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Biodegradability of nylon 4 film in a marine environment

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ABSTRACT

Biodegradability of nylon 4 in seawater from Tokyo Bay was investigated by weight loss and biochemical oxygen demand (BOD) of nylon 4 films. The remaining weight of nylon 4 film decreased with incubation time in the seawater, and the percentage of weight loss of nylon 4 film was 30% after 3 weeks. BOD biodegradability of nylon 4 film was approximately 80% within 25 days. Scanning electron microscopy images of the nylon 4 film before and after the seawater treatment revealed that the surface of the nylon 4 films was eroded after biodegradation in seawater. The average molecular weights of the nylon 4 films indicated no significant difference between before and after 30% weight loss of the film. Based on the present data, nylon 4 film was degraded on the surface of the film in the seawater. Furthermore, microbial degradation seemed to be one of the main degradation mechanisms of nylon 4.

1. Introduction

Plastic is generally a durable material that is resistant to natural biodegradation processes and does not readily break down in the natural environment such as in marine conditions [1]. Recently, plastic marine debris has become a pervasive pollution problem affecting all of the world's oceans [2–4]. Plastic degradation takes longer in the ocean than on land because of lower temperature, and plastics are degraded into smaller pieces that persist in the marine environment for a long time [5]. This marine debris is known to cause injuries and death of numerous marine animals and birds, either due to entanglement or because these animals and birds mistake the debris for prey and eat it [6,7]. This problem can be avoided by using biodegradable plastics as an alternative to non-degradable plastics [8,9]. The biodegradability of plastics such as polyesters [10–15], polyurethanes [16,17], and polyolefins [18] in the marine environment have been studied thus far; however, the biodegradability of polyamides, including nylons, in the marine environment has not yet been investigated.

Polyamides show excellent mechanical and thermal properties, high chemical resistance, and low permeability to gases [19]. Polyamides are resistant to degradation in the natural environment because of the high symmetry of their molecular structures and strong intermolecular hydrogen bonds, with the exception of poly(amino acids) such as poly(glutamic acid) and poly(aspartic acid) [20–22]. Several investigations of the biodegradation of nylons have been reported [23–30,32,33], which resulted in the conclusion that nylon 4 is one of the most promising biodegradable and bio-based polyamides. In particular, the biodegradability of nylon 4 has been widely studied using composted soil and activated sludge [30]. In addition, several kinds of bacteria and fungi were isolated as nylon 4-degrading microorganisms [32,33]. Although these results confirmed that nylon 4 is degradable in the artificial environment, its biodegradability in the natural environment, such as in the marine environment, has not yet been determined.

The present study focuses on the biodegradation behavior of nylon 4 in the marine environment to confirm that nylon 4 is a biodegradable plastic under the marine environment to reduce marine plastic debris. Biodegradation of nylon 4 in seawater from the Tokyo Bay was evaluated for its biochemical oxygen demand (BOD), weight loss, molecular weight, and surface morphology of nylon 4 films. Furthermore, the biodegradation mechanism was studied on the basis of the molecular weight and surface morphology of the nylon 4 films before and after biodegradation in the seawater.

2. Materials and methods

2.1. Preparation of nylon 4 films

Nylon 4 was prepared using the anionic ring opening polymerization of 2-pyrrolidone using *N*-acyl lactam and potassium

Abbreviations: BOD, biochemical oxygen demand; M_n, number-average molecular weight; P(3HB), poly[(*R*)-3-hydroxybutyric acid]; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy.

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tert-butoxide as an initiator and a catalyst, respectively, by following previously published methods [30,34,35]. Number-average molecular weight (M_n) of the obtained nylon 4 was estimated from the ^{1}H NMR spectra ($M_n = 38.0 \times 10^3$ g/mol). Poly [(*R*)-3-hydroxybutyric acid], P(3HB) (weight-average molecular weight, $M_w = 436 \times 10^3$ g/mol, polydispersity index = 2.4) was obtained from Imperial Chemical Industries (ICI Biological Products, Billingham, UK) [36,37].

Solvent cast film of the nylon 4 was prepared from nylon 4/2,2,2-trifluoroethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) solution (20 g/L) at 25 °C for 1 week. The sizes of the film were approximately 8 mm × 12 mm and 50 μm thick. P(3HB) was purified by a previously reported method [36]. The purified P(3HB) was dissolved in chloroform (20 mg/mL) at 60 °C to make a solvent cast film at 25 °C for 1 week.

2.2. Biodegradation test

The BOD test was performed to estimate the biodegradability of nylon 4 in seawater with a Oxitop IS-6 (WTW GmbH, Weilheim i. OBB, Germany) according to previous literature [38]. A sheet of Nylon 4 film (approximately 10 mg) was immersed in 432 mL of seawater at 25 °C for 25 days. Seawater used in this study was obtained from Odaiba Seaside Park in Tokyo Bay (Tokyo, Japan), which had a pH of 8.0 and salinity of 3.6%.

A sheet of nylon 4 film (approximately 10 mg) was immersed in the seawater (30 mL) in a Falcon tube (50 mL). The tube containing the nylon 4 film and seawater was incubated with constant shaking (180 rpm) at 25 °C for 3 weeks. The seawater was replaced with fresh seawater every week. The nylon 4 film was weighed every week after washing with Milli-Q to remove biofilms and drying *in vacuo* at 25 °C for 3 days.

2.3. Characterization of nylon 4 film after the biodegradation test

^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR analyses were performed with a VARIAN NMR System 500 (Varian Inc., Palo Alto, CA). A mixture of 2,2,2-trifluoroethanol with chloroform-d_1 (volume ratio, 1:1) was used as a solvent. Tetramethylsilane (1%) was added as an internal standard.

Scanning electron microscopy (SEM) images of the nylon 4 films recovered after biodegradation in seawater was taken with a JSM 6330F scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 3.0 kV.

2.4. Statistical analysis

Statistical analysis of weight loss and molecular weight was determined by a paired *t*-test with a two-tailed distribution, and differences were considered statistically significant at $p < 0.05$. The weight loss and molecular weight data for the nylon 4 film are expressed as means ± standard deviation ($n = 3$).

3. Results

3.1. BOD measurement

BOD measurements were performed using nylon 4 and P(3HB) films at 25 °C with stirring for 25 days to evaluate the biodegradability of nylon 4 in seawater from Tokyo Bay. P(3HB) was used as a positive control because it is known to be a biodegradable polyester in natural environments such as rivers [11] and the sea [10]. Fig. 1 shows BOD biodegradability of nylon 4 and P(3HB) films as a function of the incubation time. The degradation of the nylon 4 film started after 12 days, and then BOD biodegradability gradually

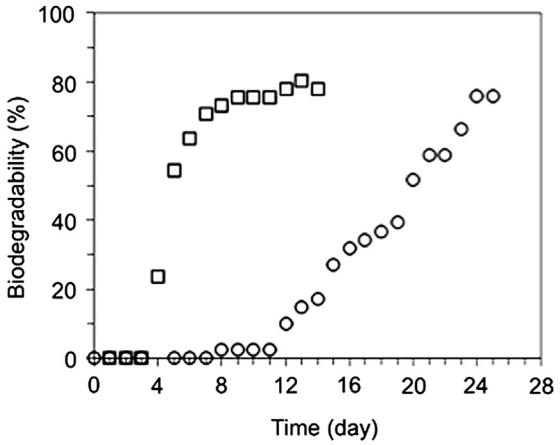

Fig. 1. BOD curves of nylon 4 (○) and P(3HB) films (□) in seawater at 25 °C.

increased with the incubation time. After 25 days of incubation in seawater, the BOD biodegradability of the nylon 4 film reached a constant value of 80%. On the other hand, P(3HB) degradation had a shorter lag phase of 4 days, compared with the lag time of nylon 4 degradation; moreover, and the degradation reached up to 80% within 2 weeks. After 80% degradation by BOD measurement, nylon 4 and P(3HB) films were completely degraded in seawater.

3.2. Weight loss and surface morphology of nylon 4 films during seawater treatment

Nylon 4 films were incubated in seawater for 3 weeks to determine weight loss and to observe the surface morphology of the nylon 4 film during biodegradation. Fig. 2 shows the remaining weight of the nylon 4 film as a function of incubation time. The remaining weight decreased during incubation in seawater, and nylon 4 film lost 30% of the original weight after 3 weeks. Fig. 3 shows SEM images of the nylon 4 films before and during the incubation in seawater at 1, 3, and 5 weeks. The nylon 4 film, which was transparent before incubation (Fig. 3a), became cloudy with the incubation time (Fig. 3b–d). In addition, a biofilm formed on the surface of the nylon 4 film during the incubation in seawater, indicating that microorganisms grew on the nylon 4 film. The nylon 4 film did not collapse even after incubation for 5 weeks (Fig. 3d). Fig. 4 shows SEM images of the nylon

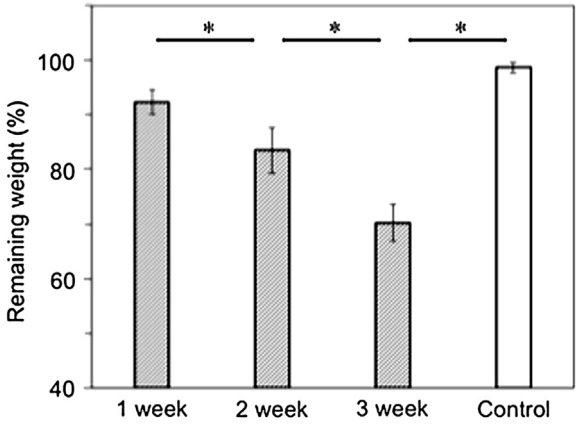

Fig. 2. Remaining weight of nylon 4 films after incubation in seawater for 1, 2, and 3 weeks at 25 °C. Nylon 4 film was incubated in sterilized seawater for 3 weeks as a negative control. Error bars represent the standard deviation of samples ($n = 3$). *Significant difference between 2 groups at $p < 0.05$.