Influence of surfactant–Tween80 on degradation of benzo(a)pyrene and its accumulated metabolites by Bacillus-07

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Abstract Two degradation metabolites of benzo(a)pyrene(BaP), cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol, were identified using high performance liquid chromatography (HPLC) with standards. Both BaP and the two metabolites were well-known toxic compounds. The effect of a nonionic surfactant-Tween80 (TW80) on the degradation of the above three compounds by *Bacillus*-07, in aqueous phase, was studied. The result showed that (1) Solubility of the three compounds was enhanced by more than 20 times and their bioavailability was markedly improved；(2) Compared with controls, degradation rate of BaP was enhanced by 25.01%, meanwhile accumulation rates of cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol were lowered 2.11% and 2.57% on the 8d, respectively, after the adding of 1000 ppm TW80; (3) TW80 could be utilized by microorganisms as energy and it exhibited no inhibitory effect on the growth of *Bacillus*-07.

Key words: benzo(a)pyrene · metabolites ·TW80 · surfactant ·

Ⅰ INTRODUCTION

As one of the most potent carcinogenic PAHs, BaP is the most studied compound of the PAHs class[1]. Cis-BP4,5-dihydrodiol, cis-BP7,8-dihydrodiol and cis-BP9,10-dihydrodiol are metabolites of BaP by some bacteria[2], and they have similar chemical structure with BaP-“K”region and “bay”region, which is relate to toxicity, mutagenesis and carcinogenesis[3]. Degradation of these metabolites is a very important step for full degradation of BaP. Therefore, the degradation, not only BaP but also its some accumulated metabolites, should be distinguishingly considered.

However, problems associated with PAHs biodegradability in environmental media have received increasing attention. The first consideration is that some surfactants are efficient in removing the contaminants by improving the solubilization and bioavailability of PAHs [4]. It is well-known that surfactants can increase the solubility of hydrophobic organic compounds by solubilizing them in the hydrophobic core of micelles. Numerous studies [5] have investigated the enhanced solubilization of a hydrophobic contaminant in the presence of surfactants above their critical micelle concentrations(CMC). Nonionic-surfactant TW80 is generally regarded as non-toxic, nonirritating, convenience, efficiency and economy desirable for biodegradation of hydrophobic organic compounds.

However, little information is hitherto available for degradation of BaP and its accumulated metabolites in aqueous phase in the presence of nonionic-surfactant TW80. This research focuses on the feasibility of using nonionic-surfactant TW80 to enhance the continued degradation of BaP and its accumulated metabolites by domestic microorganism.

Ⅱ MATERIALS AND METHODS

A Chemicals

The PAHs used in this study were BaP, cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol. They were purchased from Fluka Chemical Company. TW80 purchased from Dalian Chemical Company in China.

B *Bacteria growth conditions*

Bacteria *Bacillus*-07 was found in crude oil-contaminanted soil in the Liaohe Oil Field in China. Our experimental medium consisted of (in g/l distilled water):

NH_4NO_3, 1; $MgSO_4{\cdot}7H_2O$, 0.2; KCl, 0.2; and glucose 0.5.

C *Experiments of solubilization*

The Erlenmeyer flasks (125-ml) were divided into 5 groups and each group consisted of 3 Erlenmeyer flasks. A series of concentrations of TW80 in each group was designed as 100-, 500-, 1000-, 1500- and 2000 ppm, respectively. Superfluous cis-BP4,5-dihydrodiol was added into the Erlenmeyer flasks. Moreover, $HgCl_2$ (0.5%), as inhibitor avoiding biodegradation, was added into above flasks. Extraction was performed in a ultrasonic cleaner at 50 Hz for 1 h. After centrifugation at 8000 rpm for 20 min at room temperature, the liquid was collected. Solubility experiments of cis-BP7,8-dihydrodiol and BaP in TW80 were similar to cis-BP4,5-dihydrodiol. Experiments of controls were carried out in the same conditions except in the absence of TW80.

D Selection of concentration of TW80

A series of concentrations of TW80 was designed as 500-, 1000- and 2000 ppm, respectively. BaP (10 ppm) was added into the Erlenmeyer flasks. All other conditions were as described previously. Both BaP and the two metabolites were measured at different time intervals by HPLC. Meanwhile, density of *Bacillus*-07 and residue of TW80 in different group were measured in 600 and 620nm, respectively, by spectrophotometer.

D Analysis method

Analysis method was same with the reference[7].

Ⅲ Results and discussion

A Identify of BaP and its metabolites

Our previous study showed that concentration of accumulaed metabolites would be highest during 6~9d. This sample was extracted and analyzed on the 8d. Retention times of pure BaP, cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol were 10.361 min, 6.835 min and 5.468 min in wavelengh 254nm, respectively. Based on their retention times, qualitative analysis of our samples was implemented. Fig.1 shows chemical structures and their relationship of the three compounds.

B Solubility characteristic of the three compounds

Solubility of BaP, cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol in presence of TW80 was different. Plots of apparent solubility of the three compounds versus TW80 concentration were shown in Fig.2 (Curve a was cis-BP4,5-dihydrodiol; Curve b was cis-BP7,8-dihydrodiol; Curve c was BaP). The solubilities of cis-BP4,5-dihydrodiol, cis-BP7,8-dihydrodiol and BaP linearly increased with TW80 concentration above the CMC (15 ppm). Moreover, slopes of the curvs a and b were greater than that of c. The results show that the solubility of BaP, cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol in aqueous phase is affected by the concentration of TW80 and also by the character and state of the three compounds. TW80 do not improve their solubility when concentrations of TW80 were below or near its CMC values. While, the results were opposite when the concentrations of TW80 exceeded its CMC values. The solubility of the three compounds was increasing with the increase of TW80 concentrations. The behavior is generally attributed to the incorporation of organic solutes within surfactant micelles. The solubility of BaP in presence of TW80 (2000 ppm) was 0.078 ppm, which was more than 20 times than control experiment (0.0038 ppm). The effects of cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol were similar to BaP. But the solubility of cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol in TW80 (2000 ppm) were much higher than that of BaP, which could be seen from Fig. 2. That may be cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol could form hydrogen chemical bond with TW80, which could improve their solubility. Therefore, after adding TW80, solubility of the three compounds have been greatly enhanced, contiguity chance between *Bacillus*-07 and the three compounds remarkably increased, bioavailability would be improved. The rule of linear enhancements in solubility were consistent with solubilization data reported for other nonionic surfactants of environmental concern [8].

C Degradation of BaP and its two metabolites

It could be seen from Fig.3 that, when TW80 was 1000 ppm, degradation rate of BaP was the highest (66.25%), accumulation rate of cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol was the lowest (3.16% and 2.04%, respectively). By adding TW80, degradation rate of BaP was enhanecd 25.01%, meanwhile accumulation rates of cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol were lowed 2.11% and 2.57%, respectively, compared with controls. Although solubility of the three compounds in 2000 ppm TW80 was higher than that in 1000ppm TW80, for degradation rate, former was lower than latter. Appropriate concentration (> CMC) of TW80 could enhanced solubility of BaP and its accumulated metabolites. However, when concentration of TW80 exceeded a certain range, there were some competition between TW80 and the three compounds, which would effect degradation of the three compounds. Compared with BaP and its metabolites, TW80 was much easily degraded for microorganisms. Above results showed that degradation rate linearly increased with the solubility of the three compounds only in a certain concentration range of TW80.

D Growth of Bacillus-07

Fig.4 showed that lag period on the initial stages was short (24 h) in low concentration TW80 (500 ppm and 1000 ppm) culture medium and it was long (36 h) in 2000 ppm TW80. The higher concentration of TW80 was, the longer lag period was. But after lag period, the bacteria rapidly growed. After 48 h, absorbency of 2000 ppm TW80 was the biggest, which showed number of the bacteria was the greatest in this culture medium. The result showed the bacteria could take TW80 as one of its energy sources, and its growgh, on the whole, was not inhibited by different concentration TW80.

E Effect of subsequent addition of TW 80 on biodegradation

In order to further elucidate the effect of TW80 on biodegradation of BaP, cis-BP4,5-dihydrodiol and cis-BP7,8-dihydrodiol in aqueous phase, addition of TW80 (1000 ppm) into the systems was done at two different times (0 and 48 h). It presented two aspects merit on this: one was solving lagged period produced by high concentration of TW80; the other was solving the problem that degradation of surfactant and the key contaminants was not isochronously, which would influence terminal solubility and biodegradation of the key contaminants when degradation of surfactant was finished prematurely.

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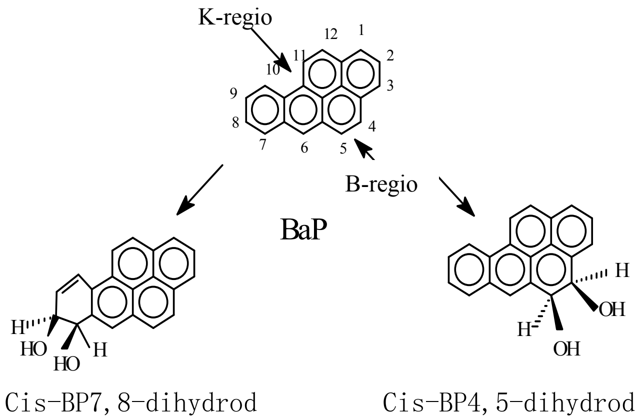

Figure. 1 **A**ccumulated metabolites of BaP by *Bacillus*-07

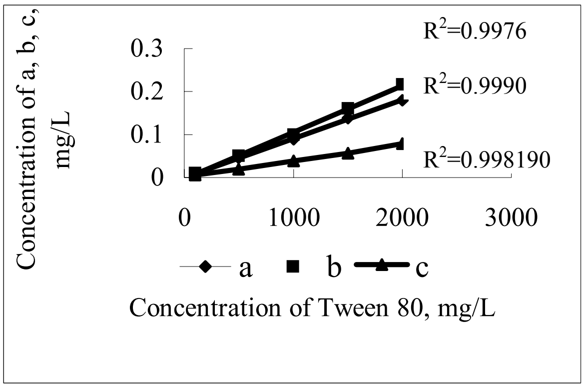

Figure. 2 Influence of TW-80 on the solubility of a,b and c. a was cis-BP4,5-dihydrodiol; b was cis-BP7,8-dihydrodiol; c was BaP.

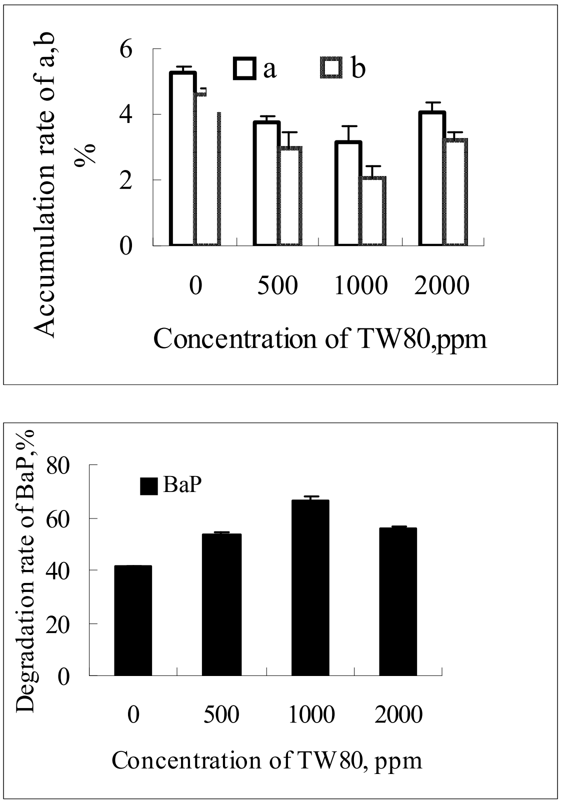

Figure. 3 Selection of initial concentration of TW80

a was cis-BP4,5-dihydrodiol; b was cis-BP7,8-dihydrodiol.

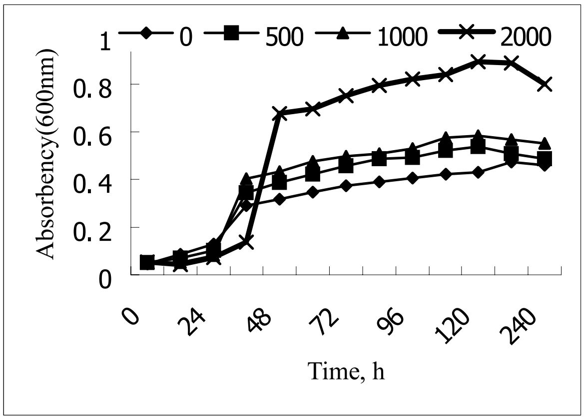

Figure. 4 Influence of different concentration of TW80 on Bacillus-07