Cultivation of Micro Alga - *Spirulina platensis* in Optimised Sugar Mill Effluent Medium

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ABSTRACT

Spirulina is one of the most explored cyanobacteria. Since ancient time it is being used as source of protein. The aim of the present study is to develop the low-cost medium from sugar mill effluent (SME) for mass production of *S. platensis* and producing high amounts of protein and create pollution free environment. Microalgae *S. platensis*, culture offers an interesting step for wastewater treatments, because they provide a tertiary bio treatment coupled with the production of potentially valuable protein biomass, which can be used for several purposes. Microalgae cultures offer an elegant solution to tertiary and quandary treatments due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth. And also, for their capacity to remove heavy metals, as well as some toxic organic compounds, therefore, it does not lead to secondary pollution. The low-cost SME medium was formulated by supplementing rice mill effluent with various concentrations of carbon, phosphorus and nitrogen sources. The best cellular growth of *S. platensis* was observed in SME medium with $NaHCO_3$ (10. g L^{-1}) as carbon source, and K_2HPO_4 (0.30 g L^{-1}) as a phosphorus source and $NaNO_3$ (2.00 g L^{-1}) as a nitrogen source, with trace of $FeSO_4$ and EDTA at pH 9.5. Parameters like specific growth rate, dry weight, chlorophyll, lipid and protein content were measured.

Key words - *Spirulina platensis*, Biomass, chlorophyll a, light intensity, and sugar mill effluent medium

Introduction

Spirulina is a microscopic blue-green aquatic plant and it is the nature's richest and most complete source of organic nutrition. Spirulina is cultivated in tropical and subtropical
bodies of water and filamentous form of cyanobacteria. *Spirulina* is a non nitrogen-fixing blue-green alga and cell wall made of mucopolysaccharide its soft and easily digestible nature, which makes it safe for human consumption. *Spirulina* is capable of growing in high alkalinity with the presence of carbonate, bicarbonates and inorganic nitrogen (Yang *et al.*, 2010).

Effluents from sugar industry, if directly released in water for irrigation, affect soil fertility as well as plant growth and seed germination (Ramkrishan *et al.* 2001). The physicochemical characteristics of sugar mill effluents discloses its organic strength i.e., chemical oxygen demand (COD) value ranges from 1500 to 4000 mg/L and biochemical oxygen demand (BOD) values from 500 to 800 mg/L (Deshmane *et al.* 2015). Because the effluent was from agro-based industry, it contains nitrogen (N), phosphorous (P), and potassium (K) in substantial quantities (Rajagopal *et al.* 2013). Pedroni *et al.* (2001) observed better results for microalgae in the wastewater treatment as compared to conventional processes such as activated sludge. Filamentous algae/cyanobacteria can be as effective as microalgae, and they may be harvested relatively easily by filtration. Moreover, some filamentous algae/cyanobacteria form aggregates and can be harvested by sedimentation or by flotation (Hori *et al.* 2002).

The aim of the present study is to develop low cost sugar mill effluent medium by supplementing with various concentrations of carbon, phosphorus and nitrogen sources for mass production of *S. platensis*.

Material and methods

Organism

The strain of Spirulina *platensis* S5 was collected or obtained from Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar, Chidambaram, Tamilnadu, which is previously maintained in Zarrouk's agar media slants in 4ºC.

Maintenance and multiplication of culture

Spirulina platensis was auxenically grown in Zarrouk's medium. Firstly, we have transferred our culture in Zarrouk's broth from Zarrouk's agar slants. Culture were incubated in a culture room at temperature of 30±2ºC and illuminated with day-light fluorescent tubes saving 4 Klux at a surface of vessels. During the process of growth, the flask was shaken 3 to 4 time per day. The experiment was run in triplicates. All manipulation involving the transfer of culture in the liquid media or on agar plates were carried out under aseptic conditions on a laminar air flow

The mass cultivation of *S. platensis* in sugar mill effluent enriched medium during summer season

Mass cultivation of *S. platensis* in the outdoors is possible under optimal conditions of nutrients, light (Sunlight), pH (9.5), temperature, agitation, culture depth and initial inoculums concentration. The production unit has to be located in areas with suitable climatic conditions and places where all culture conditions are optimum. In summer season, April- May, 2016 average day time temperature normally exceeds 39°C. The technological factors involved in the mass cultivation of best *S. platensis* multi stress tolerant strain S5 are presented in Table 1.

Table1. The mass cultivation of *S. platensis* in sugar mill effluent enriched medium during summer season

Cultivation system	**Cement tank**
Nutrients	Sugar mill effluent medium supplemented with $NaHCO_3$ (10 g L^{-1}), K_2HPO_4 (0.3 g L^{-1}), $NaNO_3$ (2.0 g L^{-1}), $FeSO_4$ (0.01 g L^{-1}) and EDTA (0.08 g L^{-1})
Light	Sunlight
pH	9.5 adjusted by sodium bi carbonate addition
Temperature	42°C
Agitation	Manual stirring by plastic stick (30 min/day)
Culture depth	15 cm to 20 cm
Seeded culture	*Spirulina platensis*- S5 strain inoculam dose 0.25 g L^{-1}
Culture period	60 days
Harvesting	Filtration through muslin cloth
Drying period	Sun drying on plastic sheets
Season	April-May, 2016
Location	Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar, Chidambaram, Tamilnadu

During cultivation period the growth parameters such as dry biomass, protein content, chlorophyll content, lipid content, total carbohydrate and phycocyanin were estimated at 10 days interval from 0 to 60 days by various methods described in table2.

Table2. Methodology adapted for estimation of biomass, chlorophyll, protein, lipid, total carbohydrates and phycocyanin content of *S. platensis*.

S. No.	**Parameters estimated**	**Methodology adapted**
1.	Biomass	Pandey *et al.* (2010)
2.	Chlorophyll content	MC Kinney (1941)
3.	Protein content	Lowry *et al.* (1951)
4.	Lipid content	Foich and Lees (1957)
5.	Total carbohydrates	Hedge and Hofreiter (1962)
6.	Phycocyanin content	Horvath *et al*., (2013)

3. Harvesting, Drying and storage

After the incubation period the algal mat was filtered through the fine nylon filter by gravity filtration. Filtered algal mats were washed in sterile distilled water to remove the impurities. After washing the excess moisture present in the algal mat was removed by pressing on filter paper. Then air dried algal mat in plastic trays covered with transparent plastic sheets were kept in bright, direct sunlight for 5 to 8 days. The sun-dried algal mats were powdered well in polyethylene lined aluminum bags for further use.

Results and Discussion

Growth of *S. platensis* S3 strain in outdoor condition (April-May, 2016)

The growth of *S. platensis* S3 strain in outdoor condition during April-May, 2016 was investigated for 60 days and the results are furnished in Table (3). The growth parameters increased with increase in growth period till 60th day. The biomass recorded on 60th day was 13.40 g DW/m^{-2} day^{-1} and the chlorophyll content were at 1.64 mg/l/d. The recorded lipid, protein, carbohydrates and phycocyanin content were 0.297 mg/1, 66.80%, 19.50% and 116.2 mg/g respectively.

The large-scale production of Spirulina biomass depends on many factors, the most important of which are nutrient availability, temperature and sunlight. These factors can influence the growth of Spirulina and the composition of the biomass produced by causing changes in metabolism, which considerably modify the time course of the accumulation of the main biomass components (Cornet *et al*., 1992).

There are two most important techniques widely used to cultivate microalgae. Namely, open raceway pond system and closed photo bioreactor system. The pond system is less favorable due to limitations in controlling contaminations from predators while photo bioreactors provide an easy system of controlling nutrients for growth, cultivation parameters such as temperature, dissolved CO_2 and pH (Ugwu *et al.*, 2008).

In the present investigation the outdoor cultivation of *Spirulina platensis* was carried out in cement tank with surface area 4m^2 (1000 L). Average daytime temperature normally exceed 42°C in summer season, which supported a biomass yield of 13.10 g DW/m^{-2} day $^{-1}$, which strongly supported the view expressed by Jimenez *et al.* (2003). They reported that during the summer season the *Spirulina* cultivation was carried out in flask and the biomass productivity obtained was 9.4 g m^2 day $^{-1}$ after 12-15 days. The study proved that summer season in India is suitable for mass production of *Spirulina* in waste waters like sugar mill effluent.

Table3. Growth of *S. platensis* S5- strains in outdoor condition (April-May, 2016)

Period (Days)	Biomass (g DW/m^{-2} day^{-1})	Chlorophyll content ($mgl^{-1}d^{-1}$)	Lipid Content ($mgml^{-1}$)	Protein content (%)	Total carbohydrate content (%)	Total phycocyanin content (mg g^{-1})
0	0.52	0.58	0.109	51.61	6.50	32.4
10	4.7 8	0.92	0.226	58.30	12.71	70.4
20	8.80	1.42	0.241	64.52	17.00	84.0
30	11.55	1.44	0.279	64.90	17.93	102.7
40	12.40	1.55	0.282	65.44	19.00	105.2
50	12.75	1.60	0.288	66.10	19.54	111.0
60	13.40	1.64	0.297	66.70	19.59	116.2
Mean	10.69	1.48	0.29	72.92	18.50	104.05
SEd	**0.31**	**0.016**	**0.0030**	-	-	**2.498**
CD (p=0.05)	**0.651**	**0.031**	**0.006**	-	-	**5.029**

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