Environmental Factors and Kinetics of Microbial Degradation of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) in an Aqueous Medium

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ABSTRACT: Environmental factors such as oxygen, temperature, and microbial species may have significant effects on decomposition of biodegradable polymers. A representative biodegradable, thermoplastic polymer, poly(3-hydroxybutyrate-*co*-hydroxyvalerate) (PHBV), was decomposed in an aqueous medium under controlled laboratory conditions by soil microbes for the intrinsic degradation kinetics and the effects of the environmental factors on polymer biodegradation. The amount of proteins, including the PHBV depolymerases, that attached to the polymer surfaces was quite constant during the period of significant mass loss of the polymer specimens. The microbial polymer degradation followed a zero-order rate model, so the residual mass fraction of PHBV films declined linearly with time. The mixed aerobic microbial organisms from fertile soil showed a higher activity of polymer degradation than an aerobic PHBV-producing bacterium and the mixed anaerobes in the same soil. The mixed anaerobic microorganisms from barren soil decomposed the polymer at a slower rate than the anaerobes from fertile soil, and this was attributed to fewer microbial cells in the barren soil instead of the difference in the microbial species. The temperature effect on PHBV degradation can be described with an Arrhenius equation, and the activation energy is around 16 kcal/mol.
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Key words: biopolymers; biodegradable; degradation

INTRODUCTION

With increased public concern about plastic waste and litter accumulating at landfill sites and in the environment, extensive efforts have been made to create biodegradable polymers, particularly packaging materials with short lifespans.[1] To claim environmentally friendly properties for various biodegradable products, we require a measurement of biodegradability under environmental conditions by laboratory tests or field tests.[2] Field tests can reveal the real situation after a material is exposed to terrestrial (e.g., soil, compost, and landfill) and aquatic (e.g., marine, river, and lake) environments.[3–6] Such tests, however, are usually time-consuming and expensive, and the results are not reproducible because of uncontrollable environmental conditions.[7,8] Laboratory tests under controlled conditions can provide reproducible data for biodegradation mechanisms and kinetics[9,10] or for the simulation of real environmental conditions in small-scale laboratory facilities.[11,12] With our current knowledge of polymer biodegradation, we are not yet able to use laboratory data to predict a material's fate in the environment.[1,13] Degradation data obtained under the conditions of a specific environment may not also be used for the degradation of the same material in other environments. The biodegradation of a polymeric material depends on properties, such as its chemical bonds and morphology; environmental factors, such as oxygen, temperature, and biological agents (microbial cells and enzymes); and the interactions between the material's properties and environmental factors.[14,15] Despite the extensive research on materials, few studies have addressed environmental factors and their effects on biodegradation. With a given polymeric material, understating its intrinsic biodegradability and the effect of various environmental factors can increase our knowledge of polymer biodegradation in the environment, which may further lead to a sound model for predicting the fate of the material in a special environment.

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) is a well-known biodegradable, thermoplastic polymer produced by bacterial species as carbon and energy storage under unbalanced growth conditions.[16] It has thermal and mechanical properties similar to those of polypropylene. More than 695 microbial strains, including both polymer producers and nonproducers, have been identified as PHBV degraders in the environment.[17] Its field tests, however, have shown quite different results because of the variations in temperature, microbial species, and speci-

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mens.[4–6,18–21] Because primary studies on PHBV biodegradation have been conducted in an aqueous medium of pure extracellular enzymes, or depolymerases,[22,23] this polymer is a good candidate for investigating environmental factors and their effects on its biodegradation by living microbial cells in an aqueous medium. The biodegradation of PHBV in an aqueous medium by soil microbes can further be used to study polymer degradation in a soil matrix, a multiphase environment. Following our previous report on the significant effect of the shaking strength (the energy dispersion rate) on PHBV film degradation in flask cultures of soil microbes,[24] this article reports the biodegradation of PHBV films under controlled conditions by microbial organisms, including a pure aerobic PHBV-producing bacterium and mixed microbial organisms extracted from fertile soil, barren soil, and an activated sludge. The PHBV biodegradation kinetics in an aqueous solution were studied to quantitatively account for the environmental factors, including the microbial population, oxygen, and temperature.

MATERIALS AND MEASUREMENTS

Polymer specimens

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV; molecular weight = 400,000, 22% hydroxyvalerate) was obtained from Aldrich (Milwaukee, WI). Specimens of the PHBV film were prepared by solvent casting. PHBV was dissolved in chloroform at an 8–10% (w/v) concentration at 60°C overnight, and the solution was left in clean glass dishes and dried slowly under room conditions for 1 week. The films were further aged for at least 2 weeks before analysis and testing. Disc-shaped specimens (15 mm in diameter, ca. 0.1 mm thick, 18–23 mg each) were cut from the casting films.

Microbial cells

Mixed microbial degraders were extracted from a local fertile garden soil according to a general procedure of soil microbiology.[25] The soil was soaked at a concentration of 0.5 kg of soil/L of tap water for 1 h, and the suspension solution was filtered through No. 1 Whatman filter paper for the removal of coarse particles (>11 μm). The filtrate (40 vol %) was mixed with a culture medium that contained (per liter) 1 g of glucose, 1 g of peptone, and 1 g of yeast extract and was incubated at 30°C for 24 h under aerobic conditions or anaerobic conditions to produce aerobes or anaerobes, depending on the following degradation tests of the oxygen effect. Aerobic conditions were maintained by the shaking of a 200-mL medium in 2000-mL Erlenmeyer flasks at 180 rpm. Anaerobic conditions were maintained by the purging of the medium with N_2 gas for 10 min (zero dissolved oxygen concentration after 4 min of purging) and the covering of the flasks with rubber stoppers during cultivation. In addition to the mixed microbial cells from the fertile soil located 10–20 cm below the ground surface, mixed microbial cells were also extracted from an activated sludge cake of a local wastewater treatment plant and a local barren soil located 1.5 m below the surface. A pure PHBV-producing bacterium, *Ralstonia eutropha* (formerly *Alcaligenes eutrophus* ATCC 17699), was also cultivated under aerobic conditions in a nutrient-rich medium containing (per liter) 5 g of yeast extract, 5 g of peptone, 2.5 g of beef extract, and 2.5 g of ammonium sulfate. The cultivated cells, containing a minimum amount of PHBV [<10% of the dry cell mass (DCM)], were resuspended in a mineral solution for PHBV biodegradation tests.

Test solution and procedure

The cultivated microbial cells were mixed with a mineral solution for the preparation of the test solutions. The mineral solution contained (per liter) 3.8 g of K_2HPO_4, 2.65 g of KH_2PO_4, 0.48 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of NH_4Cl, and 1 mL of a trace element solution that contained 200 mg of $(NH_4)Fe(SO_4)_2 \cdot 6H_2O$, 5 mg of $ZnSO_4 \cdot 7H_2O$, 5 mg of $MnCl_2 \cdot 4H_2O$, 2 mg of $CuSO_4 \cdot 5H_2O$, 2 mg of $NaB_4O_2 \cdot 10H_2O$, and 2 mg of $NaMoO_4 \cdot 2H_2O$ in 1 L of a 1*M* HCl solution. The initial concentration of seed cells in the test solutions was controlled at around 25 mg of DCM/L of test solution, equivalent to 4×10^{11} soil bacterial cells per liter.[26] This bacterial concentration was about 1/10 of the bacterial content in a typical soil (2.5×10^9/g of soil or 2.5–3.3 $\times$ 10^{12}/L of soil).[26] Erlenmeyer flasks (125 mL), each containing three PHBV discs and a 50-mL test solution, were incubated at 30°C and 120 rpm. In previous experiments, it was found that the shaking strength had a significant effect on the biodegradation rate, and a shaking strength at 120 rpm was high enough to give a negligible effect of mixing and fluid shear stress on PHBV degradation.[24] The temperature effect on the biodegradation rate was investigated with the temperature maintained at 23, 30, and 37°C, respectively. At different points in time, two or three flasks were taken and sacrificed to measure the pH, polymer mass, cell mass, turbidity, and total proteins. Two types of controls were carried out in parallel. The abiotic hydrolysis of PHBV was monitored in solutions without living cells after the test solution was sterilized at 116°C for 15 min. The lysis of seed cells in the absence of PHBV as the sole carbon source was also monitored in the experiments.

Measurements

The mass of the PHBV discs was determined with an analytical balance with a 0.1-mg accuracy after the spec-

imens were gently washed to remove the attached biomass and were dried to a constant weight. An average mass of three polymer discs in one flask was calculated to give an average residual mass fraction, $X = W/W_0$, where W is the average mass at the sampling time and W_0 is the average initial mass at the beginning of a test. In this way, the deviation due to different initial masses and the biodegradation of individual specimens in one flask was minimized. We also controlled the possible deviations among different flasks by randomly taking two or three flasks each time to give an average specimen mass of the flasks. The smallest recoverable PHBV film fragment after degradation was around 0.2 mg, about 1% of the mass of the original polymer specimens. It was assumed that the fragments of less than 0.2 mg had been degraded because their huge surface area led to much faster degradation than that for the parental specimens. The turbidity of the test solution was measured with a spectrophotometer at 620 nm and correlated with the DCM concentration. The attached proteins (extracellular and intracellular) on polymer discs were measured with a Lowry protein assay kit (Sigma, St. Louis, MO), and good linearity existed between the absorbance at 600 nm and the protein concentration up to 400 mg/L. The intracellular proteins in the attached cell mass were released with a sonic dismembrator at 20 kHz. A zoom microscope (Nikon SMZ-U, Kanagawa, Japan) was used for routine monitoring of the polymer surface morphology. The polymer surface fragmentation was observed with scanning electron microscopy (JOEL 6300 SEM, Peabody, MA) after the specimens were coated with gold in a vacuum sputter.

RESULTS AND DISCUSSION

Degradation by mixed aerobes

Soil is a rich environmental source of microbial species, providing habitat for various cultivable and non-

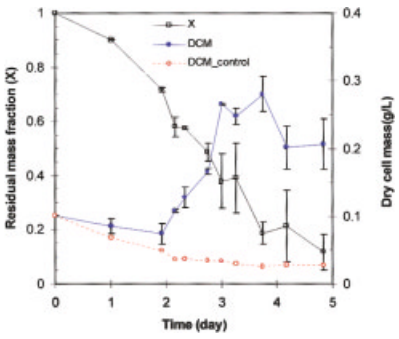


Figure 1 Typical time courses of PHBV degradation by mixed aerobic microorganisms and cell growth on the polymer as the sole carbon source at 30°C under aerobic conditions. The polymer degradation was observed as the X value of the specimen declined with time and the DCM concentration increased with time. In contrast, the seed cell mass (DCM control) declined in a control test without PHBV as the carbon source.

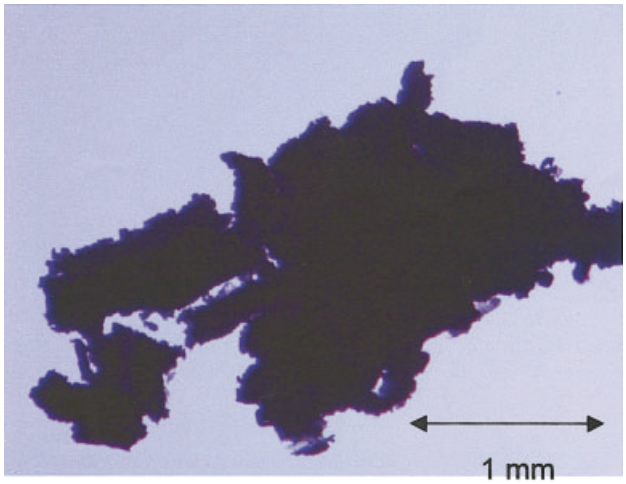


Figure 2 Typical residual piece of a PHBV film specimen that was recoverable for determining X. The dimensions of this polymer fragment were about 2.5 mm × 1.5 mm, with a thickness of about 0.04 mm and a weight of 0.2 mg.

cultivable aerobic heterotrophic strains that grow on organic carbonaceous substrates as their carbon and energy source and use oxygen as the final electron acceptor.[26] The mixed microbial cells were first extracted from a local fertile garden soil and were further cultivated in a glucose medium under aerobic conditions to produce the cultivable aerobic heterotrophic biomass as the seed for the polymer degradation tests. Figure 1 shows the typical time courses of PHBV degradation by the mixed aerobes, the cell growth on PHBV as the carbon source, and the cell lysis in the absence of PHBV. The polymer degradation was observed as X for PHBV film specimens decreasing with time. Three flasks, each containing three specimens, were sacrificed at one time point to give the average value of X and the deviations among different flasks. X had quite low and consistent deviations before 60% of the polymer mass was decomposed, and the deviations increased with further mass loss. This deviation increase was attributed to the increased difficulty in recovering the fragmented PHBV specimens. Figure 2 shows a typical polymer fragment recovered after X dropped below 80% of the original specimen mass. Some of the equivalent or smaller fragments might not have been recovered in the mass measurement. Figure 1 also reveals that with the decomposition of PHBV films, the DCM concentration of the mixed aerobic degraders increased correspondingly. It implies that the cells were using the polymer films as their sole carbon source, and this becomes even clearer in comparison with the continuous decline of the DCM concentration in the absence of PHBV as the carbon source. In the control test without PHBV, the heterotrophic seed cells did not have a carbonaceous substrate (PHBV) as their energy and carbon source for growth and maintenance and lost their integrity (lysis)

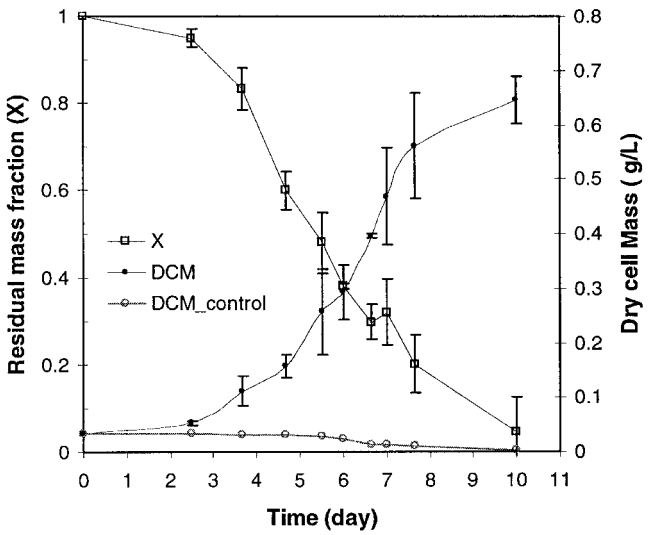


Figure 3 PHBV film degradation by the pure aerobic bacterium *R. eutropha* and cell growth on the polymer as the sole carbon source at 30°C under aerobic conditions. See Figure 1 for the legend.

with time. Three stages were observed in the cell growth on PHBV films: the adaptation stage of the first 2 days, in which the cells responded to the substrate change from glucose to PHBV films by trigging and releasing the extracellular depolymerase; the second stage, in which the cell mass increased linearly with time; and the third stationary stage, in which the cell mass concentration did not increase but declined to some extent because less polymer substrate was available for maintenance.

Degradation by a pure aerobic bacterium

R. eutropha, a soil bacterium, is a representative PHBV producer that accumulates the polymer from various organic carbons such as carbohydrates and fatty acids under aerobic conditions.[27] In starvation conditions with no organic carbon available, intracellular PHBV depolymerases are trigged to decompose the accumulated polymer as its carbon supply. Figure 3 reveals that the microbial cells could also release the depolymerases into their environment to decompose the PHBV films as an extracellular carbon source. In the absence of the extracellular PHBV films in a parallel control test, the seed cell concentration declined to undetectable level in 10 days. Compared with the mixed aerobes extracted from the fertile soil, this pure aerobic bacterium decomposed the polymer at a much slower rate, and this is further discussed in the following text. Similar to the mixed aerobes, *R. eutropha* also showed three stages in its growth: an adaptation stage of the first 2 or 3 days, in which the PHBV depolymerase was triggered and released into the environment; a second stage of cell mass increase with time; and a stationary stage after most of the polymer mass had been used. The much longer second stage implies that the polymer was degraded at a slower rate by the pure strain than by the mixed aerobes. However, the pure PHBV-producing strain seemed able to use the polymer carbon source with greater efficiency (high growth yield) than the mixed soil aerobes. Dividing the cell mass gain by the polymer mass loss in the second stage gives the growth yield of *R. eutropha* (0.57 g of DCM/g of PHBV) and the growth yield of the mixed aerobes (0.45 g of DCM/g of PHBV), the former being 27% higher than the latter. Some microbial strains in the mixed aerobes might not be highly efficient users of PHBV carbon but instead might be fast PHBV decomposers.

Degradation by mixed anaerobes

Soil is also a rich source of various anaerobic heterotrophic microbes that use organic substrates as their carbon source but use nitrate, sulfate, and organic carbon as the final electron acceptors.[26] The mixed heterotrophic anaerobes were obtained for PHBV degradation testing by the extraction of the mixed cells from fertile soil and the cultivation of the cells in a glucose nutrient medium under anaerobic conditions. Figure 4 shows the typical time courses of PHBV degradation by the mixed anaerobes, the cell growth on PHBV as the carbon source, and the cell lysis in the absence of PHBV. Compared with the mixed aerobes (Fig. 1), the mixed anaerobes had a much slower PHBV degradation rate and difficulty in decomposing the PHBV specimens to an 80% mass loss or greater. This might be attributed to the limited availability of the final electron acceptors in the closed test system.[21] Without the final disposal of the electrons released from PHBV degradation, the biochemical reactions

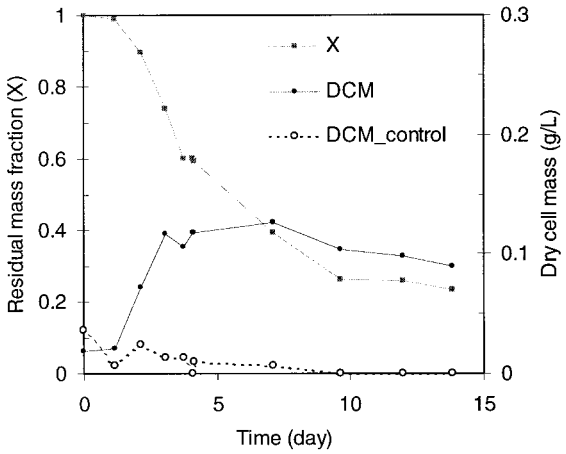
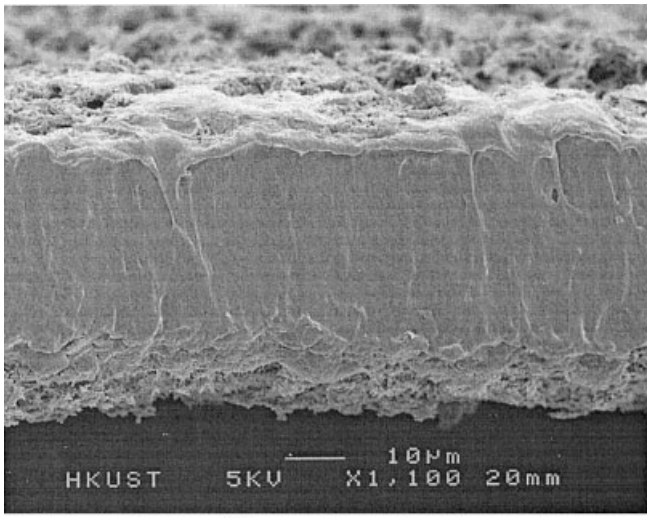


Figure 4 Typical time courses of PHBV film degradation by mixed anaerobic microorganisms and cell growth on the polymer as the sole carbon source at 30°C under anaerobic conditions. See Figure 1 for the legend.

were retarded and finally stopped. Compared with the growth yield of the mixed aerobes (0.57 g of DCM/g of PHBV), the growth yield of the mixed anaerobes was around 0.17 g of DCM/g of PHBV, this much lower value indicating the relatively poor polymer utilization for cell growth under anaerobic conditions. The pH of the test solution did not decline very much, from 6.8 at the beginning to 6.3 at the end of the tests. When the PHBV specimens were replaced by glucose (data not shown here), a common carbon source for most microbial organisms, the same mixed anaerobes had a growth yield of 0.28 g of DCM/g of glucose, and the solution pH declined from 6.9 to 4.7 because of the accumulation of fermentation acids such as acetic acid. This implies that the organic carbons in PHBV, compared with those in glucose, are not good electron acceptors in forming fermentation metabolites such as organic acids.



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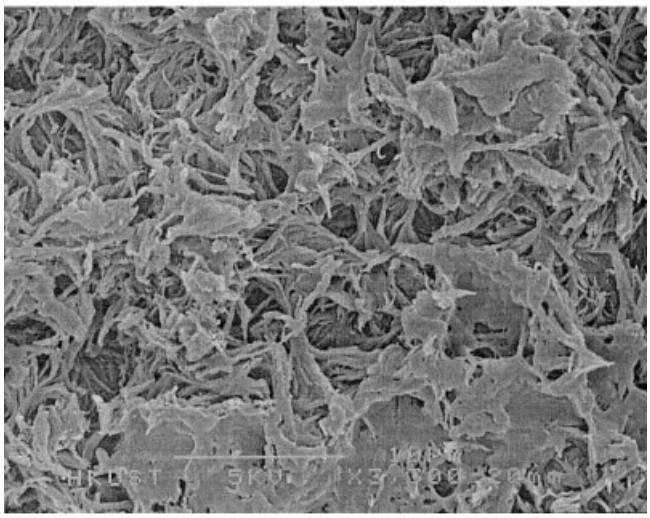


(b)

Figure 5 SEM pictures of (a) the cross-section area of a PHBV film specimen during biodegradation and (b) the PHBV film surface. The tiny fragments of the PHBV material were formed on the specimen surface under enzymatic attack.

Film morphology and the attached proteins

PHBV belongs to a family of hydrophobic polyesters that accumulate as solid granules in cell cytoplasm. Negligible abiotic hydrolysis was observed during the tests. Figure 5 shows SEM pictures of the cross section of a PHBV specimen and the surface morphology during biodegradation. It is clear that material biodegradation occurred mainly on the two surfaces (<5 μm) when we consider the disc thickness of around 50 μm, with little decomposition inside the polymer film. Very tiny fragments were formed on the polymer surface, resulting from enzymatic attack.[28] The tiny fragments could be shorn away by hydraulic shear stress and decomposed at a much faster rate than the parent specimens because of the huge surface area. The degradation of PHBV films, therefore, had a rate-limiting step, the fragmentation of the polymer surface following the attachment of extracellular depolymerases to the hydrophobic polymer surface. That the polymer biodegradation was slower by mixed anaerobes than by aerobes might be attributed to fewer degraders and depolymerases attached to the polymer surface. Figure 6 shows the total proteins, including the depolymerases attached to a unit mass of polymer films, versus the polymer degradation ($1 - X$). First, the amount of total proteins of mixed anaerobes was much less than the amount of mixed aerobes, about 1/10, as reflected by the two y-axis scales. Second, during significant polymer mass loss ($0.2 < 1 - X < 0.7$), the attached proteins, including the depolymerases, were quite constant. This implies that the polymer surfaces were covered by the attached microbial cells and enzymes and did not change very much during polymer degradation. It further implies that the PHBV degradation was independent of the concentration of the biological agents (microbial cells and enzymes) in the bulk solution during this particular period of degradation ($1 - X < 0.7$).[29] Under aerobic conditions, the PHBV specimens could be decomposed into small pieces, as shown in Figure 2. When the mass loss was up to 80% or greater it was observed that the small polymer pieces were wrapped or surrounded by biomass, which explained the very high protein (extracellular and intracellular) content per polymer mass in Figure 6 with $1 - X > 0.8$. With the mass loss being less than 60%, the original PHBV discs on which the biomass was attached were still recognizable. Figure 7 shows the overall surface morphology of the PHBV specimens under a zoom microscope. During the initiation period, the polymer surface was attacked by depolymerases, and this resulted in surface whitening

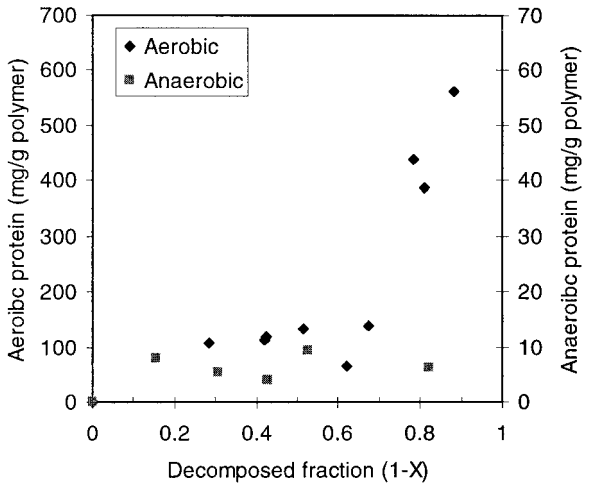


Figure 6 Total proteins attached to PHBV specimens from the mixed aerobes (Fig. 1) and the mixed anaerobes (Fig. 4) during polymer degradation ($1 - X$).

with respect to the original surface. With biodegradation proceeding, the polymer discs became thinner, and some local areas were worn away, leaving small, visible holes.

Effect of the microbial source

In addition to the fertile garden soil, mixed microorganisms were also obtained from two sources, a local barren soil and an activated sludge. Table I gives the moisture contents of the three solid matrices, glucose utilization rates, and PHBV degradation by the associated microbes under anaerobic conditions. The glucose utilization rates based on 1 g of dry solids represent the microbial activities of the three solid matrices. There was a good correlation between the glucose utilization activity and the polymer degradation activity. That there was less polymer degradation by the microbes in the barren soil might be attributed to the fewer microorganisms in the solid matrix or to the different microbial species that were more autotrophic (using CO_2 as the carbon source), that is, less heterotrophic with the use of PHBV carbons. For clarification, three types of cells were harvested after PHBV degradation tests by the three types of solid matrices and were resuspended in new test solutions at the same seed concentration. Figure 8 shows the time courses of polymer weight loss by the microbes from the three types of microbial sources. The similar weight losses by the microbes from the barren and fertile soils clearly indicate the similar microbial activities during PHBV degradation in the two soils. The activated sludge might contain different heterotrophic microbial strains that degrade and use the polymer substrate at a faster rate than those in soils.

Degradation kinetics

Figure 9 shows the decline of X with time by the mixed aerobes, the pure aerobe (*R. eutropha*), and the mixed anaerobes at three temperatures (23, 30, and 37°C). All showed good linearity between X and time as judged by the statistical determination coefficient, R^2 (0.93–0.98). It implies a zero-order PHBV degradation rate under the experimental conditions:

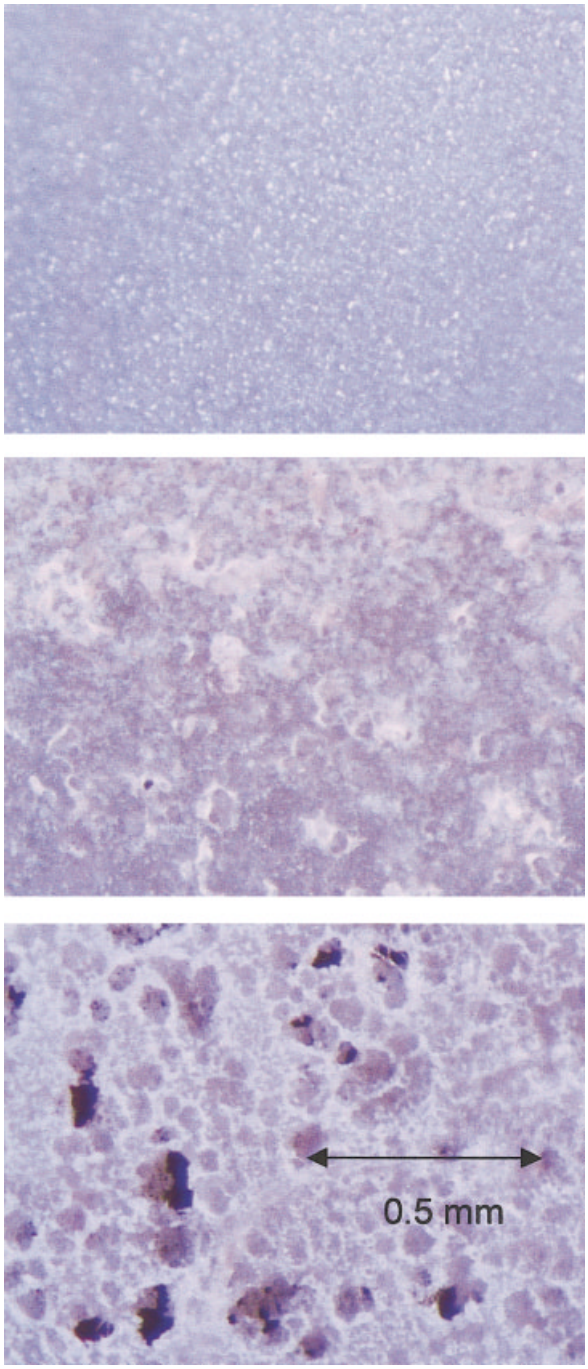


Figure 7 Surface morphologies of PHBV films during biodegradation. The top image shows the original surface ($1 - X = 0$), the middle image shows the degradation initiation ($1 - X \ll 0.1$), and the bottom image shows the degradation with constant proteins attached ($0.1 < 1 - X < 0.7$).

TABLE I
Microbial Activities in Three Solid Matrices and PHBV Biodegradation in 7 Days Under Anaerobic Conditions

Solid matrix	Moisture (wt %)	Glucose rate (mg/g of dry solid h)	PHBV degraded (mg)	PHBV degraded (wt %)
Barren soil	15.8	33.4	13.8	25
Fertile soil	40.0	63	41.0	70
Activated sludge	75.5	79.3	45.5	75

$$-\frac{dX}{dt} = -\frac{d(W/W_0)}{dt} = k \qquad (1)$$

with the initial conditions,

$$t = t_0,\ X \approx 1\ .$$

where k is the specific PHBV degradation rate (day^{-1}), and t_0 is the time required for the adaptation of microbial cells to the polymer substrate. The length of the adaptation stage depends on the microbial strains, the polymer substrate, and the environmental conditions, such as oxygen and temperature.

The integration of eq. (1) gives

$$X = -kt + (1 + kt_0) \qquad (2)$$

The two parameters of k and t_0 in eq. (2) under different conditions are estimated from the straight lines in Figure 9 and are compared in Table II. Two conclusions can be drawn from the results (Table II):

1. Mixed microbial strains versus the pure microbial strain. The mixed aerobic microbes provide not only faster polymer degradation (1.5 times) than a pure PHBV producer does but also a shorter (24%) adaptation time for starting polymer degradation. The depolymerases from various microbial strains in the mixed aerobic popu-

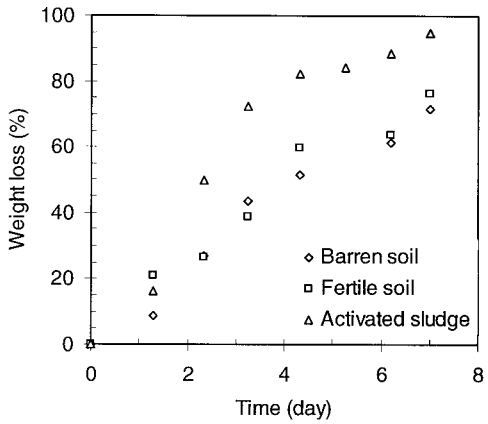
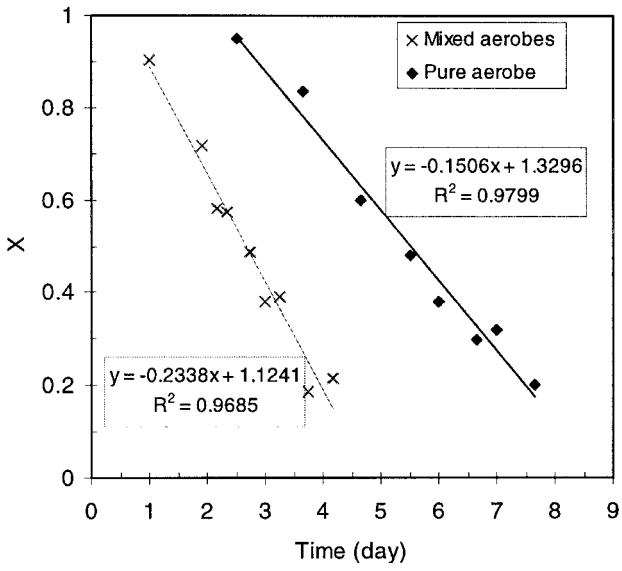
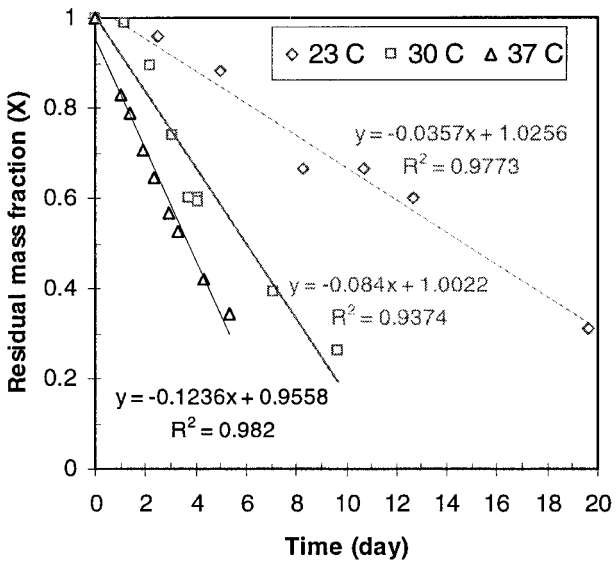


Figure 8 PHBV film degradation by anaerobic microbes that originated from barren soil, fertile soil, and activated sludge. The initial microbial seeds were controlled at the same concentration in the test solution.



(a)



(b)

Figure 9 Zero-order degradation kinetics of PHBV films (a) by mixed and pure aerobes and (b) by mixed anaerobes at three temperatures.

lation are more efficient in the enzymatic hydrolysis of polyester bonds than the depolymerase released by the pure PHBV producers.
2. The oxygen effect. The aerobic polymer degradation uses oxygen as the final electron acceptor, and this leads to a much faster polymer decomposition rate (2.7 times) than that of anaerobic degradation. The latter has to use nitrate, sulfate, and organic carbons as the electron acceptors and has a shorter adaptation time than the aerobes.

Activation energy of PHBV degradation

The dependency of the degradation rate on temperature can be described with the Arrhenius law:

$$k = k_0 \exp \frac{E}{RT} \tag{3}$$

where k_0 is the pre-exponential factor, E is the biodegradation activation energy (kcal/mol), R is the general gas constant (1.987 cal/mol K), and T is the temperature (K). The natural logarithm of eq. (3) gives

$$\ln k = \ln K_0 - \frac{E}{RT} \tag{4}$$

The straight trend of ln k versus the reciprocal of temperature is shown in Figure 10, and its slope gives an estimation on the activation energy of PHBV degradation by depolymerases:

$$\mathrm{E} = 16.2 \text{ kcal/mol}$$

$$\mathrm{k_0} = 3.9 \times 10^{10} (\mathrm{day^{-1}})$$

In physical chemistry, k_0 means the collision frequency ($\mathrm{day^{-1}}$) between the polymer ester bonds and the extracellular depolymerase. This parameter might be affected by the shaking strength in the flask cultures because strong shaking favors contact and collision between the ester bonds and enzymes. The activation energies of enzyme-catalyzed reactions are generally 4–20 kcal/mol and mostly about 11 kcal/mol.[30] The activation energy of PHBV degradation catalyzed by depolymerases estimated in this study is above the average.

TABLE II
***k* Under Different Conditions**

Oxygen	Aerobic		Anaerobic		
Microbes[a]	Pure	Mixed	Mixed		
Temperature (°C)	30		23	30	37
k ($\mathrm{day^{-1}}$)	0.15	0.23	0.036	0.084	0.124
t_0 (day)	2.2	0.53	0.72	0.03	0[b]

[a] The pure soil aerobe was *R. eutropha* (ATCC 17699); The mixed aerobes and anaerobes were extracted from a fertile garden soil followed by aerobic or anaerobic cultivation in a glucose nutrient solution.

[b] No adaptation was assumed for a negative adaptation period ($t_0 = -0.3$ day) estimated from eq. (2).

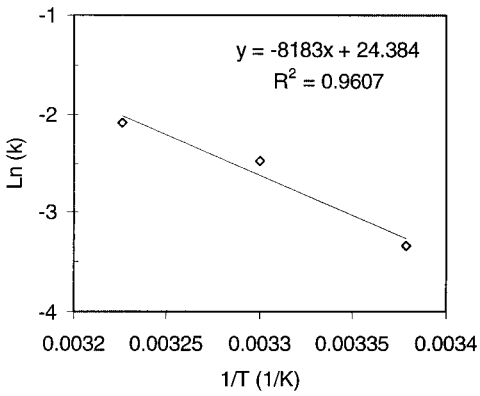


Figure 10 Arrhenius plot of k versus the reciprocal of the temperature. The activation energy of the anaerobic PHBV biodegradation was estimated from the slope (E/R).

CONCLUSIONS

1. Mixed microbial strains have a higher PHBV degradation rate and a shorter adaptation time than a pure strain does.
2. The aerobic degradation of PHBV is faster than anaerobic degradation because of the higher cell growth yield and the greater number of attached total proteins.
3. That PHBV degradation by the mixed anaerobes in barren soil is slower than that in fertile soil is mainly attributed to fewer microbes rather than a difference in the microbial species.
4. During polymer degradation, the biologically active components (proteins and cells) are attached to polymer surfaces at quite constant levels under both anaerobic and aerobic conditions, and this results in a zero-order rate model of PHBV degradation by soil microbes in an aqueous medium.
5. The polymer biodegradation relies on the first step of enzymatic hydrolysis by depolymerase. The enzyme activity and polymer degradation rate increase with temperature in a mesophilic range. The activation energy is around 16.2 kcal/mol.

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