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Disappearance of Linear Alkylbenzene Sulfonate from Different Cultures with *Anabaena sp.* HB 1017

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Linear alkylbenzene sulfonates (LAS) are the major anionic surfactants used in detergent formulations worldwide. Previous studies have shown LAS to be extensively removed during wastewater treatment and in the environment by a combination of absorption and degradation (Gard-Terech and Palla 1986; McAvoy et al. 1993). Among these processes, the biodegradation of LAS is mainly attributed to bacteria (Swisher 1987; Sigoillot and Nguyen 1992). However, the role of photosynthetic microorganisms is little known. Although the green algae have been found to have some capacity for the absorption and degradation of LAS (Davis and Gloyna 1969; Chawla et al. 1987) information on the algal degradation of LAS is far from complete. In particular, there are no reports on the cyanobacteria for LAS degradation.

Cyanobacteria are the important component of soil microflora and essential members of phytoplankton communities in aquatic ecosystem. It was reported that some species of cyanobacteria were capable of metabolizing organophosphorus and organochlorine insecticides (Boyle 1984; Megharaj et al. 1987) and aromatic hydrocarbons (Kuritz and Wolk 1995). The present study was undertaken to provide further information on the possible role of a cyanobacterium, *Anabaena sp.* HB 1017, in the removal of LAS from different culture media with the cyanobacteria respectively grown autotrophically, mixotrophically and heterotrophically.

MATERIALS AND METHODS

The filamentous cyanobacterium *Anabaena sp.* HB 1017, originally isolated from paddy field soil, was obtained as a pure isolate from the Culture Collection of Algae, Institute of Hydrobiology, The Chinese Academy of Science. This cyanobacteria strain has been demonstrated to be not only capable of autotrophic and mixotrophic growth but also chemoheterotrophic growth (Jin et al. 1995).

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Stock culture was grown in the modified mineral medium of Chu (1942) which contained ferric chloride to replace ironic citrate and citric acid, with A_6 micronutrients (Alien and Arnon 1955) and illuminated by fluorescent lamps (light intensity of about 3500 lux) with a 14 hr light : 10 hr dark cycle. Temperature maintained in an air conditioned growth chamber at 32 °C. Cells in the exponential phase of growth were collected from stock cultures and used as inocula for experiments. Linear alkylbenzene sulfonate (LAS), technical grade (80%), was obtained from Sigma Chemical Co. and added to the culture medium to the final concentration of 5,10 and 20 mg/L. All operations were carried out under sterile conditions in order to avoid contamination with bacteria or other algae.

Experiments were conducted in batch cultures, 200 mL of mineral medium as the above mentioned in 500 mL Erlenmeyer flasks (stoppered with cotton plugs) were inoculated to give an initial cyanobacteria concentration of 58 μg dry wt/mL. Four groups of batch culture flasks were set up for testing the disappearance of LAS from medium with the cyanobacteria grown under different trophic states. Among them, two groups of cultures were firstly supplemented with glucose (2 g/L) as the carbon source and then respectively incubated in light and darkness (respectively referred to as glucose medium+light and glucose medium+darkness, GL and GD) in which cyanobacteria grow mixotrophically and heterotrophically, respecitively, the other two groups of cultures were in mineral medium and respectively incubated in light and darkness (respectively referred to as mineral medium+light and mineral medium+darkness, ML and MD). The former makes the cyanobacteria grow autotrophically, the latter was used to investigate if the cyanobacteria was capable of metabolizing LAS as sole carbon source for heterotrophic growth. All the cultures maintained at 32 °C. At 0 time and at regular intervals, 10 mL culture was withdrawn from flasks and then cyanobacteria were removed by centrifugation. For LAS determination water samples were measured according to the methylene blue active substance method (MBAS) described by APHA et al. (1985).

Data in figures are the means from three replicates. Analyses of significant differences were perfomed using Student's t-tests.

RESULTS AND DISCUSSION

The disappearance of LAS from cultures with *Anabaena sp.* HB 1017 grown under different conditions is shown in Figure 1. It is interesting to note that dose and time dependent changes of LAS concentrations in medium exhibited to be in two kinds of manners. Under ML conditions, the disappearance rates of LAS from medium increased with time developing and decreased as LAS concentrations increased from 5 to 20 mg/L by the end of 4 days ($P<0.05$). This is dissimilar to the result obtained by Chawla et al. (1987) who reported that the uptake of LAS by *Scenedesmus quadricauda* was higher in 0.10% LAS than the corresponding 0.02%

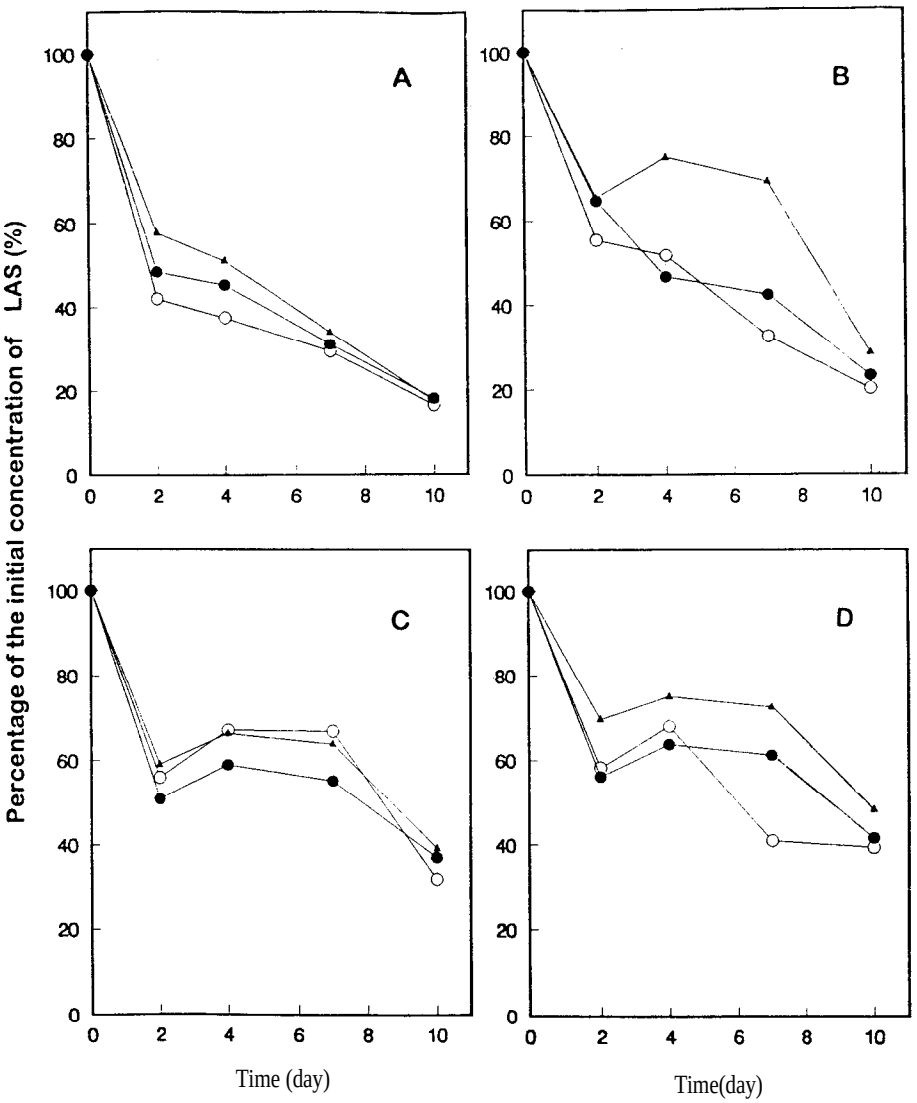

Figure 1. Disappearance of LAS from culture medium with *Anabaena sp.* HB1017 under different growth conditions. A: ML; B:MD; C:GL; D:GD. Initial concentration of LAS: (O)5 mg/L, (O) 10 mg/L, (Δ) 20 mg/L

LAS concentration. It suggests that the toxic level of LAS (10 and 20 mg/L) to *Anabaena* my be affecting the uptake and biodegradation process. Chawla et al (1988) found that toxic level of LAS to a filamentous cyanobacterium *Nostoc* muscorum is in the concentration above 0.001%. Most of the work on the toxicity mode of surfactants on algae has shown that surfactants generally denature and bind protein in the cell wall and consequently alter membrane permeability to nutrient and chemicals (Lewis 1990). But under GL and GD conditions, LAS concentrations showed a decrease (day 2) followed by slight reversion (day 4 to

day 7) and then a rapid reduction. Moreover, the time dependent changes of LAS under MD condition varied with concentration. The disappearance rates of LAS under GL condition was higher at 10 mg/L LAS than at 5 mg/L LAS by the end of 7 days ($P<0.05$). This result may be related to reduction of LAS toxicity to cyanobacteria because of the addition of glucose, which reverse organic pollutant toxicity to algae or convert it to other metabolites causing relief from toxicity, as reported for three herbicides to some nitrogen-fixing cyanobacteria (Mishra and Pandey 1989) and for two nitrophenols to *Chlorella vulgaris* (Megharaj et al. 1988).

As seen in Figure 1, under MD condition a considerable amount of LAS, more than 70%, was removed after 10 days. In general, LAS volatilization losses were very small, only 3% in 14 days under laboratory conditions (Freitag et al. 1982). The high disappearance rate mainly depend on the assimilation by cyanobacteria. It showed that *Anabaena sp.* HB 1017 was capable of utilizing LAS as sole carbon source for heterotrophic growth. Of under four culture conditions, the disappearance rate of LAS from medium followed the order: ML>MD>GL>GD. The maximum disappearance rates of LAS were observed under ML condition, at LAS concentrations of 5, 10 and 20 mg/L amounting to 58.0, 51.7 and 42.4% after 2 days and 83.4, 81.9 and 82.4% after 10 days, respectively. There were not the existense of significant difference ($P>0.05$) in the disappearance rates of LAS at three initial LAS concentrations on the 10th day. This may be attributed to the changes of LAS molecular structure during its acute biodegradation leading to a rapid decrease in its acute toxicity to cyanobacteria. LAS biodegradation rates and toxicity to aquatic organisms are properties closely related to the carbon chain length and phenyl position on the alkyl chain. Martinez et al. (1989) indicated that toxicity of LAS is directly proportional to the molecular weight of the hydrocarbon chain. Such correlation has been evidenced with fish, *Daphnia* and microorganisms (Kimerle and Swisher 1977; Gard-Terech and Palla 1986). Many studies on primary biodegradation of LAS showed that the initial stage begins with the terminal oxidation of the alkyl chain, which is then shortened by means of β-oxidation, and the intermediate biodegradation compounds of LAS are far less toxic than same concentration of the intact compound (Swisher 1987; Martinez et al. 1989).

It has been demonstrated that removal of organic compounds from water by algae or cyanobacteria cells frequently involves two stages: an initial passive accumulation nvolving a rapidly equilibrating adsorption on to the exterior surface, followed by a slow uptake which is dependent upon metabolic processes (Boyle 1984). Although the addition of glucose can stimulate algal growth which produces greater biomass and provides larger surface area (Jin et al. 1995) for sorption of LAS, the adsorption site is after all limited. In particular, glucose may also competes with LAS for accessing cell, which interferes at the level of LAS uptake by the cyanobacteria cells. This resulted in less LAS being removed from glucose

media (GL and GD) than from mineral media (ML and MD) at the corresponding LAS concentration on the 10th day ($P<0.05$).

The disappearance rates of LAS under GL condition were higher than those under GD condition at the corresponding LAS concentration. The former after 10 days amounts to 60~70% wheras the latter only 50~60% (Figure 1C and ID). With the exception to the larger surface area under GL condition, μH^+, the presumed driving force of glucose uptake, is strong affected by light, the uptake of glucose analoges is faster in light than in dark (Tanner 1969) So is the other organic compounds such as LAS. Also, it was reported that many organic macromolecules such as chlorophyll and aromatic amines formed in algal cells could sensitize and enhance photochemical reactions of a number of synthetic organics and found that flavoprotein extracted from blue-green alga in the presence of light greatly increased the photodegradation of DDT, lindane, dieldrin and etc. (Boyle 1984).

The present data showed that LAS was extensively removed from culture medium and *Anabaena sp.* HB1017 has a marked capacity to degrade LAS. Complete surfactant biodegradation is achieved by three important steps: terminal oxidation of the alkyl chain, desulfonation and aromatic-ring cleavage. A few microorganism strains were only able to carry out the first two steps. Sigoillot and Nguyen (1992) and Gard-Terech and Palla (1986) indicated that LAS mineralization is carried out by bacterial associations rather than single organisms in marine environments and that *Flavobacterium, Pseudomonas* and *Acinetobacter* seem to play a decisive role. The degradation of LAS by *Anabaena sp.* HB 1017 may only involve the initial stage which lead to the loss of surfactive properties.

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