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Removal of polycyclic aromatic hydrocarbons from contaminated soil in a two-phase partitioning bioreactor

Jae-Young Lee*,†, Tae-Soon Kwon, and Young-Chul Lee*****

*Transportation Environmental Research Team, Korea Railroad Research Institute (KRRI),
#176 Cheoldo Bangmulgwan-ro, Uiwang-si, Gyeonggi-do 16105, Korea
**Railroad Safety Research Division, Korea Railroad Research Institute (KRRI),
#176 Cheoldo Bangmulgwan-ro, Uiwang-si, Gyeonggi-do 16105, Korea
***Department of BioNano Technology, Gachon University,
1342 Seongnamdaero, Sujeong-gu, Seongnam-si, Gyeonggi-do 13120, Korea
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Abstract−A two-phase partitioning bioreactor was employed to remediate soil contaminated by a mixture of polycyclic aromatic hydrocarbons consisting of phenanthrene, anthracene, and pyrene. In this study, the transfer of three PAHs into the water-immiscible liquid phase (silicone oil or paraffine oil) from the soil was investigated during the first 24 h. And then, phenanthrene and anthracene were degraded by approximately 90% and 80%, respectively, compared with initial concentration in soil, but pyrene was not degraded during seven days of operation period. In addition, the feasibility of a soil slurry sequencing batch reactor system in terms of continuously operating a two-phase partitioning bioreactor was investigated. Phenanthrene and anthracene were degraded semi-continuously and repeatedly during two operating cycles. Pyrene was still not degraded and was just transferred into the water-immiscible liquid phase considering its solubility.

Keywords: Polycyclic Aromatic Hydrocarbons (PAHs), Two-phase Partitioning Bioreactor (TPPB), Soil Slurry-sequencing Batch Reactor (SS-SBR), Soil Remediation, Water-immiscible Liquid

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are known to be hydrophobic and xenobiotic contaminants in soils [1,2]. To treat PAHs-contaminated soil, various technologies recently have been applied [1]. In particular, biological treatment technologies have economic and ecological advantages, compared to previously physicochemical and thermal methods [3-5]. However, some difficulties are encountered in bioremediation of PAH-contaminated soil due to the poor bioavailability of PAHs to microorganisms [3]. The limited bioavailability of PAHs is caused by their low aqueous solubility and slow desorption from the soil matrix. Use of surfactants improves the bioavailability of PAHs and has shown positive, negative, or no effect on the degradation of PAHs [6-8]. Surfactants can be adsorbed onto soil, which can cause secondary contamination [1]. Since the beginning of the 1990s, a two-phase partitioning bioreactor (TPPB) using a water-immiscible liquid (WIL) has been applied to enhance the bioavailability of xenobiotics including PAHs [9-19]. Also, the TPPB system has been applied to treat volatile organic compounds [20-22]. The TPPB system is composed of an aqueous phase and a WIL phase [9]. The PAHs desorbed from contaminated soils are dissolved in the WIL, and then microorganisms degrade PAHs at the interface between the two phases and/or into the aqueous phase [9,10]. Generally, the WIL should be biocompatible and non-biodegradable. Various PAHs have been tested using the TPPB system, and the main types of WIL are silicone and vegetable oils [11-19].

Generally, the TPPB system consists of a conventional stirred tank bioreactor with batch operation mode during one cycle [9,10]. A few groups have focused on continuous operation of the TPPB system to treat PAH-contaminated soil. A continuous operation mode for a soil slurry bioreactor entails the use of a soil slurry-sequencing batch reactor (SS-SBR) with four steps: fill, react, settle, and draw [23-25]. The first step is to fill the contaminated slurry into the reactor (fill), and the second step is to operate SS-SBR (react). After settling the SS-SBR during the scheduled period (settle), the final step is to withdraw the treated slurry from the reactor (draw), and then the contaminated soil is re-injected into the reactor. From these steps, it is possible to degrade pollutants periodically from the contaminated soil in the TPPB system by integration of the SS-SBR operation mode without additional supplement of culture and the WIL. When the treatment volume of the contaminated soil is the same, SS-SBR is more economical than batch operation, which the new culture and WIL are supplied every operation cycle. In the present paper, a TPPB system was employed to degrade a mixture of phenanthrene, anthracene, and pyrene from the contaminated soil by *Sphingomonas* sp. 3Y. Two WILs were compared on the basis of the degradation efficiency of three PAHs. The feasibility of the SS-SBR as a new operation mode of a TPPB system to remediate the PAH-contaminated soil semi-continuously

†To whom correspondence should be addressed.
E-mail: iyoung@krri.re.kr

was investigated.

MATERIALS AND METHODS

1. Microorganism and Culture Conditions

The used *Sphingomonas* sp. 3Y (Korea) was isolated previously from diesel oil-contaminated soil [26]. For the preparation of the inoculum, microbial colonies were inoculated in 500 mL flasks containing 100 mL of medium with yeast extract 5 g, peptone 3 g, and manitol 25 g in 1 L of deionized water [26]. The medium was autoclaved at 121 °C for 15 min, and then after cooling in the atmosphere, the initial pH of the medium was adjusted to 7.0. After cultivation for seven days, the culture was centrifuged, washed twice, suspended with the mineral medium repeatedly, and then supplied to the TPPB system. The composition of the mineral medium was KNO_3 5 g, NaH_2PO_4 0.6 g, Na_2HPO_4 1.6 g, KCl 0.7 g, Na_2SO_4 0.28 g, $CaCl_2$ 0.002 g, $MgSO_4 \cdot 7H_2O$ 3 g, $FeSO_4 \cdot 7H_2O$ 0.03 g, and trace metal solution 5 mL ($ZnSO_4 \cdot 7H_2O$ 7 mg, $MnSO_4 \cdot 5H_2O$ 11.55 mg, $CuSO_4 \cdot 5H_2O$ 1.25 mg, H_3BO_3 0.25 mg, and $CoNO_3 \cdot 7H_2O$ 2.91 mg) in 1 L of deionized water.

2. Preparation of PAHs-contaminated Soil

Kaolinite white-O (Sancheong, Korea) was screened through 150 μm and was spiked with phenanthrene, anthracene, and pyrene (Sigma, USA) in acetone (Merck, USA). The initial PAH concentration in soil was intended to be about 1,000 mg/kg. At room temperature, acetone was volatilized from the PAH-contaminated soil. However, because the soil was manually mixed with the PAH-dissolved acetone, the initial PAH concentration in soil was not consistently uniform over time. The initial PAH concentration in soil was therefore measured for all the experiments.

3. Selection of WIL

Two WILs of silicone oil (Sigma, USA) and paraffin oil (Sigma, USA) were selected. Silicone oil is a representative WIL in the TPPB system, while paraffin oil has non-aqueous and biocompatible properties [11,12,15-19]. Table 1 summarizes the solubility of phenanthrene, anthracene, and pyrene in silicone oil and in paraffin oil as determined in previous studies [27]. Silicone and paraffin oils have a high solubilizing capacity for three PAHs considered in this study.

4. Operation Conditions of Two-phase Partitioning Bioreactor (TPPB) System

All TPPB systems with 30% (w/v) soil slurry were operated separately in a 2 L soil slurry reactor during seven days, as illustrated in Fig. 1. In the TPPB systems, 240 g of contaminated soil, 800 mL of pre-sterilized medium, and 120 mL of WIL, i.e., 15% (v/v), were mixed. The cultivating temperature was controlled at 30 °C using a water jacket. The initial pH was adjusted to 7.0 and thereafter was not controlled during the operation period because the pH did not change significantly in a preliminary test (data not shown). The initial cell concentration was about 8.6×10^7 CFU/ml and the mixing speed was 150 rpm. The change of CFU for *Sphingomonas* sp. 3Y in this study was not monitored during the operation of the TPPB system.

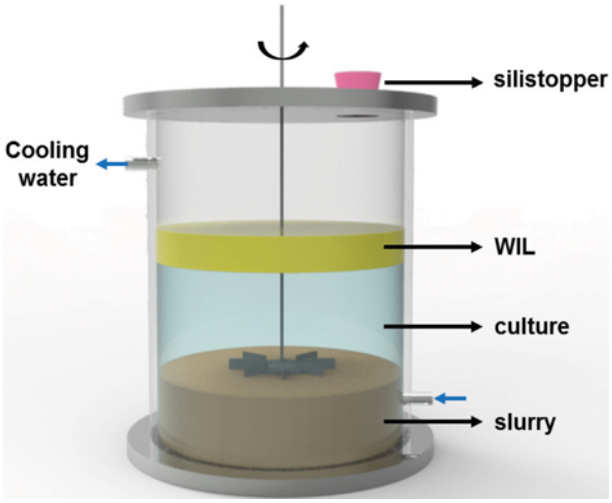

Fig. 1. Schematic diagram of TPPB system in this study.

5. Operation Conditions of Two-phase Partitioning Bioreactor (TPPB) Integrating Soil Slurry-sequencing Batch Reactor (SS-SBR)

For the first cycle operation of the SS-SBR, 240 g of the contaminated soil, 800 mL of pre-sterilized medium, and 120 mL of WIL were fed into the reactor. At one cycle operation, the react time was six days. The time for the settle, draw, and fill step was taken as one day. The draw and fill time was totally within one hour and the most of time was required for the settle step to separate slurry, aqueous and WIL phase, respectively. After one cycle operation, all the treated slurry was removed from the bottom of the reactor and the same volume of contaminated slurry was reintroduced. The WILs, medium, and microorganisms were provided only at the beginning of the experiment. One cycle time of the SS-SBR system in this study was seven days and the total operation period was 14 days. The cultivation conditions of the TPPB system integrating the SS-SBR for each cycle were identical. Residual PAH concentration in the soil and in the WIL was periodically measured.

6. Quantitative PAHs Analysis in Soil Slurry and WIL Phases

After the TPPB system settled for 30 min, 1 mL of WIL and 5 mL of soil slurry were taken periodically to measure residual PAH concentration in the soil and the WIL, respectively. The slurry samples were dried at 105 °C for 24 hr, and then 1 g of dried soil was mixed with 10 mL of methanol (Merck, USA) [28,29]. After mixing in the end-over shaker at 180 rpm for 24 hr, soil samples were

Table 1. Properties of silicone and paraffin oils as a WIL phase in the TPPB system [27]

WIL	Density (g/cm^3)	Viscosity (centistokes)	PAH solubility (mg/L)		
			Phenanthrene	Anthracene	Pyrene
Silicone oil	0.98	20	5,174±239	431±58	2,395±42
Paraffin oil	0.84	-	34,890±1,114	2,700±1,046	21,243±807

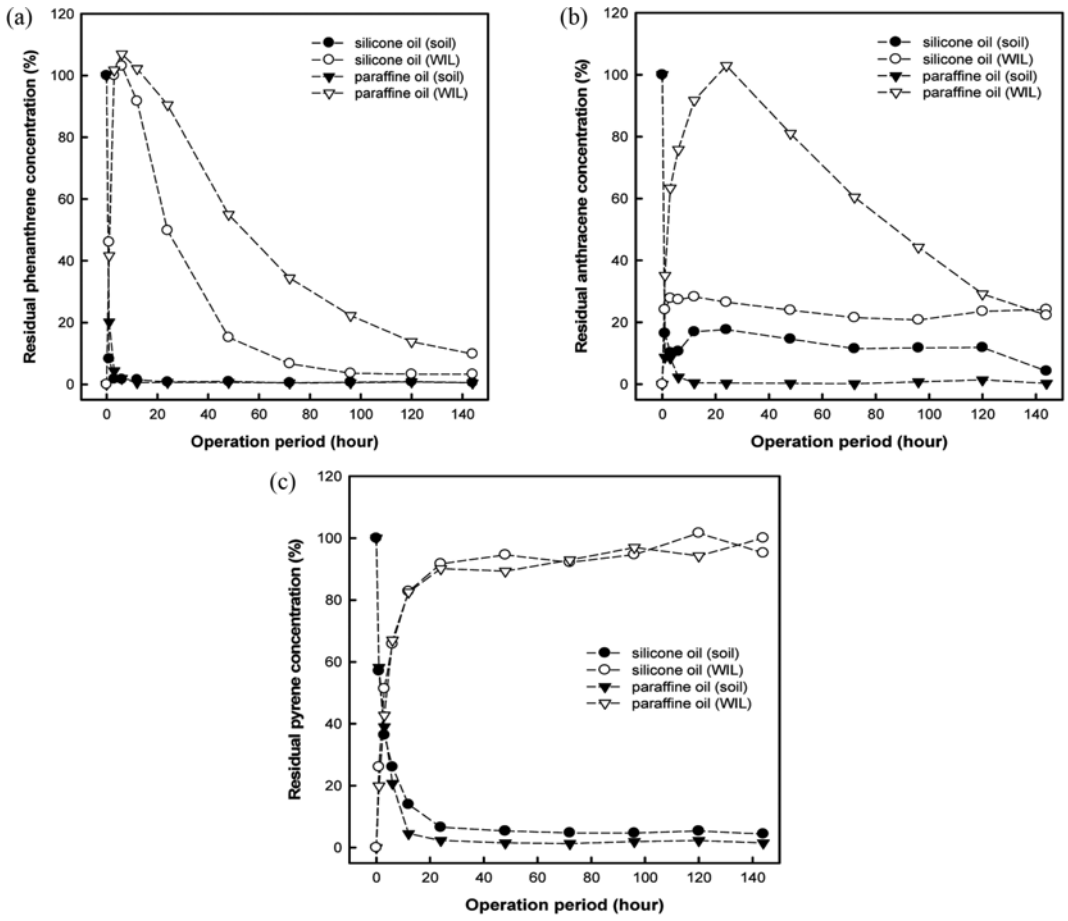

Fig. 2. Profiles for the residual concentration of (a) phenanthrene, (b) anthracene, and (c) pyrene in soil (*filled symbols*) and WIL (*open symbols*) with the operation period of the TPPB system when silicone oil (*circle symbols*) and paraffine oil (*inverted triangle symbols*) were used as a WIL phase, respectively.

centrifuged at 5,000 rpm for 5 min. 1 mL of the upper phase was taken to measure the residual PAH concentration in the soil. PAHs dissolved in WIL samples were extracted with 2 mL of N, N-Dimethyl-formamide (DMF, Merck, USA) [11]. After stagnation, 1 mL of solvent fractions was collected. The analysis was performed using a HPLC (Waters, USA) with a C18 column (4.6×250 mm) at a wavelength of 254 nm. Elution was carried out with 85% (v/v) acetonitrile at a flow rate of 0.7 mL/min. The residual PAH concentration in soil and WIL (%) was calculated separately as follows:

$$\text{Residual PAH concentration in soil (\%)} = \frac{C_{soil,\,t}}{C_{soil,\,i}} \times 100 \quad (1)$$

$$\text{Residual PAH concentration in WIL (\%)} = \frac{C_{WIL,\,t}}{C^{*}_{WIL}} \times 100 \quad (2)$$

$$C^{*}_{WIL} = C_{soil,\,i} \times \frac{m_{soil}}{V_{WIL}} \quad (3)$$

where, $C_{soil,\,t}$ is the residual PAH concentration in soil at time t (mg/kg), $C_{soil,\,i}$ is the initial PAH concentration in soil (mg/kg), C^{*}_{WIL} is the estimated maximum concentration of PAH transferred into the WIL (m/L), $C_{WIL,\,t}$ is the residual PAH concentration in the WIL at time t (mg/L), m_{soil} is the amount of soil (kg), and V_{WIL} is the volume of the WIL (L) that is used.

RESULTS AND DISCUSSION

1. PAH Degradation Test Using TPPB System

Fig. 2 shows the profiles for residual PAH concentrations in the soil slurry and WIL phases when silicone and paraffin oils, respectively, were used as the WIL phase. Within the first 24 h, the mass transfer of PAH from soil to WIL was dominant compared to the degradation of PAH. Thereafter, the phenanthrene concentration in the WIL phase was reduced rapidly until 48 h, and then decreased slowly during the cultivation periods. *Sphingomonas* sp. 3Y consumed phenanthrene as a carbon source at the aqueous-WIL interface or in the aqueous phase. The degradation efficiency of phenanthrene was more than 90%, and it was higher in silicone oil than in paraffin oil. Anthracene was degraded simultaneously with phenanthrene in the TPPB system. The degradation efficiency of anthracene was less than 10% in silicone oil and about 80% in paraffin oil. The initial anthracene concentration in the contaminated soil was higher than its solubility in silicone oil. Consequently, anthracene was not completely transferred into silicone oil. However, this limitation was not observed when paraffin oil was used as the WIL. Pyrene was transferred almost completely from soil into the WIL within 24 h, but was not degraded during the operation period of the TPPB system, irrespective of the WIL types.

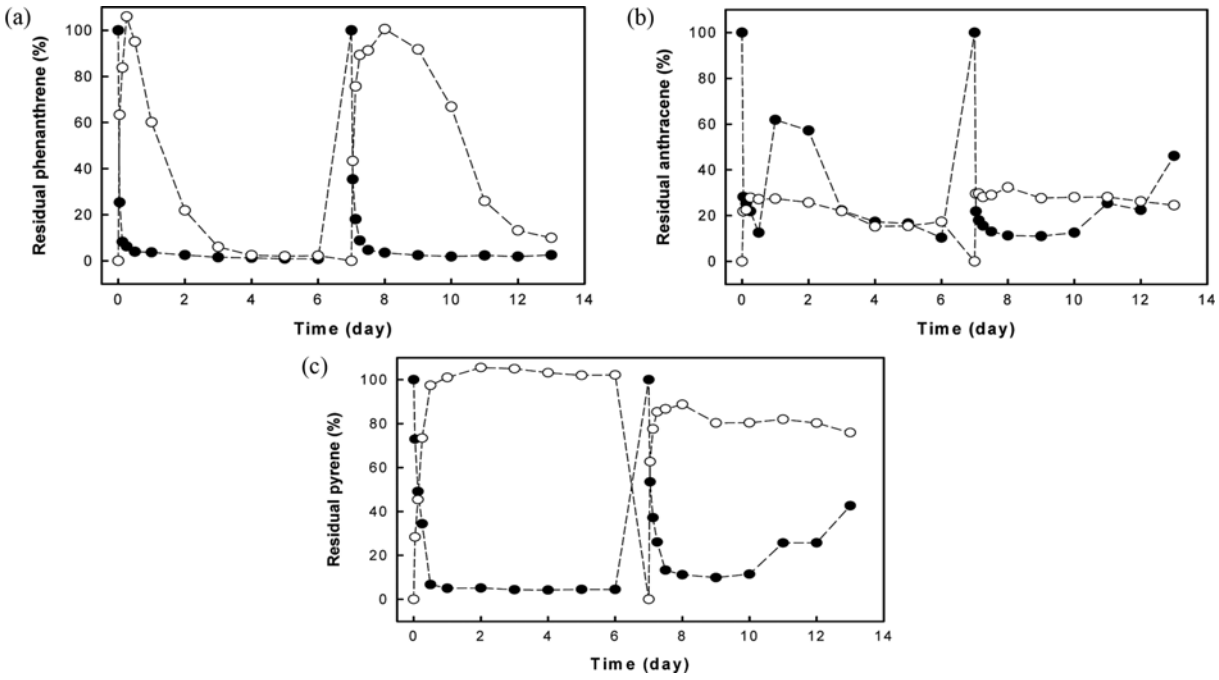

Fig. 3. Residual concentration of (a) phenanthrene, (b) anthracene, and (c) pyrene in soil (●) and WIL (○) when silicone oil was used as a WIL phase during the operation of the TPPB system integrating the SS-SBR.

2. TPPB System Integrating with SS-SBR Operation Mode

The profiles of residual PAH concentration in the soil and in the WIL are described in Fig. 3 when silicone oil was used as the WIL phase in the TPPB system with SS-SBR operation mode. More than 95% of phenanthrene was degraded during the first batch operation of the TPPB system. When fresh contaminated soil was introduced in the next cycle, the degradation rate of phenanthrene was reduced slightly at the beginning. However, the degradation efficiency of phenanthrene decreased by only about 5% at the end of the second cycle compared to that of the first batch operation. On the other hand, the residual anthracene concentration in the WIL decreased very slightly during the first cycle and was maintained

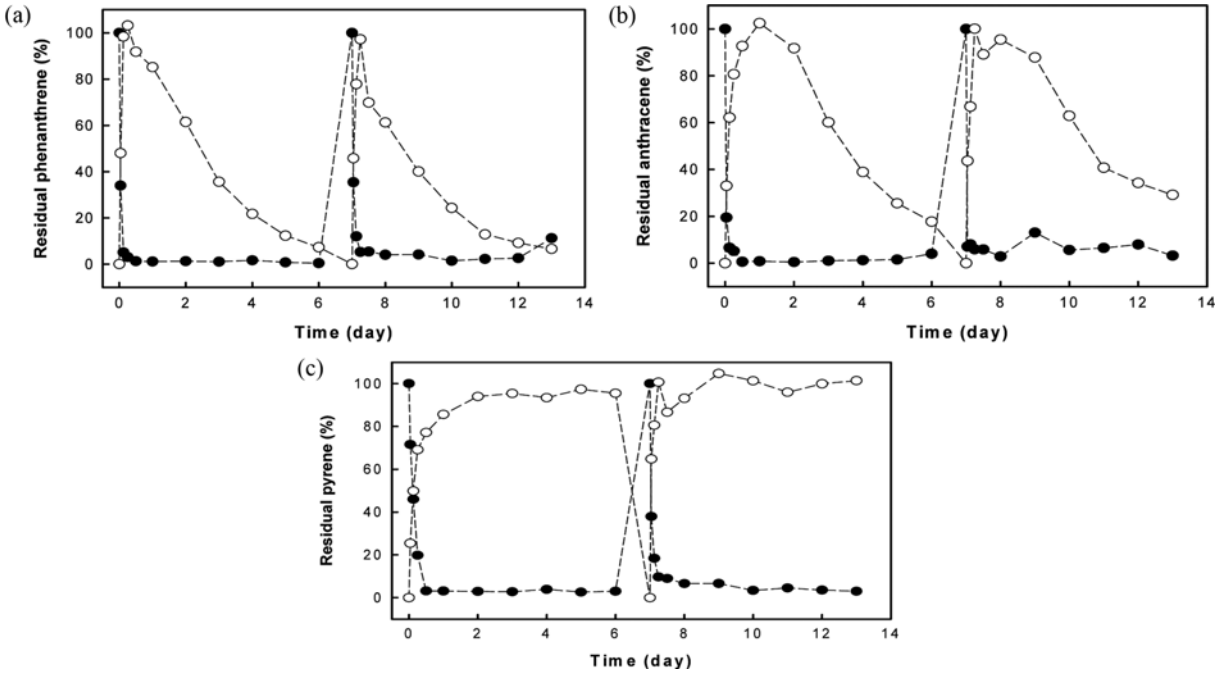

Fig. 4. Residual concentration of (a) phenanthrene, (b) anthracene, and (c) pyrene in soil (●) and in WIL (○) when paraffin oil was used as a WIL phase during the operation of the TPPB system integrating the SS-SBR.

at an almost constant level at the next cycle. Because the degradation efficiency of anthracene is very low in silicone oil, the mass transfer of anthracene was still about 30% and some amount of anthracene remained in the soil or the aqueous phase after the second operation cycle of the TPPB system. Pyrene was not biodegraded during total operation period, but its final transferred ratios into silicone oil were about 99% and 80% of the initial contents, respectively, for each operation cycle in the TPPB system. Fig. 4 depicts the residual PAH concentration in soil and in the WIL during the operation period in the TPPB system with SS-SBR operation mode when paraffin oil was used as the WIL phase. The mass transfer of pyrene as well as the degradation of phenanthrene and anthracene was observed in each cycle of SS-SBR in paraffin oil. Phenanthrene and anthracene were degraded by more than 90% and 80% for each cycle during 13 days, respectively. Paraffin oil had high degradation efficiency for anthracene in the TPPB system with SS-SBR operation mode. As mentioned previously, these trends are due to the solubility of anthracene being higher in paraffin oil than in silicone oil. The SS-SBR operation mode showed good potential to apply the TPPB system for the removal of PAHs semi-continuously from contaminated soil without additional supplement of culture and a WIL.

CONCLUSIONS

The degradation efficiency of three PAHs from the contaminated soil in the TPPB system was dependent on their maximum solubility within the WIL. The initial PAH concentration in soil was about 1,000 mg/kg, and the estimated maximum PAH concentration in the WIL by its transfer was about 2,000 mg/L. In particular, the solubility of anthracene in silicone oil was lower than the maximum concentration of anthracene transferred into silicone oil. Silicone oil showed poor degradation efficiency of anthracene. Paraffin oil with high solubility for three PAHs showed strong potential to degrade PAH in the TPPB system as a WIL phase. Pyrene was not degraded during the operation period of the TPPB system. It appeared that *Sphingomonas* sp. 3Y first utilized phenanthrene and anthracene with three aromatic rings as a carbon source, prior to pyrene with four aromatic rings. SS-SBR operation mode induced the sequential degradation of phenanthrene and anthracene with the operation cycle of the TPPB system. These results support the strong possibility of using a SS-SBR as a new option for the long-term operation of the TPPB system.

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