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Effects of concentration, head group, and structure of surfactants on the degradation of phenanthrene

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Abstract

The effects of concentration, polar/ionic head group, and structure of surfactants on the biodegradation of polycyclic aromatic hydrocarbons (PAHs) in the aqueous phase, as well as their effects on the bacterial activity were investigated. The toxicity ranking of studied surfactants is: non-ionic surfactants (Tween 80, Brij30, 10LE and Brij35) < anionic surfactants (LAS) < cationic surfactants (TDTMA). For the same head group and similar molecular structure, the toxicity to the bacteria is due to the chain length, in which the toxicity becomes lower as the chain length increases. The bacterial growth increased slightly when phenanthrene and LAS ($\leq$10 mg L^{-1}) served the sole carbon and energy resource. However, the degradation of ^{14}C-phenanthrene showed either a decrease or no obvious change with the surfactants present at all tested concentrations (5–40 mg L^{-1}). Thus, the surfactant addition is not beneficial to the removal of phenanthrene or other PAH contaminants due presumably to the preferential utilization of surfactants at low levels as the non-toxic nutrient resource and to the high toxicity of the surfactants at high levels to the microorganism activity. Biodegradation of phenanthrene was also influenced by the surfactant concentration, head group type, and structure. Much more research has yet to be completed on the use of surfactants for soil remediation due to the surfactant toxicity or biodegradation effect.

Keywords: Phenanthrene; Surfactants; Biodegradation; *Mycobacterium* spp. KR2

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced from the incomplete combustion of organic materials. Sixteen specific PAHs are listed by the Environmental Protection Agency to be among the 129 priority pollutants and five of them are listed among the 25 hazardous substances thought to pose the most significant potential threat to human health at the superfund sites. Humans may be exposed to these compounds from a wide variety of sources, such as through occupation (coke-oven or iron-foundry workers), natural environment (air pollutants, drinking water), personal habits (e.g. smoking), medical treatments (coal tar) and diets (broiled and smoked foods) [1–4].

PAHs are strongly sorbed to soil or sediments [5,6]. Consequently, the remediation of PAHs in soil–water systems depends strongly on their desorption rates from the soil surface and the subsequent containment by bulk aqueous phase. Once in the water phase, engineered treatment steps may be used to enhance the remediation. Surfactants have frequently been used to increase the rates of desorption of hydrophobic compounds from soil and the subsequent transfer into water through solute solubilization into aqueous micelles. As such, the use of surfactants may be beneficial to soil-washing or soil-flushing via a pump-and-treat operation [7–11]. Surfactants may also impact the microbial remediation of PAHs in soils by affecting the PAH accessibility to microorganisms [9]. The effect of surfactants on PAH biodegradation depends on a number of factors, including the type of surfactant and the applied level, the specificity of PAHs, and the identity of the microorganisms present.

On the other hand, surfactants are also known to exhibit negative effects on biodegradation of PAHs, due either to the surfactant toxicity or to the increased toxicity of PAHs at enhanced concentrations (due to surfactants) to microorganisms. Surfactants may also compete for PAHs and hence negatively affect the

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Table 1
Structure and physical–chemical characters of phenanthrene [17]

Molecular weight	178.2
Mp (°C[a])	101
Bp (°C[b])	340
Aqueous solubility (15 °C, mg L^{-1})	1.6
Log K_{ow}[c]	4.57
Vapor pressure (20 °C, mmHg)	6.8×10^{-4}

[a] Mp: melting point.
[b] Bp: boiling point.
[c] K_{ow}: the octanol–water partition coefficient.

PAH biodegradation. Thus, it is found that the addition of surfactants above the critical micelle concentration (CMC) inhibited or reduced PAH biodegradation in some cases despite enhancing the biodegradation in others [12,13]. These results show that the impact of surfactants on PAH degradation is complicated, requiring a further resolution [14].

The objectives of this study are to determine the toxicity of applied surfactants to bacteria and to elucidate the effects of surfactant type, concentration, and chain length on the biodegradation of phenanthrene. To achieve these objectives, three sets of experiments were conducted. The first set determined the toxicity of surfactants to the test bacteria; the second set measured the bacterial growth in the mineral solution media solution with added phenanthrene and surfactant, comparing the observed surfactant effect on the bacterial activity with the previous findings; the third set measured the biodegradation rates of ^{14}C-phenanthrene in solution with different surfactants added.

In this study, *Mycobacterium* spp. KR2, the PAHs—degradation microorganism [15,16], was used to elucidate the surfactant effects on the degradation of PAHs and their toxicity. Phenanthrene and ^{14}C-phenanthrene, a three-ring PAH, were selected as the model compounds. Three kinds of non-ionic surfactant (Brij30, 10LE, and Brij35) with different polyoxyethylene (POE) chain lengths were chosen to test the effects of chain length on their toxicity and on the biodegradation of phenanthrene. Anionic surfactant LAS, non-ionic surfactants Tween 80, Brij30, 10LE, Brij35 and a cationic surfactant TDTMA were chosen to examine the effects of ionic type on phenanthrene degradation.

2. Material and methods

2.1. Material

2.1.1. Chemicals and reagents

The 9-^{14}C-labelled phenanthrene was purchased from Biotrend Chemical (Köln, Germany) with a purity of >99% and a specific activity of 55 mCi $mmol^{-1}$. Unlabelled phenanthrene with a purity of 99.5% was purchased from Aldrich Co. The structure and physico-chemical properties of phenanthrene were shown in Table 1. The surfactants, LAS, Brij30, 10LE, Brij35 and TDTMA were obtained from Sigma–Aldrich Chemical CO., St. Louis, MO, and Tween 80 was obtained from Merck (Darmstadt, Germany). The surfactants were used as received from the suppliers without further purification. The characteristics and structures of the surfactants are presented in Table 2 and Fig. 1.

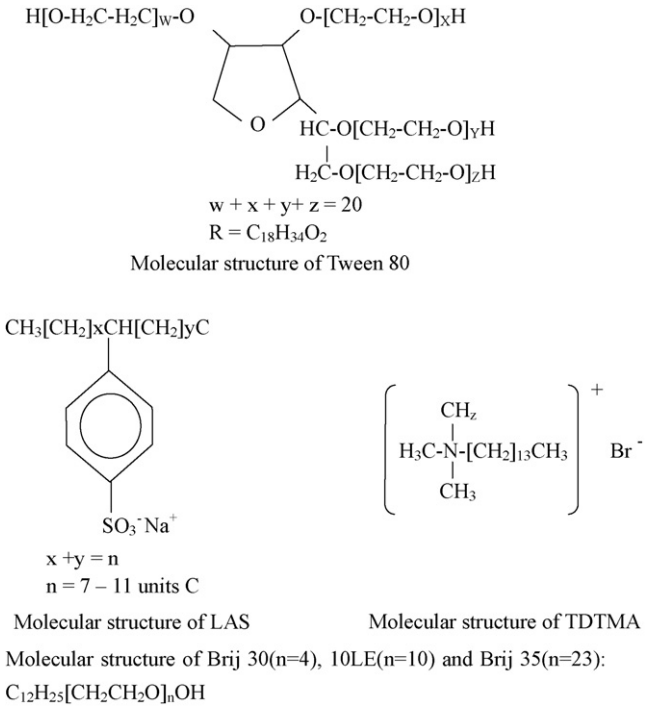

Fig. 1. Molecular structure of surfactants used in the experiments.

2.1.2. Microorganism and growth condition

Mycobacterium spp. KR2 was supplied by Dr. Rehmann from GSF/IÖC, Germany. It was isolated from PAH contaminated soils, capable of degrading phenanthrene and pyrene [15,16]. *Mycobacterium* spp. KR2 was cultured in a conical flask with Mineral Solution Media (MSM) on an orbital shaker at 20 °C/100 rpm. The compositions of growth medium MSM are shown in Table 3. R2A nutrient solution was used to study the effect of surfactant type on the growth of *Mycobacterium* spp. KR2, which has a composition (in per liter) of: 0.5 g yeast extract, 0.5 g proteose peptone, 0.5 g casein hydrolysate, 0.5 g D(+) glucose, 0.5 g starch soluble, 0.3 g pyruvic acid (sodium salt), 0.3 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7H_2O$. The pH of both nutrient media was adjusted to 7.2 ± 0.1.

2.1.3. Reagents and cocktails for measurement of ^{14}C-activity

Cocktail I for measuring ^{14}C–VOCs (volatile organic compounds) was EMME (purchased from Meriden, USA). Cocktail II for measuring $^{14}CO_2$ was a mixture of CARBO-SORB® E and PERMAFLUOR® E^+ (purchased from Gröningen, The Netherlands) in radio of 2:3 (v/v) [20].

2.2. Experimental

2.2.1. Effects of surfactant type on the growth of *Mycobacterium spp. KR2*

This test was carried out in 500 ml flasks with R2A nutrient solution. The concentrations of surfactants were 0, 5, 10, 20, 40,

Table 2
The characteristics of surfactants used in this study

Surfactant	Molecular formula	Molecular structure	Molecular weight	HLB	CMC (mg L^{-1})	Type
LAS	Linear alkykane sulfonate	$C_{21}H_{35}SO_3Na$	390	–	433.5[18]	A
Tween 80	Polyoxygethylene sorbitanmonooleate	$C_{64}H_{125}O_{26}$	1309	15.0[19]	13.4[8]	N
TDTMA	Tetradecyltrimethylammonium bromide	$C_{17}H_{38}NBr$	336.4	–	100	C
Brij30	POE(4)sorbitan monooleate	$C_{20}H_{42}O_5$	363	9.7[14]	9.7[8]	N
10LE	POE(10)sorbitan monooleate	$C_{32}H_{66}O_{11}$	626	14.1[a]	62.6a	N
Brij35	POE(23)sorbitan monooleate	$C_{58}H_{118}O_{24}$	1198	16.9[14]	74[19]	N

A: anionic surfactant; N: non-ionic surfactant; POE: polyoxyethylene; C: cationic surfactant.
[a] Provided by the supplier.

80, 100, 200, 450 and 900 mg/L. The flasks were cultured on the orbital shaker (20 °C/100 rpm, darkness). Growth of *Mycobacterium* spp. KR2 was measured at the optical density of 540 nm (OD_{540}).

2.2.2. Effects of LAS on growth of Mycobacterium spp. KR2 in phenanthrene MSM solution

This test was carried out in MSM solution with the phenanthrene concentration at 100 mg L^{-1}. LAS concentrations were 0, 5, 10, 20, 40, 80, 100, 200, 450, 900 mg/L. The following experimental steps were the same as described in 2.2.1. In this study, phenanthrene and LAS is the sole carbon and energy source for the bacterial growth.

2.2.3. Effects of surfactants on the degradation of ^{14}C-phenanthrene by Mycobacterium spp. KR2

Phenanthrene in DCM and ^{14}C-phenanthrene (4.44×10^4 in activity intensity) in methanol were added into 250 mL-cultural bottles. After evaporating off DCM and methanol, appropriate amounts of sterilized MSM solution, surfactant solution, and *Mycobacterium* spp. KR2 solution were added into the bottles. The phenanthrene concentration was 100 mg L^{-1}, and surfactant levels were at 0, 5, 10, 20 and 40 mg L^{-1}.

The test apparatus was laid out as shown in Fig. 2. With the test apparatus, 10 mL EMME was added into trap 1 to adsorb VOCs (volatile organic compounds), 15 mL Cocktail II was added into traps 2 and 3, respectively, to adsorb ultimate metabolites, CO_2. The vacuum pump was turned on so that air could be circulated to ensure that all sections in the apparatus receive the same aeration. Then the vacuum pump was shut off, and the timer was used to control the aerating time. All the cultural bottles were protected from photolysis of phenanthrene and surfactants. Samples were collected and analyzed for radioactivity by a liquid scintillation counter (LSC) to determine the masses of carbon dioxide and VOCs, respectively.

Every treatment described above was triplicated.

Table 3
Concentrations of components of mineral solution media (MSM) prescription

MSM components	Concentration (w/v)	Trace elements	Concentration (mg L^{-1})
K_2HPO_4	0.8%	EDTA-Na	0.5
KH_2PO_4	0.2%	$FeSO_4{\cdot}7H_2O$	0.2
KNO_3	0.1	H_3BO_3	0.03
$MgSO_4{\cdot}7H_2O$	0.2%	$CoCl_2{\cdot}6H_2O$	0.02
$CaCl_2{\cdot}2H_2O$	0.5%	$CuCl_2{\cdot}2H_2O$	0.01
$FeCl_3{\cdot}6H_2O$	0.01%	$ZnSO_4{\cdot}7H_2O$	0.01
NaCl	0.1%	$NiCl_2{\cdot}6H_2O$	0.006
Trace elements		$MnCl_2{\cdot}4H_2O$	0.003
		$Na_2MoO_4{\cdot}2H_2O$	0.003

3. Results and discussion

3.1. Effects of surfactant types on the growth of Mycobacterium spp. KR2

Fig. 3 shows the effects of six different surfactants on the growth of *Mycobacterium* spp. KR2. For Tween 80 and Brij35 at the applied levels of 0–900 mg L^{-1}, the growth of *Mycobactrium* spp. KR2 increased, manifesting that the surfactants present at these levels were beneficial to the bacterial growth [21]. For anionic LAS and non-ionic 10LE at levels below 40 mg L^{-1}, no obvious changes of the bacterial growth were noticed, indicating that these surfactants showed no toxicity to the bacteria [22]. However, the growth of *Mycobactrium* spp. KR2 was significantly delayed or inhibited by the added surfactants at high levels, such as LAS (≥80 mg L^{-1}), TDTMA (≥10 mg L^{-1}), 10LE (≥450 mg L^{-1}) and Brij30 (≥80 mg L^{-1}). The observed toxicity of surfactants at high levels is expressed in the form of bacterial membrane disassembling. The toxicity of non-ionic and anionic surfactants was much lower than that of the

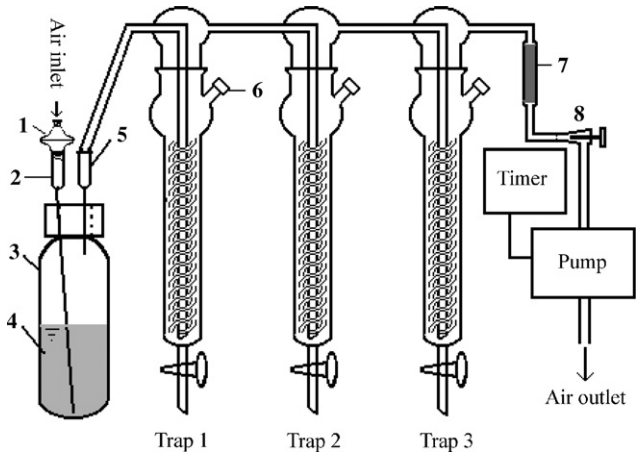

Fig. 2. Apparatus for the test of effects of LAS on the degradation of ^{14}C-phenanthrene, (1) 0.2 μm sterile membrane filter, (2) long springe needle, (3) cultural bottle, (4) culture solution, (5) short springe needle, (6) glass stopper with Teflon liner, (7) active charcoal, (8) control valve.

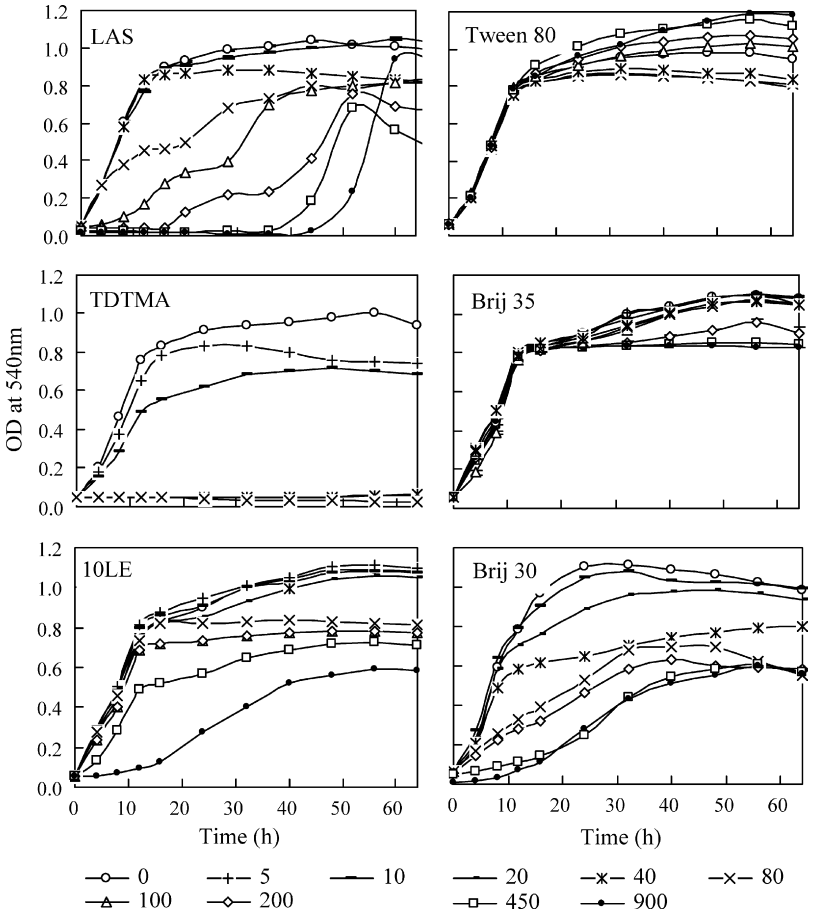

Fig. 3. Effects of surfactants on the growth of *Microbacterium* spp. KR2.

cationic surfactant. The toxic ranking of test surfactants is: Tween 80 < Brij35 < 10LE < Brij30 < LAS < TDTMA.

When the concentrations of test surfactants were below 40 mg L^{-1}, no obvious effects on the growth of bacteria could be found except with the cationic TDTMA surfactant. Therefore, to minimize the surfactant toxicity to the growth of *Mycobactrium* spp. KR2, and to elucidate the effect of different surfactants on the degradation of phenananthrene, the surfactant concentrations in the following experiments were chosen to be 0, 5, 10, 20 and 40 mg L^{-1}.

3.2. Effects of LAS on the growth of Microbacterium spp. KR2 with phenanthrene and LAS as the carbon and energy resource

Fig. 4 illustrates the effects of different concentrations of anionic surfactant LAS on the growth of *Microbacterium* spp. KR2 with phenanthrene and LAS as the carbon and energy resource.

Anionic LAS surfactant at low concentrations (<40 mg L^{-1}) did not affect the growth of bacteria KR2 in the R2A nutrient solution, as mentioned above. Its existence could increase the solubility of phenanthrene [19,23]. Normally, bacteria only utilize solubilized phenanthrene [24]. Thus, the presence of LAS at a low concentration may stimulate the biodegradation of phenanthrene. However, many negative findings were reported, such as no significant uptake of PAHs by bacteria in surfactant micelles [25,26], preferential adsorption of surfactants on the bacterial membrane to prevent the PAHs uptake [24,27], and so on. In the present test, the growth of bacteria KR2 was slightly increased with LAS concentration below 10 mg L^{-1}. Similar results also were reported by others [8,28], and these studies found the addition of non-ionic surfactant increased the oxygen consumption in the degradation experiment of PAHs. Thus, it is not certain

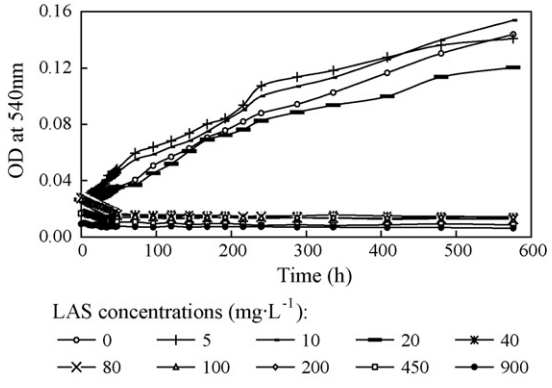

Fig. 4. Effects of LAS on the growth of *Microbacterium* spp. KR2 with the phenanthrene and LAS as the only carbon source.

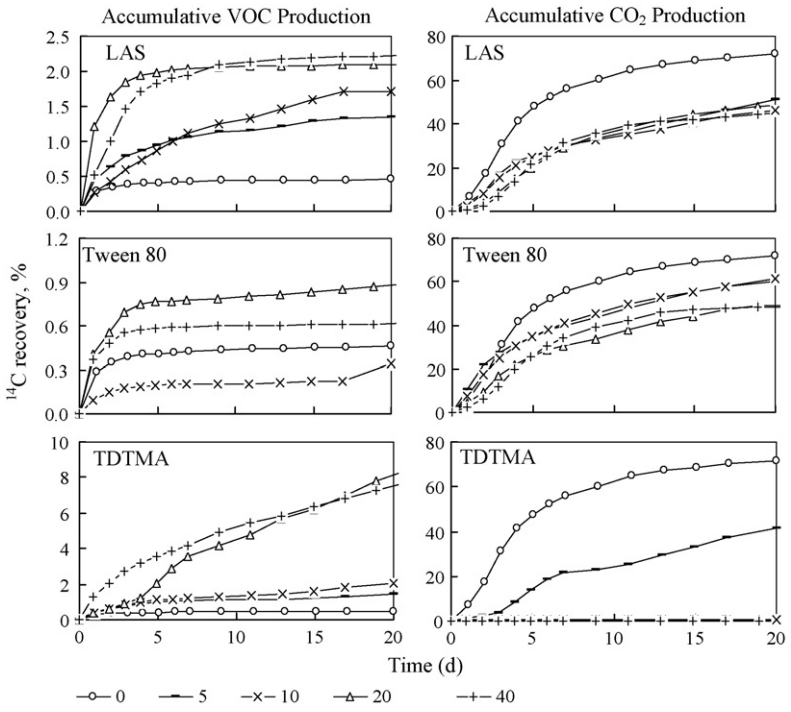

Fig. 5. Effects of Surfactants' concentrations on the ^{14}C–VOC and ^{14}C–CO_2 production of ^{14}C-phenanthrene degradation by *Mycobacterium* KR2.

whether this phenomenon was caused by the enhancement of phenanthrene bio-utilization or by the preferential utilization of LAS at low levels. It was observed that *Microbacterium* spp. KR2 did not grow when the LAS level exceeded 40 mg L^{-1}.

3.3. Effects of surfactants on the degradation of ^{14}C-phenanthrene

In order to substantiate the role of surfactant on the degradation of phenanthrene, an isotope technique was used in this study. In addition, the effects of surfactant type, structure and concentration on the degradation of PAHs were also investigated.

In the study, ^{14}C–VOCs were a mixture of the semi-metabolites of phenanthrene and a small amount of volatile phenanthrene. ^{14}C–CO_2 is the terminal metabolites of phenanthrene, its accumulative production serves to indicate the extent of phenanthrene degradation. Thus the accumulative production of ^{14}C–CO_2 and ^{14}C–VOCs of phenanthrene metabolites with the presence of surfactants were measured and presented in Figs. 5–7.

3.3.1. Biodegradation of phenanthrene with the surfactants addition

Fig. 5 shows the ^{14}C–VOC and ^{14}C–CO_2 accumulative production of ^{14}C-phenanthrene degradation by *Mycobacterium* spp. KR2 in the presence of different surfactant types and concentrations. The ^{14}C–VOCs production of phenanthrene was enhanced by the surfactant addition, but the production of its terminal metoblites-CO_2 was decreased. This means that the addition of surfactant is not beneficial to phenanthrene biodegradation, which concurred with the report of Doong and Lei [14]. Many previous studies were conducted by monitoring the oxygen consumption [28] or the optical density (OD) of bacteria [8], which could not separate the contributions of surfactant and phenanthrene on the growth of bacteria. Since the real reason for the oxygen consumption and the OD increase may be the preferential utilization of surfactants at low or non-toxic levels by bacteria, it is difficult to draw the conclusion that the biodegradability of phenanthrene is enhanced by the surfactant addition. The use of ^{14}C-phenanthrene in our experiments identified the extent of biodegraded phenanthrene through a monitoring of ^{14}C–VOCs and ^{14}C–CO_2 levels.

An increase in the VOC cumulative production in the presence of surfactant may be explained by two reasons: the first is the decrease of surface tension in the nutrient solution, which accelerated the volatilization of semi-metabolites and phenanthrene; the second is the increased toxicity of surfactants at high levels to bacteria, which leads to inhibition of phenanthrene biodegradation and to an increase in volatile semi-metabolites. A decrease in ^{14}C–CO_2 cumulative production may result from the surfactant toxicity to the bacteria KR2 at high surfactant levels [29], or from the preferential uptake of surfactants by bacteria at low surfactant levels [30,31], or from the declining biodegradability of phenanthrene as the concentration of micelles increases [14,32].

As discussed in 3.1, the growth of bacteria was not inhibited when the surfactant level was below 40 mg/L, except with the cationic TDTMA surfactant. Thus, the decreased ^{14}C–CO_2 cumulative productions may not result from the surfactant toxicity to bacteria KR2 in this experiment. The surfactant concentrations used in this experiment were below their CMC values except for Tween 80 and Brij30. The decreased ^{14}C–CO_2 cumulative production may stem directly from the declining phenanthrene biodegradability as the concentration of micelles

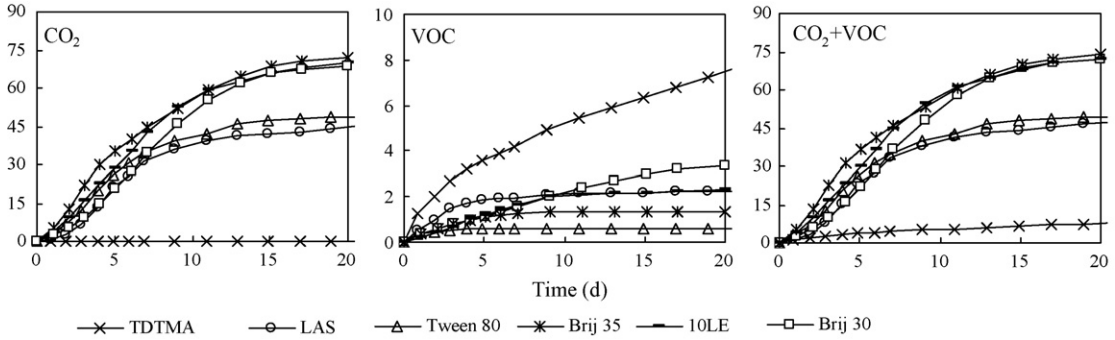

Fig. 6. Effects of ionic types of surfactants on ^{14}C-phenanthrene degradation by *Mycobacterium* KR2 at their concentration of 40 mg L^{-1}.

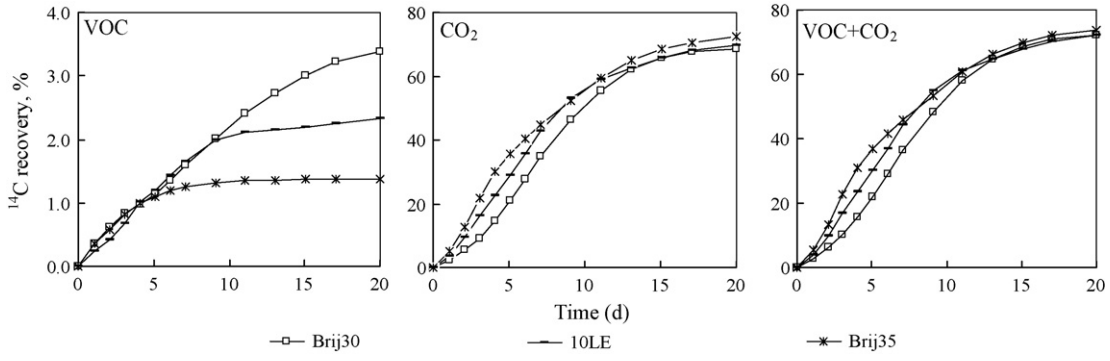

Fig. 7. Effects of surfactants with different POE chain lengths on ^{14}C-phenanthrene degradation by *Mycobacterium* KR2.

increases. Thus the only good reason for the decrease in $^{14}C–CO_2$ production is the preferential uptake of surfactants by bacteria at low surfactant concentrations. This means the surfactant addition may not be beneficial to the removal of phenanthrene and other PAHs contaminants in aqueous phase due to a preferential utilization of surfactants at low levels by microorganisms as the non-toxic nutrient resource.

3.3.2. Effects of the surfactant type, structure and concentration on phenanthrene biodegradation

As discussed above, the effects of surfactants on the biodegradation of phenanthrene were influenced by surfactant concentrations, types (or the head groups), and structures. The experimental data illustrating these effects are presented in Figs. 5–7.

Cationic TDTMA surfactant inhibited most effectively the phenanthrene degradation with the lowest $^{14}C–CO_2$ production and the highest ^{14}C–VOCs production. The next highest inhibitor was the anionic LAS surfactant, which exhibited a lower $^{14}C–CO_2$ production than the non-ionic surfactants: Tween 80, Brij30, Brij35 and 10LE.

The apparent PAH solubility was linearly related to the surfactant level when exceeding the critical micelle concentration (CMC), and increased with decreasing hydrophilic–lipophilic balance (HLB). Since only the concentrations of Tween 80 and Brij30 in this study were above the CMC, the effects of CMC on the degradation of PAHs are difficult to assess. The hydrophobicity of a surfactant can be estimated roughly from its HLB value. The lower the HLB of a surfactant, the greater is its hydrophobicity. Whereas the HLB value is not the sole factor for its solubilization capacity of a surfactant in general, it is a good indicator for surfactants within a homologous series [19]. Surfactants with a short POE chain have a relatively low HLB value and hence have a high capacity for enhancing the solubility of PAHs (Fig. 7). The sequence in the abilities of non-ionic surfactant to enhance PAH solubilization was Brij30 < 10LE < Brij35, which concurred with the results of ^{14}C–VOCs production shown in Fig. 7, indicating that the ^{14}C–VOCs production increased with reducing POE chain or HLB value. In the beginning of the experiment, the $^{14}C–CO_2$ production with surfactants was higher when the surfactant has a higher HLB value; after 13 days of bacterial adaptation to the surfactants, the $^{14}C–CO_2$ production rates were equal (Fig. 7). Therefore, the HLB value seemed to be a better factor to predict the capability of the surfactant to enhance the PAH solubility, but it was difficult to predict or describe the PAH degradation with added surfactants.

4. Conclusion

Surfactants showed negative effects on the growth of *Myrobacterium* spp. KR2 when present at high levels ($\geq$40 mg L^{-1}) and exhibited no obvious effects at low levels ($\geq$20 mg L^{-1}) except for the cationic TDTMA surfactant. Biodegradation of phenanthrene in water could not be enhanced by any kinds of surfactants, although the growth of *Mycobacterium* spp. KR2 could be enhanced in phenanthrene and LAS MSM solutions with LAS levels below 10 mg L^{-1}. It appeared that the preferential utilization of surfactants by bacteria was the main result, which led to a decrease in phenanthrene

biodegradation in water. This observation is in spite of the fact that surfactants applied to soil–water systems usually enhance the desorption of contaminants (e.g., PAHs) from soils into the water phase for suitable physical or biological treatments. The biodegradation of phenanthrene was influenced by the surfactant concentration, the polar or ionic head group, and the structure. More studies should be conducted on the utility of added surfactants for the remediation of PAH-contaminated soils.

Acknowledgments

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