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Effect of nonionic surfactants on biodegradation of phenanthrene by a marine bacteria of *Neptunomonas naphthovorans*

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ABSTRACT

Biodegradation of three nonionic surfactants, Tergitol 15-S-*X* (*X* = 7, 9 and 12), and their effects on the biodegradation of phenanthrene by marine bacteria, *Neptunomonas naphthovorans*, were studied. The experimental outcomes could be fit well with the first-order biodegradation kinetics model. It was observed that the biodegradability of these surfactants decreased with an increase in the chain length of the hydrophilic moiety of the surfactant. When surfactant concentrations initially present were less than 250 mg carbon/L, biodegradability of Tergitol 15-S-*X* surfactants is around 0.3. Reduced biodegradability of Tergitol 15-S-7 and Tergitol 15-S-9 was observed when their concentrations initially present were increased to 322 and 371 mg carbon/L, respectively. In general, biodegradation of phenanthrene was enhanced with increasing solubilization of phenanthrene by these surfactants. However, with the same initial concentration of phenanthrene, biodegradability of phenanthrene was found to decrease with an increase in surfactant concentration. For these three surfactants, more than 80% of the phenanthrene was degraded when surfactant concentrations initially present were 200 mg/L. However, less than 30% of phenanthrene could be degraded, if initial surfactant concentrations were increased to 1000 mg/L. Interestingly, the concurrent biodegradation of the surfactants reduced their effective concentrations for micelle formation and, hence, contribute to the higher bioavailability of phenanthrene by gradually releasing phenanthrene molecules into the aqueous phase.

1. Introduction

In recent years, surfactant-mediated bioremediation is a research focus [1–3]. The increasing interest is attributable to the fact that surfactant can enhance the solubilization of pollutants from contaminated soil and increase their solubility, which in turn improves their bioavailability [3–7].

Among various contaminants, considerable attention has been emphasized on remediation of polycyclic aromatic hydrocarbons (PAHs), as they are either known or suspected carcinogens and mutagens [8,9]. Moreover, their low aqueous solubility and high affinity to soils give rise to their persistence in the environment. This also makes them barely bioavailable to microorganisms and difficult for removal by bioremediation processes. Many surfactants of different kinds have been so far investigated on their possible applications in facilitating the biodegradation of organic contaminants such as PAHs. However, both enhanced and reduced biodegradation of these contaminants in presence of surfactants have been reported [4–7,10–14].

With the increasing environmental awareness, readily biodegradable surfactants are preferred in *in situ* remediation applications in terms of environmental biocompatibility [1–3]. However, their solubilization capacity and effects on the biodegradation of the environmental pollutants are also of important considerations when choosing suitable surfactants. Another advantage of using biodegradable surfactants is that bioavailability of the primary substrate might be improved with the biodegradation of surfactant [1–3]. With degradation of surfactant micelles, more solute molecules would be released from the micellar phase into the aqueous phase, making the substrates more readily available to microorganisms. The gradual release of a substrate from the micellar phase into the aqueous phase can compensate the loss of the substrate in the aqueous phase, which commonly becomes a limiting factor when a non-biodegradable surfactant is used.

Biodegradation of surfactants has been the subject of substantial research works since 1950s, when synthetic detergents came into

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widespread use [14–17]. However, concentrations of surfactants utilized in biodegradation processes were usually in the vicinity of 10 mg/L, which is approximately 10–100 times lower than their respective critical micelle concentrations (CMCs). To enhance the soil remediation process, surfactants should be applied at concentrations higher than their respective CMCs. However, information about biodegradation of surfactants at such concentrations is lacking. It is only in recent years that such studies have been carried out [11,18].

In this study, biodegradability of three nonionic surfactants Tergitol 15-S-*X* (*X* = 7, 9 and 12) at concentrations higher than their respective CMCs by marine bacteria *Neptunomonas naphthovorans* [19] was measured. Concurrently, biodegradation of phenanthrene affected by micellar solutions of these surfactants were studied. Preliminary data on the biodegradation of phenanthrene in the presence of Tergitol 15-S-7 has been reported in our previous publication [20]. Tergitol 15-S-*X* surfactants are mixtures of secondary alcohol ethoxylates. These surfactants are readily biodegradable and environmentally friendly. Selection of these Tergitol surfactants was also based on their high solubilization capacity for PAHs [21,22]. Despite of their great potential applications in *in situ* bioremediation processes, reports on biodegradation of these surfactants as well as their effects on biodegradation of PAHs have hardly been found in the open literature. Hence, it is our aim in this work to provide some insights on the biodegradation of these surfactants and their effects on the biodegradation of phenanthrene, as a model PAH, by the marine bacteria of *N. naphthovorans*.

2. Materials and methods

2.1. Materials

The commercial nonionic surfactant, Tergitol 15-S-*X* (*X* = 7, 9 and 12) were supplied by Dow Chemical Company. They are mixtures of secondary alcohol ethoxylates with the alcohol group located at various positions along a chain of 11–15 carbon atoms and with an average ethylene oxide number of 7.3, 8.9 and 12.3, respectively. These surfactants are readily biodegradable and have been accepted by the Food Safety and Inspection Service of the U.S. Department of Agriculture for general-purpose cleaning or as an ingredient of general-purpose cleaner for use in federally inspected meat and poultry processing plants. Reagent grade of phenanthrene was purchased from Aldrich Chemicals Co. Deionized water from a Milli-Q purification system (Millipore, USA) having resistivity greater than 18.2 MΩ cm was used in preparing samples. All chemicals were used as received. The marine bacteria, *N. naphthovorans* (ATCC 700638), used in the experiments was obtained from the American Type Culture Collection (ATCC). It can aerobically degrade polycyclic aromatic hydrocarbons [19]. An artificial saline water medium, with pH adjusted at 7.5 ± 0.1, based on the ionic compositions of seawater was used in the biodegradation experiments [23].

2.2. HPLC analysis of PAHs concentration

The separation and quantification of phenanthrene in the micellar solutions of these nonionic surfactants was conducted with a Shimadzu HPLC system equipped with a fluorescence detector. The analytical procedure has been reported elsewhere [21]. Values reported in this work were obtained form the average of triplicate analysis for each sample. The reproducibility of the analyses was consistent with a standard error less than 2%.

2.3. TOC analysis of bacteria and surfactant concentration

A total organic carbon (TOC) analyzer (Shimadzu TOC-5000A, Kyoto, Japan) was used for the determination of surfactant and biomass concentrations in biodegradation experiments. The oven temperature was set at 680 °C. The TOC contents of Tergitol 15-S-7, Tergitol 15-S-9 and Tergitol 15-S-12 to their molecular weights were found at about 60% (Table 1). At particular time intervals, samples were taken from each of the flasks, centrifuged at 10,000 rpm (Eppendorf 5810R, Hamburg, Germany) at room temperature (22 °C) for 10 min to remove bacterial cells, and then quantified with a TOC analyzer for surfactant concentrations. The biomass in a solution was calculated by subtracting the surfactant TOC from the total TOC of the uncentrifuged sample containing both surfactant and bacteria. The specific growth rate μ could be estimated from the time variation of the logarithms of the biomass concentration during the exponential growth phase of the bacteria, which will be illustrated in details later in this report.

2.4. CMC measurement

The CMC of the surfactant was determined with a Krüss DSA-10 tensiometer (Hamburg, Germany). The CMC value was estimated by plotting the surface tension against the logarithms of surfactant concentrations. The surfactant concentration at the transition between the descending line for surfactant concentrations less than CMC and the other straight line for surfactant concentrations greater than CMC was taken as the CMC value. The solubilization of phenanthrene by micellar solutions of the surfactants was carried out in 15 mL glass culture tubes with screwed caps. The details of the procedure have been reported [21].

2.5. Procedures for surfactant biodegradation

The mineral solutions with different surfactant concentrations were prepared by dissolving certain amounts of surfactants in 500 mL Erlenmeyer flasks, each of which had 200 mL of the saline water medium. The bacterial culture at the late exponential growth stage was centrifuged and re-suspended in a certain amount of the mineral solution. Subsequently, aliquots of the suspension were inoculated into each of the flasks with previously prepared mineral solutions containing surfactants. The flasks were placed in a

Table 1
Characteristics of surfactants and solubilization data of phenanthrene

Surfactant	Molecular formula	Mw (g/mol)	CMC (mg/L)	WSR	C_{CMC} (mg/L)	TOC (Y) vs. surf. conc. (*X*)
Tergitol 15-S-7	$C_{11-15}H_{23-31}O(CH_2CH_2O)_{7.3}H$	515	32[a]	0.0378[b]	1.15	$Y = 0.604 \cdot X + 1.367$[c]
Tergitol 15-S-9	$C_{11-15}H_{23-31}O(CH_2CH_2O)_{8.9}H$	584	45[a]	0.0253	0.98	$Y = 0.586 \cdot X + 0.975$[c]
Tergitol 15-S-12	$C_{11-15}H_{23-31}O(CH_2CH_2O)_{12.3}H$	738	75[a]	0.0207	0.95	$Y = 0.563 \cdot X + 3.390$[c]

CMC: critical micelle concentration of the surfactant; C_{CMC}: apparent solubility of phenanthrene in the mineral medium at CMC of the surfactant; WSR: mass (or weight) solubilization ratio, defined as the amount of phenanthrene solubilized by every unit weight of surfactant above its CMC in the medium. The WSR of phenanthrene by a surfactant equals to the slope of the solubilization curve of Fig. 1.

[a] Value measured in 1 wt% aqueous solution.

[b] Values reported in reference [21].

[c] Units of *X* and *Y* are mg/L.

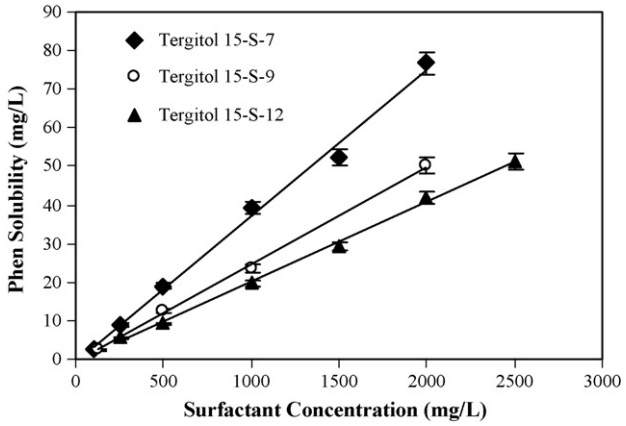

Fig. 1. Solubilization capacity of surfactant for phenanthrene in mineral medium.

water bath shaker operated at 150 rpm and 22 °C for biodegradation of the surfactant to take place. Uninoculated surfactant mineral solutions were also used in the study as control.

2.6. Procedures for phenanthrene degradation

Similar biodegradation experiments were carried out on phenanthrene in 500 mL Erlenmeyer flasks filled with 200 mL of saline water medium in each flask. To study the effect of phenanthrene solubilization by surfactant micelles on biodegradation of phenanthrene itself, solutions of the same initial phenanthrene concentration but with different surfactant concentrations were inoculated with the same mass of bacterial culture harvested at the late exponential growth stage. These flasks were agitated in an orbital shaker with thermostatic water bath operated at 150 rpm and 22 °C. Samples were periodically taken from the flasks for analyses of phenanthrene concentrations. For each solution, a control test with the sterilized uninoculated solution was also carried out. No significant loss of phenanthrene was observed.

3. Results and discussion

3.1. CMC and solubilization capacity of surfactants in mineral solution

Solubilization of phenanthrene by these three surfactants, Tergitol 15-S-*X* (*X* = 7, 9 and 12), is given in Fig. 1. It is clear that solubilities of phenanthrene in these micellar solutions are linearly dependent of surfactant concentrations above their respective CMCs. The obtained coefficients of determination (R^2) on these linear solubility curves are all greater than 0.994. That is, addition of the surfactants could effectively and linearly increase the apparent solubility of phenanthrene [21], and, hence, possibly the bioavailability of phenanthrene to the bacteria.

Mass solubilization ratios (WSRs) obtained from Fig. 1 along with the CMCs of surfactants measured with the tensiometer are also tabulated as Table 1. These measured CMC values are roughly 20% less than those given from the supplier. This is not uncommon, since surfactants used are of commercial grade, but cost-effective. Moreover, solubilities of phenanthrene in these surfactant solutions with concentrations at CMCs remained almost constant at about 0.95–1.15 mg/L, which is closed to its aqueous solubility near 1.2 and at 0.78 mg/L in the saline water medium used in this work. Moreover, the solubilization power of Tergitol 15-S-7 is the highest among these three surfactants, followed by Tergitol 15-S-9 and Tergitol 15-S-12, of which trend is coincidently as same as that of their hydrophilie–lipophilie balance (HLB) number. Indeed, solubilization capacity of homologous surfactants could be well predicted with their corresponding HLB number [21].

3.2. Biodegradation of surfactants

Biodegradation data of three surfactants at concentrations above their respective CMCs were shown in Fig. 2. Three concentrations of surfactants, ranging from *ca.* 3 to 20 CMCs, were selected for each surfactant in this study. In these biodegradation experiments, surfactants were the sole carbon sources available to bacterial culture.

It was observed that the experimental data of surfactant biodegradation were fitted well to a first-order kinetics model [24].

$$\frac{dS}{dt} = -k \cdot (S - S^*) \quad (1)$$

Integrating Eq. (1), it arrives at

$$S = S^* \cdot (S_0 - S^*) \cdot \exp(-kt) \quad (2)$$

where S_0 is the initial surfactant concentration in TOC (mg carbon/L); S is the surfactant concentration in TOC (mg carbon/L) at time t (h), and S^* is the asymptotic concentration of surfactant in the end of biodegradation experiments in TOC (mg carbon/L), and k is the first-order rate constant (h^{-1}).

Eq. (1) is based on the assumption that the biodegradation is not linked closely to bacterial growth [24]. This assumption is reasonable in the case of ultimate biodegradation of long-chain molecules, such as surfactants. Before being mineralized, large surfactant molecules need to be broken down into smaller fragments. This process is generally called "primary biodegradation" [25,26]. Primary biodegradation mainly contribute to the increase in biomass, whereas ultimately biodegradation essentially serves to maintain the bacterial growth. First-order kinetics was also observed in biodegradation of anionic and nonionic surfactants including Tergitol 15-S-12 by bacteria from an activated sludge sample [18].

The parameters S^* and k for each surfactant were obtained by fitting Eq. (2) to the experimental data of Fig. 2, and were tabulated in Table 2. Furthermore, biodegradability of surfactant could be defined as,

$$B_d = \frac{1 - S^*}{S_0} \quad (3)$$

Biodegradabilities of Tergitol 15-S-7 and Tergitol 15-S-9 were found to be close to each other (Table 2). Their biodegradability decreased when their initial concentrations are increased to *ca.* 530 and 630 mg/L, respectively. This indicates that biodegradation of surfactants is slightly retarded at higher surfactant concentrations. The mechanism of inhibition might arise from the change of micellar structure at higher surfactant concentrations. At higher surfactant concentrations, more structured and packed micelles may form, which can hamper the close contact between the micellized surfactants and bacteria. The decreased biodegradability at higher surfactant concentrations was also reported by Zhang et al. on aerobic degradation of anionic and nonionic surfactants by cultures isolated from sewage sludge [18]. The reduced biodegradability was also reflected in the bacterial growth (Fig. 3).

Fig. 3(a) shows the curves of the bacterial growth with Tergitol 15-S-7 as the carbon source. Bacterial growth was enhanced when the initial surfactant concentration was increased from 210 to 387 mg/L, i.e. equivalently from 128 to 235 mg carbon/L. However, reduced bacterial growth was observed at initial surfactant concentration of 530 mg/L (322 mg carbon/L). Similar results were obtained for Tergitol 15-S-9 and the results are given in Fig. 3(b).

The bacterial growth on Tergitol 15-S-12 is given in Fig. 3(c), from which it can be assumed that the microbial growth is not inhibited within the concentration range used in this work, i.e. from 196 to 423 mg/L or from 120 to 257 mg carbon/L. The non-inhibitory effects of Tergitol 15-S-12 could be due to the smaller concentration range used for this specific surfactant and its high CMC values (75 mg/L).

Specific growth rate μ (h^{-1}) of bacteria that characterizes the bacterial division speed during the exponential growth phase could be obtained from the biodegradation results of surfactants using the following equation,

$$\frac{dX}{dt} = \mu \cdot X - b \cdot X \quad (4)$$

where X denotes the biomass concentration in TOC (mg carbon/L); b is the first-order endogenous respiration coefficient of the bacteria (h^{-1}). Further integrating Eq. (4), the following equation is achieved:

$$\ln\left(\frac{X}{X_0}\right) = (\mu - b) \cdot t \quad (5)$$

where X_0 represents the initial biomass concentration in TOC (mg carbon/L). Consequently, the specific growth rate μ can be easily obtained from the slope of the $\ln(X/X_0)$ versus t, provided that the endogenous respiration coefficient of bacteria b was known. Similarly, if no carbon source was assuredly provided [27], the first-order endogenous respiration coefficient b was then obtained by fitting experimental data to the Eq. (6). That is, the coefficient b was determined separately from an experiment conducted with biomass undergoing endogenous respiration [27].

$$\ln\left(\frac{X}{X_0}\right) = -b \cdot t \quad (6)$$

The endogenous respiration coefficient of the bacteria in this work was found at 0.0026 h^{-1} with a determination coefficient R^2 = 0.981. Subsequently, the specific growth rates of the bacteria during the exponential growth stage on these surfactants are tabulated in Table 2. Within the concentration ranges of surfactants used in the study, the specific growth rate was estimated from 0.021 to 0.029 h^{-1} in different surfactant solutions.

It is noteworthy to point out that the inhibition observed in this study might not be induced by the toxicity of surfactants because it has been demonstrated that the bacteria can survive in surfactant solutions with surfactant concentrations up to 1 wt%. Results in Table 2 also indicate that Tergitol 15-S-12 has a lower biodegradability than the other two surfactants at initial surfactant concentrations of *ca.* 200 and 360 mg/L. Since these three surfactants have the same hydrophobic chain in their molecules, the difference might be attributable to the longer hydrophilic moiety of the Tergitol 15-S-12 molecule, which makes the micelle surface more hydrophilic and deters the contact of hydrophobic cell surface to the micelles. For secondary linear alcohol ethoxylates, a significant resistance to degradation was demonstrated on increasing the ethoxylate chain length [25]. Additionally, the hydrophilic part of a surfactant molecule is the dominant proportion of degradative scission [17,26]. The effects of molecular structure on the biodegradability of surfactants have been reported elsewhere in literature [28].

3.3. Effect of surfactant on the biodegradation of phenanthrene

Fig. 4(a–c) shows the biodegradation of phenanthrene in micellar solutions of these three surfactants. All the initial phenanthrene concentrations are well above its solubility (0.78 mg/L) in the saline water medium. The results showed that the presence of the surfactants can indeed enhance the bioavailability of phenanthrene due to the increase of apparent phenanthrene solubility by solubilization. For example, in Fig. 4(a), nearly 90% of phenanthrene, i.e. 2.0 mg/L, was degraded from a phenanthrene-spiked solution with an initial concentration at 2.13 mg/L in presence of Tergitol 15-S-7 at 200 mg/L originally. As mentioned previously, the results also indicate that increasing surfactant concentration, the biodegradability of phenanthrene is reduced, which could be mainly due to the reduced bioavailability of phenanthrene in the micellar phase at a higher surfactant concentration. The mechanisms will be discussed later. Concisely, though biodegradability of phenanthrene in the medium was decreased with an increase in the surfactant concentrations, the biodegradation amount of phenanthrene is indeed improved by this surfactant, because of the enhanced aqueous solubility and, hence, the bioavailability of phenanthrene by this surfactant.

It is shown in Fig. 4(a) that the phenanthrene concentration remained in the spiked solutions with 200 mg/L of Tergitol 15-S-7 initially drops significantly, when biodegradation process time lapses over 74–88 h, at which it is often referred as structure-breaking point of surfactant. At this point, the effective concentration of surfactant that contributes to formation of micelles and solubility enhancement of solutes is reduced considerably owing to its primary degradation, which breaks down surfactant molecules into smaller moieties. The solubilization capacity of surfactant on phenanthrene in this surfactant solution after structure-breaking point was tested again. The apparent phenanthrene solubility in this surfactant solution is still 0.78 mg/L, which is larger than the corresponding phenanthrene concentration remained (0.31 mg/L). Furthermore, measurement on surfactant concentration indicated that the losses in surfactant TOC at the end of the experiments were less than 15% all surfactant

Table 2
Parameters of first-order biodegradation kinetics of surfactants

Surfactant	Initial surfactant concentration, S_0 (mg carbon/L)	Initial surfactant concentration, S_0 (mg/L)	Rate constant, k (h^{-1})	Concentration, S^* (mg carbon/L)	Biodegradability, B_d	Specific growth rate of bacteria, μ (h^{-1})
Tergitol 15-S-7	127.6	210	0.0104	87.6	0.313	0.021
	235.4	387	0.0040	149.4	0.365	0.022
	321.8	530	0.0096	279.2	0.132	0.021
Tergitol 15-S-9	114.1	193	0.0103	79.3	0.310	0.025
	208.3	353	0.0037	138.5	0.336	0.029
	371.2	631	0.0144	341.5	0.079	0.023
Tergitol 15-S-12	120.0	196	0.0162	93.1	0.222	0.027
	213.7	352	0.0059	158.3	0.261	0.029
	257.3	423	0.0050	187.9	0.270	0.029

Initial biomass = 0.97 mg carbon/L.

solutions used. This implies that the drop of phenanthrene concentration is not due to the insufficient solubilization capacity of the surfactant caused by its biodegradation.

The significant degradation of phenanthrene after a certain period may arise from the increase of phenanthrene bioavailability with the simultaneous surfactant degradation. The breaking down of surfactant molecules by the bacteria reduces the effective surfactant concentration and, as a result, more phenanthrene was redistributed into the aqueous phase from the micellar phase. This observation also indicates that phenanthrene in the micellar phase

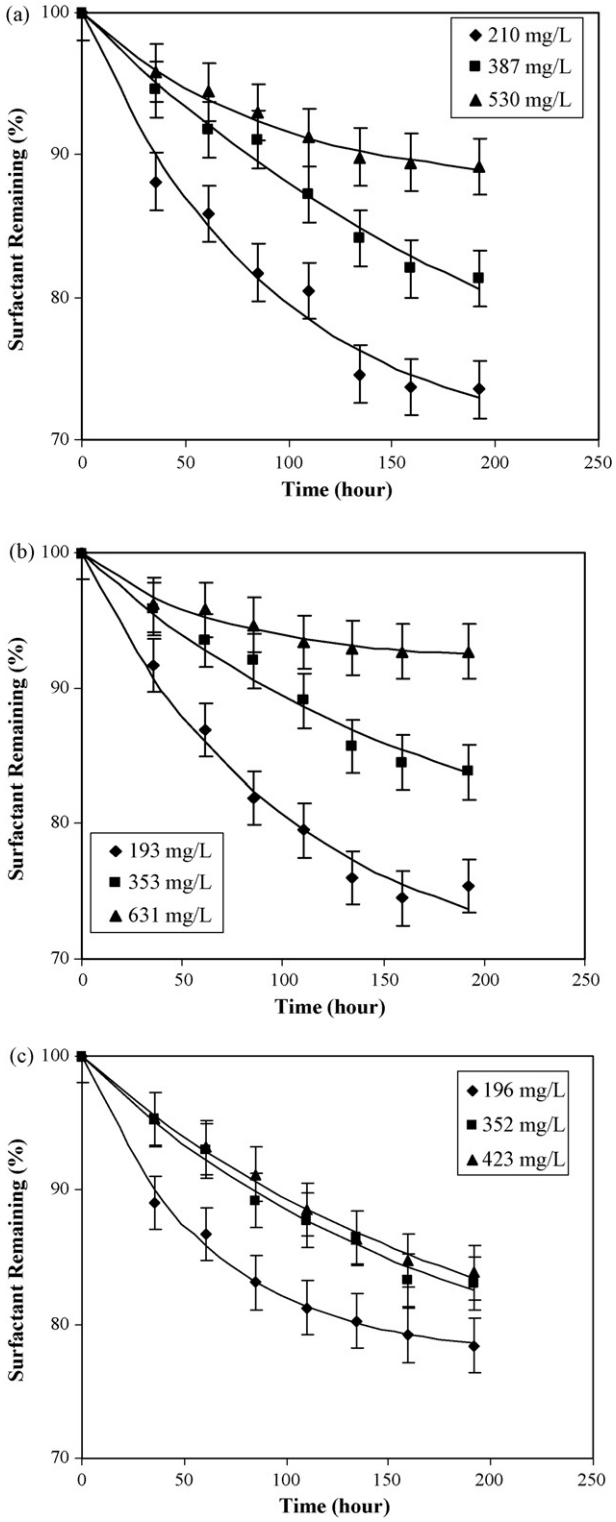

Fig. 2. Effect of surfactant concentrations on their biodegradation kinetics (initial biomass = 0.97 mg carbon/L). (a) Tergitol 15-S-7; (b) Tergitol 15-S-9; (c) Tergitol 15-S-12.

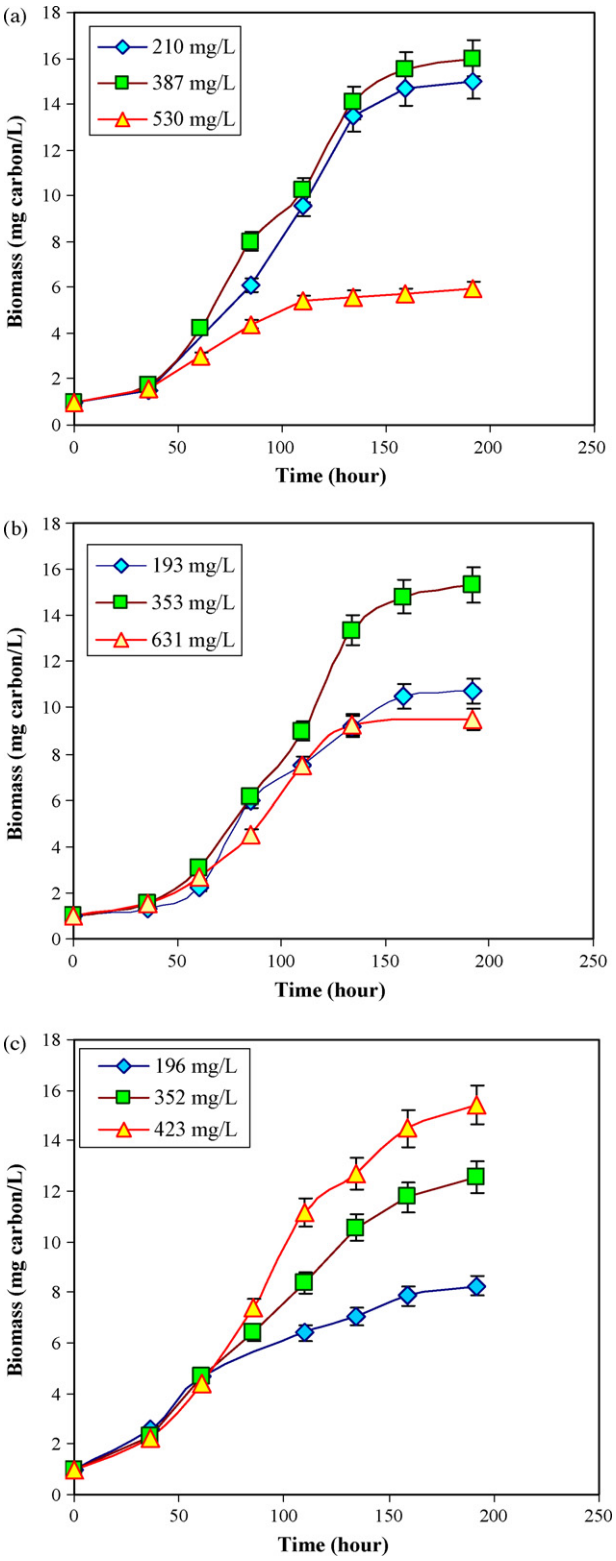

Fig. 3. Effect of surfactant concentration initially present in inoculum on bacterial growth (initial biomass = 0.97 mg carbon/L). (a) Tergitol 15-S-7; (b) Tergitol 15-S-9; (c) Tergitol 15-S-12.

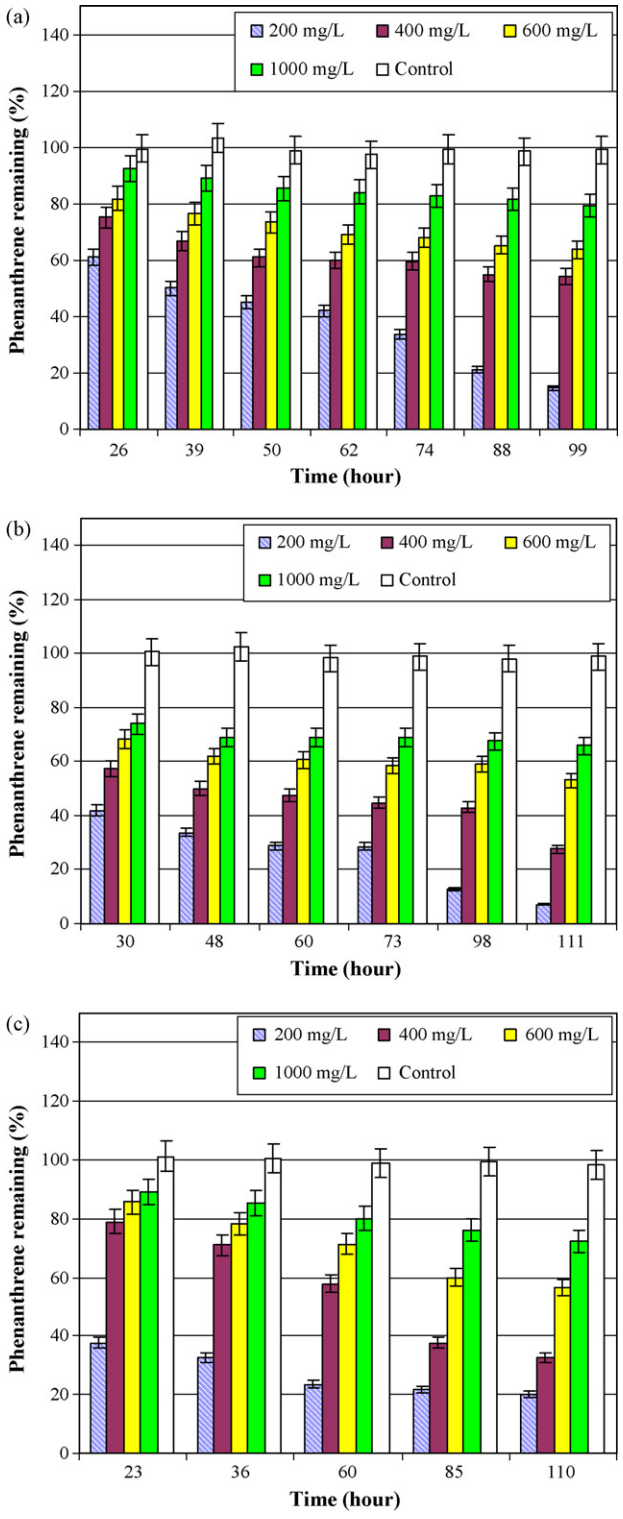

Fig. 4. Effect of surfactant concentration on biodegradation of phenanthrene. (a) Tergitol 15-S-7 (initial phenanthrene concentration = 2.13 mg/L; initial biomass = 0.22 mg carbon/L). Data obtained at 200, 400 and 600 mg/L have been reported earlier in the reference (Li and Bai [20]); (b) Tergitol 15-S-9 (initial phenanthrene concentration = 2.64 mg/L; initial biomass = 0.27 mg carbon/L); (c) Tergitol 15-S-12 (initial phenanthrene concentration = 1.64 mg/L; initial biomass = 0.97 mg carbon/L).

is not readily bioavailable, compared with pristine phenanthrene in the aqueous phase.

In Fig. 4(b), this phenomenon was observed again. The concentration of phenanthrene remained in the solution plunges considerably when the process time lapses over 73 and 98 h, respectively, for Tergitol 15-S-9 initially at 200 and 400 mg/L. Conversely, such a phenomenon is not evident in Fig. 4(c), which is possibly due to the low biodegradability of Tergitol 15-S-12 and the smaller initial phenanthrene concentration.

In a brief summary, gradual loss in solubilization power of a surfactant might improve the bioavailability of the substrate of interest. That is, micelles firstly work as depot of carbon source by enhanced solubilization of the hydrophobic phenanthrene, and gradually release out nutrients to bacteria because of gradual degradation of surfactants. However, one would expect that surfactants will not play any vital roles in assisting bioremediation processes, if surfactants are not biodegradable at all or biodegraded too fast. The former scenario will reduce the bioavailability of phenanthrene by not releasing any nutrients to bacteria and may also exterminate these bacteria as surfactants could be easily sorbed and dissolved into outer lipid cell membrane of these bacteria and, hence, alter the osmotic pressures of bacteria. The latter scenario could lead to significant loss of solubilization power, which renders the surfactant ineffective in solubilizing phenanthrene. In field applications, suitable surfactant concentrations need to be determined priorly.

3.4. Possible mechanisms of surfactant on the biodegradation of phenanthrene

The biodegradation of phenanthrene includes the degradation of free phenanthrene molecules in the aqueous phase and degradation of phenanthrene contained in the micellar phase. The biodegradation of free phenanthrene molecules is controlled by the diffusion of the molecules to the cell surface or enzyme sites. The phenanthrene in the micellar phase is degraded firstly either by diffusing into the aqueous phase and subsequently utilized by the bacteria, or by directing microbial uptake from the micelles. The first process is controlled by the kinetics of micellar aggregation. The relaxation time of the micelle commonly known as τ_2, is typically on the order of milliseconds to microseconds. Therefore, the first process is normally not a rate-limiting step. The second process is described in Fig. 5.

As shown in Fig. 5, the mass transfer from micelle into cell is composed of three steps. The first step is the transport of the micelle solubilized with a substrate to the vicinity of the cells or enzymes by mixing. The second step is the exchange of the filled micelles with the hemi-micellar layer of surfactants molecules forming

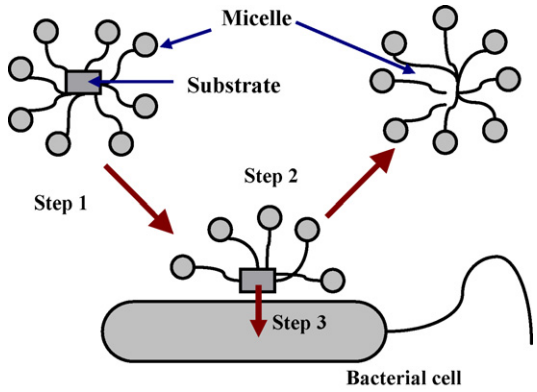

Fig. 5. Schematic on bioavailability of a substrate in the micellar phase.

around the cells. The formation of hemi-micelle layer around the cell or other substrates has been proposed and used successfully to describe the biodegradation [29]. And the third step is the transfer of the substrate from the hemi-micelle to the cell. In a well-stirred system, the first step is also not a rate-limiting step. The second and the third step normally govern the biodegradation process of a substrate in the micellar phase, which is commonly affected by the specific interactions between adsorbed micelles and cell surfaces. It has been reported that the specific interaction between the micelles and cell surfaces, such as the affinity of the two surfaces, is a limiting factor in controlling the transport of the substrate from the micelle to the cell [29].

In view of the experimental results presented above, three mechanisms are, accordingly, conjectured on the effect of surfactants on phenanthrene biodegradation:

Mechanism 1: Lower bioavailability of hydrophobic substrate such as phenanthrene at higher surfactant concentrations.

For a certain amount of phenanthrene, increasing surfactant concentrations will lead more phenanthrene to partition into the surfactant micelles because of much larger hydrophobic affinity of micelles [21]. Compared to free phenanthrene molecules in the aqueous phase, phenanthrene entrapped in the micellar phase has a lower bioavailability, because phenanthrene in micellar phase cannot be directly accessed by bacteria. In addition, higher surfactant concentrations will render the formation of more compact micelles that will deter the mass transfer rate of phenanthrene molecules from micelles to bacterial cells across the tighter palisade layers of micelles. It will result in a reduced mass-transfer of phenanthrene from the micellar phase to the aqueous phase and to the cells [11]. Therefore, when phenanthrene is being consumed, a smaller amount of phenanthrene will diffuse into the aqueous phase at a higher surfactant concentration.

Consequently, lower surfactant concentrations are more appropriate in view of achieving higher bioavailability. On the contrary, surfactant concentrations cannot bee too low to effectively raise bioavailability by enhanced solubilization of phenanthrene. To improve the bioavailability, selection of suitable bacteria is very important, because the specific interaction between bacteria and surfactant may influence significantly the bacterial uptake of solubilized substrates [30].

Mechanism 2: Competition between surfactants and phenanthrene, substrates to be catabolized, as nutrients to bacteria.

Surfactants may compete with substrates to be catabolized as nutrients to microorganisms. Since surfactants used in bioremediation work prefer to be biodegradable. Higher concentrations of surfactants may deprive catabolism of substrates from bacteria, which, consequently, will rely much less on substrates for nutrients. Therefore, biodegradation efficiency and extents by these microorganisms will be retarded significantly.

Mechanism 3: Reduced microbial activity at higher surfactant concentrations may also contribute to the lower biodegradability of substrate, phenanthrene.

More importantly, surfactants at higher concentrations may kill bacterial cells. Indeed, surfactants have been reported to have bactericidal and fungicidal properties. For example, nonionic surfactants such as polysorbates are often formulated into injectable medicine to preserve the medicine from proliferation of microorganisms. Because of the amphiphilic nature, surfactant molecules can be solubilized into the lipid membranes of bacterial cells, not only to cause cell lysis, but also to alter physicochemical properties of cell membrane, for instance, imbalanced osmotic pressure across the membrane, charge reversal on cell surface and the potential of the cell surface, causing permanent damage to enzymes and the membrane materials of cell, etc.

Finally, a successful biodegradation and bioremediation work, it really warrants our attention to the optimal process design. Selection of surfactant types are as important as choice of suitable bacterial cultures. Surfactants could really enhanced the apparent solubility of hydrophobic substrates and, hence, the bioavailability and to shorten the process time in biodegradation of these substrates. However, in the other hand, surfactants in excess will jeopardize the biodegradation process by deactivate these bacteria. Consequently, operation conditions such as applicable surfactant concentration range have to be determined priorly to ensure the success of such bioremediation and biodegradation processes.

4. Conclusions

The outcomes of this study showed that nonionic surfactants, Tergitol 15-S-*X* (*X* = 7, 9 and 12), are biodegradable by the marine bacteria of *N. naphthovorans*. The first-order biodegradation kinetics model could describe well the biodegradation behavior of surfactants as well as the biodegradation of phenanthrene in presence of these surfactants. The biodegradation behaviors of surfactants could be described well with a first-order kinetics model. The biodegradability of these surfactants, with initial concentration less than 250 mg carbon/L, was found near 0.3. However, reduced biodegradability of these surfactants was observed at a higher initial concentration. In general, biodegradability of these surfactants decreased with an increase the hydrophilic chain length of the surfactants, i.e. Tergitol 15-S-7 > Tergitol 15-S-9 > Tergitol 15-S-12. Furthermore, the endogenous respiration coefficient of bacteria was found at 0.0026 h^{-1}, whereas the specific growth rates of bacteria on these surfactants ranges from 0.021 to 0.029 h^{-1}.

The biodegradability of phenanthrene is greatly improved in presence of surfactants at proper concentrations, which could be attributable to the increased apparent solubility of phenanthrene resulted from the enhanced solubilization of phenanthrene by these surfactants. However, with the same initial phenanthrene concentration, its biodegradability was reduced with an increasing surfactant concentration, owing to the lower bioavailability of the phenanthrene in the micellar phase compared with that in the aqueous phase. For example, more than 80% of the phenanthrene was degraded when surfactant concentrations initially present were 200 mg/L. In contrast, less than 30% of phenanthrene could be degraded, if initial surfactant concentrations were increased to 1000 mg/L. Concisely, although biodegradability of phenanthrene was decreased with an increase in surfactant concentrations, the biodegradation amount of phenanthrene is indeed improved by these surfactant, because of the enhanced aqueous solubility and, hence, the bioavailability of phenanthrene by these surfactants. Lastly, an optimal concentration range of surfactants really exist for one to achieve the maximum biodegradation of phenanthrene by the marine bacteria of *N. naphthovorans*.

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