SECTION 2

Studies on *Synechocystis salina* Wislouch

CHAPTER

7

Studies on the Marine Cyanobacterium *Synechocystis salina* Wislouch

7.1 Introduction

The phytoplankton constitute an important component of any ecosystem. These mainly include algae classified into various classes based on the pigment present (Chlorophyceae, Cyanophyceae, Rhodophyceae, etc.), diatoms and the like. With the help of their pigment system which may be chlorophyll *a, b, c* or both, the algae constitute the important primary producers of the ecosystem and manufacture carbohydrate for the rest of the community. Phototrophic primary producers encompass a wide variety of cyanobacteria and eukaryotic algae. Of these the cyanobacteria abound in the world water bodies, constitute more than 50% of the autotrophic biomass and are responsible for the total primary production in the euphotic zone of the open ocean. Cyano bacteria are characterised by the presence of chlorophyll a, biliproteins gram negative cell wall, share photosystems I, II and are capable of oxygenic photosynthesis (Boekema *et al.*, 2001).

The toxicities of detergents to aquatic flora have been studied in both fresh water and marine conditions. Reported data include the production of algal blooms following the input of phosphate containing detergents into the water bodies (eutrophication) and the disturbance of the nitrogen : phosphorus ratio (Mukherjee *et al.*, 1994). The lethal concentration for fresh water species for 72-96 hr were of the range 0.9-300 ppm for C_{10-14} LAS whereas for marine species LC_{50} was between 0.025-10 ppm. For sodium alkyl sulfates, the lethal concentration for 24-48 h was 4-32 ppm for marine species. Cationic surfactant toxicity for alkyl

(C_{12-18}) tri methyl ammonium chloride was 0.1-6.1 ppm (lab *in situ* studies) and for acetyl tri methyl ammonium bromide 0.03-2.6 ppm for fresh water species for 72-96 h whereas data are lacking for the toxicities of cationics and non ionics on marine species (Kutt and Martin, 1974). The commonly tested species for toxicity studies were *Selanastrum, Microcystis, Chlorella, Navicula* etc.

The present study deals with the evaluation of toxicities of three surfactants namely sodium dodecyl sulfate (anionic), Triton X-100 (non ionic) and cetyl tri methyl ammonium bromide (cationic) on the marine cyanobacterium *Synechocystis salina* Wislouch.

7.2 Materials and Methods

Surfactants – used were the anionic sodium dodecyl sulfate (SDS), non ionic Triton X-100 and the cationic cetyl tri methyl ammonium bromide (CTAB).

Test algae - The species used for the experiment was the marine cyanobacterium *Synechocystis salina* Wislouch belonging to the order Chroococcales (Waterbury, 1989). These are small spherical cells of diameter 3 microns with bluish-green colour. They are rarely seen in pairs and jerky movements are characteristic of the cells. Within each cell the thylakoids are peripheral in 4-5 concentric layers which contain chlorophyll *a*. On outer surface of the thylakoid there are regular rows of electron dense structures that carry phycobilisomes lodging the phycobilins viz. phycocyanin and phycoerythrin. Also polyhedral polyphosphate bodies are seen. In addition small structural granules representing lipid inclusions are seen scattered among the thylakoids. These reproduce by binary fission and divisions occur in 2 or 3 planes at right angles to one another or in irregular planes.

7.2.1 Culture Medium

The cells were cultured in Allen and Nelsons medium (1910)

Solution A	Potassium nitrate 2 M	
Solution B	Di Sodium hydrogen phosphate.12 H_2O	4 g
	Calcium chloride.6 H_2O	4 g
	Con HCl	2 ml
	Distilled water	80 ml

Autoclaved A and B, added 2 ml A and 1 ml B to 1 litre sea water (sterilised) of salinity 30 ppt. The pH was adjusted to 6 as it was found to be the most optimum for algal growth in preliminary experiment.

Preparation of the test solutions: The surfactant solutions were prepared in the culture medium and the pH was adjusted to 6 ± 0.2 with 0.1 N HCl or 0.1 N NaOH. The surfactant concentrations tested include 0.2, 0.4, 0.6, 0.8 and 1 ppm of all the three surfactants. These were sub lethal concentrations which were approx. 1/6 or 1/10 of the LC_{50} of the surfactants.

Inoculation: The inoculum was taken from a culture in the exponential phase of growth so as to avoid any lag period in growth. A definite volume of the culture was taken and the cells counted using a hemocytometer. The same volume of inoculum was added to the control as well as the surfactant added test tubes. The test and control cultures were maintained in test tubes of the size 12 × 18 mm. These tubes were kept in a slanting manner and illuminated constantly from above with fluorescent light of 3000 ± 100 lux intensity. The duration of the experiment was 14 days.

The biochemical parameters studied included:

1. Estimation of Growth
2. Estimation of chlorophyll *a*
3. Estimation of protein
4. Estimation of carbohydrate
5. Estimation of lipid

7.2.2 Estimation of Growth

Monitoring of algal growth was achieved by 2 ways- by cell count using a hemocytometer and by fluorescence of algal chlorophyll. A high degree of correlation (r = 0.99) was obtained between cell count and fluorescence in preliminary experiments. These measurements were made every 24 h after inoculation and at the end of the experimental duration. The growth inhibition was evaluated as the ratio of the growth in the surfactant dosed tubes to that in the control tubes and expressed as percentage.

7.2.3 Estimation of Chlorophyll *a*

The photosynthetic efficiency was assessed indirectly by estimating the amount of chlorophyll *a* by the method of Strickland and Parson (1972). The volume of cultures in the control and the surfactant dosed tubes was noted. The cultures were then filtered through a 4.5 cm Whatman GF/C paper, the filter was drained completely under suction before removing it from the filtration equipment. The peripheral excess of the paper was trimmed using a scissors. The filter was placed in a 15 ml centrifuge tube and 8 ml of 90% acetone was added. The tube was then stoppered and the filter was dissolved by shaking the tube vigorously. The pigments were allowed to get extracted by placing the tubes in a refrigerator in complete darkness for about 20 h. The tubes were then removed from the

refrigerator and allowed to warm up in the dark nearly to room temperature. Then 90% acetone was added so as to make up the volume of acetone in the tubes to 10 ml. The tubes were then centrifuged for 5-10 min. The clear supernatant liquid was drained into a test tube. The absorbance of this liquid was read at 665, 645 and 630 nm. The chlorophyll content was calculated from the equation

$$\text{Chlorophyll a} = 11.85 \times (E_{665}) - 1.54 \times (E_{645}) - 0.08 \times (E_{630})$$

The chlorophyll content was expressed as microgram/litre of the culture volume.

7.2.4 Estimation of Protein (Lowry *et al.*, 1951)

At the end of the experimental period, the control and surfactant dosed tubes were centrifuged at 600 g to obtain a pellet of the cultured cells. These were then transferred to small glass bottles and dried at 80°C till a constant dry weight was obtained (Abou-Waly *et al.*, 1991). The dry weights of the control and surfactant dosed culture tubes were noted.

The dried cell mass was mixed with 5 ml of 1 N NaOH and finely ground in a mortar. The alkaline solution was transferred into centrifuge tubes and kept in a water bath at 60-70°C with intermittent agitation for 20 min. Later the tubes were transferred to ice-cold water and cooled. The volume of the solution was measured and readjusted to 5 ml with distilled water. This was then subjected to centrifugation for 10 min at 600 g. 1 ml of the supernatant so obtained was used for protein estimation. The protein content was estimated from a calibration curve prepared by using bovine serum albumin as standard. The protein content was expressed in μg/mg dry weight.

7.2.5 Estimation of Carbohydrate (Carroll *et al.*, 1956)

The dry cell mass for the estimation was prepared as described earlier in 7.2.4. The weight of dry cell mass was noted. To this, 5 ml of 2.5 N HCl was added and hydrolysed by keeping in a boiling water bath for 3 h. It was then cooled to room temperature and neutralised with anhydrous sodium carbonate till effervescence ceased. Then the volume was made up to 10 ml and centrifuged. The supernatant after suitable dilution was used for analysis. To 1 ml supernatant, 4 ml anthrone reagent was added and placed in a boiling water bath for 10 min after capping with glass marbles. The absorbance was read spectrosphotometrically at 620 nm. The carbohydrate content was expressed in μ gm/mg dry weight.

7.2.6 Estimation of Lipid (Frings and Dunn, 1970)

The dried tissue was prepared as in 7.2.4 and the dry weight was noted. The dry cell mass was taken in a homogeniser and 10 ml chloroform - methanol was added, mixed well and filtered. To the filtrate 2 ml of sodium chloride was added and mixed well. It was then kept overnight at 4°C for lipid extraction. The lower phase containing the lipid was made up to 10 ml and then a definite aliquot of this was dried in vacuum desiccator overnight. To the tubes 0.5 ml of sulphuric acid was added and kept in a boiling water bath for 10 min. 0.2 ml of this acid digest was taken and 5 ml phosphovanillin reagent was added and incubated for 15 min. The absorbance was read at 520 nm. The lipid content was expressed in μ gm /mg dry weight.

7.3 Results

Growth inhibition studies: The results are given in Table 7.1. Of the 3 surfactants tested, the cationic surfactant CTAB was the most inhibitory. There was only 56.87% growth at 0.8 ppm of the cationic whereas at the corresponding concentration there was 81.38% and 94.46% growth in anionic and the non ionic surfactants respectively.

Table 7.1. Percentage growth in control and surfactant exposed *S. salina*

Concentration	SDS	TRITON	CTAB
0 ppm (Control)	100	100	100
0.2 ppm	87.22 ± 5.2	97.19 ± 6.5	87.36 ± 3.8
0.4 ppm	88.69 ± 7.3	95.47 ± 8.1	72.46 ± 4.1
0.6 ppm	83.25 ± 6.5	94.76 ± 7.2	63.39 ± 7.4
0.8 ppm	81.38 ± 4.1	94.46 ± 6.3	56.87 ± 5.6
1 ppm	79.71 ± 3.8	94.05 ± 4.7	26.99 ± 8.5

Values are the mean ± SD of six separate experiments expressed as %.

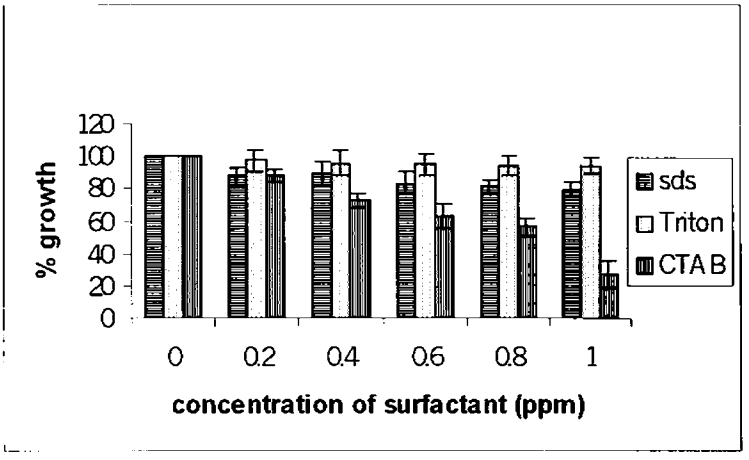

Figure 7.1. Percentage growth in *S. salina*

Table 7.1a. ANOVA for growth

Source of Variation	SS	df	MS	F	P-value	F crit
Between concentrations	1938.154	5	387.6309	2.418243	0.109999	3.33
Between surfactants	2467.982	2	1233.991	7.698276	0.009465	4.11
Error	1602.945	10	160.2945			
Total	6009.081	17				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Two way ANOVA showed that there was an overall significant difference between the surfactants with respect to growth inhibition ($F = 7.698$, $P<0.01$) (Table 7.1a). Subsequent LSD analysis (Table 7.1b) revealed that growth in CTAB treated cultures was significantly different from that of control ($P<0.01$) as well as SDS and TX-100 ($P<0.05$). However SDS and TX-100 caused similar inhibitions on growth which were insignificant.

Chlorophyll: The chlorophyll content in various cultures exposed to the surfactants are given in Table 7.2 and Figure 7.2.

Table 7.2. Chlorophyll content in control and surfactant exposed *S. salina*

Concentration	SDS	TRITON	CTAB
0 ppm (Control)	1.66 ± 0.01	1.66 ± 0.01	1.66 ± 0.01
0.2 ppm	1.45 ± 0.03	2.03 ± 0.04	1.47 ± 0.03
0.4 ppm	1.47 ± 0.02	1.51 ± 0.02	1.19 ± 0.02
0.6 ppm	1.38 ± 0.01	1.27 ± 0.03	1.17 ± 0.01
0.8 ppm	1.35 ± 0.04	0.88 ± 0.01	1.01 ± 0.02
1 ppm	1.32 ± 0.03	1.51 ± 0.03	0.77 ± 0.01

Values are the mean ± SD of six separate experiments expressed as µg/litre

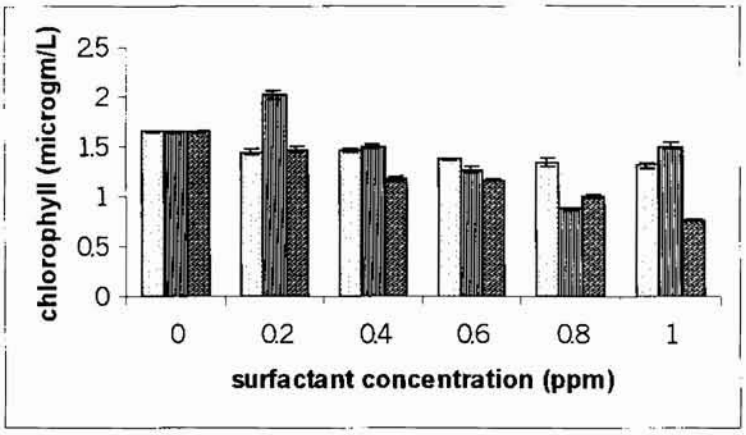

Figure 7.2. Chlorophyll content in *S. salina*

There was an overall significant change in the chlorophyll content between various concentrations of surfactants (Table 7.2a). LSD analysis (Table 7.6) revealed significant decrease in the chlorophyll content only at higher concentrations (0.8 and 1 ppm) of the surfactants ($P<0.05$) when compared to control. But there was no significant difference between the surfactants with respect to the decrease in chlorophyll content. Cationic surfactant was the most inhibitory where 50% reduction of chlorophyll was obtained between 0.8 and 1 ppm. However the reduction in chlorophyll content was not strictly concentration–dependent. For example in case of Triton dosed cells, there was only 53% chlorophyll at 0.8 ppm whereas at 1 ppm there was more of the pigment (90%).

Table 7.2a. ANOVA for chlorophyll

Source of Variation	SS	df	MS	F	P-value	F crit
Between concentrations	0.824494	5	0.164	3.254655	0.05299	3.325
Between surfactants	0.041144	2	0.020	0.40604	0.67679	4.102
Error	0.506656	10	0.050			
Total	1.372294	17				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Protein: Protein content in the control and the surfactant treated cultures are given in Table 7.3 and Figure 7.3.

Table 7.3. Protein content in control and surfactant treated *S. salina*

Concentration	SDS	TRITON	CTAB
Control (0 ppm)	20.6 ± 4.3	20.6 ± 4.3	20.6 ± 4.3
0.2 ppm	13.55 ± 2.3	9.74 ± 2.8	16.85 ± 3.1
0.4 ppm	16.76 ± 3.1	8.22 ± 1.8	14.4 ± 2.2
0.6 ppm	27.67 ± 4.2	7.1 ± 2.4	12.75 ± 3.0
0.8 ppm	5.28 ± 0.2	2.23 ± 0.1	11.59 ± 2.6
1 ppm	13.62 ± 3.1	3.23 ± 0.3	8.95 ± 3.1

Values are the mean ± SD of six separate experiments expressed as μg / mg dry weight

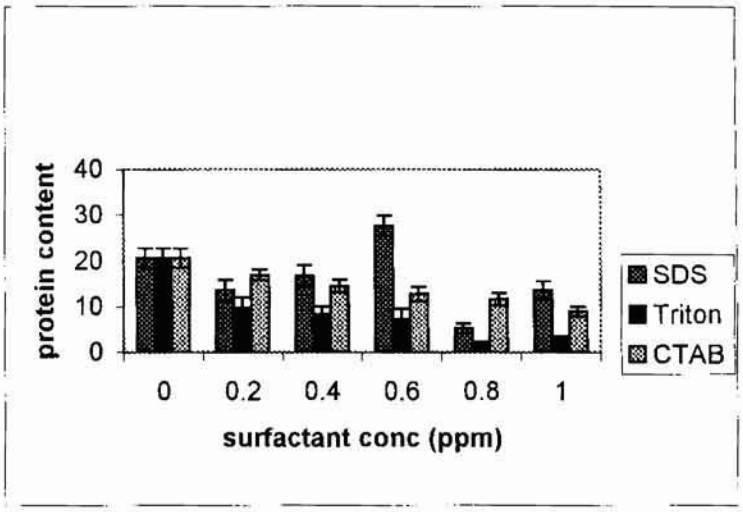

Figure 7.3. Protein content in *S. salina*

In the cationic surfactant dosed cells there was a significant concentration dependent decrease in the protein content (Table 7.3a). In SDS dosed cultures there was stimulation in protein content at 0.6 ppm and maximum inhibition was noted at 0.8 ppm, but here the inhibition was not concentration dependent. Triton dosed cultures showed no specific trend with respect to the protein content and cultures exposed to 0.8 ppm had the least protein content.

Table 7.3a. ANOVA for protein

Source of Variation	SS	df	MS	F	P-value	F crit
Between concentrations	388.0377	5	77.60754	3.925969	0.03135	3.325837
Between surfactants	192.1603	2	96.08016	4.860452	0.033525	4.102816
Error	197.6774	10	19.76774			
Total	777.8754	17				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Two way ANOVA reflected significant differences between the surfactants as well as the various concentrations with respect to protein content ($P<0.05$) (Table 7.3a). LSD analysis (Table 7.6) revealed significant differences ($P<0.05$) in protein content in all surfactant treated cultures (except SDS) when compared to control. Protein content in Triton exposed cultures were significantly decreased ($P<0.05$) when compared to SDS and CTAB. It was also observed that only higher surfactant concentrations of 0.8 and 1 ppm caused significant inhibition ($P<0.01$) of protein synthesis.

Carbohydrate: Carbohydrate content in the cultures are given in Table 7.4.

Table 7.4. Carbohydrate content in *S. salina*

Concentration	SDS	TRITON	CTAB
Control (0 ppm)	854.88 ± 29.58	854.88 ± 29.58	854.88 ± 29.58
0.2 ppm	1055.77 ± 45.6	279.12 ± 13.59	1120.65 ± 51.1
0.4 ppm	537.72 ± 30.6	332.97 ± 15.7	1453.63 ± 50.7
0.6 ppm	388.97 ± 38.9	504.72 ± 21.8	883.68 ± 28.8
0.8 ppm	295.79 ± 29.9	551.39 ± 25.7	1024.57 ± 32.3
1 ppm	395.98 ± 40.2	677.83 ± 33.9	992.51 ± 31.6

Values are the mean ± SD of six separate experiments expressed as µg / mg dry weight

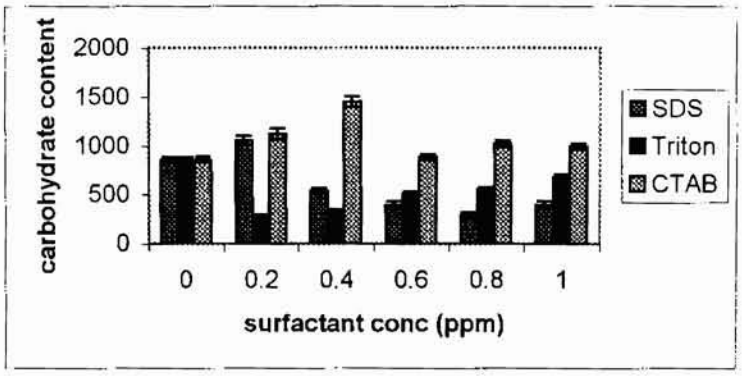

Figure 7.4. Carbohydrate content in *S. salina*

Here there was no concentration dependent decrease. On the contrary a stimulation was noted in Triton treated cultures at concentrations of 0.2, 0.4, 0.6, 0.8 and 1 ppm. In SDS dosed cultures, there was a decrease in 0.4, 0.6, 0.8 and 1 ppm which was not concentration dependent whereas there was stimulation at 0.2 ppm.

Table 7.4a. ANOVA for carbohydrate

Source of Variation	SS	df	MS	F	P-value	F crit
Between concentrations	171561.2	5	34312.24	0.45719	0.79	3.32
Between surfactants	985719.7	2	492859.9	6.56706	0.01	4.10
Error	750503.1	10	75050.31			
Total	1907784	17				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

It was observed from two way ANOVA (Table 7.4a) that there were significant differences between the surfactants with respect to the carbohydrate content ($P<0.01$). Carbohydrate content in CTAB was significantly higher

($P<0.05$) than that in control, SDS and TX-100. But there were no significant changes between various concentrations of the surfactants with respect to the carbohydrate content.

Lipid: Lipid content in control and surfactant treated cultures are given in Table 7.5 and Figure 7.5.

Table 7.5. Lipid content in *S. salina*

Concentration	SDS	TRITON	CTAB
Control (0 ppm)	542.22 ± 31.2	542.22 ± 31.2	542.22 ± 31.2
0.2 ppm	589.98 ± 35.6	85.29 ± 31.1	275.66 ± 35.1
0.4 ppm	741.89 ± 40.1	83.77 ± 29.9	481.11 ± 25.6
0.6 ppm	456.26± 21.6	1486.38 ± 68.2	272.09 ± 11.8
0.8 ppm	254.99 ± 13.7	1511.05 ± 52.5	351.9 ± 13.5
1 ppm	193.07 ± 10.7	1501.94 ± 50.7	160.76 ± 10.7

Values are the mean ± SD of six separate experiments expressed as μg / mg dry weight

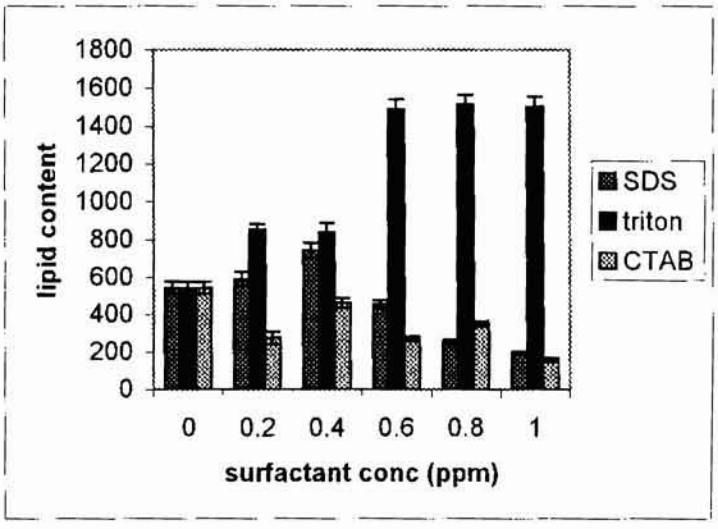

Figure 7.5. Lipid content in *S. salina*

Table 7.5a. ANOVA for lipid

Source of Variation	SS	df	MS	F	P-value	F crit
Between concentrations	91893.8	5	18378.76	0.16	0.970	3.32
Between surfactants	2095724	2	1047862	9.16	0.005	4.10
Error	1143841	10	114384.1			
Total	3331458	17				

SS – sum of squares, df – degrees of freedom, MS – mean of squares.

Two way ANOVA revealed an overall significant change in lipid content in experimental cultures (Table 7.5a). But no significant changes were noted between various concentrations of surfactants. Lipid content was significantly increased ($P<0.01$) in Triton treated cultures when compared to control, SDS and CTAB.

Table 7.6. Results of LSD analysis for growth, chlorophyll, carbohydrate, protein and lipid in *S. salina*

Groups	Growth	Chlorophyll	Carbohydrate	Protein	Lipid
Control vs SDS	NS	P<0.05	P<0.05	P<0.01	NS
Control x Triton	NS	P<0.05	NS	P<0.01	P<0.01
Control x CTAB	P<0.01	P<0.05	NS	P<0.01	NS
SDS x Triton	NS	NS	NS	P<0.05	P<0.01
Triton x CTAB	P<0.05	NS	P<0.01	P<0.05	P<0.01
SDS x CTAB	P<0.05	NS	P<0.01	NS	NS

NS – Not significant

7.4 Discussion

It may be deduced that among the three surfactants CTAB inhibited the growth of the cyanobacterium in a significant and concentration dependent

manner. But there was no significant inhibition of growth in anionic and non ionic surfactant treated cultures.

Photosynthesis in cyanobacteria is principally carried out by the major pigment chlorophyll *a*. The integrity of chlorophyll-lipid-protein complexes in the thylakoid membranes of the autotrophic species is inevitable for effective conversion of light energy to carbohydrate. In the present study, chlorophyll *a* levels were significantly decreased in a concentration-dependent manner in all concentrations of surfactant-treated groups with maximum of 46.54% inhibition in 1 ppm of CTAB. Whereas in Triton X-100, a stimulation of chlorophyll a levels was observed in cultures containing 0.2 ppm surfactant. Here the decrease in chlorophyll a was not concentration -dependent. Maximum decrease was noted in 0.8 ppm. In the anionic SDS the maximum inhibition was noted at 1 ppm though the decrease in chlorophyll content was not concentration-dependent.

This inhibition of photosynthesis might be due to the internalisation of the surfactants which can then impact the photosynthetic processes by making the chlorophyll-protein matrix more water soluble (Hicks and Neuhold, 1966; de Alda *et al.*, 1995). The penetration of the lamellae of the thylakoids by the surfactants would result in their orientation with the similarly charged polar chlorophylls and phospholipids. Transport of light energy may then be blocked as a result of their incorporation in the chlorophyll molecules, thus causing photooxidation.

Protein content was significantly decreased at higher concentrations of all surfactant treated cultures. In CTAB dosed cultures, there was concentration-dependent decrease in protein content with maximum inhibition at 1 ppm. In Triton dosed cultures, the maximum inhibition was noted at 0.8 ppm and 1 ppm. In the anionic and non ionic dosed cultures, the decrease in the protein content was not concentration-dependent. It is thought that this decrease in protein content

might be due to the membrane damage caused by the surfactants. Similar decrease in protein content was observed by Chawla *et al.* (1988) in Nostoc filaments grown in LAS in the range of 1-50 ppm.

There was an increase in the carbohydrate content in all concentrations of CTAB dosed cultures with maximum increase at 0.4 ppm. In Triton dosed cultures there was a concentration dependent increase in the carbohydrate content. But in SDS treated cultures there was a stimulation of the carbohydrate content at 0.2 ppm but decrease in all other concentrations. The increase in carbohydrate might be the response to meet the stress imposed due to surfactant exposure.

Lipid content was significantly decreased in SDS dosed cultures at all concentrations except 0.4 ppm where an increase was observed. In Triton dosed cultures, there was a reduction in the lipid content at 0.2 and 0.4 ppm whereas a highly significant increase was noted at higher concentrations of 0.6, 0.8 and 1 ppm. In CTAB exposed cultures there was a decrease in the lipid content at all concentrations, the maximum decrease was noted at 1 ppm. However this decrease was not linear with increasing concentrations of the surfactant. A change in the sterols of the red algae *Porphyridium purpureum* on exposure to detergents was observed by Nyberg and Saranpaa (1989). They have reported significant decrease in 22-dehydro cholesterol in the algal cultures exposed to the anionic LAS. And exposure to non ionic Triton X-100 at concentrations of 10 ppm resulted in an increase in cycloartenol. Here too the results were not linear with increasing detergent concentrations. This non linear pattern obtained for various biochemical parameters studied seems to be a feature often encountered in algae exposed to surfactants.

Extensive studies of toxicity tests on non ionics like polyoxy alkylene block co polymers was done by Smulikowska (1984) on the alga *Scenedesmus*

quadricauda and bacteria *Sphaerotilus natans*. A wide concentration range of 0.1 to 10000 ppm was studied. The results were difficult to interpret as it was not linear with respect to the concentrations. Thus very often at higher concentrations, lesser inhibition rates were obtained or sometimes stimulation.

The increased toxicity of cationic surfactants to algae has been attributed to the interaction of these chemicals with negatively charged moieties on the cell surface. In addition, lipid concentration in algal cell wall has a significant role in toxicity as more hydrophobic surfactants are readily solubilised in algal lipids. A positive correlation between surfactant hydrophobicity and increased algal toxicity has been demonstrated (Ukeles, 1965).

The cyanobacteria being Gram negative has an outer lipopolysaccharide (LPS) layer. The lipid constituents of LPS might have facilitated the entry of the hydrophobic surfactant viz. CTAB. Thus it may be inferred that the cationic surfactant was the most inhibitory to the cyanobacterium under study.

LITERATURE REVIEW FOR SECTION 2

TYPE OF SURFACTANT	ORGANISM STUDIED	EFFECTS OBSERVED	REFERENCE
Linear alkyl benzene sulfonate (LABS)	Freshwater and marine algae	LC 50 was 0.9-300 ppm for fresh water and that for marine- 0.025-10 ppm	Holt et.al,1992
Alkyl sulfates	Freshwater and marine algae	LC 50 was 4-30 ppm for freshwater and that for marine 1-2 ppm	Holt et.al,1992
Linear alkyl ethoxylates	bacteria	Adaptive response on prolonged exposure	Ventullo et.al, 1989
Anionics (ABS, AES, AOS)	*Selanastrum, Microcystis, Nitzschia*	Reduction of specific growth rate, Reduction in growth not always linear with the surfactant concentrations tested	Atsuko et.al, 1984
Anionic surfactant	*Gymnodinium breve*	Decrease in growth rate	Kutt & Martin, 1974
Cetyl pyridinium bromide (CPB)	Freshwater algae	inhibition of growth	Matulova , 1964
LABS, Triton X-100, Betaine	*Porphyridium*	Decrease in 22-dehydro cholesterol	Nyberg & Saraanpa, 1989
LABS	*Rhodomonas salina, Skeletonema costatum*	Decreased rate of photosynthesis	Petersons & Kokusk, 2000
Corexit	*Isochrysis galbana*	Increases bioavailability of naphthalene from water accommodated fraction of crude oil	Wolfe et al, 1998
Triton X-100	*Synechococcus* WH 7805, WH 8103	Growth activated at lower concentrations and inhibited at high concentrations	Waterbury & Ostroumov, 1994
Alcohol ethoxylates	Algae, microbes, protozoa, invertebrates	Inhibition of growth	Belanger et.al, 2000
LAS	*Nostoc*	Decrease in protein content	Chawla et.al, 1988
Poly oxy ethylene co block polymers	*Scenedesmus*	stimulation or inhibition of growth independent of the concentrations.	Smulikowska 1984