Growth performance of *Spirulina (Arthrospira) platensis* in a low cost medium: An assessment

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ABSTRACT

The unialgal culture of *Spirulina platensis* was sub-cultured in Zarrouk's medium under photoautotrophic conditions. Initially, indoor batch cultivation was carried out for a week in four different types of cultivation media *viz.*, Zarrouk's, Modified Zarrouk's, prescribed Nallayam Research Centre (NRC), and Modified NRC. In modified medium, urea and phosphoric acid of NRC medium were replaced with sodium nitrate and di-potassium hydrogen phosphate (anhydrous) and concentration of ferrous sulphate heptahydrate was reduced. The batch and airlift indoor culture experiments were carried out with an illumination of 3500±100 lux, photoperiod of 12:12 hour light and dark periods and temperature of 24±1°C. The specific growth rate value was 5.7 % higher in Zarrouk's medium as compared to modified NRC medium. However, the cost of modified NRC medium was considerably lower than Zarrouk's medium; therefore, modified NRC medium was selected for outdoor studies. The outdoor mass cultivation was done under natural conditions with the solar radiation reaching the surface of culture was between 2160 and 8450 lux and temperature ranged from 27 to 34°C. An assessment of the performance of growth in batch, airlift and FRP (Fiber Reinforced Polymer) tanks revealed that culture grown in airlift units showed best growth which was evident from higher specific growth rate and number of doublings per day. There was a 3.4-fold increase in cell density (in terms of turbidity at 750 nm) of the cultures in such units. The growth in outdoor FRP tanks was also comparable to the airlift cultures.

Keywords: *Spirulina platensis*, indoor culture, outdoor culture, medium, growth

INTRODUCTION

The cyanobacterium or blue-green microalgae, *Spirulina (Arthrospira) platensis* thrives in saline aquatic habitats of coastal and inland areas. The *S. platensis* biomass is rich in proteins (approximately 70%), amino acids, vitamins, especially B_{12} and several pigments like carotenoids, xantophylls, phycobiliproteins and chlorophyll a. This has been used in food, pharmaceutical, cosmetic industries and other high-value products. All the amino acids are present in proportions recommended by Food and Agriculture Organization except for methionine [1-3]. This microorganism also possesses considerable lipid content consisting of polyunsaturated fatty acids like gamma-linolenic and linoleic in the proportion of 1.24% and 1.04%, respectively. These fatty acids are considered important from the medical and nutritional point of view [2, 4-5]. Apart from the use of *S. platensis* for nutritional complement for humans/animals, constituent in pharmaceuticals/cosmetics and it is has been also used for wastewater treatment, recovery and reutilization of heavy metals as adsorbent materials [6].

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The conventional nitrogen source for *Spirulina* sp. is nitrate. However, Stanca and Popovici [7] have shown that there is an increase in the *S. platensis* biomass production by the use of urea as source of nitrogen. However, a limitation is that urea is hydrolyzed to ammonia in the alkaline culture medium, which can be toxic to microalgae in high concentrations [8]. Raoof [9] investigated the cost effective growth medium preparation for mass production of *Spirulina* sp. by incorporating selected nutrients of the standard Zarrouk's medium and other cost-effective alternative chemicals. Therefore, there is a thrust to evaluate different culture media to curtail the cost of production of good quality biomass of *S. platensis.* Also there is need to reduce the ammonia production in the culture medium. Present study describes the results of growth and biomass production of *S. platensis* in four different types of cultivation media. A comparison was also made among the biomass production rate of the organism grown in above media in batch, air-lift and open pond culture systems. The data of this investigation will serve as baseline information for development of low-cost technologies for outdoor cultivation of *S. platensis.*

MATERIALS AND METHODS

Microorganism and Inoculum

Unialgal culture of cyanobacterium, *S. platensis* was obtained from algal culture laboratory of Central Institute of Fisheries Education (CIFE), Mumbai, India. The pure culture was sub-cultured in Zarrouk's medium [11] under photoautotrophic conditions. The batch and airlift indoor culture experiments were carried out with an illumination of 3500 ± 100 lux using compact fluorescent lamps (Philips, 23 W). The intensity of light was measured using lux meter (LX-103, Taiwan). The photoperiod was fixed at 12:12 hour light and dark periods. The temperature was maintained at 24 ± 1°C. The outdoor mass cultivation was done under natural conditions in the month of March, 2009 when solar radiation reaching the surface of culture was between 2160 and 8450 lux and temperature ranged from 27 to 34°C.

Selection of Growth Medium

For selection of best medium for cultivation of *S. platensis,* indoor and outdoor experiments were carried out in batch cultures. Initially, indoor batch cultivation was carried out for a week in four different types of cultivation media *viz.*, Zarrouk's medium, Modified Zarrouk's medium [11]; Nallayam Research Center (Prescribed by Nallayam Research Centre, Chennai; referred as NRC) medium and Modified NRC medium. In the modified medium, urea and phosphoric acid of NRC medium were replaced by sodium nitrate and di-potassium hydrogen phosphate (anhydrous) and also the concentration of ferrous sulphate heptahydrate was reduced. The composition of various growth media is presented in table 1. The cultures were grown in triplicates in 250 ml Erlenmeyer flask containing 100 ml of cultivation medium and known inoculum size (initial turbidity: 0.07 at 750 nm). The exponential phase culture was centrifuged (R24 Research Centrifuge REMI Instruments, India) at 1358 x *g* for 10 minutes and the pellets were washed with sterilized de-ionized water by suspending in similar volume of water followed by centrifugation at the same force as mentioned earlier. The supernatant was discarded and the settled biomass was used for inoculation.

Indoor Culture of *S. platensis*

The indoor batch cultures were grown by following the earlier procedure and indoor airlift culture was grown in aspirator bottle of 20 liter capacity. The cultures were aerated by using air injection device connected to a glass pipe which releases the air bubbles from bottom of the aspirator bottle. The air-flow rate was adjusted to a level that ensures proper mixing of the culture through upward movement of air bubbles.

Outdoor Cultivation of *S. platensis*

The outdoor cultivation was done in a circular FRP tank of 1000 liter capacity and the depth of the medium was maintained between 6 to 8 inches. The culture was mixed using air injection tube and the tank was covered with polythene sheet to avoid the dust particles and droppings of the trees or animals. The perforated glass head was attached at the end of the air injection tube to achieve uniform distribution of air throughout the culture medium. The indoor and outdoor cultivation experiments were carried out for 6 days under the above mentioned cultivation condition. The duration of the experiment was from 18th to 23rd March 2009.

Growth Measurement

The samples were collected aseptically each day and the optical density of cell suspension (Turbidity) was measured using a double-beam spectrophotometer (UV 1 model, ThermoSpectronic, England) at 750 nm. The specific growth rate, generation time and number of doubling per day were calculated by using the formula of Guillard [12].

Specific growth rate (μ)

Specific growth rate was measured during exponential growth phase where the rate of increase in cells per unit time is proportional to the number of cells present in the culture at the beginning of any unit of time. This was given by the following formula:

$$N_t = N_0 e^{rt}$$

Where, N_0 is the population size at the beginning of a time interval, N_t is the population size at the end of the time interval, and r is the proportional rate of change or the intrinsic/instantaneous rate of increase. *r* is always expressed per unit time (t^{-1}). Where, *r* is equal to μ, when mortality is zero. The specific growth rate and was calculated using the formula:

$$\text{Specific growth rate } (\mu) = \frac{\ln N_t - \ln N_0}{t_t - t_0}$$

Here, N_0 and N_t are the values of absorbance at 750 nm during the exponential phase at time t_0 and time t_t respectively.

Divisions per day (k)

Doublings per day or number of divisions per day was calculated by dividing specific growth rate, μ by the natural log of 2.0 [(ln (2)] and k can be derived by using the formula;

$$\text{Divisions per day (k)} = \frac{\mu}{\ln (2)}$$

Doubling time (T_2)

Doubling time for the algal culture is expressed in the same units of time as μ and T_2 can be calculated from an estimate of μ. The doubling time or mean generation time (days) was calculated using the formula;

$$\text{Doubling time } (T_2) = \frac{\ln (2)}{\mu}$$

Dry weight

Fifty ml of the algal suspension was filtered through a pre-weighed quantitative ash-less filter paper (Merck, Advantech 5A, 0.02 mg) and the filter paper was weighed again after filtration. The difference in weight of filter paper before and after filtration shows the fresh weight of cells. The filter paper with settled algal cells was dried in a hot air oven for 6 hours at 105°C and the paper was weighed again after drying. The difference between the weight of filter paper before filtration and after the drying of the biomass settled on the filter paper gives the value of dry weight.

RESULTS AND DISCUSSION

Unialgal populations of *S. platensis* were grown in normal and modified Zarrouk's medium and in the prescribed Nallayam Research Centre medium used for outdoor cultivation of the organism. The NRC medium was modified by substituting phosphoric acid with di-potassium hydrogen phosphate and urea with sodium nitrate and referred as modified NRC medium. The concentration of ferrous sulphate heptahydrate was also reduced from 0.05 g/l to 0.01 g/l. The specific growth rate value was 5.7 percent higher in Zarrouk's medium as compared to modified NRC medium (Fig.1). However, the cost of modified NRC medium was considerably lower than Zarrouk's medium; therefore, modified NRC medium was selected for further studies.

A comparison of growth in terms of change in turbidity (750 nm) after 5 days indicated that there was about 250% change in turbidity of the cultures grown in Zarrouk's medium. The change in turbidity of culture grown in modified NRC medium was approximately 190% with reference to initial day turbidity (Fig. 2). Growth of the organism in modified NRC medium was as appreciable as in Zarrouk's medium. In spite of approximately 60% higher yield in Zarrouk's medium after 5 days of growth, the modified NRC medium was found most suitable and cost-effective because of lower price, lesser number of constituents and lower quantity of bicarbonate required (Table 1). An assessment of the performance of growth in batch, airlift and FRP (Fiber Resin Polymer) tanks revealed that culture grown in airlift units showed best growth which was evident from higher specific growth rate and number of doublings per day. There was a 3.4-fold increase in turbidity of

the cultures in such units. The growth in outdoor FRP tanks was also comparable to the airlift cultures (Fig. 3, 4).

Mass cultivation of *S. platensis* was carried out in two types of cultivation units (indoor airlift and outdoor FRP tank systems with continuous air injection). Prior to mass cultivation, the composition of growth medium (Zarrouk and NRC) was modified to enhance the biomass yield by substituting the nitrogen source (urea replaced by sodium nitrate) in the NRC medium. Further, in contrast to Zarrouk medium micronutrients were not used in the modified medium (i.e., the 1000 litre media cost for the Zarrouk's is approximately Rs. 7635whereas, the modified NRC medium costs only about Rs. 5215). The above modification in the composition of the growth medium slowed down the growth to slight extent, however based on the results obtained, the modified medium was found more cost-effective in comparison to Zarrouk's medium.

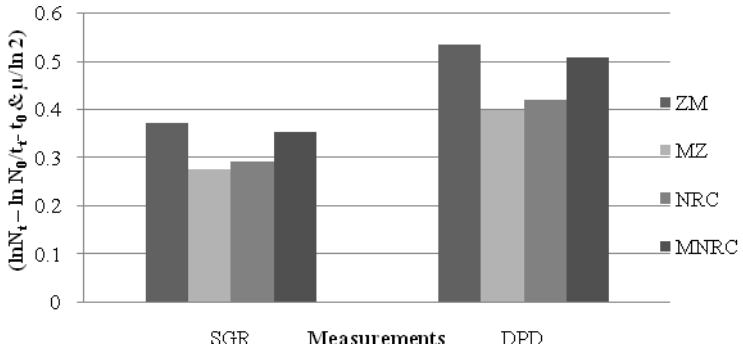

Figure 1. Specific growth rate and divisions per day of *S. platensis* in different media under photoautotrophic conditions (Light 3500 ± 100 lux; Temperature 24 ± 1°C); SGR- Specific Growth Rate; DPD- Divisions per Day; ZM- Zarrouk's medium; MZM- Modified Zarrouk's medium; NRC- Nallayam Research Center medium, and MNRC- Modified NRC medium.

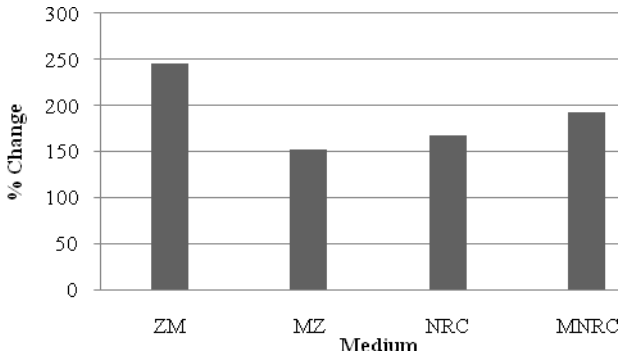

Figure 2. Percent change in turbidity of *S. platensis* (at 750 nm) in different media after 5 days of growth.

A comparison of growth on the basis of per cent increase in biomass indicates that growth in airlift unit and FRP tank was considerably higher than the batch cultures. The higher extent of growth in above units can be attributed to better aeration and proper mixing of the culture in airlift

unit and FRP tank due to continuous flow of air through the culture. It was interesting to note that the cultures grown under outdoor condition (where temperature ranged from 27°C to 34°C and light intensity ranged from 2160 to 8450 lux during the experimental period) exhibited similar extent of growth as observed in airlift culture (grown under photoautotrophic conditions, temperature: 24±1°C, light intensity 3500±100 lux), this appreciable growth of the organism under varying environmental conditions can be attributed to higher light intensity available under outdoor conditions.

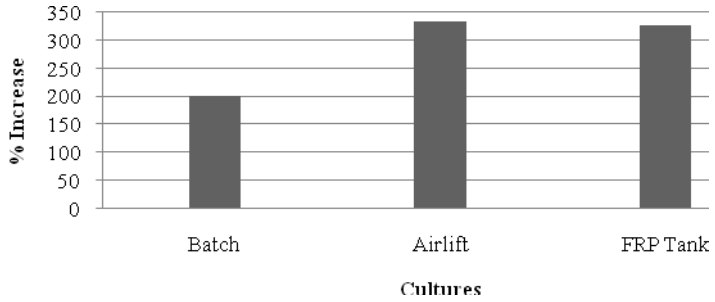

Figure 3. Change in turbidity with time (at 750nm) in batch, airlift and FRP tank grown cultures of *S. platensis*.

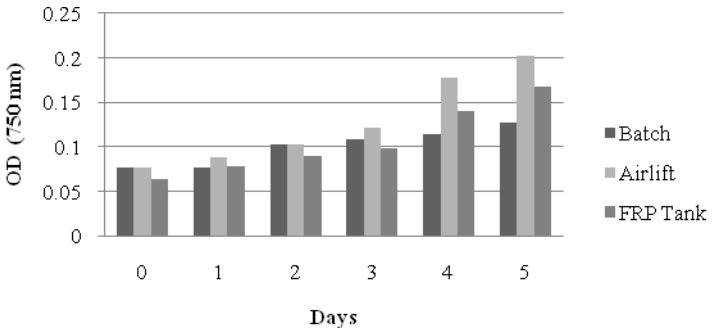

Figure 4. Percent change in turbidity (at 750nm) in batch, airlift and FRP tank grown cultures of *S. platensis*.

The present study shows that the modified NRC medium shows comparative growth as that of standard Zarrouk's medium for *S. platensis* cultivations. This clearly depicts that the replacement of urea and phosphoric acid of NRC medium by sodium nitrate and di-potassium hydrogen phosphate (anhydrous) and also by the reduction in the concentration of ferrous sulphate heptahydrate not only shows the comparative growth but also relatively low-cost for *S. platensis* cultivation.

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Table 1. Chemical composition of media for *S. platensis* cultivation.

Chemical compounds	Zarrouk's Medium	Modified Zarrouk's Medium	NRC Medium	Modified NRC Medium
Macro-elements (g/l)				
NaCl	1.0	1.0	5.0	5.0
$CaCl_2$	0.04	-	-	-
$NaNO_3$	2.5	-	-	2.5
$FeSO_4.7H_2O$ (ml)	0.01	0.01	0.5*	0.01
EDTA (Na)	0.08	0.08	-	-
K_2SO_4	1.0	1.0	0.5	0.5
$MgSO_4.7H_2O$	0.2	-	0.16	0.16
$NaHCO_3$	16.8	16.8	8.0	8.0
K_2HPO_4	0.5	-	-	0.5
Urea	-	-	2.0	-
KNO_3	-	3.0	-	-
H_3PO_4 (ml)	-	-	0.05	-
Micro-elements (1 ml of A_5 and 1 ml B_6/l)				
Trace Element Solution A_5 (mg/l)				
$ZnSO_4.7H_2O$	0.222	0.222	-	-
$CuSO_4.5H_2O$	0.079	0.079	-	-
MoO_3	0.015	0.015	-	-
H_3BO_3	2.88	2.88	-	-
$MnCl_2.4H_2O$	1.81	1.81	-	-
Trace Element Solution B_6 (mg/l)				
NH_4VO_3	0.0229	0.0229	-	-
$K_2Cr_2(SO_4)_4.24H_2O$	0.096	0.096	-	-
$NiSO_4.7H_2O$	0.0478	0.0478	-	-
$Na_2WO_4.H_2O$	0.0179	0.0179	-	-
$Co(NO_3)_2.6H_2O$	0.0439	0.0439	-	-
Ti $(SO_4)_3$	0.04	0.04	-	-
Media cost per 1000L Rs.	7635.128	7108.128	5022.46	5215.06

*10 g of $FeSO_4.7H_2O$ in 100 ml 1N HCl

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