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Interaction of *Spirulina platensis* with starchy effluent

M. Boominathan*[1] and C. Manoharan[2]

[1]P.G. and Research Department of Biotechnology, Maruthupandiyar College, Vallam Post, Thanjavur-613 403, Tamil Nadu, India.
[2]P.G. and Research Department of Botany & Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur District, Tamil Nadu, India

Abstract

In order to develop an efficient and integrated waste treatment and recycling process, as a preliminary step, economically important cyanobacterium, *Spirulina platensis* was tested to treat the starchy effluent. Conversely, the effect of starchy effluent on the cyanobacterium has also been studied. Initial physico-chemical analysis of the effluent showed the presence of higher content of phosphorus, calcium and magnesium along with alkaline pH. Treatment of effluent by *Spirulina* reduced the above chemical constituents of the effluent to a considerable amount and brought down the pH to near neutrality and increased dissolved oxygen content from 1.4 to 6.7 mgl^{-1}. On the other hand, the effect of effluent on the biochemical constituents of *Spirulina* was strikingly different. Level of carbohydrate was found to be reduced in the effluent treated cyanobacterium when compared to control. Some of the fatty acids reported in the control were missing in the effluent treated cyanobacterium, while some other fatty acids which were not found in the control were recorded in the effluent treated *Spirulina*.

Keywords : fatty acid, physico-chemical characters, *Spirulina*, starchy effluent, TLC

INTRODUCTION

With increasing growth of urban population and industrialization, the demand for clean water has been on the increase. This warrants immediate and effective steps to treat the ever increasing waste water generated from a variety of sources. In recent years, the importance of biological waste treatment system has caught the attention of researchers all over the world and has helped in the development of relatively efficient, low cost waste treatment systems. Microalgal cultures offer interesting alternatives for waste treatment as secondary or tertiary biotreatments to remove inorganic nutrients, such as nitrogen and phosphorus, while producing potentially valuable biomass.

Usefulness of cyanobacterium (*Oscillatoria*) in the treatment of domestic sewage as well as the industrial effluents such as ossein, paper mill etc., and their reciprocal effects on the physiology and biochemistry of the cyanobacterium has amply been understood (Manoharan and Subramanian, 1992 a & b). But works on the treatment of industrial effluents using *Spirulina* are very little. *Spirulina platensis,* a microalga which has recently been included in the bacterial classification as cyanobacteria, has lot of medicinal properties. Recycling of waste materials using *Spirulina* should not only minimize pollution problem of water, but also revitalize the inherently rich nutrients of waste. The biomass obtained from cultivation of *Spirulina* in the waste water media may be used as a pigment protein supplement in animal feed and as raw materials for certain chemicals.

Of the different effluents, effluent from the starchy industry, because of its white colour and chemical constituents, creates lot of pollution problems when it is let into the surrounding water bodies and contaminate the entire ecosystem. The treatment of this effluent using cyanobacteria especially *Spirulina* has not been studied so far. By keeping all these in mind, an attempt has been made to study the interaction of *Spirulina platensis* with starchy effluent.

MATERIALS AND METHODS

The effluent sample was collected from a starch factory, Namagiripettai, Namakkal district, Tamil Nadu, India. The organism used for the study was the freshwater cyanobacterium *Spirulina platensis*, obtained from Indian Agricultural Research Institute (IARI), New Delhi, subcultured and maintained in *Spirulina* medium at 27 ± 2°C in an illuminated culture room. As the organism failed to grow in the effluent as such (undiluted effluent), the effluent was diluted to 50 per cent. Initial physico-chemical analyses of the effluent were made following the Standard Methods (APHA, 1975).

EXPERIMENTAL CONDITIONS

The following treatments were employed in order to study the interaction of *Spirulina* with the effluent: (1) Effluent (50 % diluted) inoculated with *Spirulina* - as treatment; (2) Effluent (50 % diluted) uninoculated – as control for physico-chemical analysis and (3) *Spirulina* medium inoculated with *Spirulina* – control for

*Corresponding Author
email: *c_manoharan2004@yahoo.com*

biochemical studies. Inoculation was made by 2.0 ml of uniform suspension of *Spirulina*. The experiment was conducted under controlled conditions (Temperature 27 ± 2°C with light intensities of 1500 lux provided from overhead cool white fluorescent tubes) for one month (since the growth of *Spirulina* was very slow in the effluent, a long duration was provided to get a culture of exponential growth). The cultures were harvested on 30th day by filtration through ordinary filter paper and washed repeatedly with distilled water. The filtered effluents (inoculated and control) were used for physico-chemical analyses. *Spirulina* obtained both from the medium and effluent were used for the biochemical studies *viz.,* estimation of pigments (Mackinney, 1941), carbohydrates (Dubois *et al.*, 1956), total proteins (Lowry *et al.*, 1951), free amino acids (Jayaraman, 1981), total lipids (Sato and Murata, 1988) and fatty acids (Miller and Berger, 1985).

RESULTS AND DISCUSSION

Initial pH of the effluent was 9.5. After 30 days, the pH of the *Spirulina* treated effluent was brought down to 7.4, contrary to the pH of control (9.1) (Table 1). This showed that the typical capacity of cyanobacteria to bring about changes in the pH to suit their requirement.

High amounts of CO_2 and HCO_3 (Table 1) were noted in the effluent. But there was no carbonate even on the 30th day. The microalga seems to be able to take up free CO_2 which is extensively utilized as a substrate for RuBP carboxylase and some microalgae utilize HCO_3 (Beardall *et al.*, 1976). Cyanobacteria are capable of utilizing HCO_3^- as a source of inorganic carbon for photosynthesis and they have high CO_2 affinity and low CO_2 compensation point (Colman, 1989). This may be due to the dehydration activities of carbonic anhydrase to produce CO_2. In the present study, effective removal of these carbon sources by *Spirulina* was observed.

There was a low level of dissolved oxygen (DO) in the starchy effluent. An increase in DO content was noted on 30th day in the effluent inoculated with *Spirulina* (Table 1). This increase in DO content was due to the photosynthetic activity of the *Spirulina*. Kalisz (1974) also reported that algae are the producers of oxygen through the process of photosynthesis. A similar increase in DO content has already been noted with different effluents inoculated with cyanobacteria (Manoharan and Subramanian, 1992 a&b, 1993a).

Nitrogen and phosphorus are the main nutrients of cyanobacteria. That is why cyanobacteria could effectively remove these nutrients from the effluent. Moreover they have high nutrient uptake capabilities as they can accumulate inorganic phosphate and nitrogen and store them as phosphate and cyanophycin, respectively (Fay, 1983). Both free and immobilized cyanobacteria in the efficient removal of different forms of combined nitrogen and phosphorus was also reported earlier (Proulx and De la Noue, 1988). Removal of nitrogen and phosphorus from different effluents, using *Oscillatoria*, has been reported already (Manoharan and Subramanian, 1992 a&b, 1993a). Efficient nitrogen and phosphorus uptake by *Phormidium bohneri* from the effluent has also been reported when temperature is in the range of 20-30°C (Blier *et al.*, 1995; Sylvestre *et al.*, 1996). In the present study, a substantial removal of nitrite and almost a complete removal of nitrate in the *Spirulina* treated effluent was observed (Table 1). Though, a complete removal of phosphate from the effluent treated with *Spirulina* was not observed, atleast 50 per cent removal was noted when compared to control. This might be due to the initial high level of phosphates in the effluent. The capacity of cyanobacteria to remove large amount of phosphorus from waste waters was demonstrated by several earlier researchers also (Chan *et al.*, 1979; Tam and Wong, 1989; Tadros and Phillips, 1992; Manoharan and Subramanian, 1992 a&b, 1993a).

Besides the macronutrients, algae require a sufficient supply of micronutrients such as calcium, magnesium, manganese and potassium for their vigorous growth. In the present study, both calcium and magnesium were found to be removed considerably from the *Spirulina* treated effluent. Although calcium and magnesium are undoubtedly required for growth (Fogg *et al.*, 1973), substantial reduction in calcium and magnesium levels cannot be explained by uptake. The property that divalent cations such as calcium and magnesium are known to be essential for flocculation and would coflocculate (Richmond and Becker, 1986) could explain their observed reduction.

Chlorides are generally considered to be one of the major pollutants in effluents which are difficult to be removed by conventional biological treatments. In the present investigation substantial removal of chloride level was not observed in *Spirulina* treated effluent (Table 1).

Inoculated cyanobacteria are known to grow fairly efficiently in different types of effluents (Sallal, 1986; Manoharan and Subramanian, 1992 a&b, 1993a). Growth in the present study was measured interms of biomass (Fig.1). Though *Spirulina* grow fairly well in the starchy effluent, its growth in the control was far better than in the effluent. This slow growth or low biomass content might be due to high CO_2 concentration in the effluent (Table 1) as Gordillo *et al.* (1999) observed that high CO_2 and N limitation showed the lowest yield of *Spirulina platensis*.

Pigments such as chlorophyll-a, carotenoids, phycocyanin, allophycocyanin and phycoerythrin showed a substantial reduction in the effluent treated *Spirulina* than in control (Fig.2). This could be due to higher concentration of CO_2 and low nitrogen level in

Table 1. Physico-chemical characteristics of initial, control and *Spirulina platensis* treated starchy factory effluents

Characteristics	Initial	Control	Treated
Colour	White	White	Colourless
pH	9.5	9.1	7.4
Carbon-di-oxide	66	66	44
Carbonate	Nil	Nil	Nil
Bicarbonate	900	900	200
Dissolved oxygen	1.4	2.2	6.7
Nitrate	0.184	0.184	0.011
Nitrite	8.21	8.21	4.11
Total phorphorus	220	220	127
Inorganic phosphorus	178	178	80
Organic phosphorus	48	48	42
Calcium	643	643	330
Magnesium	1027.20	1027.20	630.00
Chloride	39.99	38.99	38.79

Except colour and pH, all the other parameters are mgl^{-1}.

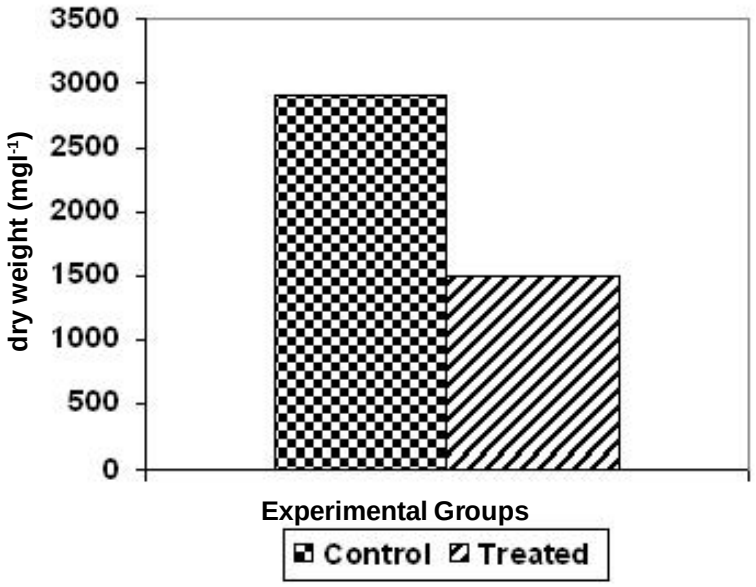

Figure 1. Biomass content in *S. platensis* when grown in starchy effluent (treated)

Table 2. Qualitative and quantitative analysis of fatty acids in *Spirulina platensis* (mg/g lipid) grown in control medium and starchy effluent

Fatty acids	Control	Treated
Capric acid (C:10)	37.8950	2.6503
Lauric acid (C:12)	Nil	1.0671
Tridecanoic acid (C:13)	3.1111	2.0207
Myrestic acid (C:14)	4.3378	1.2014
Pentadecanoic acid (C:15)	0.0193	0.0251
Palmitic acid (C:16)	0.0817	6.4696
Heptadecanoic acid (C:17)	2.3095	Nil
Stearic acid (C:18)	0.2454	0.1741
Non-decanoic acid (C:19)	6.3840	0.0324
Arachidic acid (C:20)	Nil	13.1837
Heneicosanoic acid (C:21)	Nil	0.1152
Palmitoleic acid (C:16.1)	59.8237	1.9274
Oleic acid (C:18.1)	0.9503	2.5716
Cis linoleic acid (C:18.2)	9.5826	2.0714
Linolenic acid (C:18.3)	40.2993	Nil

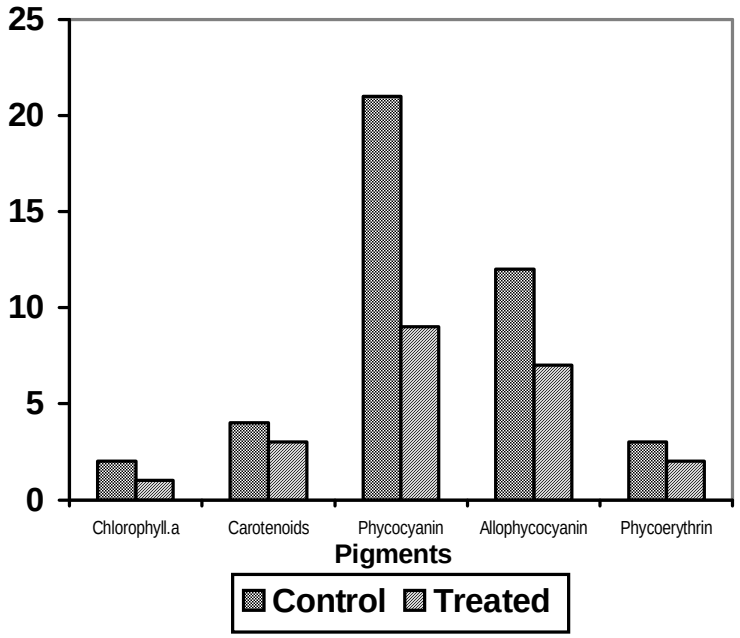

Figure 2. Pigments level in *S. platensis* when grown in starchy effluent (treated)

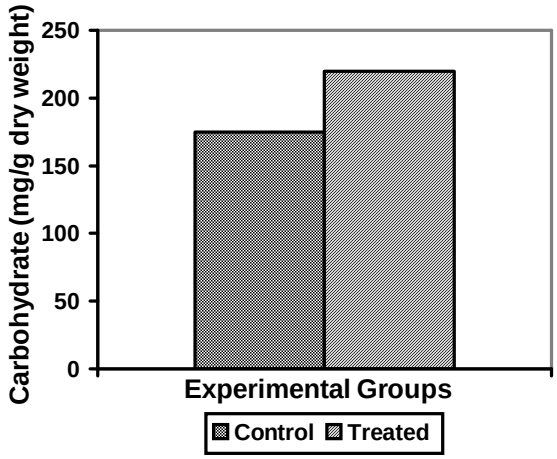

Figure 3. Total carbohydrate level in *S. platensis* when grown in starchy effluent (treated)

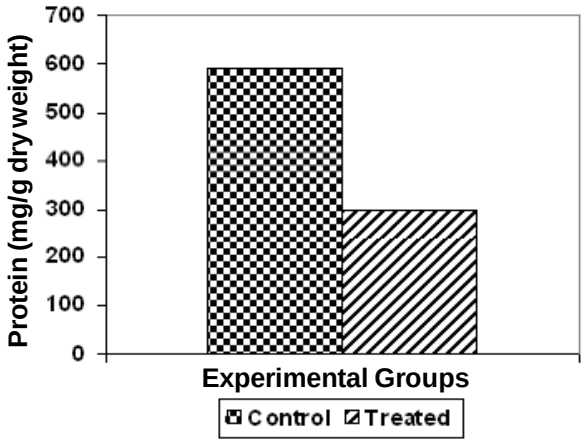

Figure 4. Total protein level in *S. platensis* when grown in starchy effluent (treated)

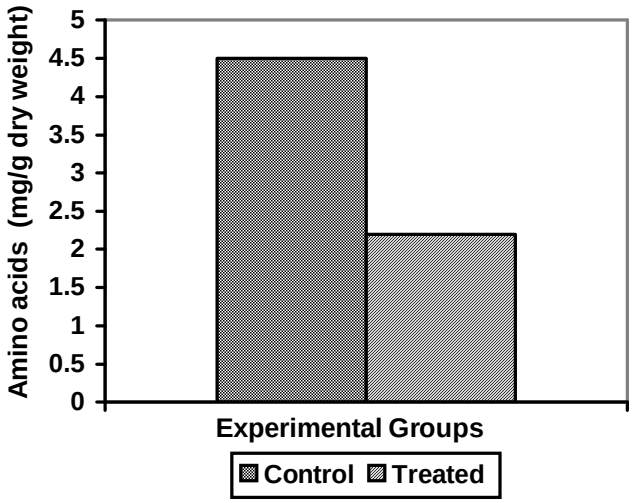

Figure 5. Free amino acid level in *S. platensis* when grown in starchy effluent (treated)

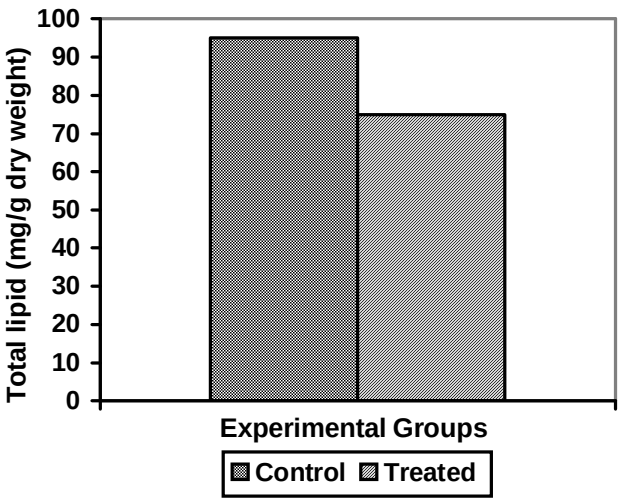

Figure 6. Total lipid level in *S. platensis* when grown in starchy effluent (treated)

the effluent (Table.1). Increase in CO_2 in the medium and limited nitrogen supply were reported to cause significant decrease in chlorophyll-a (20-25 %), carotenoid (50 %) and phycocyanin (14 %) by Gordillo *et al.* (1999) also. Decrease of pigment content and protein are a typical response in N limited algae (Turphin, 1991). Nitrogen limitation caused photoassimilated carbon to be redirected towards the synthesis of carbohydrates instead of protein and chlorophyll. This response has been widely observed in many algal species (Turphin, 1991). Protein and chlorophyll decrease and carbohydrate increase by CO_2 enrichment have been previously observed in a number of species (Lochle, 1995). These reports are in accordance with the present investigation, where there was an increase in carbohydrate level in the effluent treated *Spirulina* than in the control (Fig. 3) and conversely, there was a reduction in protein and pigment contents in the cyanobacterium treated with starchy effluent (Figs. 2 & 4).

The total protein level in the effluent treated *Spirulina* showed a reduction compared to the control (Fig.4). Similar results were also observed with regard to free amino acid level (Fig.5). This could probably be due to very low amount of combined nitrogen and increase in CO_2 concentration in the effluent and is further supported by a substantially higher level of carbohydrate accumulation. Similar observations were made in *Oscillatoria* treated with different effluents (Manoharan and Subramanian, 1992 a & b). Environmental stress induced modification of proteins resulting in the inhibition of the synthesis of several proteins or enhancement of certain others has been reported already in Cyanobacteria (Apte *et al.*, 1987; Bhagwat and Apte, 1989).

An increase in total carbohydrate is not only reflected in the reduction of total proteins and amino acids but also in the reduction of lipids (Fig. 6). Similar observations were made when *Oscillatoria* treated with different effluents also (Manoharan and Subramanian, 1992 a and b). Variation in lipid contents and composition under different environmental conditions including variations in light and dark incubation have been observed in a number of cyanobacteria (Al-Hasan *et al.*, 1989). The reduction in lipid content (Fig.6) with effluent treated *Spirulina* when compared to the control and differences in the classes of lipids between control and effluent treated cyanobacteria, had been observed in the present study (Table 2). All the classes of lipids that have been observed in the present study have been reported in cyanobacteria by different workers earlier (Sallal *et al.*, 1987; Al-Hasan *et al.*, 1989). Influence of environmental factors including light on the fatty acid composition is also known (Al-Hasan *et al.*, 1989). There are earlier reports on the presence of a variety of long chain saturated and unsaturated fatty acids in cyanobacteria (Jahnke *et al.*, 1989). In the present study, twelve different fatty acids from the control and thirteen from the effluent treated *Spirulina* have been detected by gas chromatograph using standards (Table 2). Though, most of the fatty acids in the effluent treated *Spirulina* showed a reduction in their content when compared to control, there was an increase in oleic acid (C:18.1) and palmitic acid (C:16) in the treated sample. Remarkable increase in oleic acid and palmitic acid content has been observed by Manabe *et al.* (1992), when *Spirulina* growth was interrupted. The linolenic acid (C: 18.3) was not recorded in the treated sample, instead long chain saturated fatty acids such as arachidic acid (C:20) and heneicosanoic acid (C:21) and short chain fatty acid lauric acid (C:12) have been observed only in the effluent treated *Spirulina*. The absence of linolenic acid in the effluent treated cyanobacterium could be attributed to the fact that the unsaturated fatty acid might be transformed into other saturated fatty acids *viz.* arachidic acid (C:20) and heneicosanoic acid (C:21) due to environmental stress, especially the effluent. Considerable and significant changes in the levels of fatty acids were observed by Manoharan and Subramanian (1993b), when *Oscillatoria* treated with different effluents and even in light and dark incubations (Al-Hasan *et al.*, 1989). Considering the above, changes observed in the present study in the levels of different fatty acids with starchy effluent is quite remarkable.

In the present study, an attempt has been made for the bioremediation of starchy effluent utilizing *Spirulina*. The results showed that *Spirulina* can grow only in 50% diluted effluent and proved its efficiency with regard to few parameters tested. Further studies are essential in this aspect to identify efficient microalgal candidates capable of growing luxuriantly in the undiluted effluent also.

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