Biodegradation of Nylon4 and Its Blend with Nylon6

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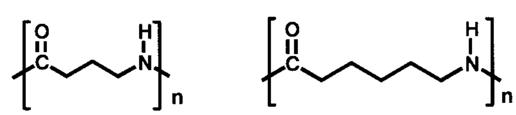
ABSTRACT: The nylon4 portion in the blend films composed of nylon4 and nylon6 was degraded and completely disappeared within 4 months in two kinds of composted soils gathered from different university farms as well as pure nylon4 film reported previously, while the nylon6 portion remained even after the burial test for 15 months. Nylon4 powder was also degraded to carbon dioxide in the degradation test in an activated sludge obtained from a sewage disposal institution in Kogakuin University. Three species of microoganisms (i.e., ascomytous fungi) were isolated through the inoculation from the nylon4 film partially degraded in the soil on a medium containing nylon4 powder as a carbon source. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 2307–2311, 2002

Key words: biodegradable; nylon4; composted soil; activated sludge; microorganism

INTRODUCTION

Native biodegradable polymers, such as polysaccharides, proteins, nucleic acids, biopolyesters, and so on, exist on the earth now as a result of evolution developed during millions of years. Thus they are naturally put into a carbon circulation system on the earth through degradation by microorganisms. On the other hand, artificial synthetic polymers have been produced through human research for only a century, and most of them are essentially difficult to decompose under naturally occurring conditions on the earth. In particular, the degradation of synthetic polyamides (nylons) is known to be generally slower than that of the corresponding synthetic polyesters through both biological and hydrolytic processes under neutral conditions.[1–8] The high resistance to the degradation of nylons may be caused mainly by high symmetrical molecular structures and strong intermolecular hydrogen bonds, which result in a highly ordered crystalline morphology. Therefore, only a few investigations on the biodegradation of nylons having relatively simple repeating units have been reported to date.[9–12] ε-Caprolactam and its oligomers were degraded by several microorganisms.[10,11] Nylon fibers were reported to become brittle by *Aspergillus* and *Penicillium* molds.[13] Recently, a white matter decay germ (IZU-154 strain), a kind of microorganism-degrading lignin, was found to decompose nylon66 film.[12]

In 1994 we found that a film of nylon4 (**1**), obtained easily through the ring-opening polymerization of α-pyrrolidone, was decomposed in the composted soil gathered from the Nagoya University Farm and disappeared within 4 months.[14,15] Recently, Nakayama et al. reported that nylon4 was also degraded in an activated sludge.[16] The present study is concerned with further research on the degradation of nylon4 and its blend with nylon6 (**2**) in composted soil and in an activated sludge, and the isolation of microorganisms participating in the degradation of nylon4 in the composted soil.



1 **2**

EXPERIMENTAL

Polyamides

Nylon4 (**1**) was prepared by the ring-opening polymerization of α-pyrrolidone in a high-vacuum line by a method similar to that described in our previous study.[14] Commercially available potassium *t*-butoxide

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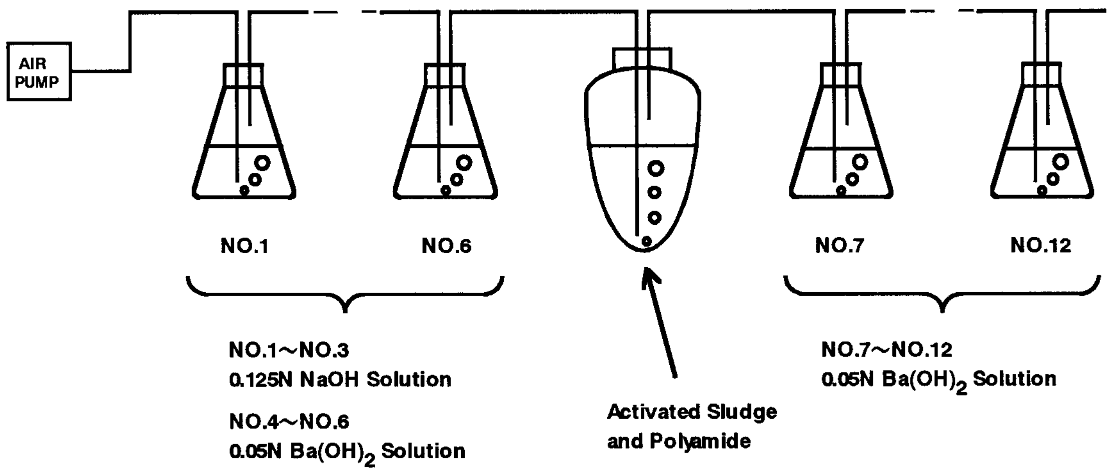

Figure 1 Apparatus for determination of carbon dioxide evolving from an activated sludge during the bubbling of the carbon dioxide–free air.

was used instead of potassium α-pyrrolidonate as catalyst. The intrinsic viscosity [η] of **1** in *m*-cresol at 25°C was 3.67 dL/g, corresponding to about 1.4×10^5 weight-average molecular weight.[17] Nylon6 (**2**) was provided by Teijin Co. (Tokyo, Japan), which was purified by reprecipitation using 2,2,2-trifluoroethanol (TFE) and acetone as a solvent and a precipitant, respectively. The value of [η] in *m*-cresol at 25°C was estimated to be 1.26 dL/g, which corresponded to 3.7×10^4 weight-average molecular weight.[18] The ^{13}C-NMR spectrum was taken by a JEOL JNM-EX270 Fourier transform NMR spectrometer (JEOL, Tokyo, Japan) operating at 67.8 MHz. Tetramethylsilane was used as internal standard in a deuteriochloroform–TFE mixture.

Soil burial test

Nylon4, nylon6, and their mixture (wt ratio 3 : 7) solutions in TFE were cast on flat glass plates and dried at room temperature to give the corresponding films with about 50–70 μm thickness. The resulting polyamide films were immersed and rinsed in deionized water and dried *in vacuo*. Several pieces of polyamide films (1 × 5 cm) were buried in the composted soils packed in containers made of polypropylene (Tupperware; Orlando, FL). The soils, gathered from the Nagoya and Tokyo University Farms, were composted mainly with cow manure and grass for more than 10 years (pH 7.5–7.6). The containers were kept in an atmosphere controlled at 80% relative humidity at 25°C. At an appropriate time, the films were removed from the composted soil and washed with water. After drying *in vacuo*, they were weighed and analyzed by ^{13}C-NMR spectroscopy.

Determination of carbon dioxide generated during the degradation of nylon4 in an activated sludge

An apparatus for the degradation test of polyamides in an activated sludge is illustrated in Figure 1, which is a modification of that reported in a previous study.[19] Nylon4 powder (about 0.33 g) was charged into a flask containing 200 mL of an activated sludge (pH 6.9), which was obtained at the sewage disposal institution at Kogakuin University. The carbon dioxide–free air was prepared by passing through 0.125*N* aqueous sodium hydroxide solution and 0.05*N* aqueous barium hydroxide solution in six flasks connected in series (No. 1 to No. 3 and No. 4 to No. 6, respectively), and bubbled into the activated sludge at a constant flow rate (about 75 mL/min) at 25°C. The air was passed through 0.05*N* aqueous barium hydroxide solution in six flasks connected in series (No. 7 to No. 12), and the resulting carbon dioxide was quantitatively converted to barium carbonate and captured. At appropriate intervals (1 or 2 days), barium carbonate precipitated in flasks 7–9 was collected on a filter, dried, and weighed. No precipitation of barium carbonate was observed in flasks 10–12. A control run was also carried out in a similar way in the absence of the polyamide. The amount of carbon dioxide generated by the degradation of the polyamide was estimated from the amount of barium carbonate educing in the presence and absence of the polyamide.

Preparation of agar media for the plating method

Agar (15–20 g), KH_2PO_4 (0.14 g), NaCl (0.03 g), $MgSO_4{\cdot}7H_2O$ (0.14 g), $CaCO_3$ (0.01 g), yeast extract (trace), and a mixture of trace elements (1 mL) were dissolved in deionized water and diluted to 1.0 L (final pH, 7.2). After addition of 0.1 wt % of nylon4 powder

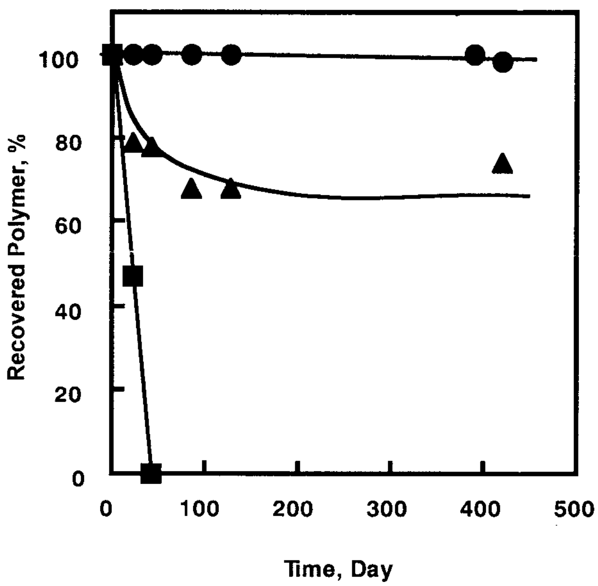

Figure 2 Change in the weight of polyamide films containing nylon4 and nylon6 recovered after the soil burial test (relative humidity, 80%; 25°C). The soil was composted for more than 10 years at the Nagoya University Farm (pH 7.6). Amount of nylon4 in the film: ●, 0%; ▲, 30%; ■, 100%.

(80 mesh) or γ-amino-*n*-butyric acid as a carbon source, the solution was sterilized in an autoclave at 126°C for 30 min, and charged into sterilized disposable flat Petri dishes.

Isolation of microorganisms inhabiting the surface of nylon4 film buried in the composted soil

A nylon4 film was buried in the composted soil gathered at the Nagoya University Farm. The surface of the partially degraded film was rubbed with a sterilized platinum stick, and its tip was touched on the media containing nylon4 powder as a carbon source. After the inoculation, the media were incubated at 25°C. The multiplying microorganisms were streaked onto fresh media. After the repeated streaking, a single colony was finally obtained on the media.

Isolation of microorganisms in the composted soil

The composted soil (0.1 g) used for the soil burial test of nylon4 was dispersed in 10 mL of sterilized physiological saline. The suspension was diluted to 1 L, and 0.1 mL of the dilute suspension was inoculated on the media containing nylon4 with a spreader. After incubation at 25°C for 1 week, a white colony grew on the medium. It was inoculated on fresh medium several times, and finally a single colony was obtained.

RESULTS AND DISCUSSION

Degradation of polyamide films in the composted soils

Three kinds of polyamide films [nylon4, nylon6, and nylon4/nylon6 blend (3/7 wt)] were buried in the soil composted for more than 10 years at the Nagoya University Farm (pH 7.5–7.6). As shown in Figure 2, the weight of the recovered nylon6 film was almost constant during the burial test for more than 6 months, whereas the nylon4 film was found to be decomposed in the soil and disappeared within 4 months. The term necessary for the complete disappearance may depend on the conditions of the soils, such as the amount of the compost in the soils, season and place in which the soils were gathered, and so on.

The weight of the nylon4/nylon6 blend film (3/7 wt) was also found to decrease during the burial in the soil, although the weight loss was only up to about 30% of the weight of the original blend film, which corresponded to the weight of the nylon4 portion in the film. In addition, the blend film recovered after the burial test for 6 months was confirmed to contain only nylon6 by ^{13}C-NMR spectroscopy. Such results indicate that the nylon4 portion in the blend film was exclusively degraded in the soil and the neighboring nylon6 portion was recovered without degradation. Since nylon4 has been reported not to be hydrolyzed in a phosphate buffer controlled at pH 7.5 in our previous study,[14] some microorganisms living in the composted soils are expected to degrade nylon4.

A similar burial test of polyamide films was tried in the composted soil gathered from the Tokyo University Farm. Only the nylon4 portion in the film was again decomposed exclusively, and completely disappeared within 4 months. Therefore, some microorganisms leading to the decomposition of nylon4 may be common in the composted soils.

Degradation of polyamides in the activated sludge

As described in the introduction section, nylon4 was recently reported to be degraded in an activated

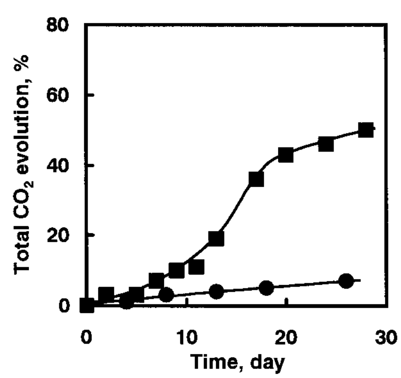

Figure 3 Evolution of carbon dioxide on the degradation of polyamides in the activated sludge: ●, nylon6; ■, nylon4.

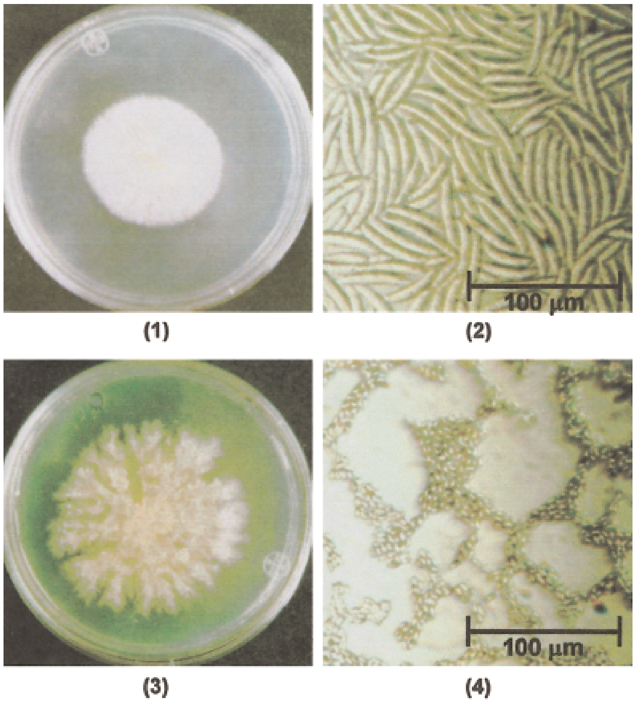

Figure 4 Photographs and microphotographs of the propagating colonies of microorganisms participating in the degradation of nylon4: (1) and (2), *Fusarium solani* (Martius) Saccardo; (3) and (4), *Clonostachys rosea* (Link: Fr.) Schroers et al. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sludge.[16] We tried a similar degradation test of nylon4 powder in an activated sludge obtained from a sewage disposal institution operating at the Hachioji campus in our university.

As shown in Figure 3, carbon dioxide was found to generate in the activated sludge (pH 6.9) in the presence of nylon4 powder during the bubbling of carbon dioxide–free air at 25°C. After an induction period, the amount of the evolving carbon dioxide increased enormously with the bubbling time, and after 28 days reached 49% of the theoretical amount of carbon dioxide generated during the complete degradation of nylon4. On the other hand, a similar significant evolution of carbon dioxide from the nylon6-containing sludge was not observed during the bubbling for 28 days. The pH values of the sludge after the test were determined to be 7.2 and 6.7, respectively. Because the hydrolytic degradation of nylon4 for several months is known to be negligible at the pH range from 4.5 to 7.5,[14] the degradation of nylon4 through biological processes is expected again to proceed not only in the composted soil but also in the activated sludge.

Therefore the isolation of microorganisms leading to the degradation of nylon4 in the composed soil was investigated by several methods.

Isolation of microorganisms

As described in the experimental section, the nylon4 film was partially degraded in the composted soil. After the inoculation from the surface of the film into the media containing nylon4 as a carbon source and incubation for 2 days, microorganisms were observed to grow in the media. Colonies of the microorganisms were obtained by the repeated streaking on the media containing nylon4. The isolated microorganisms were found to propagate on the media containing γ-amino-*n*-butyric acid as a carbon source. They were identified as *Fusarium solani* (Martius) Saccardo, *F. oxysporum* Schlechtendahl, and *Clonostachys rosea* (Link: Fr.) Schroers et al., which belong to the ascomycetous fungi. In Figure 4 are summarized photographs and microphotographs of the propagating colonies of the microorganisms, *F. solani* [(1) and (2)] and *C rosea* [(3) and (4)].

The suspension of the composted soil was also used as inoculum. After incubation at 25°C, a colony was obtained on the media. It was identified to be *F. solani*. Thus, *F. solani* is a candidate for microorganisms participating in the degradation of nylon4. *F. solani* and *F. oxysporum* are known to be typical microorganisms for the degradation of lignin.[20]

CONSLUSIONS

Polyamide blend films composed of nylon4 (**1**) and nylon6 (**2**) were buried in two kinds of composted soils gathered from different university farms. The nylon4 portion in the films was degraded and completely disappeared within 4 months in both soils as well as pure nylon4 film reported previously,[14] whereas the nylon6 portion remained even after the burial test for 15 months. Nylon4 powder was also degraded to carbon dioxide in the degradation test in an activated sludge obtained from a sewage disposal institution at Kogakuin University. Three species of microorganisms (ascomycetous fungi) were isolated through the inoculation from the nylon4 film partially degraded in the soil on a medium containing nylon4 powder as a carbon source. Because the isolated microorganisms are common in soils, the synthetic polyamide investigated in the present work, nylon4, may be one of the useful biodegradable polymeric materials.

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