 4. However, the modified Sturm test results indicated about 20% of carbon of the smooth PHBQ films was assimilated in 28 days. It seems to be partly due to that the degradation rate of the PHB film by the activated sludge was faster than that by *P. pinophilum* as compared in Fig. 6. CO_2 was emitted without any lag time when PHBC and PHBQ with rough surfaces were degraded by the activated sludge. The amount of CO_2 emitted from the smooth PHBQ films showed a sigmoidal curve with a lag time of 5~10 days. The lag time in degradation of PHBC film by *P. pinophilum* was also about 10 days. The long lag time might have been caused in part as *P. pinophilum* was inoculated in spores.

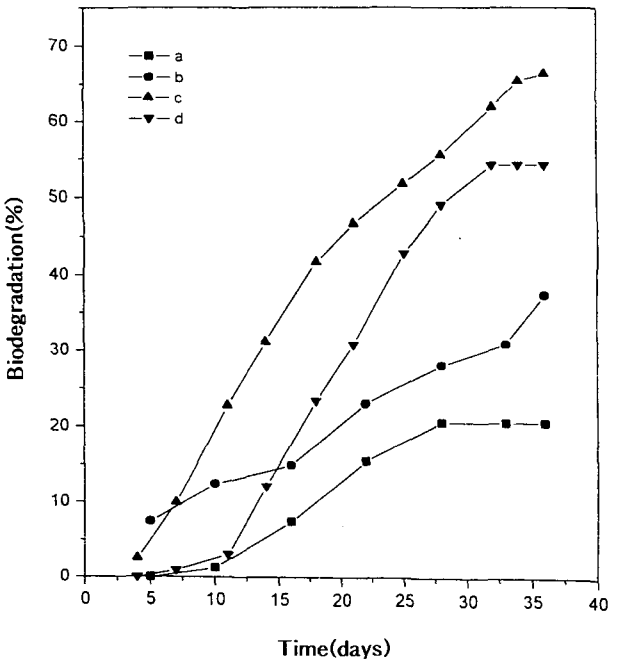

Fig. 6. The modified Sturm test results of PHB biodegradation by activated sludge. a: smooth, b: rough, c: solvent cast, d: solvent cast film (degraded by *P. pinophilum*).

ACKNOWLEDGMENTS

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Poly(3-hydroxybutyrate) 표면 형태가 생분해에 미치는 영향

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적 요 - Poly(3-hydroxybutyrate)의 표면 형태가 생분해성에 미치는 영향을 조사하였다. 용융 상태의 PHB를 서냉(−0.5°C/min)시켰을 때 PHB의 결정화도는 액체질소 속으로 급냉한 후 90°C에서 2시간 동안 숙성한 PHB의 결정화도보다 높게 나타났으나 전자의 PHB 시료는 미세 균열의 존재로 인하여 후자의 PHB 시료보다 더 빠르게 생분해 되었다. 거친 표면을 가진 평판 위에서 hot press하여 표면을 거칠게 만든 PHB film의 생분해는 표면이 평활한 film보다 훨씬 더 빠르게 진행되었다.