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Original Research Paper

Tolerance Limit of the Alga *Spirulina platensis* to Linear Alkyl Benzene Sulphonate Polluted Wastewater

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ABSTRACT

Domestic detergents carried by the urban wastewater normally accumulate in the surface water bodies in most of the Indian cities. One of the major chemical components of these detergents is linear alkyl benzene sulphonate (LAS) and its accumulation in water bodies may become toxic to aquatic flora and fauna. In the present study, the growth of a Cyanobacterium *Spirulina platensis*, generally used as a nutraceutical source of protein, was studied in CFTRI medium spiked with 5 levels of LAS (2, 4, 6, 8, 10 ppm). The growth of *Spirulina* gradually increased and became maximum at 6 ppm of LAS beyond which there was a gradual decline with the minimum growth recorded at 10ppm level. At 6 ppm level of LAS, maximum biomass yield (109.5 mg/50mL), protein (587.5 µg/mL) and nitrate reductase activity (79.2 NR µM NO_2 /hr/g FW) were observed. Maximum content of chlorophyll-*a* (4.324 mg/g FW) and carotenoid (0.451 mg/g FW) were observed at 4 ppm level of LAS. The results thus, showed that *Spirulina platensis* is able to tolerate up to 6ppm of LAS in wastewater bodies.

INTRODUCTION

The two important chemical components of detergents, sodium tripolyphosphate (STPP) and linear alkyl benzene sulphonate (LAS) are the common contaminants in wastewater in most cities. Due to unplanned urbanization wastewater percolates in water bodies causing STPP and LAS to accumulate in varying concentrations in the water and aquatic biodiversity. The degree of accumulation may, however, depend on the physico-chemical characteristics of wastewater and genetic make-up of the species.

Spirulina is a blue green alga widely cultivated around the world for its nutritional value. It is used as a human dietary supplement as well as a whole food. It is also used as a feed supplement in aquaculture, aquaria, poultry and also as a biofertilizer. *Spirulina* is used as bioindicator of the LAS and STPP pollution and its population exhibits varied trends depending upon the concentration of these pollutants in aquatic ecosystems (Forlani et al. 2011). In order to assess the effect of different concentrations of LAS on the growth, biomass, protein content, nitrate reductase, photosynthetic pigments of *Spirulina*, an experimental study was conducted using CFTRI growth medium containing different levels of LAS (2, 4, 6, 8,10 ppm).

MATERIALS AND METHODS

Experimental Set-up

Twenty four Erlenmeyer flasks (500 mL) containing 200mL of nutrient medium and 2mL inoculum of *Spirulina platensis* were taken. The flasks were arranged in 6-series each containing 4-flasks. First series was kept as control and rest 5-series were treated with 2, 4, 6, 8, 10 ppm of LAS. The whole set-up was placed in screen house for observing growth and other physiological parameters.

Algal Collection

The *Spirulina platensis* was obtained from the stock available in the IARI, New Delhi. Repeated transfer to liquid CFTRI medium maintained the culture axenically. The composition of nutrient medium (g/L) for culturing *Spirulina platensis* is as: $NaHCO_3$ (4.5); K_2HPO_4 (0.5); $NaNO_3$ (1.5); K_2SO_4 (1.0); NaCl (1.0); $MgSO_4.7H_2O$ (1.2); $CaCl_2.2H_2O$ (0.04); $FeSO_4$ (0.1); pH-9.0

Growth Measurement and Biomass Estimation

The algal growth measurements were carried out in test tubes containing 5mL of nutrient medium and *S. platensis* inoculum. The growth was monitored at 2-day intervals by colorimetric analysis by measuring the optical density using distilled water as reference (APHA 1995).

Total biomass was estimated by filtering 50mL sample through optipure fibre glass filters using microfilter assembly through weight taken. After filtration, glass fibre filters

having *Spirulina* were sun dried at a temperature of 38°C for about 3-hours. The weights of fibre filters with algal cells were taken and finally biomass of algal cells was deduced (APHA 1995).

Estimation of Physiological Parameters

Protein estimation: Protein estimation was carried out following Lowry et al. (1951) through cell breakage by ultrasonication. Spectrophotometer Cecil CE2031 was used for the measurement of absorbance at λ = 660 nm.

Nitrate reductase activity: Nitrate reductase (NRA, EC.1.6.6.1) activity was measured by method of Hagerman & Hucklesby (1971) using KNO_3 as standard at 540nm.

Photosynthetic pigments: Pigment contents (chlorophylls & carotenoids) were analysed spectrophotometrically by adopting the non-macerating procedure described by Hiscox & Israelstam (1979) using Lambda 20-UV/Visible Spectrometer of Perkin Elmer make. The pigments were calculated by using the formula given by Arnon (1949).

Statistical Analyses

Results of the analyses were submitted to statistical analysis of least significant design (LSD) in order to verify significant differences among different treatments.

RESULTS AND DISCUSSION

Effect of LAS on the growth of *Spirulina platensis*: The specific data (Fig. 1) shows that the 5-treatments of LAS were given to the *Spirulina* culture and each of them were analysed for growth measurements after the interval of every 2nd day. The data show that in all the treatments the growth of *Spirulina platensis* was successful. All the treatments showed uniform (constant) growth during lag and log phases while during stationary phase the maximum growth was found in treatment LAS-2ppm and LAS-4ppm showing 2.39 and 2.21 respectively (LAS seems to be the source of carbon for cyanobacterium at lower levels) as compared to the treatment LAS-8ppm and LAS-10ppm showing minimum growth of 1.82 and 1.87 in the form of absorbance. Under treatment LAS-8ppm and LAS-10ppm *Spirulina* immediately showed the death phase i.e., 1.07 and 0.98 (also seen by visual observations). Higher concentrations of LAS seem to be toxic to the growth of *Spirulina*.

Carbon is the principal nutrient required by *Spirulina,* and in alkaline lakes this organism is the dominant species because of the presence of high concentrations of bicarbonates and carbonates. LAS has a linear alkyl chain of 10-14 carbon units which is consumed by the cyanobacteria i.e., bacteria are capable of chewing up the straight main branched chain of LAS. These environments contain significant quantities of heterotrophic microorganisms, which are capable of biodegrading a range of detergent chemicals, including LAS (Larson et al. 1989).

Variation of protein content in LAS treated culture: The data in Fig. 2 show that maximum concentration of protein is present in LAS 6-ppm. i.e., 587.5 µg/mL, and the minimum (350 µg/mL) in LAS-2ppm. The protein content increased from LAS-2ppm to LAS-6 ppm (350 350 µg/mL-587.5 350 µg/mL) and decreased in LAS-8ppm and 10ppm (506.2 350 µg/mL-431.2350 µg/mL). The decrease in protein contents might be due to lesser growth as well as biomass

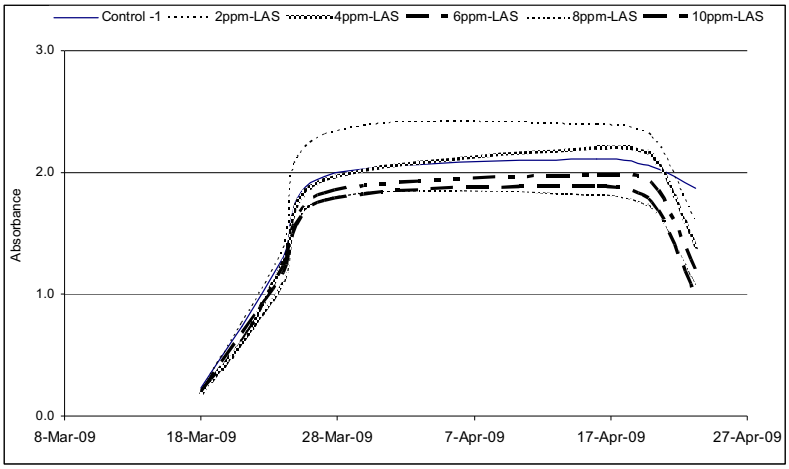

Fig. 1: *Spirulina* growth curve depicting the effect of LAS levels.

Table 1: Growth of *Spirulina platensis* as affected by different treatments of LAS in CFTRI medium.

Treatment	Biomass yield after10 days (mg/50 mL)	Chlorophyll-*a* content (mg/g FW)	Nitrate reductase activity (μM NO_2 /hr/g)	Total carotenoid (mg/g FW)	Protein content (μg/mL)
Standard CFTRI medium with P as KH_2PO_4 (Control)	69.2	3.489	67.6	0.350	575.0
Standard CFTRI medium with 2-ppm LAS	76.9	3.830	50	0.446	350.0
Standard CFTRI medium with 4-ppm LAS	78.9	4.324	50.4	0.451	362.5
Standard CFTRI medium with 6-ppm LAS	109.5	4.317	79.2	0.368	587.5
Standard CFTRI medium with 8-ppm LAS	88.1	3.480	51.6	0.333	506.2
Standard CFTRI medium with 10-ppm LAS	79.7	3.473	44.8	0.284	431.2
LSD (P≤0.05)	4.19	0.19	2.86	0.02	23.44

due to high LAS-8 and 10ppm treatments. The good biomass production also favours protein production that was found less in these two treatments (Rafiqul et al. 2005).

These results suggest that the protein content of blue green alga *S. platensis* changes due to the treatment of LAS (LAS treated *S. platensis* contains almost 25- 42% protein) as compared to the pure strains of *S. platensis* that contains 47% protein. Cifferri (1983) claimed that the culture conditions like temperature, light intensity and pH etc. were known to change the protein content of blue green alga *S. platensis*.

Activity of nitrate reductase in different levels of LAS: The enzyme activities of the alga under various treatments has been shown in Fig. 3, which shows that highest activity of nitrate reductase was present in LAS-6ppm (79.2 NR 350 μM NO_2 per hr/g FW), which seems to be obvious because the alga recorded maximum growth, maximum protein content and biomass in this treatment. On the other hand lower concentration of nitrate reductase activity was found in LAS-10ppm (44.8 NR μM NO_2 per hr/g FW) because the alga recorded minimum growth, protein and biomass in this treatment.

Variation of chlorophyll contents in different LAS levels: The data on the chlorophyll contents are presented in Fig. 4. Data show that chlorophyll-*a* content of *S. platensis* was highest in all the treatments as well as in control than chlorophyll-*b*, as the photosystems in cyanobacteria contain only chlorophyll-*a* and not chlorophyll-*b* and chlorophyll-*c*. A cyanobacterium is a prokaryote, and it is similar in performing photosynthesis as eukaryotes (Carr & Whitton 1982, Soundarapandian & Vasanthi 2008).

In the present study highest chlorophyll content (4.324 mg/g FW) was recorded in treatment LAS-4ppm, which is due to the fact that LAS acts as source of carbon at low treatments, and in turn yields good growth. The least chlorophyll content (3.473 mg/g FW) was recorded in treatment LAS-10ppm, which is due to the fact that higher dose of the LAS suppresses growth of the alga. Soundarapandian & Vasanthi (2008) also found chlorophyll-*a* content of five different cultures whose values support our results. However, chlorophyll-*b* and chlorophyll-*c* were also recorded but in very small amounts.

Abd El-Baky et al. (2008) have also reported total chlorophyll content of 7.69 ± 0.54 mg/g in the pure culture of *Spirulina maxima*. Our study has shown that total chlorophyll content of 5.61mg/g FW in pure culture of *Spirulina platensis*. Paoletti et al. (1980) reported chlorophyll content of *Spiru*lina as 8-15mg/g. Henrickson (1998) obtained 10 mg/g chlorophyll-*a* in *S. platensis* at the Earthrise Farm. In the present study chlorophyll content in pure culture (4.575 mg/g FW) was found lesser than these findings (Fig. 4).

Effect of LAS on the total carotenoid content: The carotenoid contents of *S. platensis* in LAS treated cultures are presented in Fig. 5. Highest carotenoid content (0.451 mg/g FW) was recorded in LAS-4ppm followed by 0.446 mg/g FW in LAS-2ppm. The least carotenoid content (0.284 mg/g FW) was observed in treatment LAS-10ppm.

Soundarapandian and Vasanthi (2008) also estimated the carotenoid content of five cultures of *S. platensis* whose values match with our results. Abd El-Baky et al. (2007) showed that *S. plantensis* stimulated the protective system in the form of carotenoids when grown under stress and the total carotenoid content of *S. platensis* was gradually increased under stress in dependency on concentration of LAS.

Biomass estimation in different LAS levels: The specific data presented in Fig. 6 show the 5-treatments of LAS given to *Spirulina platensis* and after full growth of 10 days, its biomass in 50 mL of suspension. The maximum biomass was found in LAS-6ppm i.e., 109.5 mg/50 mL, and minimum in LAS-2ppm i.e., 76.9 mg/50 mL. The biomass increases from treatment LAS-2ppm to LAS-6ppm, which is due to higher growth activity of the alga in these treatments. It decreases from treatment LAS-8ppm to LAS-10ppm, which seems to be obvious because of lower growth in these treatments, and its suppressing effect on the growth at higher treatment. Soundarapandian & Vasanthi (2008) also estimated the biomass of *S. platensis* in five different cultures at

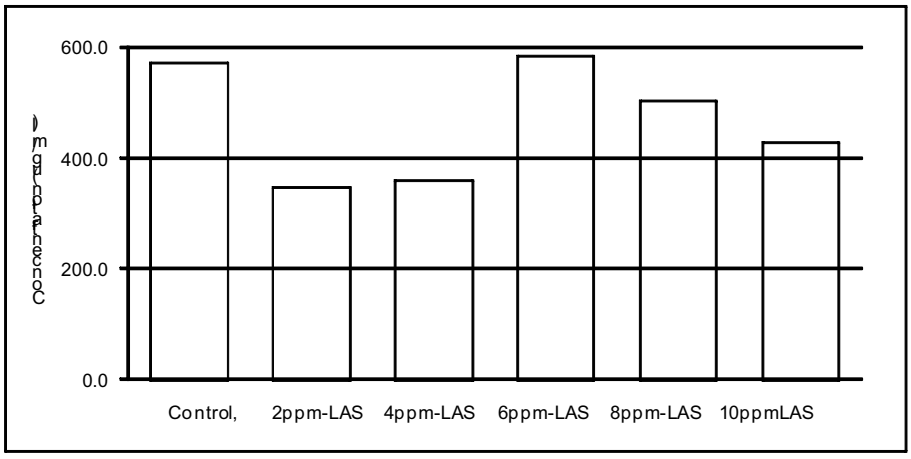

Fig 2: The effect of LAS levels on the total protein concentrations.

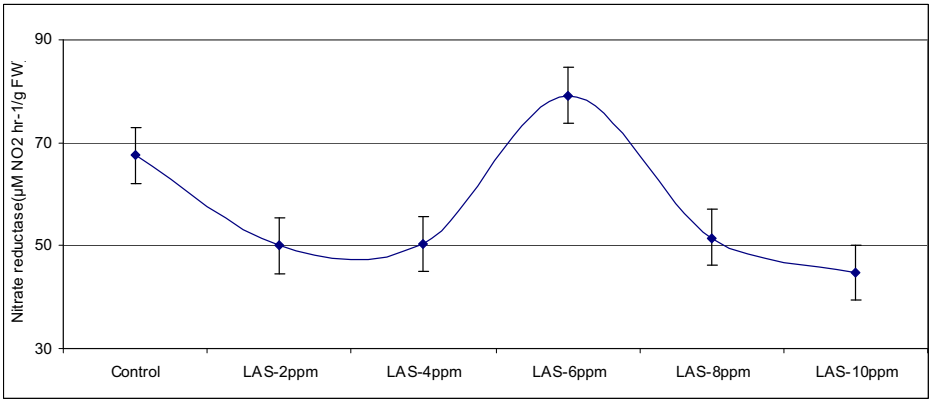

Fig 3: The nitrate reductase activity of *Spirulina platensis* in different treatments of LAS.

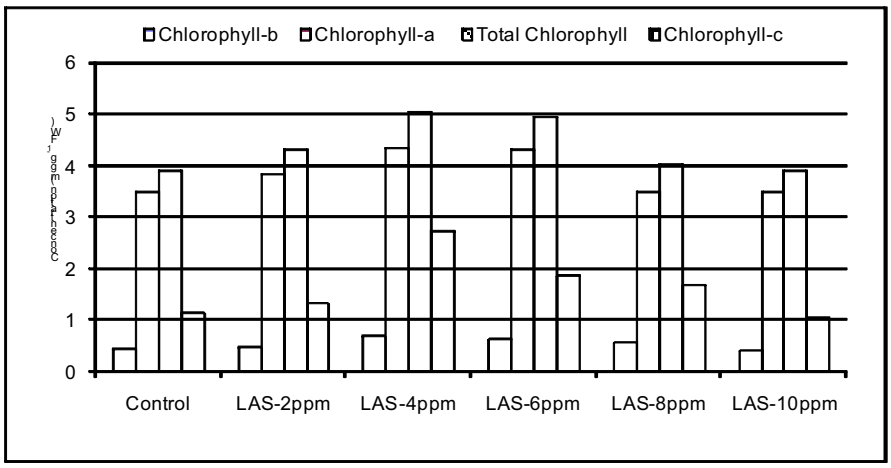

Fig 4: The effect of LAS levels on the chlorophyll content.

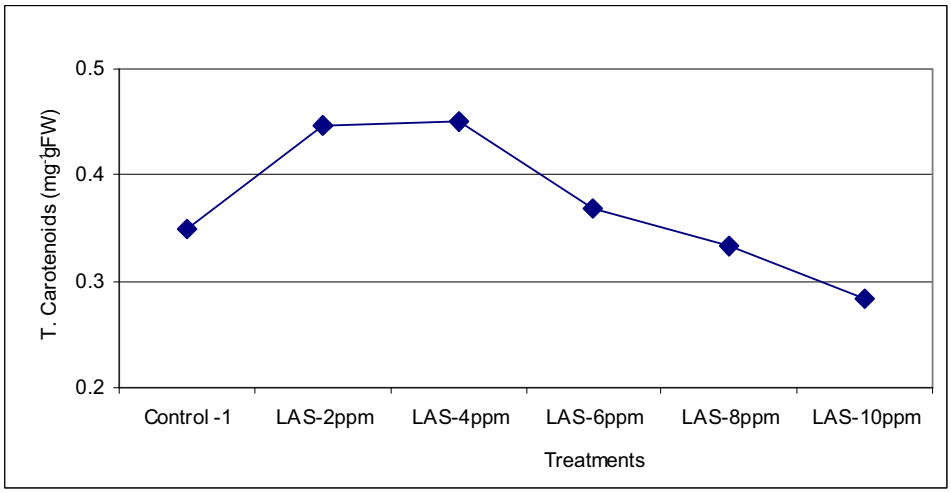

Fig 5: Effect of LAS levels on the total carotenoid content.

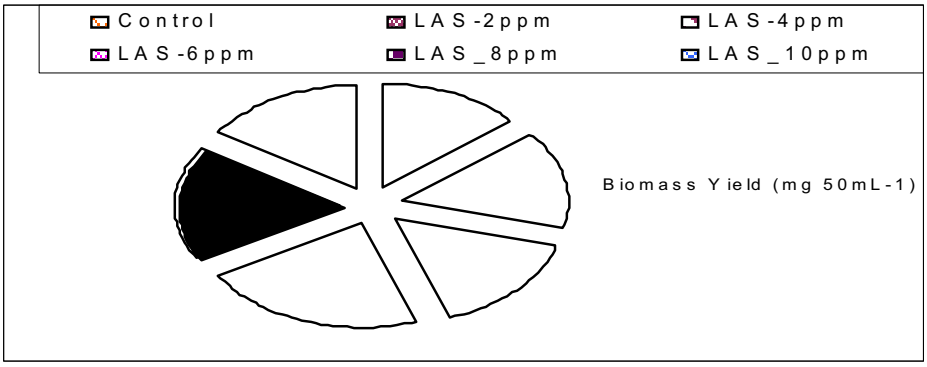

Fig. 6: Effect of LAS levels on the total biomass.

a regular interval of 10, 20, 30 days after inoculation and the biomass estimated was very less as compared to our results.

Also during the biomass estimation, the temperature was approximately 38°C. The higher biomass could be due to the increased activity of metabolic enzymes at that temperature, ultimately leading to higher biomass production, which is in conformity with the findings of Vonshak (1997).

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