

Biodegradation of Aromatic Compounds from Soil by Drum Bioreactor System

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Abstract A drum bioreactor was used for the treatment of sandy soil contaminated with three kinds of aromatic compounds (phenol, naphthalene, and phenanthrene), and its performance was evaluated in two different operation modes; intermittent and continuous rotation of drum. When the drum bioreactor was operated with one rotation per day, the microbial growth was relatively low, and most of the compounds remaining in soil, except naphthalene of 90 mg/kg dry soil, disappeared mainly due to volatilization. In contrast, when the drum was continuously rotated at 9 rpm (rotation/min), the number of microorganisms was drastically increased and nitrate was consumed for growth as a nitrogen source. Phenol and phenanthrene were removed at rates of 56.7 mg/kg dry soil/day and 3.2 mg/kg dry soil/day, respectively.

Key words: Bioremediation, drum bioreactor, soil contamination, aromatic compounds

Bioremediation has often been used for the treatment of soils contaminated with hazardous organic compounds [2, 3, 6, 10, 11]. Among the various bioremediation techniques, soil treatment with a bioreactor is considered to be one of the fastest methods, since substrates (contaminants) and oxygen can readily be transported to microbial cells by mixing [9, 16, 18, 20]. This technique would be powerful especially in the case when the fast treatment is required at sites where time rather than cost is critical, such as small and heavily contaminated working areas.

However, the use of vertical slurry bioreactor has been limited to the treatment of fine soils, because heavier soils such as sand cannot be suspended and adequately mixed in this reactor [14]. Sandy soil should be separated from fine

soils and cleaned by washing method. But this washing method requires an extra process such as biological treatment to completely degrade the toxic chemicals in the large quantity of wastewater [17]. Thus, it would be more economical and efficient to completely treat the target compounds in sandy soil in one single bioreactor process.

A horizontally rotating bioreactor can be an attractive alternative to the soil washing method, especially for the treatment of sandy soil. Unlike the vertical slurry bioreactor, the drum bioreactor can handle a wide range of particle sizes of soil and requires much less amount of water compared to the soil washing method. There have been a few reports regarding bioremediation of soil using drum bioreactors. These previous studies focused on the treatability of total petroleum hydrocarbons and total polyaromatic hydrocarbons [12] or single compound [5]. Therefore, the studies did not investigate the relationships between chemical property and operational mode. Also, Gray *et al.* [5] used relatively high water content (60 wt%), which is very similar to a vertical slurry bioreactor except for the mixing mechanism. Such a horizontally rotating bioreactor could not exactly satisfy the original advantages (large size of soil and small amount of water) of a drum-type bioreactor.

The rotation strategy of a drum in the bioreactor is one of the most important operational conditions, since it is highly related with the treatment efficiency as well as cost. The objective of this study was to demonstrate, through the operation of a medium-scale drum bioreactor system, the relationship between the treatment efficiency or removal mechanism and operational mode. Under the different operational conditions, the fate of the target compound may be variable according to its physical property. Thus, in this study, three kinds of aromatic hydrocarbons (phenol, naphthalene, phenanthrene) with highly different physical properties were chosen as target pollutants.

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MATERIALS AND METHODS

Microorganisms and Culture Conditions

All chemicals, of the highest purity available, were variously obtained from Aldrich, Sigma, Merck, or Difco. The mineral salt medium (SM) for enrichment and isolation contained 40 mM KNO₃, 5 mM NaH₂PO₄, 11.3 mM Na₂HPO₄, 10 mM KCl, 2 mM Na₂SO₄, 1.25 mM MgSO₄·7H₂O, 0.02 mM CaCl₂, 0.1 mM Na₂MoO₄·2H₂O, 1 mM EDTA, and 5 ml/l of a trace solution containing 0.12 mM HCl, 5 mM ZnSO₄·7H₂O, 20 mM FeSO₄·7H₂O, 10 mM MnSO₄·H₂O, 1 mM CuSO₄·5H₂O, 2 mM CoCl₂·6H₂O, 0.8 mM H₃BO₃ per liter deionized water [4]. The final pH was 7.0.

Soil samples contaminated with polyaromatic hydrocarbons were obtained from a railroad site at Pohang, Korea. Five grams of this soil were cultivated in a 250-ml Erlenmeyer flask containing 100 ml of SM with 100 mg of phenanthrene. The enrichment of phenanthrene-degrading bacteria was obtained by subculturing every two weeks for 3 months with 10% inoculation in a rotary shaker (150 rpm; 30°C). The subculture was plated on naphthalene- or phenanthrene-coated agar medium containing 15 g bacto agar per liter SM. After about 7-day incubation, the colonies of PAH-degrading bacteria could be visualized as a clearing zone surrounding colonies on the agar surface coated with PAHs, which resulted from the bacterial uptake of PAHs as substrates. These colonies were aseptically mixed with deionized water and cultivated in a 250-ml Erlenmeyer flask containing 50 ml SM with 5 mg phenanthrene and 0.3% (w/w) Triton X-100 to enhance phenanthrene solubility. This mixed culture (PM) was subcultured every two weeks and used as an inoculum for drum bioreactor operation after cultivation in nutrient broth (Difco) for 3 days. Among the strains of the mixed culture, four pure bacterial cultures were isolated and identified to be *Burkholderia cepacia*, *Corynebacterium* sp., *Candida* sp., and *Curtobacterium* sp. by the fatty acid analysis conducted by the Korean Collection for Type Cultures.

A phenol-degrading bacterium (PNM) was isolated from the coke-plant wastewater of Pohang Iron and Steel Company and identified to be *Pseudomonas* sp., as described in our previous work [7]. This bacterium was cultivated in SM-containing medium with 500 mg/l of phenol as a sole carbon source for 3 days and used as an inoculum for the drum bioreactor operation.

The extent of microbial degradation was tested with individual aromatic compounds in liquid flask culture. The compounds were phenol, naphthalene, and phenanthrene, and their properties are summarized in Table 1. A 250-ml Erlenmeyer flask was supplemented with microbial inoculum and 100 ml SM containing the target chemical and cultivated at 150 rpm in a shaker for 7 days at 30°C. The initial microbial inoculum was adjusted at 0.015 of OD₆₀₀ for all tests but with the corresponding microorganisms;

Table 1. Properties of target chemicals in this study [13].

	Mol. formula	Mol. wt	C _{w,sat} ^a	log K _H ^b	log K _{ow} ^c
Phenol	C ₆ H ₆ O	94.1	59,400	-3.39	1.45
Naphthalene	C ₁₀ H ₈	128.2	31.5	-0.31	3.36
Phenanthrene	C ₁₄ H ₁₀	178.2	1.12	-1.45	4.57

^aAqueous solubility at 25°C [mg/l].

^bHenry's law constant at 25°C [l atm/mol].

^cOctanol-water partition coefficient at 25°C [(mol/l octanol)/(mol/l water)].

PM for naphthalene and phenanthrene, and PNM for phenol. Initial concentrations of the target chemicals were 500, 50, and 50 mg/l for phenol, naphthalene, and phenanthrene, respectively. In order to solubilize the target chemicals completely in liquid, Triton X-100 was used at a final concentration of 0.3 wt%. OD₆₀₀ and the concentration of the target chemicals were analyzed in duplicate flask tests.

Soil

Soil was obtained from the playground (10–40 cm beneath the surface) of the Pohang University of Science and Technology, Korea. The soil was air-dried for 3 days and sifted with a U.S. standard No. 10-mesh (2 mm) sieve. This sifted soil was loamy sand containing 81% of sand, 18.4% of silt, and 0.6% of clay. The content of organic matter was 1.3%, and the pH of soil slurry (0.1 g soil/ml deionized water) was 7.5.

Operation of Drum Bioreactor

A schematic diagram of the drum bioreactor system is shown in Fig. 1. The drum was made of stainless steel with a total volume of 12.6 l (200 mm i.d.×400 mm length). Three baffles with 3 cm width were connected to the inner wall of the drum. The lid of the bioreactor consisted of two parts: The movable outer part was made of stainless steel, and the stationary center part was made of acrylic polymer to see the inside of drum reactor, and equipped with air inlet and outlet ports and a thermocouple. The body of the drum

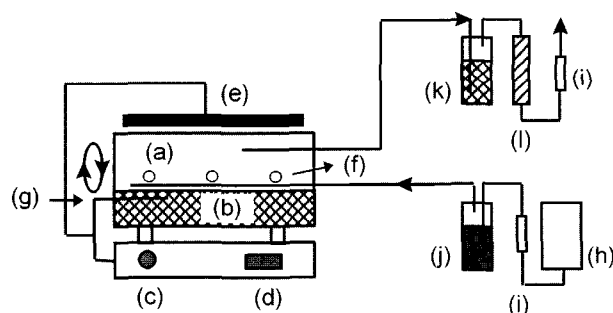


Fig. 1. Schematic diagram of drum bioreactor system.

(a) Drum bioreactor, (b) soil, (c) temperature controller, (d) rpm controller, (e) ceramic heater, (f) sampling ports, (g) thermocouple, (h) air blower, (i) flow meter, (j) prehumidifier, (k) water trap, (l) PAH adsorption column.

had three sampling ports at regular intervals. The temperature was detected in soil of the reactor and controlled by heating a ceramic plate near the outer surface of the drum.

Five kg of dried soil were artificially contaminated by adding 0.75 g of naphthalene, 1.0 g of phenanthrene, and 5.0 g of phenol dissolved in 1 l of methylene chloride. The final concentrations of the chemicals were chosen, based on the commonly contaminated ranges and their biodegradability obtained from liquid flask cultures. The completely wet soil with solvent was dried in a hood at 30°C. To start the operation, 5 kg of artificially contaminated soil along with three-fold concentrated SM of 0.4 l and 4.5 g Triton X-100 to enhance the solubility of aromatic compounds were added to the reactor. This was then inoculated with 0.2 l PAH-degrading bacteria (PM) and 0.2 l phenol-degrading bacteria (PNM) and 1.3 l deionized water. Individual bacterial cultures previously grown in Nutrient Broth for 3 days were used for the inoculum. Air was supplied in the headspace at a rate of 1.2 l/min through a 0.2 µm filter (Millipore Co.). The outlet air was passed through a column packed with PAH adsorbent (OrboTM-43, Supelco Co.) to trap the volatilized compounds.

The reactor was operated in two different operation modes; intermittent and continuous rotation of drum. For the initial 22 days (operation A), the reactor was rotated at 9 rpm for 10 min once a day. For the next 38 days (operation B), the drum was continuously rotated at 9 rpm.

Analyses

About 1 g of soil sample was taken out from three different locations of the reactor at each time and accurately weighed. Total nine samples were individually withdrawn for the analysis of water capacity, CFU, concentration of nitrate, and aromatic compounds. Water capacity of soil sample was determined by measuring the weight difference of sample before and after drying in an oven for 24 h at 105°C. This water capacity was also used for the conversion of the data into dry soil basis. Each sample was mixed with 10 ml sterile water, vortexed for 5 min, and the slurry supernatant was used for the analysis of colony forming units (CFU) and nitrate. The CFU was counted on nutrient agar (Difco) after incubation for 7 days at 30°C as the total number of heterotrophic microorganisms in the soil. Nitrate was analyzed by an ion chromatography (BioLC, Dionex, U.S.) with a conductivity detector. The column was eluted with mobile phase (0.191 g Na₂CO₃ and 0.143 g NaHCO₃/l deionized water) at a flow rate of 2.0 l/min. The analytical column was an IonPac A4 (Dionex, U.S.A.).

Soil samples for extraction of aromatic compounds were stored at -4°C and extracted within 7 days. The soil was basically extracted with methylene chloride by sonication according to the EPA method 3550 [15]. First, the wet soil sample was mixed homogeneously with anhydrous sodium

sulfate, and a known amount of fluoranthene dissolved in methylene chloride was added as a surrogate standard. The extraction yields were 87.6±8.5% for naphthalene, 90.3±4.7% for phenol, and 85.4±5.3% for phenanthrene. The samples were extracted in 15 ml methylene chloride using a sonicator (Sonic Dismembrator, Fisher Co., U.S.) with a power of 375 J/s for 3 min and then centrifuged at 2,000 ×g (Sorvall, DuPont, U.S.A.) for 20 min. Samples were extracted twice with 15 ml each of methylene chloride. The extract was evaporated at 45°C to the volume of about 5 ml, about 10 ml acetonitrile were added to the concentrated extract, and it was re-evaporated to approximately 1 ml. The exact volume of the concentrated extract was measured, filtered, and injected into a detector inlet of the HPLC (BioLC, Dionex, U.S.A.).

HPLC analysis for quantification of phenol, naphthalene, and phenanthrene was performed using an RP SupelcosilTM LC-PAH column (150 mm×4.6 mm) connected to a UV detector (254 nm). The mobile phase for elution of naphthalene and phenanthrene was a mixture of acetonitrile and water (75:25) at a flow rate of 1.5 ml/min. Phenol was analyzed separately from naphthalene and phenanthrene, but using the same method except mobile phase (30:70, acetonitrile: water). The standard curves were obtained with the chemicals of over 99% purity. The minimum detectable concentrations were 0.1 mg/g dry soil for naphthalene and phenol and 0.01 mg/g dry soil for phenanthrene.

The amount of aromatic compounds volatilized was measured after the operations A and B. The adsorbent column (0.8 cm×8 cm) was removed from the outlet line and extracted totally with methylene chloride using the same method as described above for the soil sample.

The oxygen transfer coefficients were separately determined with the same initial conditions of the reactor operation, as described above, except for contamination and inoculation at 0 rpm and 9 rpm, respectively. Before starting the measurement, the slurry in the reactor was deoxygenated by N₂ bubbling. After switching N₂ with air, the change of dissolved oxygen concentration was monitored using a polarographic oxygen electrode (Ingold, Mettler-Toledo, U.S.A.).

RESULTS AND DISCUSSION

Biodegradation in Liquid Flask Cultures

The cell growth and biodegradability of individual aromatic compounds were examined in liquid flask cultures, as summarized in Table 2. Phenol was completely degraded within 3 days in parallel with dramatic cell growth. As expected, naphthalene and phenanthrene were degraded more slowly than phenol. In the case of phenanthrene, approximately 40% of initial concentration still remained without biodegradation after 7 days of culture, while naphthalene

Table 2. Biodegradation of individual compounds in liquid flask cultures.

Target compound/ Microbial inoculum	Cell growth (OD ₆₀₀)			Remaining concentration (mg/l)		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Phenol/PNM	0.11 (0.016)	0.28 (0.027)	0.21 (0.019)	348 (27.6)	0	0
Naphthalene/PM	0.020 (0.002)	0.14 (0.018)	0.12 (0.013)	42.7 (0.566)	13.5 (0.919)	0
Phenanthrene/PM	0.015 (0.001)	0.033 (0.002)	0.13 (0.02)	47.7 (1.98)	40.5 (4.67)	20.5 (4.10)

Initial concentration of each chemical was 500, 50, and 50 mg/l for phenol, naphthalene, and phenanthrene, respectively. PNM and PM represent the phenol-degrading bacterium and PAH-degrading bacteria as described in Materials and Methods. Initial OD₆₀₀ was 0.015 for all conditions. Data presented are means and standard deviations (in the parentheses) of duplicate samples.

was degraded completely. There was no appreciable degradation (below 10% of initial concentration) of all the compounds without bacterial inoculation (data not shown). Although each strain of the mixed culture (PM, PAH-degrading bacteria) was able to degrade naphthalene and phenanthrene, the pure single culture was found to be not as much effective as the mixed culture in the degradation of the PAHs. Therefore, the mixed culture (PM) instead of a single strain was used for PAHs degradation in subsequent drum bioreactor operations.

Biodegradation in Drum Bioreactor Operations

Figure 2 shows the disappearance of aromatic compounds in soil of the drum bioreactor operations. The mixed chemicals composed of three kinds of aromatic hydrocarbons (phenol, naphthalene, and phenanthrene) with highly different physical properties were used as target pollutants. Naphthalene has high volatility, and phenol has high water solubility, and phenanthrene has high organic-water partition coefficient (Table 1). In the operation A, 53% of initial phenol was removed at a rate of 16.1 mg/kg dry soil/day; 24% (900 mg) by evaporation and 29% (1,080 mg) by biodegradation (Table 3). The phenol degradation rate began to increase after about 15 days, possibly because of enhanced oxygen

transfer due to decreased water capacity and increased microbial activity. In the case of naphthalene, the complete removal was achieved at a rate of 5.7 mg/kg dry soil/day within 15 days. This disappearance of naphthalene was mainly due to volatilization rather than biodegradation. Approximately 19% of phenanthrene was removed; 1% (6.5 mg) by volatilization and 18% (130 mg) by biodegradation. Although CFU increase was not observed during this operation, approximately 50 mg nitrate/kg dry soil disappeared after 15 days (Figs. 3 and 4). Nitrate seemed to have disappeared not by abiotic processes such as adsorption, volatilization, and chemical reaction, but rather due to uptake by microorganisms, which were not enough to be detected by the plate count method. Thus, the disappearance of phenol and phenanthrene after 15 days would have been mainly due to biodegradation.

From day 23, the mode of operation was changed to continuous rotation of drum (operation B). The rate of phenol removal further increased to 56.7 mg/kg dry soil/day. The amount of phenol removed by volatilization was 18% (350 mg) of total disappearance and that by biodegradation was 82% (1,445 mg) in this period. About 579 mg of remaining phenanthrene was completely removed during the entire period at an average rate of 3.2 mg/kg dry soil/day, mainly by biodegradation. The maximum degradation rate of phenanthrene was 15–20 mg/kg dry soil/day at the beginning of the operation B, which was similar (about 25 mg/kg/day, [12]) or lower (about 60 mg/kg/day, [1]) than that of other studies. It seemed to be partly due to low degradability of the microorganisms used in this study, nonoptimized water content, and mixing condition, particularly at the later part of the operation. Therefore, in order to obtain more efficient operation of the drum bioreactor, optimization and automation of water content control would be desirable.

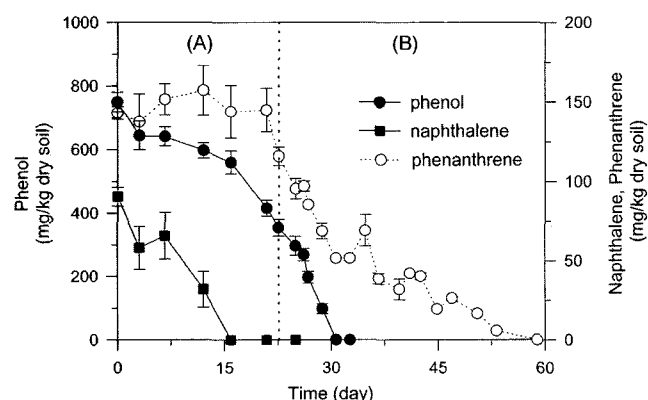


Fig. 2. Changes of phenol, naphthalene, and phenanthrene concentrations in soil of drum bioreactor.

Error bars represent standard deviation of triplicate samples. A: the reactor was rotated once per day for 10 min at 9 rpm. B: the reactor was continuously rotated at 9 rpm.

Cell Growth

Time course of CFU change in the drum bioreactor is shown in Fig. 3. The number of microorganisms drastically decreased from 2.4×10^6 /g dry soil to 4.6×10^4 /g dry soil and remained at this level during the period of the operation A. At the beginning of the operation B, additional culture of the PAH-degrading bacteria was inoculated to improve the

Table 3. Starting and final amounts of aromatic compounds in the operations of drum bioreactor.

	Phenol	Naphthalene	Phenanthrene
Initial (day 0) (mg)	3,750 (± 152) ^a	453 (± 30)	715 (± 20.8)
After operation A (day 22)			
Volatilized (mg)	900	350	6.5
Disappeared except for volatilization (mg) ^b	1,080 (± 197)	153 (± 30)	130 (± 35.1)
Remaining in soil (mg)	1,770 (± 126)	Nd ^c	579 (± 28.3)
After operation B (day 60) ^d			
Volatilized (mg)	325	Na ^e	4.2
Disappeared except for volatilization (mg) ^b	1,445 (± 126)	Na	575 (± 28.3)
Remaining in soil (mg)	Nd	Na	Nd

^aData presented are means of triplicate samples \pm standard deviations.

^bAmount disappeared = Initial amount of each operation - amount volatilized - amount remaining in soil.

^cNot detected.

^dInitial amount of the operation B was the same as the amount remaining in soil after operation A.

^eNot applicable. Naphthalene was not detected at the beginning of operation B since it was removed during operation A.

degradation of phenanthrene, however, the initial decrease of CFU from 1.0×10^7 /g dry soil to 1.3×10^6 /g dry soil was still observed. This initial decrease of CFU was presumed to be due to unfavorable growth conditions such as existence of heavily toxic compounds, and low mass transfer rates of substrates and oxygen. However, in the operation B, the initial decrease of CFU was less than that in the operation A and microorganisms began to grow again within a short time (about 3 days). After this adaptation period, the CFU was rapidly increased to 3×10^7 /g dry soil, corresponding to rapid degradation of phenol and maintenance of remaining phenanthrene degradation at around 10^7 /g dry soil.

Water Capacity

The water capacity of soil in the reactor continuously decreased due to evaporation (Fig. 4). The water capacity is defined as the percentage of saturation of water content

in soils. The saturation level (i.e., water capacity is 100%) for the soil used in this study were 0.38 ml/g. During the operation A, the water capacity decreased from 109% to 78% at an evaporation rate of 0.0068 ml/kg dry soil/day. As expected, the evaporation rate increased about two folds (0.013 ml/kg dry soil/day) due to enhanced mixing by drum rotation during the operation B. In the present study, the soil began to be aggregated and compacted onto the inside wall after about 40 days. This pellet formation has been reported to cause decrease of mass transfer and consequent decrease of microbial activity [12]. Because of this reason, water was manually added to the reactor on the 40th day.

Nitrate Consumption

As shown in Fig. 4, nitrate decreased during the operation, confirming the biotic removal. Nitrate was not consumed

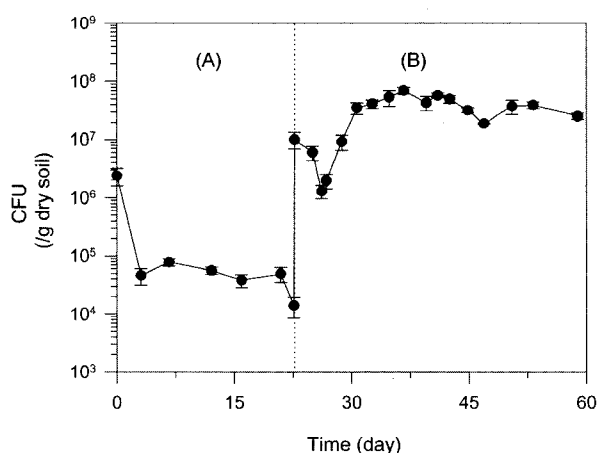


Fig. 3. Change of microbial number in soil of drum bioreactor. Error bars represent standard deviation of triplicate samples. A: The reactor was rotated once per day for 10 min at 9 rpm. B: The reactor was continuously rotated at 9 rpm.

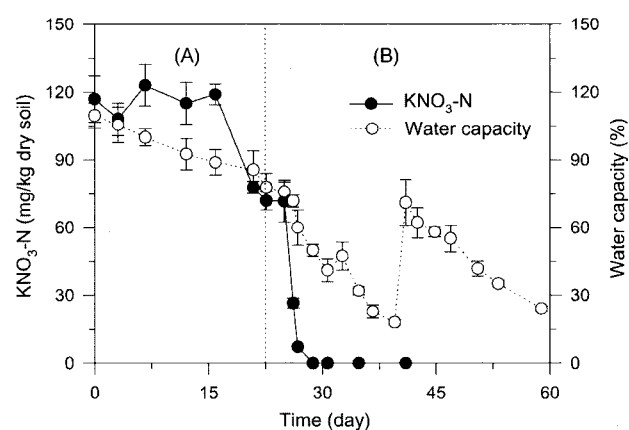


Fig. 4. Changes of nitrate concentration and water capacity in drum bioreactor operation.

Error bars represent standard deviation of triplicate samples. A: The reactor was rotated once per day for 10 min at 9 rpm. B: The reactor was continuously rotated at 9 rpm.

for the initial 15 days, which coincided with no microbial growth. However, nitrate began to decrease from the end of the operation A and more rapidly during the operation B. It was also observed that about 50 mg of phenanthrene/kg dry soil was slowly but continuously degraded after 30 days, even in the absence of nitrate. This would possibly be due to other nitrogen sources such as ammonia and amino acids from soil organic components or lysed cells. The C/N ratio was estimated to be 3.81 (mg C/mg N) up to day 30 in the operation B, where carbon consumption was assumed to be the amount of phenol and phenanthrene removal, except for the volatilized amount. This value was somewhat lower than that (5.0) reported as a cell composition of typical microorganisms [8]. It is highly possible that part of the nitrate was assimilated with consumption of other carbon substrates such as natural organic matter originally bound to soil. Thus, in order to effectively degrade the target chemicals, more nitrogen sources than expected would be required due to possible consumption of other carbon sources such as natural organic matter rather than target compounds.

Mixing and Mass Transfer

In separate tests, oxygen transfer coefficients ($k_L a$) were determined to be very low (0.16 h^{-1}) at 0 rpm, compared to that (1.9 h^{-1}) at 9 rpm. The maximum amount of carbon consumption (ΔC_{\max}) was estimated from the following equation as 101 mg C/kg in the operation A;

$$\Delta C_{\max} = k_L a \cdot O^* / Y_{O_2/C} \cdot V_w / M_s \cdot \Delta t$$

The values used in this calculation were the yield coefficient of oxygen ($Y_{O_2/C}$) = 2.1 mg O_2 /mg C [19], the water volume (V_w) = 1.5 l, the soil mass (M_s) = 5 kg, the saturated concentration of oxygen (O^*) = 8.25 mg/l, and Δt = 22 days. This means that the amount of carbon substrates by aerobic biodegradation cannot be higher than 101 mg/kg in the operation A. This may not exactly reflect the real situation of the bioreactor operations, because the water capacity in soil was variable during the operation. Nevertheless, it could not be difficult to expect that the transport of oxygen would be very limited in the operation A in spite of intermittent rotation. In contrast, oxygen could be effectively transported to the microbial cells by mixing through continuous rotation in the operation B. Thus, the improved oxygen transfer in the operation B could be a main reason to enhance the biodegradation of aromatic compounds.

Naphthalene is known to be the most volatile among the three compounds, and it could be removed simply by air blowing even under relatively poor mixing condition (operation A). This continuous air blowing would drive gas phase concentrations, and mass transfer of naphthalene from soil into air would continuously occur via water, almost without attack of microorganisms. Phenol is highly soluble and the most available to microorganisms. Thus,

the extent of biotic removal, as compared to abiotic removal, was the highest with phenol. The solubility of phenanthrene in water as well as Henry's Law constant are extremely low (Table 1); moreover, phenanthrene is most tightly bound onto soil. Consequently, it is not difficult to expect the lowest volatilization rate of phenanthrene among the three compounds.

In conclusion, a drum bioreactor system was used for the treatment of sandy soil contaminated with three kinds of aromatic compounds (phenol, naphthalene, phenanthrene). The reactor was operated by two different operation modes, intermittent and continuous rotation of drum, but with continuous air blowing in both cases. The extent of biodegradation of aromatic compounds was found to drastically increase by applying appropriate mixing of soil with continuous rotation of drum rather than intermittent rotation.

In a conventional vertical slurry bioreactor system, the amount of water required ranges from 3 to 20 times more than the drum bioreactor system. Also, the vertical slurry bioreactor requires high energy cost for mixing and suspending soil particles. Higher biodegradation rates might be attained by the vertical slurry bioreactor. Therefore, the optimal bioreactor type appears to depend on the soil texture, and time and cost allowed. The horizontally rotating drum bioreactor would be more appropriate for treating soils with large size rather than fine soils and for relatively long-term remediation projects.

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