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ORIGINAL PAPER

Co-degradation with glucose of four surfactants, CTAB, Triton X-100, SDS and Rhamnolipid, in liquid culture media and compost matrix

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Abstract Strengthened biodegradation is one of the key means to treat surfactant pollution in environment, and microorganism and surfactant have significant effects on degradation. In this paper, co-degradation of CTAB, Triton X-100, SDS and rhamnolipid with glucose by *Pseudomonas aeruginosa*, *Bacillus subtilis* and compost microorganisms in liquid culture media, as well as the degradation of rhamnolipid in compost were investigated. The results showed that CTAB was recalcitrant to degrade by the three microorganisms and it also inhibited microorganisms from utilizing readily degradable carbon source. Non-ionic surfactant Triton X-100 could also hardly be degraded, but it was not toxic to microorganisms and would not inhibit the growth of the microorganisms. Anion surfactant SDS had no toxicity to microorganisms and could be co-degraded as carbon source with glucose. Biosurfactant rhamnolipid was a kind of particular surfactant, which had no toxicity and could be degraded by *Bacillus subtilis* and compost microorganisms, while it could not be utilized by its producing bacterium *Pseudomonas aeruginosa*. Among these three bacteria, the compost consortium had the strongest degradation capacity on the tested surfactants due to their microorganisms' diversity. In compost matrix rhamnolipid could be degraded during composting, but not preferentially utilized.

Keywords Biodegradation · Composting · Microorganism · Surfactant

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Introduction

In recent years surfactants are widely applied in industries and daily life for their surface or interfacial functions (Nicholas and Raymond 1999; Mulligan et al. 2001). The rapid removal of them from the environment to avoid secondary pollution will make its application more safe and wide. The strengthened degradation of surfactants by microorganisms is one of the promising methods. There are primarily three circumstances of the surfactant degradability: (1) Readily degradable. The surfactant can be used by a microorganism, no matter preferentially or non-preferentially on other carbon source. (2) Hardly degradable. It is difficult for a microorganism to take up and metabolize the surfactant, but it does not inhibit the degradation of other carbon

sources. (3) Toxic. The surfactant is not only undegradable by a microorganism, but also inhibits the microorganism's growth on other degradable carbon source or kills it. The degradability is affected by surfactant properties, microbial degradative capability and environmental conditions, among which the surfactant properties and microorganism activity are key factors. The surfactant properties include its electric property (cationic, anionic or non-ionic), molecular structure complexity (simple chain or with polymeric structure) and source (chemically synthesized or microbially produced). Studies showed that anionic surfactants generally have no toxicity to microorganisms and are degradable. Linear alkylbenzene sulphonates (LAS), the typical anionic surfactant, is readily degradable with a half-life of 1–87 days once applied to the aerobic soil environment (Scott and Jones 2000). Lee et al. used stream periphyton to degrade anionic surfactant and found that stream periphyton were able to rapidly degrade the anionic surfactant C12-alkyl sulfate (SDS) at environmentally relevant concentrations (Lee et al. 1997). Abd-allah et al. also demonstrated that biodegradation of anionic surfactant (XP-100) was enhanced in the presence of yeast and organic contaminants using two different steps for microbial adaptation (Abd-allah and Srorr 1998). The non-ionic surfactants usually have low toxicity however its degradability depends on the complexity of the molecular structure. Mezzanotte et al. reported that while six linear alcohol ethoxylates with different HLB (Hydrophilic lipophilic balance) and two fatty acid esters were degraded in both aerobic and anaerobic conditions, the degradability were related with numbers of ethoxy groups and length of the alkyl chain (Mezzanotte et al. 2003). The type and capability of microorganisms strongly affected the degradation process of these compounds as well (Chen et al. 2005). The degradability of cationic surfactants varies with the surfactant and microorganism species. Because they are positively charged, they have strong affinity to negatively charged microorganisms and are considered highly biologically available (Van Ginkel 1995). Games et al. reported half-life of 2.5 h for octadecyltrimethylammonium chloride in the presence of wastewater microorganisms (Games et al. 1982). Similar result was obtained for alkylbenzyldimethylammonium chloride (Krzeminski et al. 1973). However, the inherent recalcitrance and toxicity of cationic surfactants is also evident. Janicke and Hilge reported that quaternary ammonium salts exhibited little or no degradation during anaerobic wastewater treatment (Janicke and Hilge 1979). Battersby and Wilson observed that concentrations of 200 mg/1 hexadecyltrimethylammonium bromide were inhibitory to the resident microbes in the similar conditions (Battersby and Wilson 1989). Aquatic toxicity in laboratory tests of monoalkylquats was nevertheless reported to be high in comparison with other surfactants and an increase in the alkyl chain length or the substitution of a benzyl group for a methyl group reduced the biodegradation rate (García et al. 2001). In contrast to synthetic surfactants, degradability of biosurfactants was supposed to be stronger. In a survey of eight synthetic and nine biosurfactants, the biosurfactants were found to be overall less toxic and more biodegradable than the synthetic compounds (Poremba et al. 1991). Providenti et al. investigated degradation of rhamnolipid and found that it could be easily utilized by bacterium consortia from sandy loam, silt loam and creosote contaminated soil, but immune to *Pseudomonas aeruginosa* strain when it was present as the sole carbon source (Providenti et al. 1995).

Although the degradability of surfactants has been totally revealed, however, in most of the previous studies the sole carbon source of surfactant was used and whether the surfactant is readily degradable, hardly degradable or toxic to the degradation microorganisms is not fully exposed. The present study investigated co-degradation of surfactants with glucose, a highly degradable carbon source, and the degradation status reflected by concentrations of the two carbon sources could basically disclose the more specific degradability of the surfactants. The typical cationic, non-ionic, anionic and microbial surfactants, CTAB, Triton X-100, SDS and rhamnolipid, were selected as the surfactant carbon source. A Gram-negative *Pseudomonas aeruginosa* strain, a Gram-positive *Bacillus subtilis* strain and the microbial consortium from municipal solid waste compost

are selected as the degradation microorganisms. This experimental design permitted us to study effects of different classes of surfactants and bacteria on the degradation process. Finally the biodegradation of biosurfactant rhamnolipid in composting matrix was also basically investigated to verify their supposed friendliness in its application (Ahimoua et al. 2000; Pooja and Swaranjit 2004; Sim et al. 1997; Zhang et al. 2004; Vipulanandan and Ren 2000).

Material and methods

Surfactants and chemicals

Triton X-100 (Scintillation Grade, purity >99%) was purchased from BDH Chemicals Ltd. (Poole, England). SDS and CTAB (G. R., Sigma Chemical Co., Shanghai, China) were used as received. Other reagents were of A.R. grade without further purification. Ultra pure water with an initial resistivity of 18.2 MΩ cm^{-1} produced by Laconco Water Pro PS (Kansas, USA) was used through the experiment.

Microorganisms

The *Pseudomonas aeruginosa* strain (No. AB93066) used as RL (rhamnolipid) production and substrate degradation bacterium as well as *Bacillus subtilis* (No. AB93108) in the experiment were from China Center for Type Culture Collection and stored at 4°C on trypcase soy agar until use. Compost microorganisms were taken from the compost in its secondary fermentation stage in Shanghai Meishang solid waste treatment plant which was stored at 4°C in sealed plastic sack until use.

Rhamnolipid production and purification

RL was produced by *Pseudomonas aeruginosa* AB93066. Briefly, the strain was inoculated to the seed culture medium (SCM) in a 250-ml Erlenmeyer flask containing the following mineral salts and carbon source per litre (Zhong et al 2003): $NaNO_3$ 2.0 g, KH_2PO_4 1.5 g, $Na_2HPO_4{\cdot}12H_2O$ 1.5 g, $MgSO_4$ 0.1 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, yeast extract 0.5 g and pH 6.5, and enriched at 37°C, 200 rpm on an orbital incubator shaker for 24 h. Then the SCM was transferred at 2% (v/v) inoculation to the 200 ml minimal mediums with the same mineral salts as SCM and 20 g l^{-1} glucose in a 1,000-ml Erlenmeyer flask. The culture was grown at 37°C, 200 rpm in the shaker for another 72 h until it reached the highest RL yield. A modified acid precipitation method of Noordman et al. (2000a) was used for the extraction of RL in the medium. The pH of the medium was adjusted to 8.0 with 2 N HCl and centrifuged at 10,733*g* for 15 min to remove bacteria and other impurities. The supernatant was treated with 6 N HCl to pH 2.0 and kept overnight at 4°C. Then it was centrifuged at 10,733*g* for 15 min. The supernatant was decanted and the pellet was washed by deionized water for three times and freeze-dried. Then the product was dissolved in chloroform and filtered to remove protein insolubles. After vacuum evaporation of the chloroform by a rotoevaporator at 40°C, brownish paste rhamnolipid, which was supposed mainly to be the mixture of rhamnolipid and neutral lipids, was obtained and then further purified by column chromatogram. A column 25 cm × 2 cm (dia.) was prepared with activated silica gel (100–200 mesh) chloroform slurry. Crude rhamnopilid extract was dissolved in chloroform and loaded with separatory funnel. The column was washed with chloroform until neutral lipids were completely eluted. Chloroform: methanol mobile phases were then applied in sequence: 10:1 v/v (200 ml); 1:2 v/v (200 ml) at a flow rate of 1 or 2 ml min^{-1} and 20-ml fractions were collected in test tube. TLC (Thin-Layer Chromatography) was used to determine the existence of rhamnolipid in collection tubes, and the tubes containing rhamnolipids were stored. After the liquid volatilized entirely, pure rhamnolipid, which is suggested to be the mixture of mono-rhamnolipid and di-rhamnolipid at a ratio of 4:1(Arino et al. 1996), were obtained and stored for further use.

Degradation experiment in liquid culture medium

The experiment was conducted in 500 ml Erlenmeyer flasks containing 100 ml culture media

with following mineral salts and carbon source per litre: NH_4Cl 1.0 g, K_2HPO_4 1.0 g, KH_2PO_4 1.0 g, $MgSO_4{\cdot}7H_2O$ 0.05 g, $CaCl_2$ 0.02 g, trace $FeSO_4{\cdot}7H_2O$, glucose 1.0 g and surfactants1.0 g, and pH was adjusted to 7.2. After the mediums were autoclaving sterilized, bacterium solutions, whose concentration were adjusted to 10^8 CFU/ml by OD (optical density) adjustment according to linear relation of OD value and viable cell number (presented by CFU), were inoculated at 1% inoculation. The degradation experiment was performed at 37°C, 200 rpm in a gyratory shaking incubator. For analytical purposes, 2 ml solutions were drawn from the culture medium at time intervals. After centrifuged at 10,733*g* for 10 min, the supernatant was sucked out and the TOC and surfactant concentration were determined. Methylene blue spectrophotometric method was used to measure SDS concentrations. Triton X-100 concentrations were determined using a UV–visible spectrophotometer at 224 nm after extraction by chloroform. Picric method was performed to determine CTAB concentrations (Al-Tahhan et al. 2000). Rhamnolipid concentration was measured by phenol-sulfuric acid (Monticone et al. 1994) after extraction by ethyl acetate. For the biomass determination, the pellet of *Pseudomonas aeruginosa* and *Bacillus subtilis* after centrifugation was re-dissolved into water, and the OD was determined by spectrophotometer.

Degradation experiment in compost matrix

The 20-day compost sample was taken from Shanghai Meishang solid waste treatment plant and the components were made up of 8.02% paper, 13.93% plastic, 1.43% bamboo, 2.87% fiber, 54.67% kitchen residue, 13.69% pericarp and 5.41% clay. Rhamnolipid solution was added into the compost matrix, and the ratio of compost solid and rhamnolipid reached 800:1 (w:w). Water was added to adjust water content to about 60%. Then the compost matrix was incubated at constant temperature of 37°C in a 500 ml Erlenmeyer flask. Every 2 days, water content in the matrix was determined and water was added to keep the water content at 40–60%. For analytical purpose, the sample was taken every 2 days and biomass was measured by the pour plate technique on plate count agar immediately after the sample was mixed up with water at the ratio of 1:20 (m/m) and incubated on an end-over-end shaker at 100 rpm for 30 min until suspension was formed. The residual suspension was contrifuged at 10,733*g* for 10 min and TOC and rhamnolipid concentration in the supernatant was measured as described above.

Sample assay

OD and the concentration of surfactants were recorded on a dual wavelength UV-2552 spectrophotometer (Shimadzu Company, Japan). TOC value was obtained by 1010-TOC instrument (O I Analytical Company, USA).

Results and discussion

Effect of surfactants on degradation

Surfactants' degradation degree and their toxicity to the microorganisms can be illuminated from the change of surfactants concentration, glucose concentration which can be reflected by TOC concentration and the growth of microorganisms, since the co-degradation of surfactants and glucose, which was readily degraded, was investigated in the liquid culture medium. Surfactants' toxicity and availability varied with the properties of surfactants and microorganisms despite of the similar culture conditions. As shown in Fig. 1, in the culture medium containing CTAB as co-degradation carbon source, the concentration of CTAB as well as TOC kept the initial concentration (about 1,000 mg/l and 900 mg/l, respectively) unchanged, which showed that neither glucose nor CTAB could be utilized well by the selected strains. On the other hand, no growth of *Bacillus subtilis, Pseudomonas aeruginosa* and compost microorganisms was observed in the medium containing CTAB, which showed that CTAB had toxicity to the three strains in the tested condition. The result supported the antibacterial property of cationic surfactants.

During the co-degradation of nonion surfactant Triton X-100 and glucose, TOC concentration in

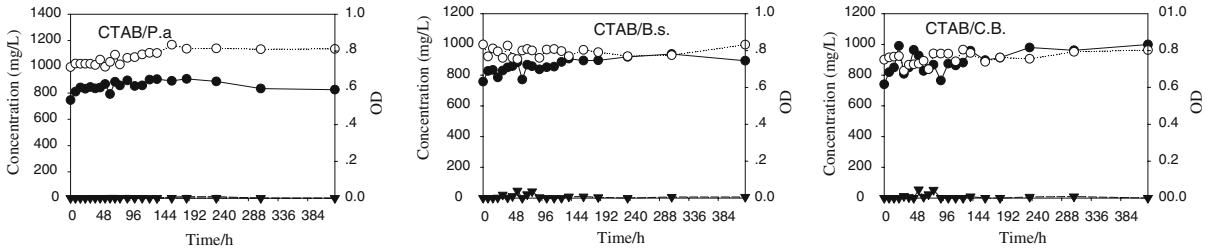

Fig. 1 Co-degradation of CTAB with glucose by *Pseudomonas aeruginosa, Bacillus subtilis* and compost microorganisms in culture media. (○) surfactant concentration in the culture medium, (●) TOC in the culture medium and (▼) bacteria OD in the culture medium

the medium decreased from about 900 mg/l to about 700 mg/l and OD values of culture mediums increased to 0.3 for *Bacillus subtilis* in 72 h, 0.5 for *Pseudomonas aeruginosa* in 48 h and 0.5 for compost microorganisms in 24 h, indicating non-toxicity of Triton X-100 to the three strains. However, under this condition, the concentration of Triton X-100 decreased little in the whole culture process, which showed that all of the three microorganisms could not use Triton X-100. The results indicated that Triton X-100 was difficult to degrade though it had no toxicity to the three strains. This may be due to aromatic ring and polymeric ethylene oxide structure in the molecule which are hardly degradable parts (Fig. 2).

The concentration of anion surfactant SDS decreased significantly in the media containing the three strains and in the medium amended with compost microorganism the decrease is the most remarkable (Fig. 3). Margesin et al. also found rather good effect of SDS degradation when SDS was used as the carbon source in mineral salt medium (Margesin and Schinner 1998). These results demonstrated that anion surfactant SDS was a readily degradable surfactant.

Degradation of different microorganism on RL is different (Fig. 4). RL concentration changed little when degraded by *Pseudomonas aeruginosa*, which indicated that it cannot be degraded by its source bacteria. However, the stimulated effect of RL on growth of *Pseudomonas aeruginosa* and degradation of glucose was evident since there was no stagnant phase for bacterium growth. For *Bacillus subtilis* and compost microorganisms, RL was readily degradable compounds. It seemed to be even more readily degradable than glucose for *Bacillus subtilis* since the RL concentration decrease was earlier than that of TOC decrease. For compost microorganisms, RL and TOC consumption was more rapid and complete. The two-step growth of microorganisms was also observed. We supposed that in the first step glucose was used as the main carbon source and RL was

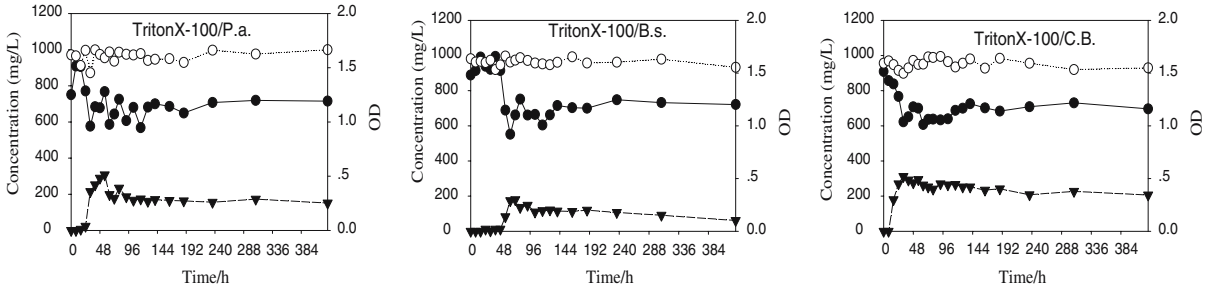

Fig. 2 Co-degradation of Triton X-100 with glucose by *Pseudomonas aeruginosa, Bacillus subtilis* and compost microorganisms in culture media. (○) surfactant concentration in the culture medium, (●) TOC in the culture medium and (▼) bacteria OD in the culture medium

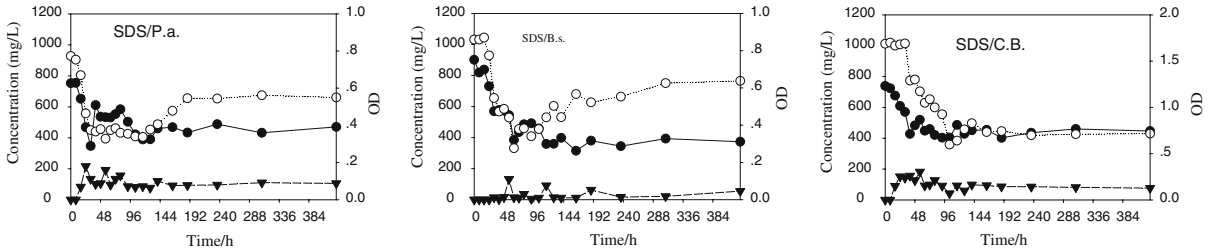

Fig. 3 Co-degradation of SDS with glucose by *Pseudomonas aeruginosa, Bacillus subtilis* and compost microorganisms in culture media. (○) surfactant concentration in the culture medium, (●) TOC in the culture medium and (▼) bacteria OD in the culture medium

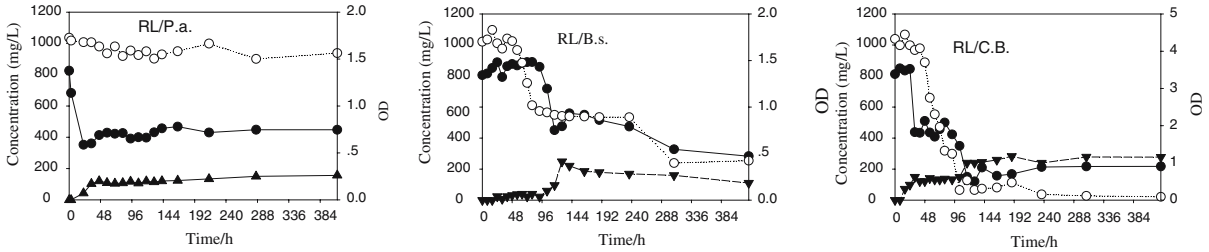

Fig. 4 Co-degradation of RL with glucose by *Pseudomonas aeruginosa, Bacillus subtilis* and compost microorganisms in culture media. (○) surfactant concentration in the culture medium, (●) TOC in the culture medium and (▼) bacteria OD in the culture medium

metabolized into less available organic molecules by some bacteria. By the end of this stage, TOC reached the first plateau. In the second step, the further degradation of the organic molecules caused regrowth of some bacteria. Over 90% degradation rate demonstrated the strong degradability of compost consortium on RL.

Effect of different microorganisms on degradation

Effects of microorganisms on degradation efficiency were not as significant as that of surfactants. All the three microorganisms could not utilize CTAB and TritonX-100 in the tested media. SDS and rhamnolipid were degradable surfactants and degradation capabilities of the three strains were different. Degradation capability of compost microorganisms was the strongest among the three strains. The lag phase of the microorganism was less than 8 h and 60% SDS and 90% rhamnolipid were degraded within 104 h. The microbial growth was also the most remarkable. *Pseudomonas aeruginosa* could only degrade SDS but the degradation was rapid and over 50% SDS was degraded at 32 h. *Bacillus subtilis* could degrade SDS and rhamnolipid but the degradation capability was not so strong, and the lag phase was longer for all the three degradable surfactants. Compost microorganisms could degrade surfactants more rapidly and completely due to the diversity of microbial species. *Pseudomonas aeruginosa* had stronger capability to degrade SDS but could not utilize rhamnolipid produced by itself as carbon source, which complies with the study of Providenti et al. (1995).

Degradation of rhamnolipid in composting

Composting organic granules are of multi-phase media composted of organic granules, air and water whose physicochemical characters are quite different from that of liquid culture media. The degradation of RL in compost matrix was affected by substrate availability, size of pores and porosity, water and microorganisms' distribution status, et al. As shown in Fig. 5, RL

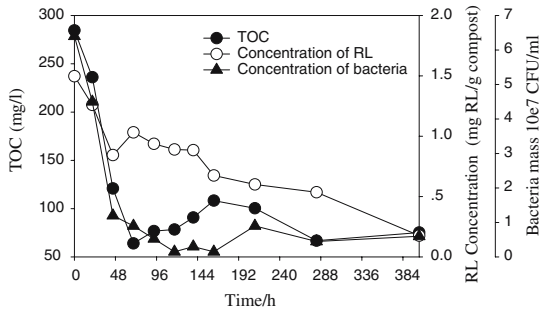

Fig. 5 Degradation of rhamnolipid in compost matrix

concentration in the matrix decreased slowly from the beginning of biodegradation and 90% rhamnolipid was degraded at 400 h. TOC and microbial mass of the compost extract decreased fast at the beginning and then stabilized at 72 h. This was because the composting matrix was taken at the secondary stage of composting and readily degradable nutrient had been exhausted. The results showed that rhamnolipid was not the main organic material which contributed to the dissolved TOC in the composting matrix and it could not be degraded preferentially, but could be finally degraded.

Conclusions

Totally there are three degradability statuses for surfactants to a certain microorganism, readily degradable, hardly degradable and toxic, and it can be clarified by co-degradation test of surfactants with other carbon sources. In liquid culture media, the degradability depends on surfactants and microorganisms. Toxicity of cationic surfactant CTAB is identical since it inhibited the growth of all the tested microorganisms. Non-ionic surfactant Triton X-100 is hardly degradable surfactant, but it is not toxic to the three microorganisms because about 300 mg/l TOC was degraded when glucose was co-existent. Basically anion surfactant SDS is readily degradable by the three strains though it varies with microorganisms to some extent. Over 70% and 90%degradation rates of RL by *Bacillus subtilis* and compost microorganisms indicated that it is the most readily degradable among the four surfactants, though it can not be used by its producing strain, *Pseudomonas aeruginosa*. For the three microorganisms, compost consortium has the strongest degradability due to the highly complexity of microbial species. *Bacillus subtilis* has the lowest degradability, demonstrated by its always longest lag phase. In the composting matrix RL is readily degradable, indicating its potential compatibility in environmental applications.

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