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Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes

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Abstract

The aerobic and anaerobic biodegradation of polyethylene glycols (PEGs) in a model wastewater was investigated employing sludge microbes from Xiamen Terylene plant. The effect of molecular weight (MW) on the aerobic/anaerobic biodegradation of PEGs was assessed by performing shake flasks/sealed flasks experiments, respectively, using organic model wastewater containing PEG 600, 6000 and 20,000. In aerobic biodegradation, although there were differences between the degradation processes, the three kinds of PEG were all degraded by about 80% in 5 days, regardless of the wide diversity of MW. In anaerobic biodegradation, about 50% degradation of PEG 600 in 9 days, 40% degradation of PEG 6000 in 10 days and 80% degradation of PEG 20,000 in 6 days were obtained. The effect of nutrition on anaerobic degradation was also investigated. The biodegradation rate of PEG 6000 increased sequentially in the organic, inorganic and enriched organic media. The latter two were similar to each other. In the enriched organic media PEG 6000 was degraded by 50% in 10 days and 70% in 14 days, while in the organic media the degradation rate was 23% in 10 days and 50% in 14 days. It was notable that the anaerobic microbes could use PEG 6000 as sole carbon source, and PEG MW up to 20,000 was efficiently biodegraded in this study, either in aerobic or anaerobic way.

Keywords: Biodegradation; Polyethylene glycol (PEG); Aerobic process; Anaerobic process

1. Introduction

Polyethylene glycols (PEGs, $HO(CH_2CH_2O)_nH$) are an important group of non-ionic synthetic water-soluble polymers of ethylene oxide. These compounds are widely used in the production of pharmaceuticals, cosmetics, lubricants and antifreeze for automobile radiators, in the conservation treatment of ancient waterlogged wood, and in the manufacture of non-ionic surfactants. Every year, millions of tons of PEG are manufactured worldwide and most of them reach conventional sewage disposal systems after industrial utilization. From the last three decades, concern has been expressed about the fate of these polymers in the environment and several studies have been performed on their biodegradability.

Haines and Alexander [1] studied the aerobic degradation of PEGs using soil microorganisms and reported that a strain of *Pseudomonas aeruginosa* was able to degrade mono-, di-, tri- and tetra-ethylene glycol and PEGs up to 20,000 MW. Unfortunately, the strain was said to be lost. Obradors and Aguilar [2] reported aerobic biodegradation of PEGs up to 14,000 MW, using a bacterium isolated from river sediments. PEGs can also be degraded by the synergistic action of two or more strains of organisms. Sewage bacteria have been used by Watson and Jones [3] to degrade PEGs up to 1500 MW, while Cox and Conway [4] were able to degrade aerobically PEGs up to 4000 MW. Kawai et al. [5–7] investigated the symbiotic degradation of PEGs by mixed culture of *Flavobacterium* and *Pseudomonas* species. Neither of the bacteria alone could utilize PEGs for growth, but when both cultures were grown together they degraded PEGs ranged from 300 to 20,000 MW. According to their experiments, enough growth of symbiotic bacteria on PEG 20,000 was obtained in 7 days (1.7 of optical density at 610 nm). A mixture of PEGs with molecular weights of 400–6000 was degraded by 97% in 4 days and 100% in 6 days.

Dwyer and Tiedje [8,9] studied the anaerobic degradation of PEGs and found that PEG 20,000 could be metabolized

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by methanogenic consortia isolated from sewage sludge, and the rate of degradation was inversely proportional to the number of ethylene glycol units presented in the molecule. 82% degradation of PEG 20,000 and 83% degradation of PEG 10,000 in 12 days, 100% degradation of PEG 400 in less than 4 days were obtained. In another study, Schink and Stieb [10] investigated the anaerobic biodegradability of PEG by *Pelobacter Venetianus* sp. nov. They found that PEGs with molecular weight from 106 to 20,000 were degraded completely in enrichment cultures of the bacteria.

Although the above studies reported that even high MW PEGs were amenable to biodegradation, quantitative assessment of the application of these strains to the wastewater containing PEGs was not given. Little attention has paid to aspect of total removal of PEGs from wastewater by biological methods. Research work on the removal of PEGs from wastewater totally by biological methods is scarce. Only some combined treatments were reported. Suzuki et al. [11,12] reported that a significant improvement in biodegradability occurred after ozonation pretreatment. They studied the ozonation and subsequent fragmentation of PEG 8000 and found that a soil microorganism, which was not capable of utilizing PEG 8000, could easily degrade PEG 300. Otal et al. [13] studied the integrated treatment of a model wastewater containing PEG 10,000 by means of a continuous WAO (wet air oxidation) process followed by continuous aerobic biodegradation and found the efficiency was enhanced compared to the direct biological treatment. However, chemical oxidation processes (i.e. ozonation, wet air oxidation) are generally considered to be less environmentally friendly and more costly than biological treatment processes.

The treatment of wastewater containing PEGs attracted concern because the resistance of PEGs to normal biodegradation results in their long time existence in the natural environment. This threatens the health of mankind. Understanding the biodegradation process of PEGs and finding possible candidate microbes to remove these pollutants from our environment are of paramount importance. The aims of this work are to screen for microbes with high degradability on PEGs, investigate their aerobic and anaerobic biodegradation processes, and make possible the totally biological treatment of wastewater containing PEGs by taking advantages of both aerobic and anaerobic processes.

2. Materials and methods

2.1. Microbiological methods

Microbes were separated from the sludge of Xiamen sewage treatment plant (XP) and Terylene plant (TP), respectively. Aerobic cultivation was carried out in 500 ml shake flasks with 80–100 ml media at the temperature of 30 °C, on a rotary shaker (120 r/min, 25 mm stroke), while anaerobic cultivation involved 500 ml sealed flasks with 250 ml media at 37 °C and 40 r/min. The biomass of microbes was monitored by counting cells in a hemocytometer. Degradation of PEGs was determined by analyzing the PEG concentration in the culture media after microbe growth.

2.2. Media (g/l)

2.2.1. The trace elements solution contained (mg/l)

$CaCl_2$ 40, $FeSO_4{\cdot}7H_2O$ 40, $MnSO_4{\cdot}4H_2O$ 40, $ZnSO_4{\cdot}7H_2O$ 20, $CuSO_4{\cdot}5H_2O$ 5, $CoCl_2{\cdot}6H_2O$ 5, $Na_2MoO_4{\cdot}2H_2O$ 5.

2.2.2. The inorganic medium contained (g/l)

K_2HPO_4 0.625, KH_2PO_4 0.375, $MgSO_4{\cdot}7H_2O$ 0.25, NH_4Cl 0.5, NaCl 1 and trace element 1 ml.

2.2.3. The organic medium (1 l)

Yeast extract of 0.25 g was added to the inorganic medium.

2.2.4. The enriched organic medium (1 l)

Glucose of 20 g, 0.25 g yeast extract and 4.0 g peptone were added to the inorganic salts medium. To perform PEG biodegradation experiments, a certain amount of PEG (1.0–2.0 g) was added to the medium described above.

2.3. Analytical methods

PEG was determined by spectrophotometric analysis in an ammonium ferrothiocyanate aqueous–chloroform system [14] or by thin layer chromatography (TLC) [3].

3. Results and discussion

3.1. Isolation of microbes

In order to screen and obtain the goal microbes efficiently, attention should be paid to the original environment where they are living. Sewage from a Terylene plant often contains organic acids, ethylene glycol, and some polymers. Microbes adapted to this kind of sewage are supposed to be extraordinary and will exhibit special biodegradation capacities. Therefore, comparative experiments were conducted with the sludge microbes from Xiamen domestic sewage treatment plant (XP) and the sludge microbes from a Terylene plant on their ability to biodegrade PEG 600. Organic and inorganic media were used, respectively.

TLC results show that in the aerobic experiments, there was no perceptible degradation on PEG 600 except in experiments using sludge microbes from TP and the organic media. PEG 600 almost disappeared in 5 days. In the anaerobic experiments, however, the results were similar. Sludge microbes from both plants could degrade the PEG 600 to some extent in 9 days, no mater whether the media were organic or inorganic. It is confirmed that the anaerobic sludge microbes in both plants were mostly composed of methanogenic

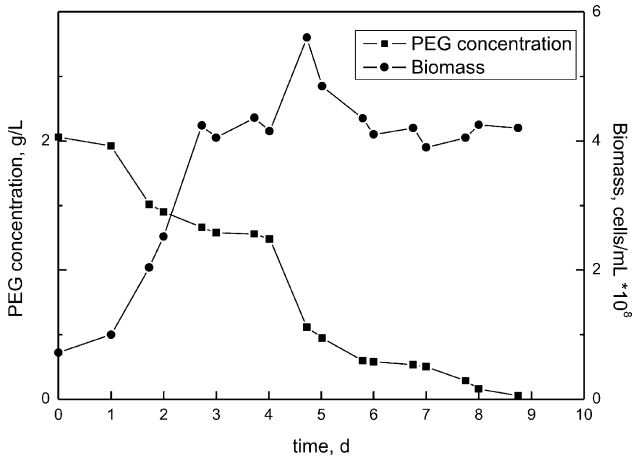

Fig. 1. Biomass growth and PEG 600 concentration during shake flasks experiments for PEG 600.

consortia. The similar results in our experiments are supposedly due to the same functional microbes, according to the report from Dwyer and Tiedje [8,9] that methanogenic consortia isolated from sewage sludge could metabolize PEGs up to 20,000 MW.

3.2. Aerobic biodegradation of polyethylene glycols in organic media

The comparative experiments above indicated that the aerobic sludge microbes from TP were extraordinary. Therefore, further experiments were performed to assess quantitatively their biodegradability on PEGs 600, 6000 and 20,000. The results are shown in Figs. 1–3, where the concentration of PEG 600, 6000 and 20,000 together with the biomass is plotted against time, respectively.

Growth of microbes on PEG 600 exhibited a lag phase of about 1 day, during which neither significant biomass growth nor PEG removal was found (Fig. 1). However, within the next day, biomass increased rapidly while PEG 600 decreased smoothly. On the 4th day the microbes had a

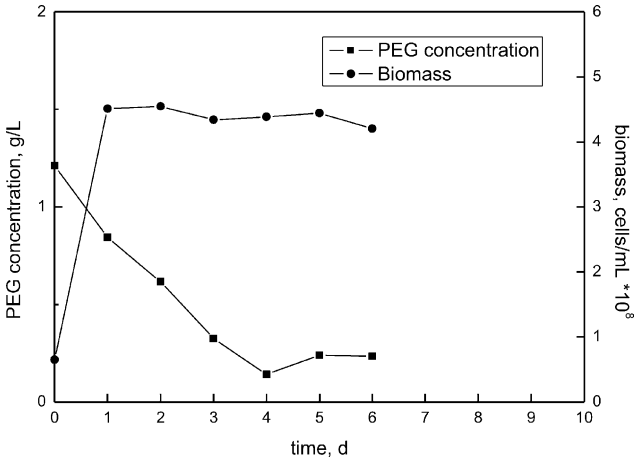

Fig. 2. Biomass growth and PEG 6000 concentration during shake flasks experiments for PEG 6000.

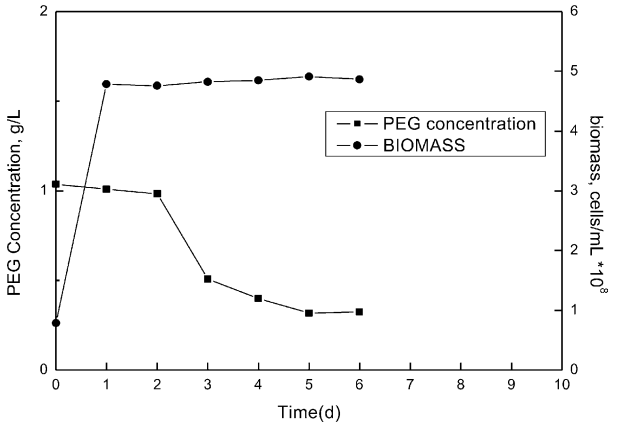

Fig. 3. Biomass growth and PEG 20,000 concentration during shake flasks experiments for PEG 20,000.

second crest of growth, while the PEG 600 degraded rapidly. 85% degradation could be reached after 5 days. The growth of microbes accurately correlated with the biodegradation of PEG 600. Fig. 2 shows that PEG 6000 was biodegraded evenly and the growth of microbes reached a stationary phase after 1 day. 88% degradation could be obtained in 4 days. Although PEG 20,000 was not degraded in the first 2 days (Fig. 3), a significant decrease could be observed from the 3rd day and 77% degradation could be obtained in 5 days.

It is notable that PEGs with a high molecular weight could also be biodegraded aerobically. MW did not affect the biodegradability significantly in the long term. pH value changes were similar in the above experiments, falling from 7.0 to 6.0 and then remaining constant.

3.3. Anaerobic biodegradation of PEGs

Anaerobic microbes were adapted to PEG containing wastewater for 46 days before they were employed in the anaerobic biodegradation experiments.

Results of anaerobic biodegradation on PEGs are shown in Fig. 4. As to PEG 600, 23% degradation in 5 days and 45% degradation in 9 days were obtained. These results indicate that the anaerobic biodegradation of PEG 600 did not show any advantages compared to its aerobic biodegradation. This conclusion was also applicable to PEG 6000. Only 40% degradation on PEG 6000 was obtained in 10 days. However, an exciting result came from PEG 20,000. After 4 days' stagnant, PEG 20,000 was decreased by 70% in the next 2 days. No linear relationship between the degradability and PEG MW could be deduced in this study. These results were quite different from the report of Dwyer and Tiedje [8,9].

Biomass in the anaerobic experiment (Fig. 5) is about half less than that in the aerobic experiments (Figs. 1–3). The lower biomass might be responsible for the lower degradation rates in the anaerobic experiments, whereas the degradation of PEG 20,000 was exceptional. The metabolic rate in anaerobic environment was normally lower.

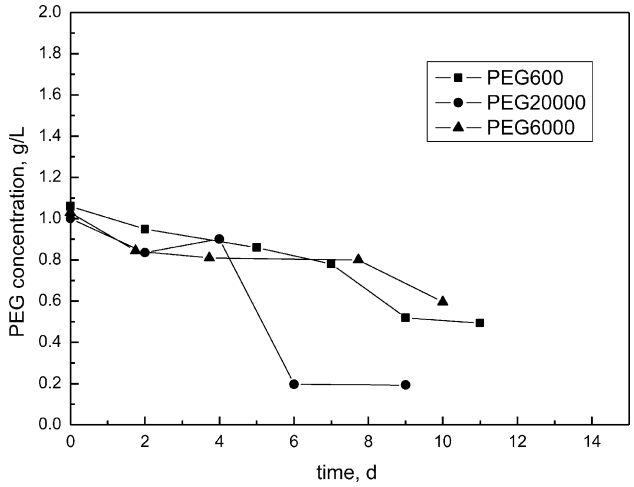

Fig. 4. PEG concentration during the anaerobic experiments.

The pH changes in the anaerobic experiments were similar to the aerobic ones. pH fell from 7.0 to 6.0 and remained constant at this level. Since these pH values were quite different from those optimal for the adequate metabolism of methanogenic bacteria, it might be another cause of the lower degradation rates observed in these experiments.

3.4. Anaerobic biodegradation of PEG 6000 in different media

To improve the degradation rate, the effect of nutrition on anaerobic degradation was investigated. Biodegradation of PEG 6000 by anaerobic bacteria was performed in enriched organic, organic and inorganic media.

Fig. 6 shows the biomass during the experiments using various media. It is evident that the biomass in the inorganic media was lower than that in the other two and remained low. Biomass in the organic media increased significantly at the 10th day and remained high. In the enriched organic media, biomass concentration reached its maximum value on around the 10th day.

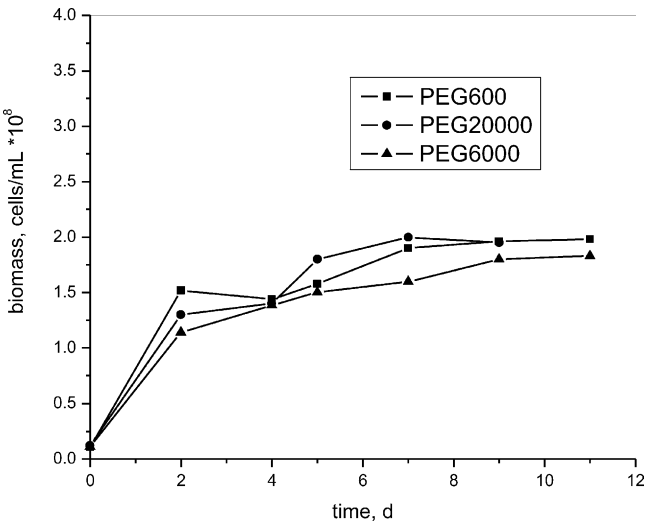

Fig. 5. Biomass during the anaerobic experiments.

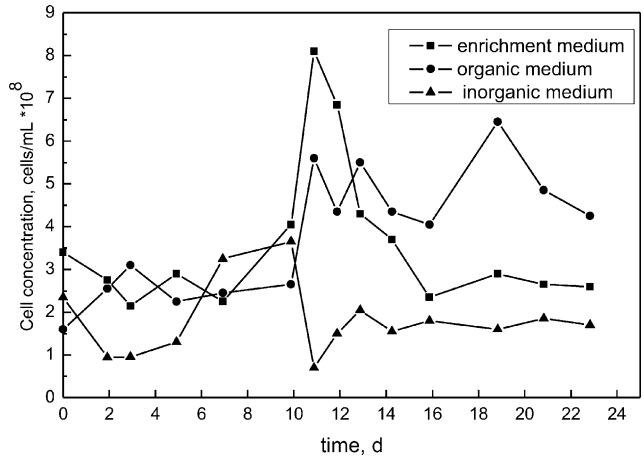

Fig. 6. Biomass during anaerobic degradation experiments in various media.

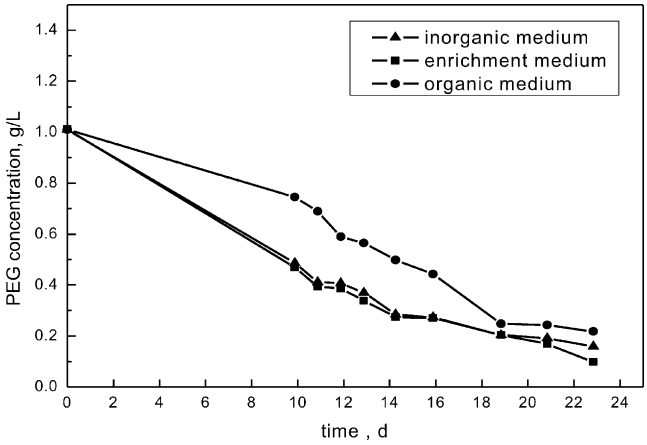

Fig. 7. PEG 6000 concentration during anaerobic degradation experiments in various media.

Fig. 7 shows the PEG 6000 concentration during experiments using various media. The biodegradation rate increased sequentially in the organic, inorganic and enriched organic media. The latter two were similar. It is notable that some anaerobic microbes could use PEG 6000 as sole carbon source. Higher the efficiency of the biodegradation could be achieved using the enriched organic medium, in which PEG 6000 was degraded by 50% in 10 days and 70% in 14 days, while in the organic medium the degradation rate was 23% in 10 days and 50% in 14 days.

4. Conclusions

Aerobic and anaerobic biodegradation of PEG 600, 6000 and 20,000 was investigated. PEGs were more likely to be degraded by an aerobic process and its efficiency is much higher than that of the anaerobic process. This is

somewhat different from the general conception [15]. A reasonable explanation for it is the employment of efficient microbes. These results also suggest an innovative treatment for wastewater containing PEGs by a totally biological method. For the optimization of the aerobic process on PEGs, knowledge of the properties of the microbes, the reaction mechanism and pathway is necessary. Research on these topics is ongoing in our laboratory.

Acknowledgements

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