Enhancement of Biodegradation of Oil Adsorbed on Fine Soils in a Bioslurry Reactor

Tetsuji Okuda, Martha E. Alcántara-Garduño**, Masahiro Suzuki**, Chikara Matsui**, Tomohiro Kose***, Wataru Nishijima*, Mitsumasa Okada*****

**Corresponding author*
Environmental Research and Management Center, Hiroshima University
1-5-3 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8513 Japan
Tel.&Fax +81-82-424-6195
aqua@hiroshima-u.ac.jp
***Department of Material Science and Chemical System Engineering, Graduate School of Engineering, Hiroshima University*
****Graduate School of Natural Science and Technology, Okayama University*
*****Director-Vice president, Hiroshima University*

Abstract

Techniques for enhancing the biodegradation of oil-contaminated fine soils in a slurry-phase bioreactor were investigated. Using a model system consisting of kaolin particles containing adsorbed *n*-dodecane as a diesel fuel surrogate, we investigated how increasing the temperature and adding a surfactant and various hydrophobic support media affected the biodegradation rate of *n*-dodecane. Increasing the temperature from 25 to 35°C decreased the time required for complete degradation of *n*-dodecane by 30%, from 110 h to 80 h. Addition of the surfactant polyethylene glycol *p*-1,1,3,3-tetramethylbutylphenyl ether decreased the degradation time to less than 48 h at 35°C, although a high concentration of the surfactant (3000 mg l^{-1}) was required. We suspect that the surfactant increased the degradation rate by solubilizing the *n*-dodecane into the solution phase in which the microorganisms were suspended. We tested five types of organic polymers as support media for the microorganisms and found that the biodegradation time could be reduced by approximately 50% with a support medium made from polyurethane; in the presence of this medium, only 36 h was required for complete decomposition at 35°C. The reduction in the degradation time was probably due to transfer of the *n*-dodecane from the soil to the support medium, which improved contact between the *n*-dodecane and the microorganisms. The polyurethane support medium bearing the microorganisms was stable and could be reused.

Keywords: *n*-dodecane; remediation; support media; surfactant; temperature

Introduction

Petroleum products such as diesel fuel, heavy oil, gasoline, fuel residues, and mineral oil are common soil contaminants. Although these contaminants are not regulated by the Soil Contamination Countermeasures Law (2003) in Japan, remediation of oil-contaminated soils to eliminate oily odors and oil film on runoff water and in groundwater is required.

The U.S. Environmental Protection Agency (EPA) introduced soil washing as a remediation technology for oil-contaminated soils (US EPA, 2001). Soil washing is quicker than other remediation methods, such as bioremediation, chemical remediation, and combustion (Anderson et al., 1999; Mouri et al., 2004).

Washing can be used to remove oils on large particles such as sand and gravel, but removing oils from fine particles such as silt (particle diameter, 5–75 μm) and clay (< 5 μm) is difficult. Oils are concentrated on the surface of finer particles owing to its high specific surface area (Okita et al., 1999; Mouri et al., 2004). After washing, soils composed mainly of large particles can be used as backfill at the original contamination site. However, soils consisting of fine particles are disposed of at landfill sites, because oils are concentrated on it. Fine particles make up 20% to 50% of some soils (Anderson et al., 1999; Shiratori, 2003; Mouri et al., 2004), and if these soils are to be reused, they must undergo further treatment after washing.

The biodegradation of oils in a slurry-phase bioreactor (SPB) has a higher degradation rate than other biological treatment methods (US EPA, 1990; Puskas et al., 1995). Various modes of SPB operation have been tested in laboratories and pilot-scale plants, and one of the most common and best performing modes involves a soil slurry–sequencing batch reactor (SS-SBR). For example, Cassidy et al. (2000) found that the concentration of diesel oil on soil (real contaminated sandy loam) could be reduced from 12 g $kg_{\text{-soil}}^{-1}$ to less than 1 $kg_{\text{-soil}}^{-1}$ in 4 d by means of an SS-SBR under conditions of controlled dissolved oxygen (DO) levels and pH. If an SPB process could be developed for the degradation of oils on fine particles, soil washing following by an SPB process would be a useful method for complete remediation of soils containing fine particles and would allow for the reuse of such soils. However, the biodegradation rate of oil on the fine fraction of soil is lower than that on the larger fraction in SPB (Bhadari et al., 1994). Geerdink et al. (1996) reported that reducing the concentration of diesel oil in soil with high clay and silt contents from 17 g $kg_{\text{-soil}}^{-1}$ to 0.05 g $kg_{\text{-soil}}^{-1}$ took more than 10 weeks in an SPB. Therefore, technologies for accelerating the degradation of oils on fine soils in SPBs must be developed.

Nano et al. (2003) found that diesel oil contamination in soil (sieved natural soil) could be reduced from about 15 $kg_{\text{-soil}}^{-1}$ to approximately 1 g $kg_{\text{-soil}}^{-1}$ in a week if sand and a surfactant were added to the soil. Cassidy and Hudak (2001) found that the production of biosurfactant by microorganisms enhanced the biodegradation of diesel oil. These results suggest that biodegradation could be enhanced by increased bioavailability, that is, by an increase in the concentration of dissolved oil (Rijnaarts et al., 1990).

In this study, we adopted a different method for enhancing the biodegradation of oil: we transferred the oils from the fine soil and increased contact between the microorganisms and the oil. We attempted to enhance the biodegradation by adding a surfactant and various support media and by increasing the temperature. The microorganisms must be separated from the soil so that both can be reused. The use of a support medium bearing the microorganisms also permitted the separation of the microorganisms from the soil after purification. We also investigated the reusability of the support media.

Materials and Methods

In this study, we used a model system consisting of kaolin particles with adsorbed *n*-dodecane as a diesel fuel surrogate to investigate a method for the purification of oil from fine soil fractions in an SPB. Although oil-polluted soils obtained from the environment can behave differently than artificially polluted soil samples, we nevertheless used a model system to ensure repeatability. *n*-Dodecane was used as diesel fuel surrogate because its biodegradation behavior is

similar to that of diesel fuel (Nano et al., 2003). Kaolin particles (Kanto Chemical; average diameter, ~3 μm) were used to model a fine soil, and *n*-dodecane (Trade TCI Mark) was used as a diesel fuel surrogate. *n*-Dodecane (2 g, 2.7 ml) was added dropwise to 198 g of kaolin, and then the mixture was mixed well and aged for 3 d.

The biodegradation of *n*-dodecane adsorbed on the kaolin surface was carried out in an SPB (Fig. 1). The operating conditions for the SPB are summarized in Table 1. In a glass reactor with three ports and a working volume of 2 l, the kaolin with adsorbed *n*-dodecane (10 g $kg_{\text{-kaolin}}^{-1}$) was mixed with enough tap water to bring the total slurry volume to 500 ml. Activated sludge (500 ml) containing 8000 mg l^{-1} of mixed liquor suspended solids (MLSS) was added, affording 1 l of kaolin slurry with a final MLSS concentration of 4000 mg l^{-1}. A fresh activated sludge was obtained from a municipal sewage treatment plant in Japan for each batch experiment. One of the reactor ports was fitted with a bubble diffuser stone through which humidified air was supplied at 1 l min^{-1}. The DO level was kept at least 2 mg-O_2 l^{-1} in all the experiments. A mechanical mixer with Teflon impeller blades occupied the central port of the reactor, and the third port was used for off-gas and for the sampling of slurry. Nutrients (K_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$) were added at the beginning of each experiment to obtain a C:N:P ratio of approximately 10:10:1. The water temperature was controlled with a water bath, and the pH was maintained at 7.0 ± 0.5 with HCl and NaOH solutions. No antifoaming agent was added.

Polyethylene glycol *p*-1,1,3,3-tetramethylbutylphenyl ether (Triton X-100) was selected as the surfactant because it is less prone to blister formation during aeration than sodium dodecyl sulfate and polyoxyethylene lauryl ether. The concentration of Triton X-100 used as surfactant was set at 3000 mg l^{-1} to avoid excessive foaming. It was added at the beginning of each experiment with the nutrients.

We transferred the oils from the fine soil to a support medium bearing microorganisms, and this transfer increased contact between the microorganisms and the oil. Hydrophobic support media such as organic polymers (plastics) are favorable media in the adsorption of both oil and microorganisms. Five support media, including three types of polyurethane (PU-1, -2, and -3) and two types of polyvinyl alcohol (PVA-1 and -2), were used. Microorganisms from the activated sludge were attached to the support media (cube; each 5 mm) by mixing of the support media and activated sludge before the experiments. The adhesion of microorganisms onto the support media was conducted at a medium concentration of 100 ml l^{-1} in 10 l of activated sludge (8000 mg l^{-1} MLSS) with aeration and mixing (approximately 1 l min^{-1} and 50 rpm, respectively) at room temperature (around 20°C). The microbial concentration on each support medium was adjusted to 2000 or 4000 mg $l_{\text{-medium}}^{-1}$ (1000 or 2000 mg $l_{\text{-slurry}}^{-1}$ of MLSS) by adjusting the mixing period (6–24 h). A control experiment without a support medium was carried out simultaneously with the experiments with the five support media.

Slurry samples were removed from the reactor at various intervals. Kaolin particles and the support media were separated from the solution by filtration through a 1 μm glass filter (Whatman, GF-B) and flatten out the media to squeeze out inside solution. The support media could be separated by hand sorting on the filter on basis of particle size. Suspended and emulsified *n*-dodecane was also trapped by the filtration and measured. The *n*-dodecane trapped on the filter with

the kaolin and the support medium was extracted by Soxhlet extraction with hexane/acetone (1:1) for 16 h. The *n*-dodecane dissolved in the filtrate was extracted by liquid–liquid extraction with hexane/acetone (1:1). *n*-Dodecane contained in the off-gas was also measured after being trapped in hexane solution. The methods used for extraction of *n*-dodecane adsorbed and dissolved were based on an EPA method and a Japanese Industrial Standard (JIS) method, respectively (US EPA, 1994; JIS, 1998). The concentrations of *n*-dodecane in the hexane/acetone solutions were determined by gas chromatography with flame ionization detection (Shimadzu, GC-18A). Phenanthrene-*d101* was used as an internal standard. The recovery percentage was more than 80%, and the detection limit was approximately 0.01 g-dodecane $kg_{\text{-kaolin}}^{-1}$.

Dissolved organic carbon (DOC) in the solution was determined using a total organic carbon analyzer (Shimadzu, TOC-V_{CSN}) after the suspended materials were separated by centrifugation. The microbial biomass on the support media was calculated from the weight difference between the dried weight of the fresh support media and that of the support media after incubation with the microorganisms.

Results and Discussion

The results for the biodegradation of *n*-dodecane in solution (that is, in a suspension with activated sludge without kaolin particles) and for the biodegradation of *n*-dodecane adsorbed on kaolin particles at 25°C are shown in Fig. 2a. The time required for complete degradation (to a concentration lower than the detection limit) of *n*-dodecane was 48 h in the absence of kaolin particles. In contrast, more than 110 h was necessary for the complete biodegradation of *n*-dodecane on kaolin particles. The amounts of *n*-dodecane in the off-gas were only 9% and 3% of initial amount in the reactor after 50 h of reaction without kaolin and after 140 h with kaolin, respectively. The percentage of *n*-dodecane in the off-gas was less than 5% in subsequent experiments, so it was neglected throughout this research. In addition, there was no significant difference (less than 15%) in the reproducibility of the time necessary for complete biodegradation. The biodegradation of *n*-dodecane in the absence of kaolin was much faster than that of *n*-dodecane in the presence of kaolin, indicating that adsorption of *n*-dodecane on kaolin hindered biodegradation. In fact, the *n*-dodecane concentration in the solution fraction was always below the detection limit in the presence of kaolin. The faster degradation performance in the absence of kaolin was due to enhancement of the contact between *n*-dodecane and microorganisms in solution.

We tried to enhance the biodegradation of *n*-dodecane on kaolin by three methods: we increased the temperature to enhance *n*-dodecane dissolution and to increase the bioactivity of the activated sludge; we added a surfactant to enhance *n*-dodecane dissolution; and we added support media to increase contact between *n*-dodecane and the microorganisms on the support media.

Temperature

The effect of temperature on the biodegradation of *n*-dodecane was evaluated at 20, 25, 30, and 35°C (Fig. 2b). The time required for complete degradation was shortened from more than 130 h at 20°C to less than 85 h at 35°C; the difference between the biodegradation rates at 25 and 30°C was especially large. The higher

bioactivity and the enhanced dissolution of *n*-dodecane at higher temperature clearly enhanced the biodegradation rate of *n*-dodecane.

Surfactant

Figure 2c shows the results for the biodegradation of *n*-dodecane on kaolin at 25°C with and without the surfactant Triton X-100. The rate of biodegradation was markedly enhanced by the addition of 3000 mg l^{-1} of Triton X-100. The time required for complete degradation was shortened from about 110 h to less than 48 h. The enhancement was likely due to the dissolution of *n*-dodecane and to better contact with the microorganisms. *n*-Dodecane was detected at a concentration of approximately 86 mg l^{-1} in the solution (approximately 6% of the total *n*-dodecane) at 2 h after Triton X-100 was added. The *n*-dodecane concentration in the solution phase was almost the same throughout the experiment, and the concentration had fallen below the detection limit by the end of experiment (48 h). In contrast, no *n*-dodecane was detected in the solution without Triton X-100.

These results confirm that the transfer of the *n*-dodecane from the kaolin surface into the solution enhanced the biodegradation. However, a high concentration (3000 mg l^{-1}) of the surfactant was required, because no *n*-dodecane was detected in the solution with 1000 mg l^{-1} of Triton X-100. The surfactant remained in the reactor after biodegradation; approximately 200 mg l^{-1} of DOC remained even after 140 h, whereas only 20 mg l^{-1} of DOC was detected in the control experiment without Triton X-100. The residual DOC, which was due to Triton X-100 and intermediates of Triton X-100 biodegradation, could contaminate the purified soils. In addition, microorganisms were mixed with the purified soils, owing to the difficulty of separating the microorganisms from the purified fine soils. The presence of organic materials and microorganisms in the purified soils makes the soils unsuitable for reuse.

Support Media

To enhance contact between the *n*-dodecane and microorganisms and to facilitate the separation of the microorganisms from the purified fine soils, we experimented with adding various support media. The support medium bearing the microorganisms could be easily separated from the soil owing to the size difference between the medium and the soil particles. The SPB performance was evaluated at 35°C with the support media bearing the microorganisms. Figure 2d shows the biodegradation performance for *n*-dodecane on kaolin with and without PU-1 as a support medium. Complete degradation was achieved within 36 h in the SPB with PU-1. The time required for complete degradation was approximately half the time required without PU-1. The *n*-dodecane concentration in the solution fraction was always below the detection limit in each experiment.

The transfer of *n*-dodecane from the kaolin to PU-1 was evaluated in the absence of microorganisms on PU-1. Approximately 10% of the *n*-dodecane applied with the kaolin was detected on the surface of PU-1 after 10 h, which indicates that the *n*-dodecane was transferred from the kaolin surface onto PU-1. *n*-Dodecane would come into contact with microorganisms on the surface of PU-1 and would be biodegraded rapidly.

The biodegradation performances with five support media were compared on the basis of the time required for complete degradation. The ratios of the time required with each support medium to the time required without a support

medium are shown in Fig. 3. PU-1 showed the lowest ratio among the five media, and PU-3 and PVA-2 showed no remarkable enhancement of the biodegradation. The ratios for PU-1 and PU-3 differed markedly, as did the ratios for PVA-1 and PVA-2, even though these pairs of support media were made from the same materials. This result indicates that the performance cannot be explained in terms of the chemical properties of the base polymers. PU-1 and PU-3 have different surface characters, owing to the presence of dopants and coating agents related to hydrophobicity. Expressing the hydrophobicity numerically is difficult because the support media are poromeric, and they have different pore sizes. However, visual observation revealed that there were more bubbles on the PU-1 surface than on the PU-3 surface. The adsorbability of *n*-dodecane and the microorganisms on the surface of PU-1 may have been higher than on the surface of PU-3, which may have resulted in the higher performance with PU-1.

We also evaluated the reusability of PU-1 by carrying out multiple runs. The microbial concentration on PU-1 was set at 2000 mg $l_{\text{-mediun}}^{-1}$ (about 1000 mg l^{-1} of MLSS in the reactor) in the first run. The time required for complete degradation decreased until the third run, owing to the increase in microbial concentration on PU-1, and then the time leveled off after the fourth run (Fig. 4). This result indicates that the PU-1 bearing microorganisms could be reused without substantial decrease in bioactivity. The specific degradation activities in these five runs were 65–89 $\times$ 10^{-3} mg min^{-1} mg^{-1}, and no significant differences were observed. This result indicates that the decrease in the time required for complete degradation during the first three runs was mainly due to increases in the microbial concentration, not to increases in microbial activity.

Conclusions

The goal of this study was to enhance the biodegradation of *n*-dodecane adsorbed on fine soils (kaolin) in a slurry-phase bioreactor by adding a surfactant and support media and by increasing the temperature. On the basis of the results, we made the following conclusions:

1) Raising the temperature from 25°C to 35°C decreased the time required for complete degradation of *n*-dodecane by approximately 30%.
2) The addition of Triton X-100 as a surfactant also reduced the time required for biodegradation, but a high concentration of the surfactant (3000 mg l^{-1}) was required.
3) The biodegradation time was shortened by the addition of a support medium. The degree to which the support media reduced the biodegradation time varied, and a support medium made from polyurethane reduced the biodegradation time by 50%. We suggest that the support medium enhanced the biodegradation by assisting in the transfer of *n*-dodecane from the soils to the support medium and by improving contact between *n*-dodecane and microorganisms on the medium.
4) The support medium made from polyurethane with microorganisms was stable and could be reused.

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Figures and Tables

Captions

Figure 1. The schematic of the experimental slurry-phase bioreactor.

Table 1. Operating conditions for the slurry-phase bioreactor.

Figure 2. Biodegradation of n-dodecane. a: with (on) and without kaolin at 25°C, b: at 20–35°C, c: with and without 3000 mg l^{-1} of Triton X-100 at 25°C, d: with and without PU-1 at 35°C. (2000 mg l^{-1} of MLSS for a-c, 4000 mg l^{-1} of MLSS for d)

Figure 3. Ratio of biodegradation time relative to control for the five support media. (35°C, 2000 mg l^{-1} of MLSS)

Figure 4. Degradation times and microbial concentrations for five runs. (35°C)

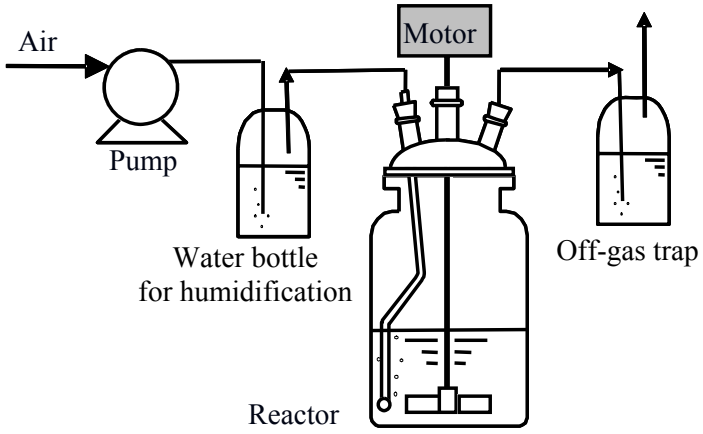

Figure 1

Table 1.

Reactor volume	2 l
Slurry volume	1 l
Kaolin concentration	200 g $l_{\text{-slurry}}^{-1}$
Oil concentration	10 g $kg_{\text{-kaolin}}^{-1}$
Slurry solvent	tap water
Temperature	20–35°C (±1°C)
Aeration	1 l min^{-1}
pH	7.0 ± 0.5

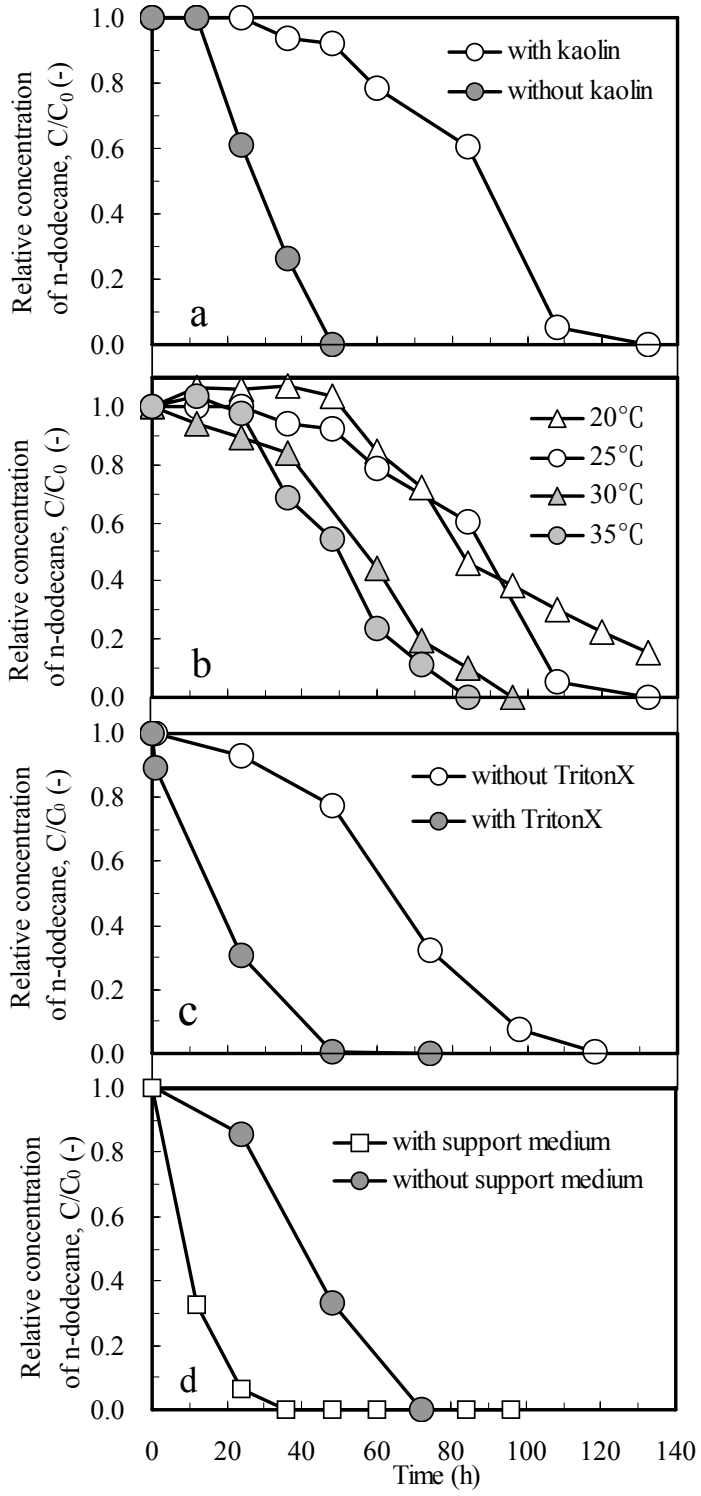
Relative concentration of n-dodecane, C/C0 (-)
1.0
0.8
0.6
0.4
0.2
0.0
a
with kaolin
without kaolin
b
20°C
25°C
30°C
35°C
c
without TritonX
with TritonX
d
with support medium
without support medium
0
20
40
60
80
100
120
140
Time (h)

Figure 2.

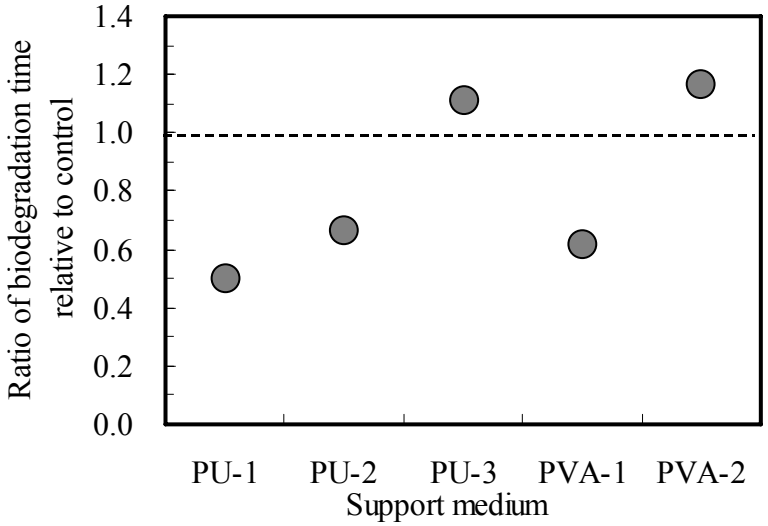

Figure 3.

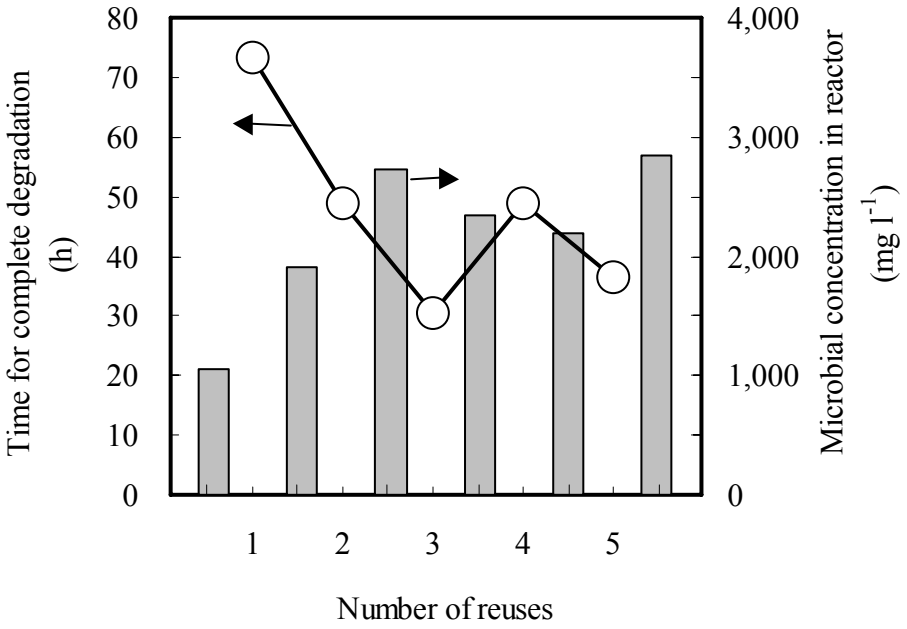

Figure 4.