The response of polycyclic aromatic hydrocarbon degradation in coking wastewater treatment after bioaugmentation with biosurfactant-producing bacteria *Pseudomonas aeruginosa* S5

Tingting Zang, Haizhen Wu, Yuxiu Zhang and Chaohai Wei

ABSTRACT

The polycyclic aromatic hydrocarbons (PAHs) that accumulate during the coking wastewater treatment process are hazardous for the surrounding environment. High molecular weight (HMW) PAHs account for more than 85% of the total PAHs in coking wastewater and sludge, respectively. The degradation of total PAHs increased by 18.97% due to the increased bioavailability of PAHs, after the biosurfactant-producing bacteria *Pseudomonas aeruginosa* S5 was added. The toxicity of total PAHs to humans was reduced by 26.66% after inoculation with S5. The results suggest biosurfactant-producing bacteria *Pseudomonas aeruginosa* S5 not only increase the biodegradation of PAHs significantly, but also have a better effect on reducing the human toxicity of PAHs. Kinetic analyses show that PAHs biodegradation fits to first-order kinetics. The degradation rate constant (k) value decreases as the number of PAH rings increases, indicating that HMW PAHs are more difficult to be biodegraded than low molecular weight (LMW) PAHs. The results indicate the bioaugmentation with the biosurfactant-producing strain has significant potential and utility in remediation of PAHs-polluted sites.

Key words | bacteria, biosurfactant, degradation, PAHs, toxicity, wastewater

Tingting Zang
Haizhen Wu (corresponding author)
Yuxiu Zhang
Chaohai Wei
State Key Laboratory of Organic Geochemistry,
Guangzhou Institute of Geochemistry, Chinese Academy of Sciences,
Guangzhou 510640,
China
E-mail: *hzhwu2@scut.edu.cn*

Haizhen Wu
School of Biology and Biological Engineering,
South China University of Technology,
Guangzhou 510006,
China

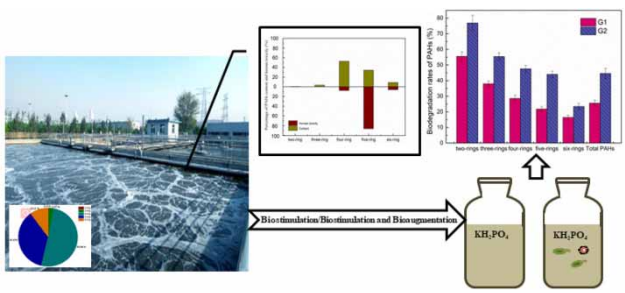
Chaohai Wei
School of Environment and Energy,
South China University of Technology,
Guangzhou 510006,
China

Tingting Zang
Yuxiu Zhang
University of Chinese Academy of Sciences,
Beijing 100049,
China

HIGHLIGHTS

- Removal of PAHs mainly occurred in sludge phase.
- Biosurfactant-producing bacteria S5 increased the biodegradation of PAHs.
- Biosurfactant-producing bacteria S5 reduced the human toxicity significantly.

GRAPHICAL ABSTRACT



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INTRODUCTION

Coking wastewater is produced during the process of coal coking, gas purification and chemical product refinement. It is characterized as containing a high organic load, a complex composition, and strong toxicity (Ou *et al.* 2014). To date, more than 250 million tons of coking wastewater discharges each year in China (Yuan *et al.* 2020). Coking wastewater is a typical industrial wastewater containing inorganic and organic toxic pollutants, such as ammonia, sulfides, cyanides, thiocyanate, phenols, and Polycyclic Aromatic Hydrocarbon (PAHs) (Ou *et al.* 2014).

PAHs are one of the most harmful substances in coking wastewater, which pose a great threat to human health due to their toxicity, carcinogenicity and mutagenicity (Wu *et al.* 2019). PAHs have received significant attention because of their pervasive presence in the environment and can enter and enrich in the human body through the food chain (Guo *et al.* 2017).

PAHs have poor solubility in water, which results in low bioavailability, and inhibits microbial utilization (Zhao *et al.* 2018). Surfactants are frequently added to PAHs-polluted sites to facilitate their biodegradation. Surfactants having hydrophilic and hydrophobic parts could significantly reduce the surface and interfacial tension when they interact with the interfaces of various polarities, thus increasing the solubility of hydrophobic compounds (Lee *et al.* 2018). Biosurfactants produced by bacteria attracted more attention compared with synthetic surfactants, due to more biodegradability, low toxicity and higher foaming (Pi *et al.* 2017). Biosurfactants also increase bioavailability of hydrophobic compounds by changing the surface properties of microbes. Lai *et al.* (2009) applied biosurfactants – rhamnolipids and surfactins – produced by *P. aeruginosa* and *Bacillus subtilis*, respectively; and synthetic surfactants – Tween 80 and Triton 100 – to remove petroleum hydrocarbon from contaminated soil. The result showed that biosurfactants exhibit much higher remediation than that of synthetic surfactants. Biosurfactants and surfactants were used to facilitate the phytoremediation of crude oil-polluted soil, and the findings suggested that biosurfactants may be an efficient and green biotechnological approach for the remediation of hydrophobic compounds-contaminated sites (Liao *et al.* 2016).

In this study, we added *Pseudomonas aeruginosa* S5 (Sun *et al.* 2019) to the coking wastewater treatment system, which could produce biosurfactants to increase the bioavailability of PAHs and facilitate their biodegradation effectively. Strain S5 originated from a coking wastewater treatment plant (WWTP) could quickly adapt to the environment and make effects on PAHs bioremediation. The objectives of the current work are (1) to demonstrate the distribution of PAHs during treatment processes; (2) to compare the efficiency of PAHs biodegradation with or without S5 added; (3) to analyze the biodegradation kinetic of PAHs after biosurfactant treatment; and (4) to evaluate the human toxicity of PAHs under different treatments.

MATERIALS AND METHODS

Materials

A standard solution of 16 PAH mix in dichloromethane: benzene (50:50), at 2 mg/mL, including naphthalene (Naph), phenanthrene (Phe), acenaphthene (Ace), acenaphthylene (Acy), benz[a]anthracene (BaA), fluorene (Fle), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), chrysene (Chr), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[g,h,i]perylene (BgP), dibenzo[a,h]anthracene (DBA), benzo[k] fluoranthene (BkF) and indeno[1,2,3-cd] pyrene (Inp) was obtained from Accustandard Inc. (USA). A deuterated PAH Mix containing Acenaphthene-d10, Chrysene-d12, 1,4-Dichlorobenzene-d4, Perylene-d12, Naphthalene-d8 and Phenanthrene-d10 dissolved in dichloromethane at 4 mg/mL was obtained from Accustandard Inc. (USA). Hexamethylbenzene purchased from Aldrich Co. (China) was used as an internal standard for gas chromatography-mass spectrometry (GC-MS) analysis. Solid phase extraction cartridges (ENV+, 0.5 g), used to extract PAHs from wastewater, were obtained from Supelco Co. (USA).

Pseudomonas aeruginosa strain S5 was used to produce biosurfactants to enhance the bioavailability of PAHs (Sun *et al.* 2019). Strain S5 was grown in LB medium at 30 °C and kept in a shaker at 150 rpm for 72 h. The cells were harvested by centrifugation at 10,000 g for 5 min, washed twice by phosphate-buffered saline, and then the pellet was inoculated into the reactor directly.

Sludge and wastewater sampling

Coking sludge and coking wastewater were sampled from the coking wastewater treatment system (A/O1/H/O2) in the Baowu Steel Group, Shaoguan, China, with an average

treatment capacity of 1,600 ± 200 m^3/d. Coking wastewater samples were collected from the WWTP after biological treatment. Coking sludge and wastewater samples were transported to the lab on ice.

Operation strategy

The sludge from the WWTP was aerated for 12 h and then inoculated into the reactor. Wastewater (600 mL) was introduced directly into a 2 L narrow mouth brown bottle after 400 mL of sludge was seeded to the microcosms. The reactor was aerated by blast aeration, with an aeration rate of 200 mL/min. KH_2PO_4 was added to the sludge to stimulate the growth of microorganisms as the coking sludge was nitrogen rich and phosphorus deficient. The reactors were operated with two protocols: (1) G1, biostimulated group with 0.5 g KH_2PO_4; and (2) G2, bioaugmented and biostimulated group with 0.5 g KH_2PO_4 and 0.5 g activated S5.

Analysis methods

The PAHs of wastewater and sludge analyses were conducted using the methods described by Zhang *et al.* (2012). The mixture of wastewater and sludge was filtered with glass filters to separate the liquid and particle phases. Deuterated PAH Mix (100 μL, 100 μg/mL) was added to the aqueous samples to estimate the recovery rates. The aqueous sample (400 mL) was extracted onto C18 cartridges. Then, the column was eluted with 30 mL dichloromethane three times. The eluent was concentrated to 1 mL by rotary evaporation, and 5 mL n-hexane substitute solvent was added to continue the concentration to 0.5 mL. The sludge particle (0.5 g) was freeze-dried and soxhlet-extracted with 200 mL dichloromethane in a water bath at 50 °C for 48 h. Prior to extraction, the sludge was spiked with 50 μL deuterated PAH Mix (100 μg/mL).

The extracts of the wastewater and sludge samples were purified and fractionated by an alumina/silica gel glass column. The column consisted of absorbent cotton, 6 cm alumina (3%), 12 cm silica gel (3%), and 1 cm anhydrous Na_2SO_4 from bottom to top. First, aliphatic hydrocarbons were removed by 15 mL of hexane. Then, the eluent containing PAHs was collected by 70 mL dichloromethane/hexane (3:7, v/v). The eluent was concentrated to 0.5 mL in a mild N_2 stream. Internal standard (5 μL) was added to the sample before GC-MS analysis.

PAHs analysis was performed by GC-MS (Agilent 7890A, 5975C) with HP-5 MS column (Agilent) (30 m × 0.25 mm × 0.25 film thickness). Helium was used as carrier gas at a flow of 1 mL/min. The injection port and ion source temperatures were 250 °C and 230 °C respectively. The GC oven temperature was set from 45 °C to 310 °C at 5 °C/min (10 min hold). Aqueous samples (1 μL) were analyzed using the splitless injection method. The mass spectrometer was set in electron impact ionization mode at 70 eV over a scan range of 60–640 m/z. The determination coefficient (R^2) of all calibration curves meet the requirement of $R^2 \geq 0.99$.

Surface tensions of solutions were determined by Wilhelmy plate technique, using a tension-meter (BZY-3B, Shanghai).

Kinetics analysis

PAHs degradation kinetic was explored by first-order kinetic model (Equation (1)) (Chettri & Singh 2019):

$$\ln C = -kt + A \quad (1)$$

where C is the concentration of PAHs at time t; k is the apparent kinetic constant of PAHs degradation; and A is a constant.

The theoretical half-life ($t_{1/2}$) value of PAHs was determined using Equation (2):

$$t_{1/2} = \ln 2/k \quad (2)$$

Human toxicity of PAHs analysis

Rosenbaum *et al.* (2008) carried out a comparison of seven toxicity characterization models (CalTOX, IMPACT 2002, USES-LCA, BETR, EDIP, WATSON and EcoSense) applying a chemical test set comprising 45 organic substances to identify the most influential model choices and develop a scientific consensus model – USEtox. USEtox is a tool for estimating the toxicity of PAHs to humans, developed from a comparison of all existing life cycle impact assessment models (Dong *et al.* 2014; Rosenbaum *et al.* 2015; Piekarski *et al.* 2017). The impact score (IS) was estimated according to Equation (3).

$$IS = \sum\sum CF_{x,i} \times M_{x,i} \quad (3)$$

where IS is the impact score for human toxicity, expressed at the endpoint level as the number of disability-adjusted life years [DALY × 10^{-9}/g sludge]; $CF_{x,i}$ is the characterization

factor of PAHs x emitted to compartment i [DALY/kg emitted]; and $M_{x,i}$ is the emitted mass of PAHs x to compartment i (μg/g sludge).

RESULTS AND DISCUSSION

Distribution of PAHs during treatment processes

The percentages of PAHs concentration in coking wastewater treatment processes after 432 h are displayed in Figure 1. As shown in Figure 1, four-, five-, and six-ring PAHs were dominant PAHs in the coking wastewater and sludge phase under different treatments. HMW PAHs (four or more aromatic rings) accounted for more than 85 and 95% of the total PAHs in coking wastewater and sludge, in G1 and G2, respectively. The concentrations of 16 PAHs during coking wastewater treatment processes are drawn in Figure 2. Flu (four-ring), BbF (five–ring) and Inp (six-ring) accounted for 21.65%, 9.53% and 6.20% of the total PAHs concentration in wastewater phase in G1 respectively and did not change significantly in G2. Flu, BbF and Inp accounted for 15.23%, 13.72% and 7.21% of the total PAHs concentration in sludge phase in G1 and accounted for 15.32%, 14.79% and 8.84% of the total PAHs concentration in the sludge phase in G2 respectively. The HMW PAHs accumulated in coking wastewater and sludge may be due to the large amount of HMW PAHs produced during the coking process, which could not be degraded easily by microorganisms during the biological stage.

Figure 2 shows that the concentrations of 16 PAHs in the wastewater phase did not change significantly after bioaugmentation with S5. LMW PAHs (two or three aromatic rings) decreased slightly in wastewater. This is because they could be degraded easily by microorganisms, resulting in few LMW PAHs remaining in the wastewater phase. The concentrations of HMW PAHs in wastewater increased after S5 added, possibly due to a lower biodegradation rate compared to the enhanced solubilization rate. The increased solubility of PAHs leads to an increase in bioavailability of

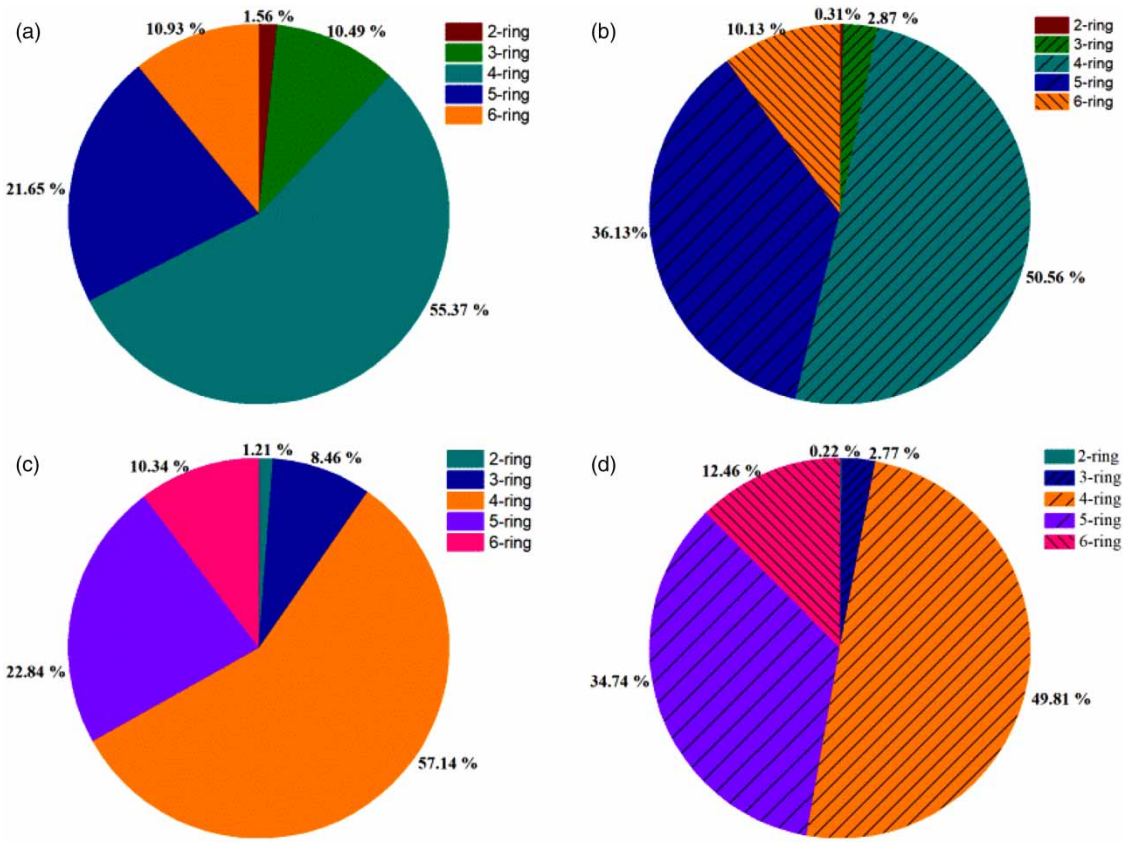

Figure 1 | Percentages of different number rings PAHs concentration in wastewater and sludge in G1 (biostimulated group with 0.5 g KH_2PO_4) and G2 (bioaugmented and biostimulated group with 0.5 g KH_2PO_4 and 0.5 g activated S5). (a) percentages of PAHs concentration in wastewater in G1; (b) percentages of PAHs concentration in sludge in G1; (c) percentages of PAHs concentration in wastewater in G2; and (d) percentages of PAHs concentration in sludge in G2.

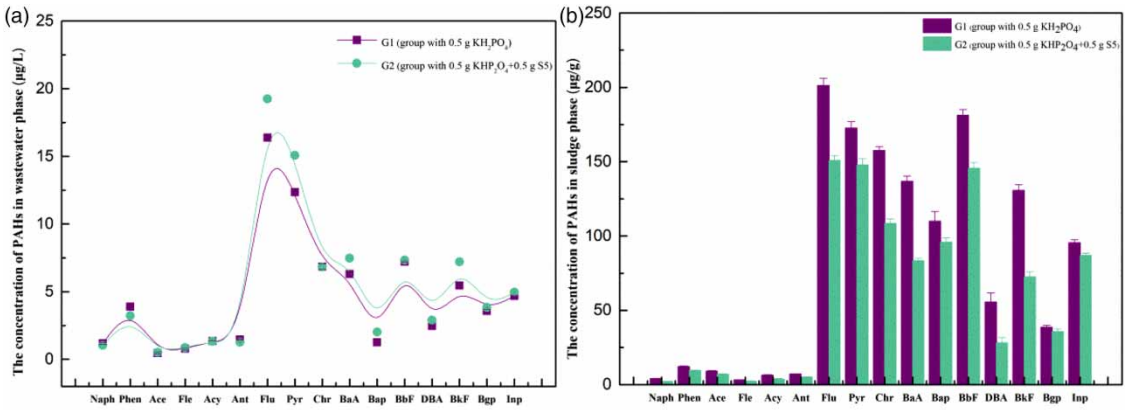

Figure 2 | Concentrations of 16 PAHs during coking wastewater treatment processes.

PAHs, which means that microorganisms in the system may be subjected to a greater toxic load. In fact, the refractory of PAHs mainly depends on its macromolecular structure and large Π bond structure, but the structure of PAHs is not very toxic to microorganisms. Most PAHs degrading bacteria can tolerate 100 mg/L PAHs (Bezza & Chirwa 2017; Chettri & Singh 2019; Sivaram *et al.* 2019), and in this experiment, the concentration of PAHs in the solution is much less than this value. Moreover, biosurfactants changed the phase transfer rate of PAHs and improved the kinetics of PAHs by increasing the concentration of the dissolved state. More conversion of carbon sources increased the number of microorganisms, which in turn increased their resistance to toxicity.

The concentrations of all 16 PAHs decreased in G2 sludge phase as the biosurfactants produced by S5 could change the partition of PAHs between the adsorbed and dissolved phases. The results suggest that the removal of PAHs in the system is mainly from the sludge phase. This may be due to the solubility of PAHs decreasing with an increasing number of benzene rings and tending to accumulate in sludge, and their desorption from sludge limiting their bioavailability in G1. Biosurfactants produced by S5 could relieve this limitation in G2. Biosurfactants enhanced the solubility of the HMW PAHs and subsequently initiated the degradation process of ring cleavage, side chain and central aromatic, which eventually enters into the TCA cycle (Wolf *et al.* 2020).

Efficiency of PAHs biodegradation

The biodegradation rates of PAHs in G1 and G2 are shown in Figure 3. The biodegradation of total PAHs increased from 25.60% in G1 to 44.57% in G2. During the PAHs

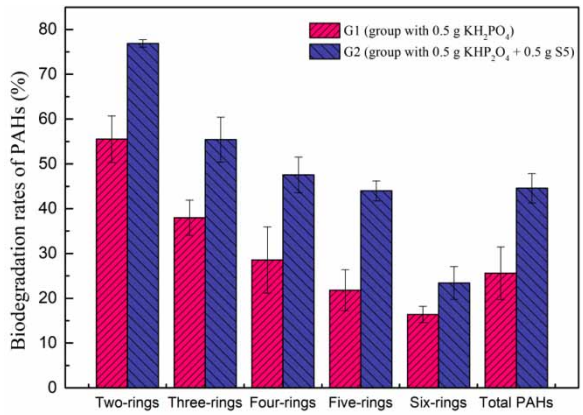

Figure 3 | Biodegradation rates of PAHs in G1 and G2.

bioremediation process, the solubilization of PAHs is a key factor. Bioremediation of PAHs increased in G2 due to the biosurfactants produced by S5 enhancing the solubilization of PAHs and facilitating their biodegradation. In addition, biosurfactants can change the hydrophobicity of the cell surface, resulting in the increased association between bacterial cells and PAHs, which may be another reason for the enhanced bioremediation (Pacwa-Plociniczak *et al.* 2011). Ortega-Calvo *et al.* (2013) also found that the bioavailability of PAHs and the metabolic potential of the bacteria could be increased when adding biosurfactant-producing bacteria to the bioremediation system. The results showed bioaugmentation with biosurfactant-producing bacteria is a feasible method for removing PAHs from contaminated sites.

The biodegradation of PAHs decreased with increasing ring number: degradation for two-, three-, four-, five-, and six-ring PAHs was 55.51%, 37.99%, 28.55%, 21.80%, and 16.40% in G1, increased to 76.88%, 55.42%, 47.52%,

43.98%, and 23.43% in G2, respectively. The HMW PAHs could not be easily degraded during the bioremediation process, which may be accounted for the following reasons: (1) the solubilization rates of HMW PAHs are low (Liang *et al.* 2016); (2) the HMW PAHs are thermodynamically stable and recalcitrant to microbial attack (Cerniglia 1992); (3) the steric effects of HMW PAHs limit the binding of degradation enzymes to PAHs and inhibit their biodegradation (He *et al.* 2013); and (4) microorganism species capable of degrading the HMW PAHs are fewer than the LMW PAHs in coking wastewater treatment (Haritash & Kaushik 2009).

The surface tensions in G1 and G2 were measured. As shown in Figure S1, the surface tension of the solution in G1 was 69.2 ± 0.12 mN/m and did not fluctuate with time. The surface tension of the solution in G2 decreased on days 0–6 from 69.3 mN/m to 58.8 mN/m. The result indicated that S5 gradually adapted to the environment and began to produce and accumulate biosurfactants. Subsequently, the surface tension began to rise. This may be due to the decreased S5 activity or the increased uptake of biosurfactants by other microorgansims, or both. Although the surface tension gradually increased after 6 days in G2, the degradation rate of PAHs still increased with time, increasing due to the residual biosurfactants. The total PAHs degradation rate at different time interval is shown in Figure S2.

Pseudomonas aeruginosa is one of the most common biosurfactant-producing bacteria. Although the addition of S5 greatly improved the degradation of PAHs in G2, *Pseudomonas aeruginosa*, as it is a class of conditional pathogenic bacteria, its pathogenicity has to be considered. As far as we know, *Pseudomonas aeruginosa* generally infects the population with immunodeficiency. Hosts with normal immune function could automatically recognize and eliminate *Pseudomonas aeruginosa* from the body. Moura-Alves *et al.* (2019) proposed that by spying on interbacterial communication, the aryl hydrocarbon receptor is capable of sensing the status quo of the *Pseudomonas aeruginosa* community during infection, allowing the host to mobilize the most appropriate defense mechanism according to the severity of threat. Besides, *Pseudomonas aeruginosa* also needs to overcome high concentrations of reactive oxygen species (ROS) before successfully infecting host cells. ROS could change the expression level of downstream genes controlled by the virulence proteins of *Pseudomonas aeruginosa*, leading to reduced pathogenicity (Chen *et al.* 2011). Even so, if *Pseudomonas aeruginosa* is to be applied in factories in future, we will test the virulence of the strain and knock out its virulence factors genes before use.

Kinetics of PAHs biodegradation

The kinetic analysis of PAHs degradation is helpful to explore the bioremediation model of PAHs, in order to improve efficiency. The first-order model was used to study the biodegradation kinetic of PAHs. The kinetic parameters of PAHs biodegradation were determined by Equations (1) and (2). The kinetic parameters of PAHs degradation in G1 and G2 are displayed in Table 1. The high R^2 values indicated that the biodegradation of PAHs in G1 and G2 followed the first-order kinetics. Several other studies also reported that the biodegradation fitted to first-order kinetics when using pure bacteria or a bacterial consortium containing several consortia to remove PAHs (Chettri & Singh 2019; Bianco *et al.* 2020).

Figure 4 shows the kinetic curves of PAHs in G1 and G2. The half-life values ($t_{1/2}$) were 18.77 days, 28.55 days, 41.91 days, 54.41 days and 67.04 days for two-, three-, four-, five- and six-ring, respectively, and 46.36 days for total PAHs, in G1. It was observed from the kinetic analysis that the $t_{1/2}$ values of two-, three-, four-, five-, six-ring, and total PAHs in G2 were all less than those in G1. The estimated $t_{1/2}$ values decreased to 8.57 days, 15.30 days, 18.61 days, 21.04 days, 45.45 days, and 20.48 days for two-, three-, four-, five-, six-ring, and total PAHs, respectively, in G2. The addition of strain S5 could effectively enhance the biodegradation of the PAHs, suggesting this strain has great potential and advantages in the bioremediation of PAHs-polluted sites. This data provides valuable information for modelling the kinetics of PAHs degradation in contaminated environments.

Human toxicity of PAHs

The characterization factors (CFs) represent the impact by a unit emission of substance to certain environmental recipients through different exposure pathways, and was applied to estimate the human toxicity of organic matters at specific concentration. The CFs of the USEtox model are developed on the basis of an environmental model, which simulates the behavior of organic matters. The USEtox model is used to quantify the potential impacts of human toxicity at special exposure concentrations by predicting the fate and exposure to chemicals (Nordborg *et al.* 2017).

The impact score for human toxicity (IS-HT) of 16 PAHs in G1 and G2 were estimated using Equation (3)

Table 1 | The kinetic parameters of PAHs biodegradation in G1 and G2

		G1			G2		
Rings		**Rate constant (k) (day^{-1})**	**R^2**	**$t_{1/2}$ (days)**	**Rate constant (k) (day^{-1})**	**R^2**	**$t_{1/2}$ (days)**
Two-	Naph	0.0369	0.8748	18.77	0.0809	0.9878	8.57
Three-	Phe	0.0115	0.8933	60.06	0.0275	0.9913	25.19
Three-	Ace	0.02450	0.9868	28.30	0.0413	0.9572	16.79
Three-	Fle	0.0505	0.9604	13.73	0.0696	0.9094	9.96
Three-	Acy	0.0296	0.9417	23.43	0.0652	0.9912	10.64
Three-	Ant	0.0238	0.8342	29.11	0.0473	0.9917	14.65
Four-	Flu	0.0155	0.8379	44.81	0.0375	0.9950	18.47
Four-	Pyr	0.0154	0.8545	45.16	0.0272	0.9824	25.46
Four-	Chr	0.0143	0.8946	48.51	0.0383	0.9903	18.12
Four-	BaA	0.0223	0.9949	31.05	0.0501	0.9912	13.85
Five-	Bap	0.0093	0.9821	74.69	0.0174	0.9384	39.81
Five-	BbF	0.0127	0.9750	54.66	0.0265	0.9828	26.14
Five-	DBA	0.0187	0.9607	37.05	0.0590	0.9913	11.74
Five-	BkF	0.0129	0.9149	53.86	0.0476	0.9829	14.57
Six-	Bgp	0.0102	0.9564	68.02	0.0150	0.9964	46.09
Six-	Inp	0.0104	0.9862	66.58	0.0154	0.9880	45.16

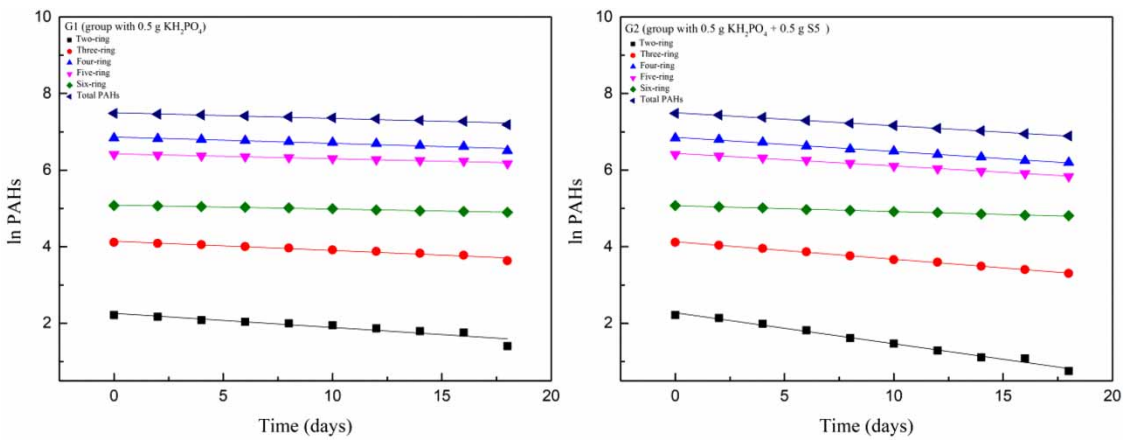

Figure 4 | Kinetic curves of PAHs in G1 and G2.

and are displayed in Table 2. The CFs of PAHs vary with the number of aromatic rings of PAHs, so the IS-HT could not be simply correlated to the concentration of PAHs. For example, BkF ranked sixth and eighth in terms of concentration in G1 and G2 respectively; however, the ranking of IS-HT of BkF was highest in both G1 and G2. The ranking of Flu content was highest in both G1 and G2; however, the IS-HT of Flu ranked tenth in both G1 and G2.

The percentage of concentration of PAHs and its corresponding percentage of IS-HT in different systems are presented in Figure 5 (the PAHs concentration in wastewater is much less than that in sludge, so this work only estimated the concentration of PAHs in sludge). Figure 5(a) shows that the IS-HT of 16 PAHs mainly concentrated on the HMW PAHs. Figure 5(b) shows that five-ring PAHs accounted for 34.38% of total PAHs concentration, while accounting for 86.44% of IS-HT of total PAHs. Therefore,

Table 2 | The ranking of concentration of 16 PAHs and their corresponding ranking of IS-HT after the two different treatments

	G1				G2			
	Concentration (μg/g)	Ranking	IS-HT (DALY × 10^{-9}/g sludge)	Ranking	Concentration (μg/g)	Ranking	IS-HT (DALY × 10^{-9}/g sludge)	Ranking
Naph	4.08	15	8.6088E-06	16	2.12	16	4.4732E-06	16
Phe	12.18	11	0.0001486	13	9.58	11	0.0001169	13
Ace	9.03	12	0.0001192	14	6.86	12	0.0000906	14
Fle	3.21	16	0.0000570	15	2.25	15	0.0000401	15
Acy	6.39	14	0.0002134	12	3.57	14	0.0001192	12
Ant	7.15	13	0.0023667	11	5.03	13	0.0016649	11
Flu	201.17	1	0.0083083	10	150.79	1	0.0062276	10
Pyr	172.57	3	0.0126839	9	147.66	2	0.0108530	9
Chr	157.47	4	0.1921134	7	108.4	4	0.1322480	6
BaA	136.66	5	0.1954238	6	83.37	7	0.1192191	7
Bap	109.92	7	0.3561408	5	95.77	5	0.3102948	5
BbF	181.28	2	1.2544576	2	145.58	3	1.0074136	2
DBA	55.49	9	0.9100360	3	28.09	10	0.4606760	3
BkF	130.6	6	2.5205800	1	72.49	8	1.3990570	1
Bgp	38.55	10	0.0129528	8	35.61	9	0.0119650	8
Inp	95.31	8	0.3698028	4	86.98	6	0.3374824	4

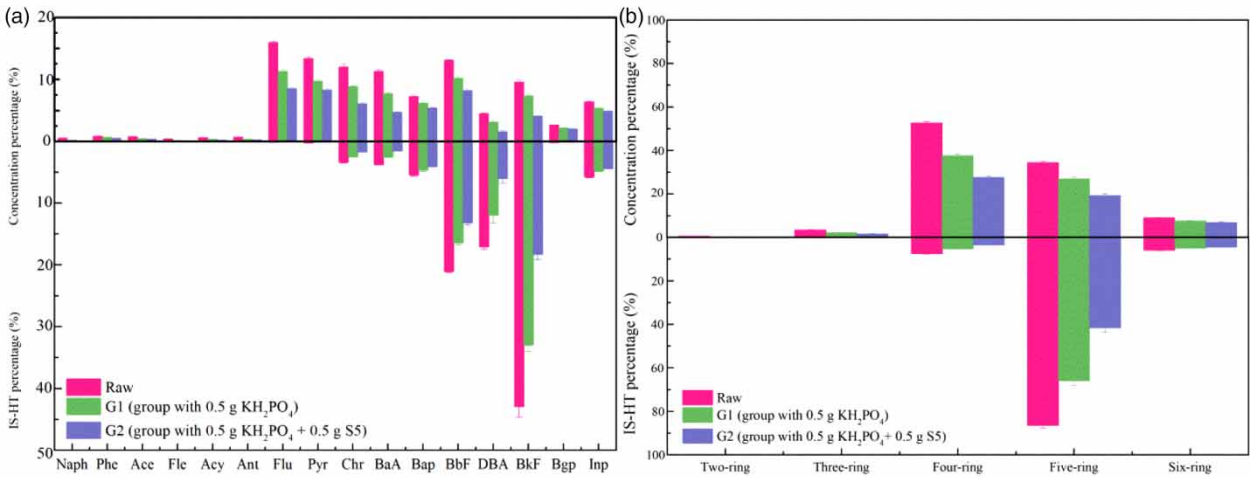

Figure 5 | The percentage of concentration of PAHs and its corresponding percentage of IS-HT in different systems. (a) the percentage of concentration of 16 PAHs and its corresponding percentage of IS-HT in different systems; (b) the percentage of concentration of different-rings PAHs and its corresponding percentage of IS-HT in different systems.

the removal of HMW PAHs should be an urgent priority for PAHs remediation.

The IS-HT of two-, three-, four-, five-, six-ring, and total PAHs are displayed in Table 3. The IS-HT of LMW PAHs is low in G1 and G2, which may be due to the low concentration and CFs of LMW PAHs. The concentration of four-ring PAHs declined most, decreasing by 10.00%; however, the IS-HT of four-ring PAHs only decreased by 1.83%. The content of five-ring PAHs decreased by 7.62%; however, the IS-HT of five-ring PAHs decreased by 24.38%. The results suggest biosurfactants increased the biodegradation of four-ring PAHs and decreased the IS-HT of five-ring PAHs significantly. The concentration of total PAHs decreased from 1,775.6 μg/g to 1,321.06 μg/g in G1, and to 984.15 μg/g in G2. The IS-HT of total PAHs decreased from 7.6442 DALY × 10^{-9}/g sludge to 5.8354

Table 3 | IS-HT of two-, three-, four-, five-, six-ring PAHs, and total PAHs

	Raw		G1				G2			
Rings	Concentration (μg/g)	IS-HT (DALY × 10^{-9}/g sludge)	Concentration (μg/g)	Ratio (100%)	IS-HT (DALY × 10^{-9}/g sludge)	Ratio (100%)	Concentration (μg/g)	Ratio (100%)	IS-HT (DALY × 10^{-9}/g sludge)	Ratio (100%)
Two-	9.17	1.93E-05	4.08	0.23	8.61E-06	0.0001	2.12	0.12	4.47E-06	5.85E-05
Three-	61.22	0.0050	37.96	2.14	0.0029	0.04	27.29	1.54	0.0020	0.03
Four-	934.68	0.5764	667.87	37.61	0.4085	5.34	490.22	27.61	0.2685	3.51
Five-	610.37	6.6076	477.29	26.88	5.0412	65.95	341.93	19.26	3.1774	41.57
Six-	160.11	0.4551	133.86	7.54	0.3828	5.01	122.59	6.90	0.3494	4.57
Total	1,775.6	7.6442	1,321.06	74.40	5.8354	76.34	984.15	55.43	3.7975	49.68

DALY × 10^{-9}/g sludge in G1, and to 3.7975 DALY × 10^{-9}/g sludge in G2. The concentration of total PAHs decreased by 18.97% after inoculation with S5 and the corresponding IS-HT of total PAHs decreased by 26.66%. The results suggest that when S5 increases the biodegradation of PAHs, it also significantly reduces the toxicity of PAHs.

CONCLUSIONS

In the present study, we have added biosurfactant-producing strain S5 to the wastewater treatment process. The HMW PAHs were predominant PAHs in the wastewater and sludge phase during wastewater treatment. The HMW PAHs accumulation may be due to the large amount of HMW PAHs produced during the coking process and that could not be degraded easily by microorganisms. The results indicated that the removal of PAHs in the system mainly occurred in the sludge phase. The synergistic degradation of PAHs by inoculating strain S5 is mainly the removal of components with high human risks, and the toxicity of residual components to human health is significantly reduced. The high R^2 values suggested that the biodegradation of PAHs followed the first-order kinetic. K values of total PAHs in G1 and G2 were 0.0150 day^{-1} and 0.0338 day^{-1}, and the corresponding half-life values ($t_{1/2}$) of total PAHs were 46.36 days and 20.48 days, respectively. The results indicated that the strain S5 enhanced the PAHs biodegradation effectively. The concentration of total PAHs decreased by 18.97% after inoculation with S5 and the corresponding IS-HT of total PAHs decreased by 26.66% after inoculation with S5. These results suggest that bioaugmentation with biosurfactant-producing bacteria is a feasible and effective method for remediation of PAHs from contaminated sites. The degradation of PAHs is strongly influenced by sludge microbial activities. Therefore, in the future, it will be necessary to explore the microbial community structure in sludge after S5 addition to further increase the degradation of PAHs.

NOTES

The authors declare no competing financial interest.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTION

Tingting Zang: Investigation, Writing-Original Draft, Visualization; Haizhen Wu: Conceptualization, Supervision, Writing-Review & Editing; Yuxiu Zhang: Formal investigation; Chaohai Wei: Writing-Review & Editing, Supervision. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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