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Bioplastic biodegradation activity of anaerobic sludge prepared by preincubation at 55 °C for new anaerobic biodegradation test

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ABSTRACT

The new method to evaluate the anaerobic biodegradability of bioplastics, such as polycaprolactone (PCL) and poly (lactic acid) (PLA), under aquatic (slurry) conditions at 55 °C is applying. For this method, we prepared the sludge at 55 °C from the sludge at 37 °C by the method in which the sludge from the real tank operating at around 37 °C using cow manure and vegetable waste as the feed stock was preincubated at 55 °C. It was unknown at which stage the sludge during preincubation has the optimized anaerobic biodegradation activity of plastics. Four different stage sludges during preincubation (the sludge at 7 days after the start of preincubation at 55 °C, at 12 days, at 18 days, and at 40 days) were compared by the anaerobic biodegradation activity of PLA. The preincubated sludge at around 18 days (a gradual decrease in biogas evolution and a methane ratio over 60%) showed the highest biodegradation activity of PLA. In addition, the bacterial population in each sludge was analyzed by the denaturing gradient gel electrophoresis (DGGE) analysis of the amplified 16S rRNA gene fragments, however, the newly grown bacteria bands at 55 °C were not clearly detected.

1. Introduction

Anaerobic fermentation has some advantages when compared to aerobic fermentation, such as composting. The anaerobic fermentation plant is a nearly closed system compared to the more odorous aerobic one with a shorter processing time, and produces CH_4 as an energy source. In anaerobic fermentation plants, the non-biodegradable garbage collection bags are currently separated from the feed stocks for anaerobic fermentation. The product, such as a garbage collection bag, made with biodegradable plastics and its waste is thought to be anaerobically degraded with household organic waste or animal manure in the anaerobic fermentation plant. To verify whether biodegradable plastics are degraded in an anaerobic fermentation plant, their biodegradability must be measured in a laboratory, preferably by standard test methods.

The new ISO method to evaluate the anaerobic biodegradability of bioplastics, such as polycaprolactone (PCL) and poly (lactic acid) (PLA), is being verified in Japan. In this method, the anaerobic biodegradabilities of bioplastics were measured by the new system 'MODA–B apparatus' (Fig. 1) [1,2]. This method is more convenient than using a pressure transducer or inverted graduated cylinder submerged in water because the evolved biogas is collected in a gas sampling bag under atmospheric pressure. In these tests, we prepared the sludge at 55 °C from the sludge at 37 °C by the method in which the sludge from the real tank operating at around 37 °C using cow manure and vegetable waste as the feed stock was preincubated at 55 °C [1,2]. The microorganisms working at 55 °C were newly grown during the preincubation at 55 °C, and the mature anaerobic sludge at 55 °C was gradually formed. The preincubated sludge at 55 °C was used for the anaerobic biodegradation test in previous experiments when the biogas evolution from the sludge decreased, because the biogas evolution from the blank vessel was low with this stage sludge [1,2]. With the sludge in this stage, the PLA powder (125–250 μm) degradation at 55 °C was reproducible, about a 60% degradation in 30 days, about 80% in 40 days, and about 90% in 60 days (Fig. 2a). However, the sludge in this stage already passed the logarithmic phase of the microorganism's growth, so there is a possibility that the sludge activity decreased when compared to that of the logarithmic phase. It is unknown which stage's preincubated sludge condition is the most active for the anaerobic biodegradation test of plastics. The preincubated sludge condition (the sludge condition in the vessel of the MODA–B apparatus at the start of the anaerobic biodegradation test) is the most important factor for the plastic anaerobic biodegradation test. It is necessary to determine the sludge in which stage during preincubation is better to be used for the anaerobic biodegradation test of plastics.

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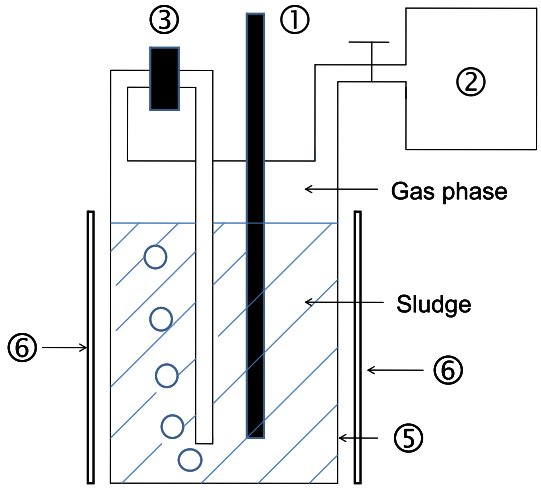

Fig. 1. System of MODA–B apparatus. ① Temperature sensor, ② gas sampling bag, ③ pump (gas phase in head space is exhausted from the bottom of the vessel by this pump), ⑤ test vessel, ⑥ panel heater.

In the present ISO for plastic anaerobic biodegradation (ISO 14853 and 15985), cellulose is used as the reference material, and the validity of the anaerobic biodegradation test is evaluated using cellulose anaerobic biodegradation. This idea is based on the assumption that the cellulose degradation reflects the plastic biodegradation activity of the anaerobic sludge. However, the cellulose anaerobic biodegradation does not reflect the sludge condition including the plastic biodegradation activity. Cellulose was easily degraded even if without the optimized sludge (for example, high H_2S concentration and methane ratio below 50% in the biogas evolved from the sludge, or decreasing anaerobic biodegradation activity due to the temperature reduction of the sludge or incubation (keeping) for a long time in the absence of the carbon source, etc., in the sludge). On the other hand, the normal condition sludge (evolved gas volume from the sludge is not very small and the methane ratio in the gas is over 60%) degrades the PLA to about 90% in 60 days, and non-optimized sludge degrades the PLA below 60% in 90 days. One sludge degrades the PLA (90% degradation in 60 days) while another does not adequately degrade the PLA (50–60% degradation in 90–100 days) even if both sludges adequately degraded the cellulose (80–90% degradation) in some of our preliminary and previous experiments (Fig. 2). Cellulose is very easily degraded, therefore the cellulose degradation does not reflect the sludge condition including the plastic biodegradation activity. Therefore, the sludge condition is not able to be judged by the cellulose degradation. Microorganisms taking part in the biodegradation of cellulose seem to be not very sensitive. On the other hand, the PLA degradation rate decreased in the non-optimized sludge condition. The PLA degradation reflects the sludge condition including the plastic biodegradation activity. Some of the microorganisms taking part in the biodegradation of PLA seems to be sensitive. To judge the sludge condition during each stage of the preincubation, the PLA degradation was used as criteria for the sludge condition.

In this study, the PLA biodegradation was measured using the four different stage sludges during preincubation (at 7 days after the start of preincubation at 55 °C, at 12 days, at 18 days, and at 40 days). With the anaerobic biodegradation activity of plastics, the bacterial population in each sludge was analyzed by denaturing gradient gel electrophoresis (DGGE) analysis of the amplified 16S rRNA gene fragments.

2. Experimental section

2.1. Plastic sample

Cellulose powder of thin-layer chromatography grade with a particle size of less than 20 μm (Cellulose microcrystalline, Merck, Germany) and PCL (Aldrich, USA) were purchased. PLA was obtained from Unitika (Japan). The PLA and PCL powder was prepared using mechanical crushing by previously described methods [3,4]. Powder with a particle size between 125 and 250 μm (Av. 180.7 μm) was used for the test. The averaged molecular weight and the polydispersity of PCL were 43,700 and 2.48, respectively. The melting temperature and the enthalpy of fusion of PCL crushed powders measured by DSC first scanning were 61.3 °C and 83.8 J/g, respectively. PCL powder used in this study was an averaged crystallized PCL. The averaged molecular weight and the polydispersity of PLA were 100,500 and 1.99, respectively. The melting temperature and the enthalpy of fusion of PLA crushed powders measured by DSC first scanning were 165.5 °C and 40.5 J/g, respectively. By this enthalpy value of PLA, this used PLA had a general crystallinity for a crystallized PLA.

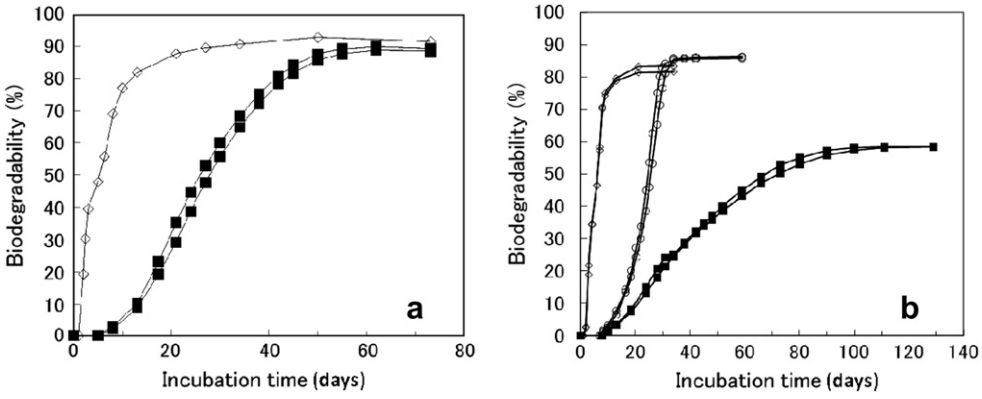

Fig. 2. Cellulose and poly (lactic acid) (PLA) anaerobic biodegradation for normal and non-optimized sludge in previous studies. ◇: cellulose 10 g, ■: PLA 10 g, ○: polycaprolactone (PCL) 10 g. Biodegradability (%) was calculated as $((\Sigma V(s) - \Sigma V(b))/V(\max)) \times 100$). The gas volumes from the blank vessel at each measuring point are assumed to be the values that the volume from the blank vessel between the measuring points was proportionally divided by the incubation time between the measuring points. (a) In normal condition sludge (TS = 2.07%). Biogas volume from blank vessel was 0.87 (L).The sludge in 27 days after the start of the preincubation at 55 °C was used. (b) In non-optimized sludge (TS = 0.83%). Biogas volume from blank vessel was 0.03 (L). The sludge in 21 days after the start of the preincubation at 55 °C was used. The sludge was diluted twice with water bubbled with N_2 gas.