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Cultivation of two species of *Spirulina (Spirulina platensis and Spirulina platensis* var *lonar)* on sea water medium and extraction of C-phycocyanin

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ABSTRACT

Spirulina has been cultivated worldwide for their commercially valuable products like crude protein, bio-pigments and other food supplements. Commercial Spirulina Producers tired to cultivate it in cheap natural resources and thus reduce the cost of production and environmental pollutions. In this experiment there were two species of Spirulina viz., Spirulina platensis (filamentous type) and Spirulina platensis var lonar has been tried to cultivate on cultivation medium formulated with sea water. The cultivation was carried out for a period of 15 days, at 27 ℃ and at constant light intensity of 1.7 klux. The physico-chemical characteristics of seawater were analyzed before media formulation as per the standard protocols provided by American Public Health Association (APHA-1989). The growth of the microalgae was monitored by Direct Microscopic Count (DMC), Optical Density (OD) at 560nm and Biomass estimation. The generation time of the two micro-algae and their C-phycocyanin yield in control as well as seawater media were also determined. The results revealed that the sea water medium forms better alternative natural cheap resource to cultivate the Spirulina. As for as biomass yield is concern, the Spirulina platensis (filamentous type) provided increased yield (2.72g/l) when compared to the control (2.48g/l) (Zarrouk) medium.

Keywords: Seawater medium, *Spirulina* species, Physico-chemical characteristics, Generation time, Phycocyanin.

INTRODUCTION

Spirulina is a microscopic, filamentous Cyanobacterium, has a long history of use as food. Species of *Spirulina* have been isolated from tropical waters to North Sea, Thermal springs, Salt pans, Warm waters from Power plants, Fish ponds etc., Thus the organisms appears to be capable of adaptation to very different habitats and colonizes certain environments in which life for other organism is difficult. Typical example is the population by alkalophylic *S.platensis* of certain alkaline lakes in Africa and by *S.maxima* of Lake Texcoco in Mexico[1]. The optimal temperature for *Spirulina* growth is in the range of 35°C-38°C. In addition *Spirulina* requires relatively high pH, which effectively inhibits the growth of other algae in the culture medium. In this respect high amounts of sodium bicarbonate must always be present in the culture medium to sustain the high pH and prevent fluctuation. Zarrouk medium which is rich in bicarbonate has successfully served as a common culture medium in *Spirulina* culture for years [2]. *Spirulina* has been cultivated commercially for their valuable natural pigments and also used as dietary supplements. Although alterations were made in the basic composition, the media so developed commercially were inorganic in nature and not economical. Zarrouk medium is not feasible for the commercial production due to its high production cost. Hence many investigators tried to cultivate the *Spirulina* on cheap resources such as swine dung [3], spent-wash [4], cow dung [5] etc., and also various supplementation have been made to achieve enhanced biomass yield and bio-products [5]. In this study the cultivation media is formulated by using sea water to reduce the production cost commercially.

MATERIALS AND METHODS

Culture collection and Maintenance

In the present study the growth of two species of *Spirulina*, viz., *Spirulina platensis* (filamentous) and *Spirulina platensis* var *lonar* were used to cultivate on the formulated sea water medium. The *Spirulina platensis* was obtained from C.A.S Botany Department, University of Madras, Tamilnadu, India. *Spirulina platensis* var *lonar* was collected from P.G Research Institute, Kattupakkam, a Unit of Tamilnadu Veterinary and Animal Science University, Tamilnadu, India. The culture was maintained in Zarrouk medium in a 1000ml Erlenmeyer flask in the normal room temperature, with 12 hours light and 12 hours dark photo period with normal white light and the flask were aerated artificially.

Physico-chemical analysis of seawater

The seawater was collected from the Bay of Bengal, Kovalam near Chennai, Tamilnadu, India. The physico-chemical parameters such as Turbidity, Total Dissolved Solids (TDS), pH, Hardness, Calcium, Magnesium, Sodium, Potassium, Chloride, Sulphate and Silica content (mg/l) were determined according to standard methods as published by American Public Health Association [6].

Cultivation

The cultivation was carried out in 1 liter Erlenmeyer flasks containing sea water medium. The medium was prepared by diluting the seawater in the ratio of 1:4, 2:4, 3:4 and the initial pH was adjusted to 8.5. The flask containing the medium was inoculated with 10% of the 15 days old culture of *Spirulina platensis.* The flasks were maintained under laboratory conditions and provided with artificial light source and the medium was continuously aerated. The growth of the culture was monitored as per the protocol [7] for a period of 15 days and the generation time was calculated [8].

Phycocyanin extraction

The C-phycocyanin was extracted from fresh biomass by the following procedure [9]. Fresh biomass was homogenized with 50mM sodium phosphate buffer, the homogenate was subjected to alternate freezing and thawing(3 to 4 cycles) and centrifuged at 5000rpm for 10 minutes. The phycocyanin content was estimated [10].

RESULTS AND DISCUSSION

Production of Spirulina in sea water medium

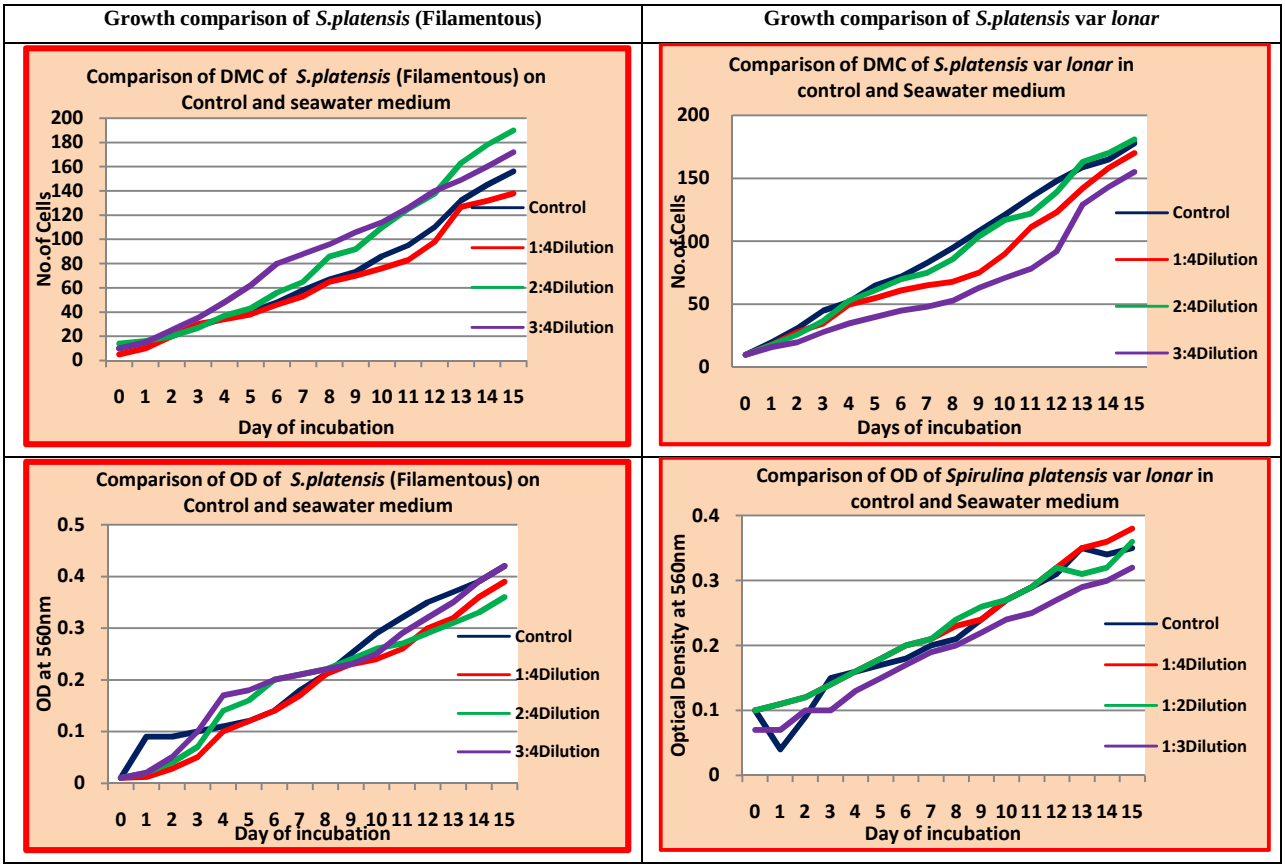
Sea water is one of the cheapest medium for cultivation of *Spirulina platensis*. The possibilities of utilization of sea water enriched with urea as the culture medium for the blue green alga *Spirulina platensis* pretreatment by precipitation with $NaHCo_3$ and Na_2CO_3 was found essential to remove the excess amount of Ca^{2+} and Mg^{2+} present in the sea water prior to cultivation. A culture medium as good as the synthetic medium has been reported in the literature for the growth of *Spirulina maxima* was obtained, ie., the sea water treated with $NaHCO_3$ at pH 9.2 and 35°C for 2 hours, filtering to remove precipitates and enriched with K_2HPO_4, $NaNO_3$ and $FeSo_4$ has been used for Spirulina cultivation. It was conducted on the 130 L cultivation open pond also confirmed as well as the best known synthetic medium [11] and the outdoor mass cultivation of S*pirulina maxima* in sea water was also reported[12]. The sea water medium was used for SCP production, after adjusting the pH to 9-11 by adding $NaHCo_3$ @ 1g/ litre[13]. Similar trend was observed in three sea water based media used for biomass production by *Arthospira maxima*. *Arthospira maxima* reached a biomass concentration of $<1.2 \pm 0.09$ g l^{-1} in the sea water based media.

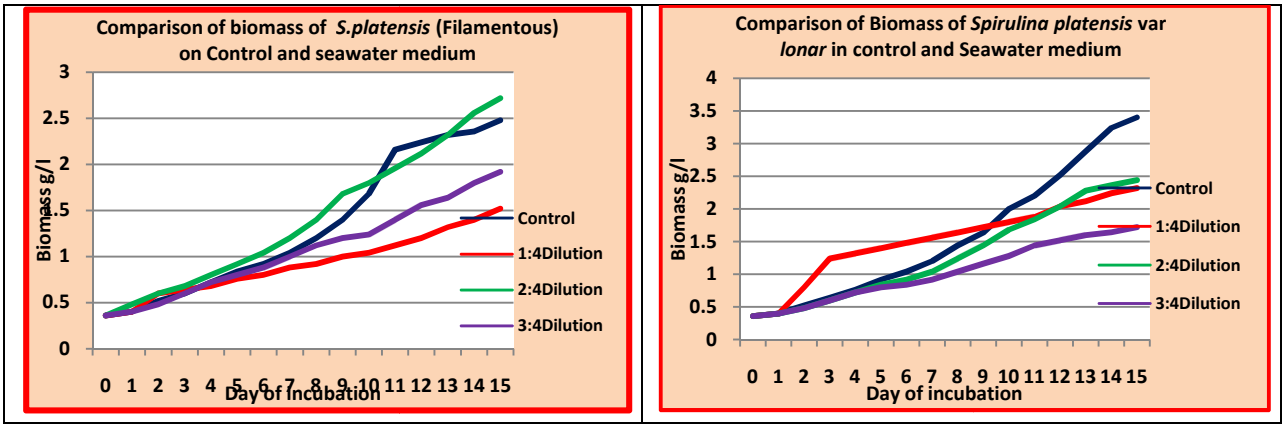
In the present study *Spirulina platensis and platensis var lonar* were cultivated on three concentration of sea water medium. The pH of the medium was adjusted with $NaHCO_3$ as adopted by the previous researcher[13]. The cultivation was carried out *in-vitro* for a period 15 days. The growth was monitored during cultivation as per the protocol [7]. A biomass yield of 1.2g/lit in the sea water based medium was also obtained [13]. In our study the obtained biomass of *Spirulina platensis* (filamentous type) in sea water medium was 1.52g/l,2.72g/l and 1.92g/l in the dilutions of 1:4, 2:4 and 3:4 respectively. The biomass of *S.platensis* var *lonar* was 2.32g/l,2.44g/l and 1.72g/l in the dilutions of 1:4, 2:4 and 3:4 respectively (graph 1-6). All the dilutions of sea water medium provided an increased yield when compared to the previous results [13]. Of the two species used in this study the *S.platensis* filamentous type provided higher yield in the 2:4 dilution of sea water medium.

Table 1: The physico-chemical analysis of seawater

S. No	Parameters	Results
Physical examinations		
1.	Appearance	Colourless and clear
2.	Colour	None
3.	Turbidity NTU	2.6
4.	Total dissolved solids mg/l	34240
5.	Electrical conductivity	49588
Chemical examinations		
6.	pH	7.26
7.	Alkalinity pH(as CaCO3)mg/l	0.0
8.	Alkalinity total(as CaCo3)mg/l	132
9.	Total hardness(as CaCo3)mg/l	6800
10.	Calcium (as Ca)mg/l	1520
11.	Magnesium (as Mg) mg/l	720
12.	Sodium(as Na) mg/l	7800
13.	Potassium (asK)mg/l	900
14.	Iron(as Fe)mg/l	0.01
15.	Manganese(as Mn)mg/l	0.0
16.	Free ammonia(as NH3)mg/l	0.02
17.	Nitrite(as NO2)mg/l	0.01
18.	Nitrate(as No3)mg/l	1.0
19.	Chloride(as Cl)mg/l	18068
20.	Fluoride(as F)mg/l	0.21
21.	Sulphate(as So4)mg/l	1101
22.	Phosphate(as PO4)mg/l	0.07
23.	Tidy's test (as O)mg/l	0.7
24.	Silica(as SiO2) mg/l	23.14

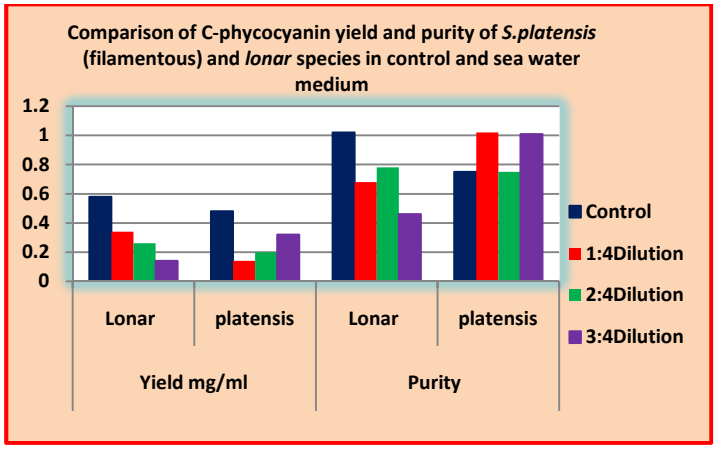
Graph 1-6: shows the growth characteristics of *S.platensis*(filamentous) *S.platensis* var *lonar* by Direct Microscopic Count, Optical Density and Biomass





The phycocyanin was extracted from the culture as per the protocol [6]. The C-phycocyanin yield of *S.platensis* filamentous type was 144mg/ml, 200mg/ml and 320mg/ml in the dilutions of 1:4, 2:4 and 3:4 respectively and the purity was 1.02, 0.75 and 1.01 in respect of the dilutions 1:4, 2:4 and 3:4. The C-phycocyanin yield of *S.platensis* var *lonar* was 340mg/ml, 260mg/ml and 140mg/ml in the dilutions of 1:4, 2:4 and 3:4 respectively and the purity was 0.68, 0.78 and 0.46 in respect of the dilutions 1:4, 2:4 and 3:4(Graph 7).

Graph 7: sowing the comparison of the C-phycocyanin yield and purity of *S.platensis*(filamentous) *S.platensis* var *lonar* on seawater medium and control



CONCLUSION

From the study it was inferred that, the seawater medium forms better alternative and natural resource for the cultivation of *S.platensis.* Further the seawater medium is commercially feasible and also does not have environmental pollution hazards.

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