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Biotreatment of oily wastewater by rhamnolipids in aerated active sludge system*

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Abstract: Oily wastewater generated by various industries creates a major ecological problem throughout the world. The traditional methods for the oily wastewater treatment are inefficient and costly. Surfactants can promote the biodegradation of petroleum hydrocarbons by dispersing oil into aqueous environment. In the present study, we applied rhamnolipid-containing cell-free culture broth to enhance the biodegradation of crude oil and lubricating oil in a conventional aerobically-activated sludge system. At 20 °C, rhamnolipids (11.2 mg/L) increased the removal efficiency of crude oil from 17.7% (in the absence of rhamnolipids) to 63%. At 25 °C, the removal efficiency of crude oil was over 80% with the presence of rhamnolipids compared with 22.3% in the absence of rhamnolipids. Similarly, rhamnolipid treatment (22.5 mg/L) for 24 h at 20 °C significantly increased the removal rate of lubricating oil to 92% compared with 24% in the absence of rhamnolipids. The enhanced removal of hydrocarbons was mainly attributed to the improved solubility and the reduced interfacial tension by rhamnolipids. We conclude that a direct application of the crude rhamnolipid solution from cell culture is effective and economic in removing oily contaminants from wastewater.

Key words: Oily wastewater, Rhamnolipid, Aerated active sludge system, Biodegradation
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INTRODUCTION

Since petroleum products are commonly used as energy sources and raw materials in a wide variety of industries, hydrocarbons-containing oily wastewater generated by various industries poses a threat to the environment in the world. Crude oil excreted from the oil and gas industries and lubricating oil commonly released from metal processing industry as rinsing baths are the main contaminants in oily wastewater (Allard and Neilson, 1997; Qing *et al*., 2005; Tellez *et al*., 2002).

Although dissolved air flotation, flocculation, oxidation and membrane filtration have been developed for treatment of the hydrocarbons-containing oily wastewater, these physico-chemical processes are expensive and complex (Al-Shamrani *et al*., 2002a; 2002b; Chang *et al*., 2001; Zouboulis and Avranas, 2000). Moreover, the physico-chemical treatment does not really degrade, but only displaces, the hydrocarbons in concentrated wastewater.

Biological treatment by the aerobic active sludge system can degrade the petroleum hydrocarbons effectively and has thus been regarded as an efficient method to deal with the hydrocarbons-containing oily wastewater. Nevertheless, the low solubility and bioavailability of the petroleum hydrocarbons limit the biodegradation of oily wastewater (Tellez *et al*., 2002; Abalos *et al*., 2004; Lee *et al*., 2005; Yang *et al*., 2000; Soda *et al*., 1998). The addition of biosurfactants into aerobically-activated sludge has been highly regarded to enhance the solubility and emulsification of hydrophobic substrates and thus

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proposed as a promising strategy for biotreatment of petroleum hydrocarbons (Uysal and Turkman, 2005). Compared with the chemically synthetic surfactants that could become a secondary waste due to their low biodegradability in the activated sludge system (Rosso *et al*., 2006; Carvalho *et al*., 2004; Tang *et al*., 2003), the environment-friendly biosurfactants as a sort of naturally produced products are more desirable for application in biotreatment (Bai *et al*., 1997; Mohan *et al*., 2006). However, the application of biosurfactants in industries has been limited by their high cost compared with the chemically produced surfactants. In addition, the application of biosurfactants is also limited by low product concentration and complicated isolation. Rhamnolipid is one of such products that are effective but costly (Costa *et al*., 2006).

In our previous studies, the waste frying oil was used as the sole carbon source to cultivate *Pseudomonas* sp. zju.um1 for producing rhamnolipids (Zhu *et al*., 2007) and the addition of its cell-free fermentation broth significantly improved the biotreatment of waste frying oil-containing wastewater (Zhang *et al*., 2009). The use of the rhamnolipids directly from cell-free culture broth in biotreatment of hydrocarbons-containing oily wastewater using aerobically-activated sludge would save the isolation cost. However, its effectiveness on treating more recalcitrant crude oil and petroleum hydrocarbons is still unknown. Therefore, the goal for this study was to address the feasibility of directly using the rhamnolipid-containing cell-free culture broth for enhancing the biodegradation of crude oil and lubricating oil in a conventional aerobically-activated sludge system.

MATERIALS AND METHODS

Production of rhamnolipids

Rhamnolipids were fermented by *Pseudomonas* sp. zju.um1 in a 50-L reactor (Zhu *et al*., 2007). The cell-free culture broth contained 35 g/L of rhamnolipids and the critical micelle concentration (CMC) was detected to be 28.8 mg rhamnolipids/L with a minimal surface tension of 30.1 mN/m by the ring method (Abouseoud *et al*., 2008). Two components of rhamnolipids were identified as α-rhamnopyransyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-C_{10}-C_{10}) and 2-*O*-α-rhamnpyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-Rha-C_{10}-C_{10}) (Zhu *et al*., 2007).

Activated sludge and synthetic oily wastewater

The activated sludge was kindly provided by Hangzhou Sipu Sewage Plant, Zhejiang, China. In each treatment, the mixed liquor suspended solid (MLSS) of the active sludge was set at around 2000 mg/L with the sludge volume index (SVI) of around 25 g/L.

The crude oil and lubricating oil were individually added into the sludge system. As the crude oil (Shengli Oilfield, Shandong, China) is sticky at below 30 °C, 2 g crude oil was weighted and put into the activated sludge system. The commercial liquefied lubricating oil (500 mg/L) was directly added into the activated sludge system. $NaNO_3$ (250 mg/L) and KH_2PO_4 (40 mg/L) were added to provide sources of nitrogen, phosphorus, and potassium for microorganisms in the activated sludge.

Bench-scale activated sludge system

The bench-scale activated sludge treatment was conducted in glass tanks (Zhang *et al*., 2009). Each glass tank was filled with 4 L of synthetic oily wastewater and aerated by two air distributors at a flow rate of 0.3 L air/min. The two distributors sat oppositely at the bottom of the tank. The volume of evaporated water was examined every 12 h and the additional tap water was supplemented.

Analysis of residual hydrocarbon

As crude oil was low dispersible at below 30 °C, two types of crude oil presented in the activated sludge before or after biotreatment, some being attached to the glass wall and the other being dispersed into the water. Two types of samples were simultaneously taken. Around 1000 ml oily wastewater mixed with the dispersed crude oil was put into a wide-opening glass bottle with a Teflon cap, acidified to pH <2 with 4 ml 1:1 (v/v) H_2SO_4 and stored at 4 °C. For the attached crude oil, a cotton ball with pre-immersed chloroform was used to transfer it into chloroform solution. Upon analysis, two types of samples were extracted by 10 ml of CCl_4 twice, brought to a final volume of 25 ml by adding CCl_4,

and subsequently analyzed by infrared spectrum (IR) (Nicolet, IR 560) at 2930 cm^{-1} to detect the residual crude oil either dispersed in water or attached to glass wall (Tellez *et al*., 2002). For lubricating oil, the samples were taken and analyzed following the same procedure as that for the dispersed crude oil. The residual oil concentration was determined by dividing the total amount in extracts by the volume of the synthetic wastewater, while the removal efficiency was expressed as a percentage relative to the initial oil concentration before biotreatment.

In addition, the compositions of crude oil before and after biotreatment were analyzed using gas chromatography (GC). The total crude oil extract before or after biotreatment was obtained by combining two extracts, one from the aqueous solution and the other from the attached form in the activated sludge system. 2 μl of the total crude oil extracts were injected into GC1690 (Kexiao, China) equipped with a flame ionization detector (FID) and a capillary column (SE-30, 0.25 mm). The operating conditions of the GC1690 included an initial temperature of 60 °C, an increasing rate of 5 °C/min, and final temperatures of 300 °C in column and 330 °C in FID. Similarly, the compositions of lubricating oil extract were determined as those of crude oil by GC.

Measurement of surface and interfacial tension

Surface tension and interfacial tension were determined with a JYW-200A tensiometer (ChengDe, China) using the ring method according to American Society for Testing and Materials (ASTM D971-99a, 2004).

Measurement of solubility of lubricating oil

Aqueous solutions of the rhamnolipids (0~22.5 mg/L) were prepared by adding the cell-free culture broth into water with pH adjusted to 7.0 using NaOH. 1 ml of lubricating oil was added into 100 ml of rhamnolipid solution at various concentrations, resulting in biphasic systems. The mixtures were agitated at 180 r/min on an orbital shaker for 12 h under either 20 °C or 25 °C. A 50-ml sample on the bottom aqueous layer after being settled for 12 h was taken and subsequently extracted with 5 ml of CCl_4 (Clifford *et al*., 2007). The bottom extracts were analyzed by IR at 2930 cm^{-1} (Noordman *et al*., 2002).

Data analysis

All values for bench-scale studies are mean± standard error of mean (SEM) of 3 independent experiments.

RESULTS

Biodegradation of crude oil

The biotreatment was individually investigated under four temperatures ranging from 20 °C to 37 °C when rhamnolipid concentrations varied from 0 to 22.5 mg/L. The rhamnolipid concentration over 25 mg/L was not recommended because of the severe foaming. After aerobic treatment for 48 h, the samples in either aqueous solution or attached form were individually collected for analysis of the final residual petroleum concentration.

The effect of rhamnolipid on biodegradation of crude oil is shown in Fig.1a. The addition of rhamnolipids reduced the residual concentration of crude oil and thus enhanced the corresponding removal efficiency at 20 and 25 °C. At 20 °C, the removal efficiency of crude oil was 70% at rhamnolipid concentration of 22.5 mg/L and was only 17.7% in the absence of rhamnolipids. At 25 °C, the removal efficiency of crude oil was over 80% with the presence of rhamnolipids in comparison with 22.3% in the control group. This biodegradation was confirmed by GC analysis shown in Fig.2. At a high temperature over 30 °C, the removal efficiency was over 90% in both presence and absence of rhamnolipids.

The distribution of residual crude oil varied among different treatments as shown in Figs.1b and 1c. At 20 °C, the residual crude oil mostly adhered to the glass wall in the absence of rhamnolipids, and the addition of rhamnolipids at 11.2 mg/L greatly increased the residual petroleum concentration in water from 7.2 to 67.9 mg/L (Fig.1b). At 25 °C without the presence of rhamnolipid, the residual crude oil distributed more in water, indicating the positive effect of enhanced temperature on dispersion of crude oil.

Biodegradation of lubricating oil

According to our preliminary findings that lubricating oil was more biodegradable than crude oil, a short treatment for 24 h was selected in evaluation of

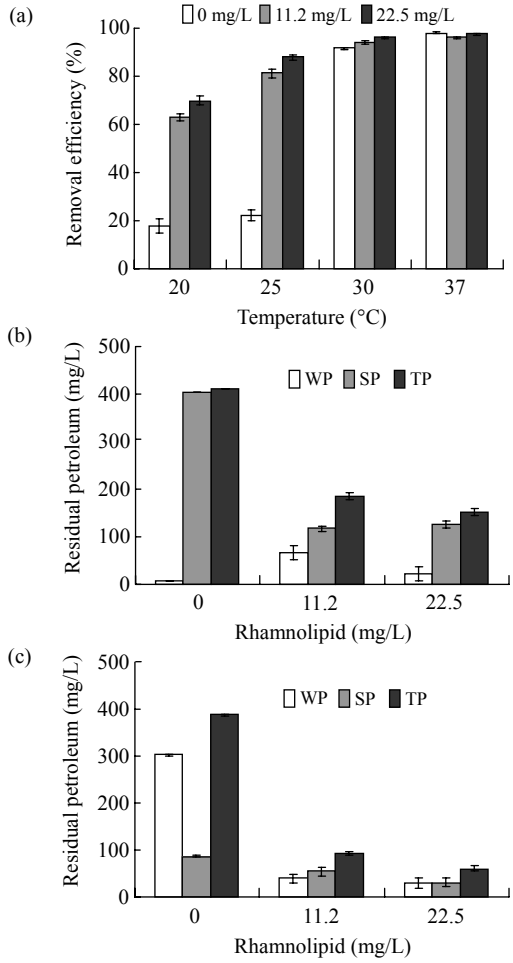

Fig.1 Effect of rhamnolipids on biodegradation of crude oil

(a) The removal efficiency of crude oil when treated with or without the presence of rhamnolipids at the temperature range of 20~37 °C; (b) The residual distribution of crude oil at 20 °C; (c) The residual distribution of crude oil at 25 °C. WP indicates the petroleum dispersed in aqueous solution; SP indicates the petroleum adhered to the glass wall; TP indicates the total residual petroleum comprising WP and SP

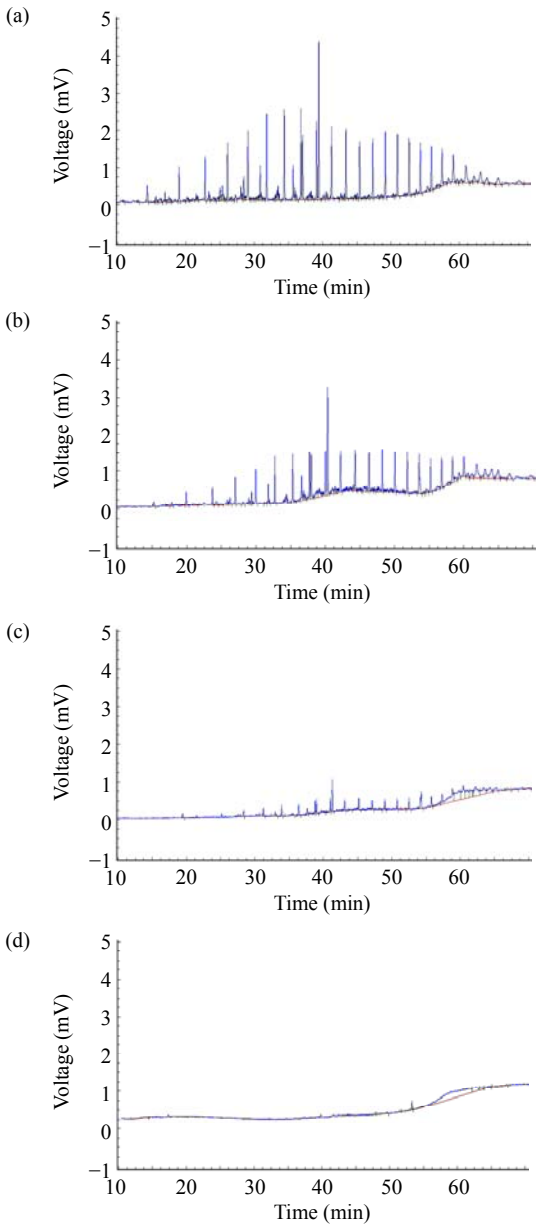

Fig.2 GC-FID chromatogram of crude oil from activated sludge system at 25 °C

(a) Before biotreatment; (b) After biotreatment in the absence of rhamnolipids; (c) After treatment in the presence of rhamnolipids at 11.2 mg/L; (d) After treatment in the presence of rhamnolipids at 22.5 mg/L

its biodegradation. As indicated in Fig.3a, rhamnolipids greatly facilitated the biodegradation of lubricating oil at 20 and 25 °C, but did not show significant improvement at 30 °C. The time course of biodegradation was then detected under the three different temperatures with addition of rhamnolipids (11.2 mg/L). Rhamnolipid treatment (22.5 mg/L) for 24 h at 20 °C significantly increased the removal rate of lubricating oil to 92% compared with 24% in the absence of rhamnolipids, while the effect of rhamnolipid was not so obvious due to a high removal of over 70% at a temperature higher than 25 °C (Fig.3b).

Similarly, there was no significant difference between the presence and absence of rhamnolipids when the temperature was higher than 25 °C. The GC analyses of lubricating oil before and after treatment at 25 °C are illustrated in Fig.4, showing the obvious effect of rhamnolipids in enhancing the removal efficiency. Like crude oil, the lubricating oil components

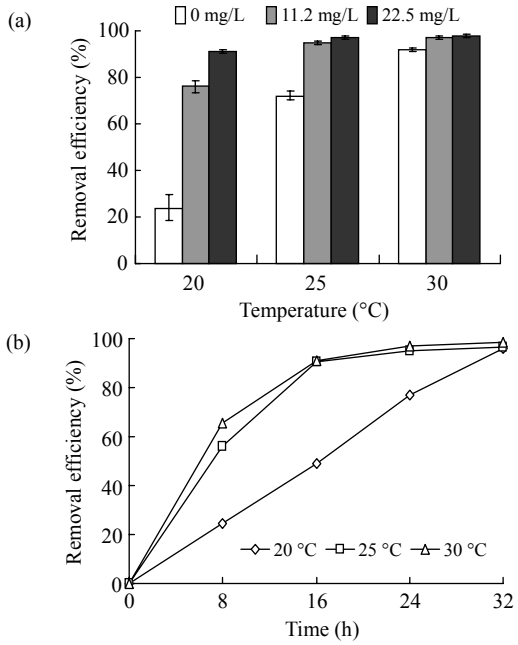

Fig.3 Effect of rhamnolipids on biodegradation of lubricating oil

(a) The removal efficiency of lubricating oil at temperature range of 20~30 °C; (b) The time course of lubricating oil in activated sludge systems with the presence of rhamnolipids at 11.2 mg/L at varying temperature

were greatly reduced after rhamnolipid treatment with no significant peak detected at 22.5 mg/L (Fig.4d), which is in a good correspondence with IR analysis in Fig.3a. It is thus concluded that the presence of the rhamnolipids facilitated the biodegradation of lubricating oil.

Mechanisms on enhanced biodegradation of hydrocarbons in the presence of rhamnolipids

Both the solubility and the interfacial tension of the lubricating oil were examined at 20 °C and 25 °C and the results are shown in Fig.5. We excluded the treatment at 30 °C in this experiment due to the ineffectiveness of rhamnolipid on enhancing biodegradation at this high temperature.

As shown in Fig.5a, the solubility of the lubricating oil was proportionally enhanced with rhamnolipid concentration at a range of 0~22.5 mg/L. By comparison of the treatment at 25 °C with that at 20 °C, it was evident that the increase of temperature could significantly enhance the solubility of lubricating oil. Compared to the linear increase of solubility with rhamnolipid concentration, more drastic decreases on interfacial tension between lubricating oil and water were detected (Fig.5b). For an example, at 25 °C, the interfacial tension decreased from 32.2 mN/m to 8.3 mN/m with rhamnolipid concentration increasing from 0 to 22.5 mg/L. Nevertheless, the enhanced temperature had no effect on the interfacial tension.

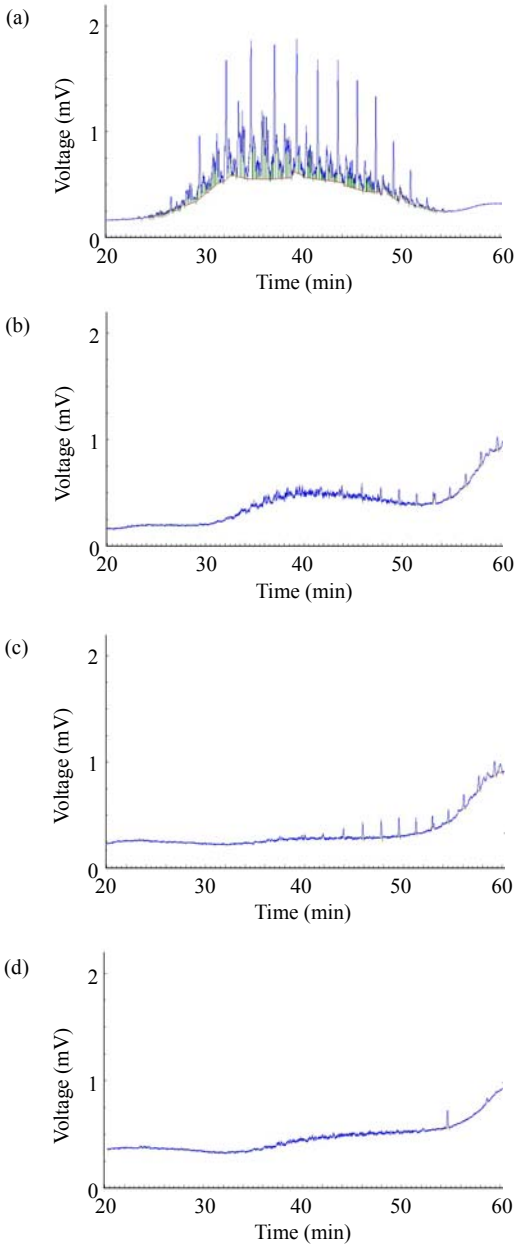

Fig.4 GC-FID chromatogram of lubricating oil from activated sludge system at 25 °C

(a) Before biotreatment; (b) After biotreatment in the absence of rhamnolipids; (c) After treatment in the presence of rhamnolipids at 11.2 mg/L; (d) After treatment in the presence of rhamnolipids at 22.5 mg/L

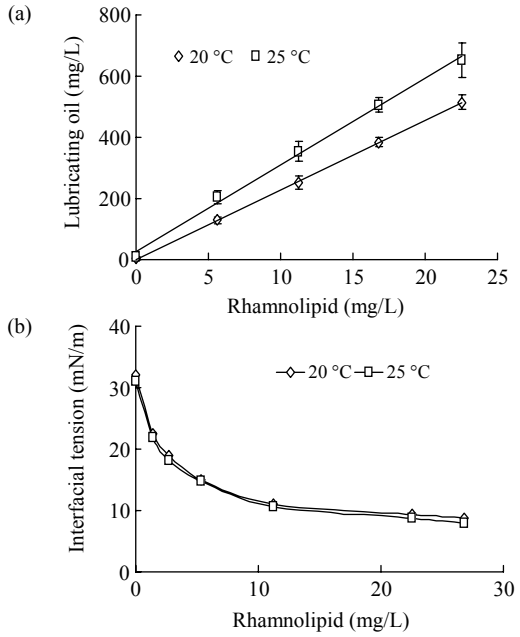

Fig.5 Effect of rhamnolipids on physico-chemical properties of lubricating oil at temperature of 20 and 25 °C

(a) Enhanced solubility of the lubricating oil in aqueous solution; (b) Interfacial tension between lubricating oil and water

DISCUSSION

Efficient treatment of oily wastewater at a low cost has always been a major concern in the oil and gas industries, and effective and economic environment-friendly biosurfactants are desired. Therefore, this paper addressed the feasibility of applying less costive rhamnolipid solution from cell culture for enhancing the removal efficiency of petroleum hydrocarbons in an activated sludge system. Crude oil and lubricating oil were selected as two types of petroleum hydrocarbons.

Corresponding with the previous result that rhamnolipids facilitated biodegradation of 2,4-dichlorophenol in an activated sludge system (Uysal and Turkman, 2005), the addition of rhamnolipids significantly increased the biodegradation of crude oil as reflected by both IR and GC analyses (Figs.2 and 3) when the treatment temperature was around the range of 20~25 °C. Over 30 °C, crude oil was largely degraded in the absence of rhamnolipids due likely to the higher metabolic activities of microbials and a good dispersion of crude oil at this high temperature. Although high temperatures over 30 °C can enhance the biodegradation via increasing their bioavailability, the aerobically-activated sludge system is typically operated at the range of 20~25 °C. Enhancement of the treatment temperature is unlikely feasible in consideration of energy cost. As an alternative, the cost-effective rhamnolipids could significantly improve the biodegradation of the major crude oil components in a normally-operated aerobic sludge.

Lubricating oil, the fractional compositions of petroleum oil, is another typical contaminant in oily wastewater in large quantities. Similar to the biodegradation of crude oil, rhamnolipids greatly enhanced its removal rate at a temperature below 25 °C. The high removal efficiency in the absence of rhamnolipids at a high temperature could be due most likely to the increasing dispersion of oil and metabolic activities of microorganisms. Under 20 °C, as previously reported, a long retention time of 60 h was needed for a higher removal of petroleum products at an initial concentration of 126 mg/L in normal sludge treatment (Tellez *et al*., 2002). In comparison, although with a higher initial lubrication oil of 500 mg/L, the addition of rhamnolipid could greatly reduce the required treatment time to only 24 h to reach a high removal efficiency, which is superior to normally-operated activated sludge system in biotreatment of oily wastewater. It has been well known that the positive effect of rhamnolipids on enhancing biodegradation of crude oil is due to its capability in dramatically lowering the interfacial tension between crude oil and water (Patel and Desal, 1997) and increasing the solubility of crude oil into water (Abalos *et al*., 2004). However, the mechanism of rhamnolipids on biodegradation of lubricating oil has never been examined. In the present study, the effect of rhamnolipids on enhancing solubility and interfacial tension of lubricating oil is consistent with the previous reports on crude oil (Patel and Desal, 1997; Abalos *et al*., 2004). Moreover, the corresponding enhancement of solubility of crude oil with temperature (Fig.5) further illustrated the positive effect of temperature on enhancing biodegradability of crude oil. Nevertheless, the enhanced temperature had no effect on the interfacial tension, which well agrees with the previous results that the surfactant of 10-molecule ethoxylate caused no changes of the interfacial tension between water and liquid paraffin by regulating temperature (Mitsui *et al*., 1971).

Overall, the bioavailability of petroleum pollutants was greatly enhanced via increasing solubility and decreasing the surface tension by rhamnolipids. It has been well recognized that the resistance of hydrocarbon to microbial biodegradation was due to its low bioavailability (Miller and Bartha, 1989) that could be enhanced by rhamnolipids via increasing the aqueous dispersion of crude oil and the solubility of lubricating oil (Zhang and Miller, 1992). This could explain the enhanced biodegradation efficiency of crude oil and lubricating oil.

Although increasing temperature can also enhance bioavailability, it is practically difficult to raise temperature for an aerated active sludge system in a sewage treatment plant. In contrast, the rhamnolipids from cell culture showed a promising feasibility in cleaning up the oily contaminants in a sewage treatment plant. A low concentration (11.2 mg/L) of rhamnolipids, with a cost of only about 0.05 USD/m^3, has been proven to be effective, which is also very economic compared with the cost of about 0.1~0.2 USD/m^3 in common wastewater treatment in China.

CONCLUSION

We conclude that the addition of rhamnolipid-containing culture broth can greatly improve the efficiency of biodegradation of petroleum in an aerobically-activated sludge system in the normal operation temperature of 20~25 °C. And the enhanced removal efficiency by rhamnolipid treatment is closely associated with its capacity in increasing the solubility of petroleum hydrocarbons and reducing interfacial tension between two aqueous solutions. The addition of crude rhamnolipid solution in an aerated active sludge system could be both effective and economic for treatment of oily wastewater.

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