Research paper

Comparative study on the growth performance of Spirulina platensis on modifying culture media

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ABSTRACT

India faces a severe challenge to ensure adequate nutrition for children and women. Chronic child nutrition deficiency is more prevalent in Madhya Pradesh state of India. *Spirulina*, multicellular and filamentous cyanobacterium are considered an absolute food supplement to combat malnutrition in Asian and African countries. Spirulina cultivation requires sufficient aeration, agitation and proper light intensity for enhanced biomass yield, cell productivity, specific growth rate, and protein content. This paper presents a novel experimental approach to maximize biomass yield, minimize evaporation rate and respiration losses in a laboratory scale closed reactor and open pond system. Lab scale open pond and closed reactor system were designed for spirulina cultivation under dry climatic conditions at Bhopal, India. Zarrouk media was used as standard and modified organic media was prepared by changing the nitrogen source. Temperature and other input parameters were maintained. Aeration was done manually in an open pond, and the air pump was used in the case of a closed reactor system. Biomass yield obtained from an open pond system was 11.34 g/l, and 12.28 g/l in the closed reactor system. Doubling time was also less in the closed reactor in comparison with the open pond system. Urea seems to be a promising alternative source of low-cost nitrogen for Spirulina cultures. From the experimental results, it is concluded that modified organic media and closed reactor system could be used for better biomass yield.

1. Introduction

Photosynthetic microorganisms are one of the most promising sources of energy as they are renewable and CO_2 neutral. Species belonging to the genus Spirulina, now called Arthrospira, are among the photosynthetic microorganisms of commercial importance (Salunke et al., 2016; Pedrosa Bezerra et al., 2011; Gonçalves et al., 2016; Huesemann et al., 2016; Falkowski et al., 1985; Geider et al., 2004). Spirulina is a multicellular and filamentous blue–green alga shown in Fig. 1, which has gained considerable attention in the health care and food sector as a protein and vitamin supplement. It grows in water, can be harvested and processed easily. It also contains very high amount of micro and macronutrients (Platt and Jassby, 1976; Eppley, 1972; Goldman and Carpenter, 1974; Yoder, 1979). Cyanobacteria has been commercially explored owing to its capacity to generate great amount of important products, such as phycocyanin. It is also being used for the production of food supplements, animal feed, and pharmaceutical products. The mass cultivation of Spirulina depends on a number of factors, including the availability of nutrients, temperature, and light. Spirulina also requires a relatively high pH, which inhibits the growth of other algae in the system. In order to maintain high pH and avoid fluctuations, high amounts of sodium bicarbonate must always be there in the culture medium (Yoder, 1979; Soni et al., 2016; Sudhakar and Premalatha, 2012, 2015; Coles and Jones, 2000; Montagnes and Franklin, 2001).

The light and temperature are the main factors of growth in nutrient-operated outdoor pond (N, P, CO2, etc.) and well-mixed conditions. The specific growth rate of the selected strain must be determined based on these two variables (Huesemann et al., 2016). Different photobioreactors and ponds have been designed and modeled for biomass growth. These models generally estimate light attenuation inside the culture and predict the growth rate as a function of incident or absorbed light. With few exceptions, most models rely on Beer–Lambert's law to determine the light intensity depending on the depth of culture and the concentration of biomass. However, this is problematic for cultures with high density where light dispersal can be important. However, present models are not reliable for selection of new

Fig. 1. Microscopic view of spirulina . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

strains based on higher productivity of biomass in an outdoor system. This is because the majority of models are difficult and require the introduction of a huge number of parameters, most of which are complicated, costly or time-consuming to establish or, worse, have values that merely have to be postulated (Boyd et al., 2013; Geider and Osborne, 1989; Ryther and Guillard, 1962; Grobbelaar and Soeder, 1985; Torzillo et al., 1991; Ogbonna and Tanaka, 1996).

The purpose of this experimental study is to grow spirulina in open pond and closed reactor using zarrouk media and modified media

(i) To analyze the different growth parameters such as biomass productivity, doubling time and specific growth rate.

(ii) To examine the influence of climatic parameters as incident light intensity, water temperature on growth including pond depth, pH, nutrients, and media composition.

(iii) To compare the open pond and closed reactor system for maximum biomass growth

2. Experimental methodology

2.1. Location and cultivation conditions

The strain of Spirulina was obtained from Gerophyta Nutraceuticals, Illupur, Tamil Nadu and cultivated at Energy Centre, MANIT, Bhopal, which was previously maintained in Zarrouk's medium at ambient temperature, with 12 h light and 12 h dark photoperiod with standard white light and the flask were aerated manually. All the reagents used for preparing media were of food grade. The thin orange curve is the current trajectory of the sun, and the yellow area is the variation of the path of the sun throughout the year. The closer to the center is the higher the sun above the horizon shown in Fig. 2.

2.2. Experimental setup

A laboratory scale open pond and closed system shown in Fig. 3 was used as an experimental setup for spirulina growth. The open pond is **a** $30 * 23$ cm ^{2} glass structure like an aquarium, having an adequate depth of 27 cm. The closed reactor is a Long and cylindrical plastic structured with 34 cm height and 25.5 cm diameter.

2.3. Experimental procedure

Different input parameters as temperature, pH, water level and Optical density, transmittance and concentration were determined at 450 nm for open pond and closed reactor with two different nutrient media. Light intensity was kept between 1500 lux to 3500 lux. The following conditions are maintained throughout the experiment.

Inoculum: Both the system was inoculated with concentrations of 10 mg l^{-1}.

Lighting: Spirulina requires lots of sunlight, but since we are growing the mother culture with very little concentration we choose a nearby place to the window, which ensures a right amount of sunlight with minimum direct radiation. If the light intensity is below 1000 Lux Spirulina could grow, and if the light intensity is higher spirulina cells may die. The best range for growth of spirulina is 1500 lux to 4500 lux (Sukenik et al., 1991). Lutron LX-105 was used and to measure light intensity.

Aeration system: Proper aeration of spirulina is required to fulfill its CO_2 requirement and also aeration does not allow algae to settle down and form a layer at the bottom. Aeration can be achieved by either mechanical stirring or air pump system. Experiments were carried out in an aquarium with manual stirring and a closed reactor with a continuous air pump. When stirrer is used for aeration, the rpm should not be more than 20. If rpm is increased cells may break.

pH and temperature measuring device: PCSTestr 35 multi-parameter instrument and pH strips are used to determine pH value and temperature of media.

Acclimatization: Spirulina grows typically in sea water and in a humid climate, but in Bhopal, there are extreme climatic conditions with a temperature of 45–50 degrees in summer and below 10 degrees during winters. Spirulina cultivation was successfully carried out at MANIT Bhopal by adjusting the input levels and allowing it to maintain performance across a range of environmental conditions.

2.4. Analysis

Biomass and kinetic parameter analysis

The concentration of cells (X) was determined daily using optical density measurements at 450 nm and was plotted on a standard curve based on dry weight (g l^{-1}). Chlorophyll was determined by a spectrophotometric method (mg g^{-1} biomass) from fresh biomass, and its concentration was calculated using a standard curve based on chlorophyll and SIGMAJ. The biomass productivity, specific growth rate and doubling time were determined spectrophotometrically at 450 nm from fresh biomass using a visible spectrophotometer. The conversion factor of nitrogen cells (YX / N) was estimated based on nitrogen content in the nitrogen sources (KNO_3 and urea). The productivity of chlorophyll (Pcl) was calculated using chlorophyll content measured on the day of Maximum cell concentration (X_{max}). Cell productivity, Cell concentration, Chlorophyll productivity, Specific growth rate and doubling time were calculated based on the following relations.

Productivity

Cell productivity (PX) is based on the independent variable described as the lowest difference in cultivation time (TC) (Behrenfeld and Falkowski, 1997; Goldman, 1979).

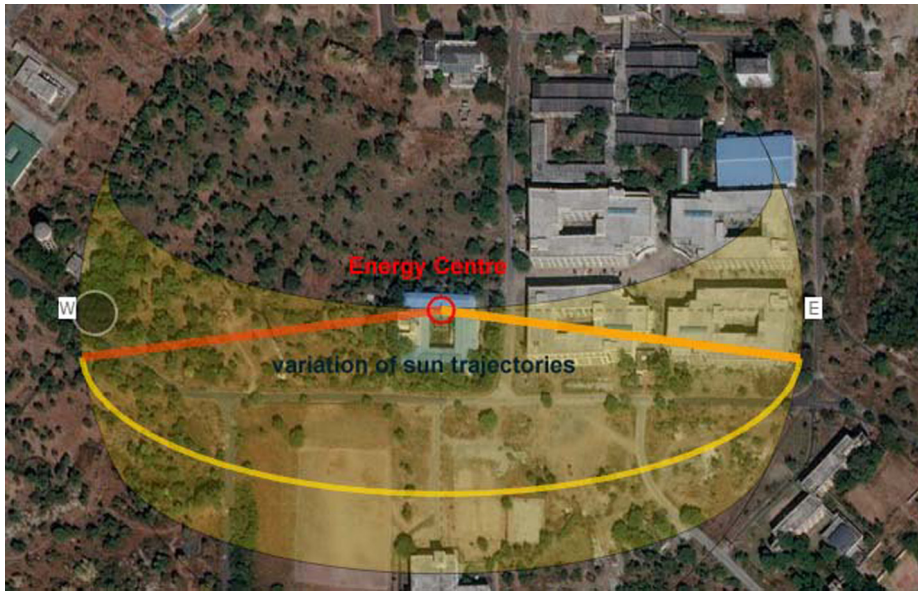The system productivity γ is defined as

$$\gamma = \mu x \tag{1}$$

Fig. 2. Location for spirulina cultivation at Energy Centre, MANIT, Bhopal.

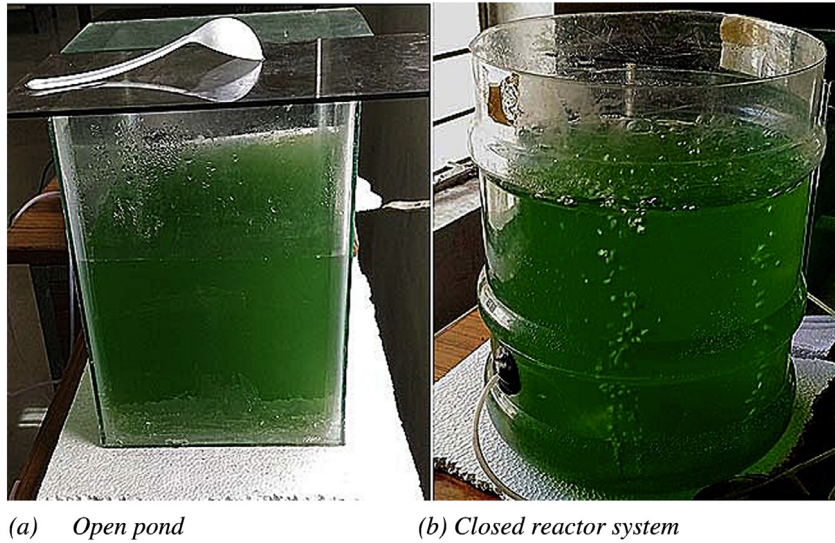(a)   Open pond   (b) Closed reactor system

Fig. 3. Open pond and closed reactor for spirulina cultivation at MANIT, Bhopal.

Where x is the biomass concentration and μ is the specific growth rate

Cell productivity (P_X) is calculated as the ratio of the variation in the concentration of cells (X_m-X_i) to cultivation time (TC)

$$P_X = (X_m - X_i)/T_C \qquad (2)$$

The cell productivity (P_X) was calculated on the basis of the ratio between (X_f-X_i) and the corresponding time for X_f, where X_f and X_i are the final concentration of cells at the time of cultivation T and the concentration of inoculum cells. The cultivation time (T) is denoted as the time of observation of the highest total chlorophyll.

Specific growth rate and doubling time

The specific growth rate of spirulina under normal growth conditions is given by the following equation:

$$\mu = \frac{t}{x}\frac{dx}{dt} \qquad (3)$$

Most commonly used relation to estimate the specific growth rate has been described using the formula

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1} \qquad (4)$$

Where the concentration of biomass at the time interval t_1 and t_2 is x_1 and x_2. The simple equation that combines the specific growth is (μ) and the doubling time or the production time (g) of the crop. is:

$$g = \frac{\ln}{\mu} == \frac{0.693}{\mu} = d.t \qquad (5)$$

Chlorophyll content

Chlorophyll can be obtained in sizeable quantities from Spirulina biomass. It is a pigment used in the food, pharmaceutical, and cosmetic industries as a colorant.

Chlorophyll a mg/l = 12.7 × O.D 663–2.69 × O.D 645
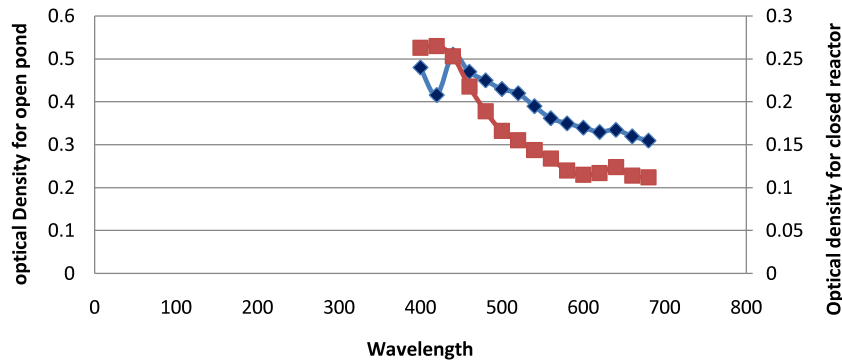Chlorophyll b mg/l = 22.9 × O.D 645–4.68 × O.D 663

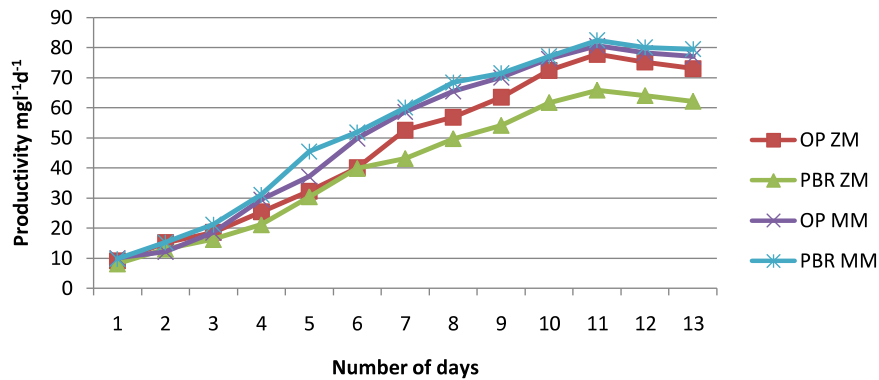**Fig. 4.** Variation of optical density for open pond and closed reactor with the wavelength.

Fig. 5(a). Variation of productivity in open pond and closed reactor with Zarrouk's and modified media.
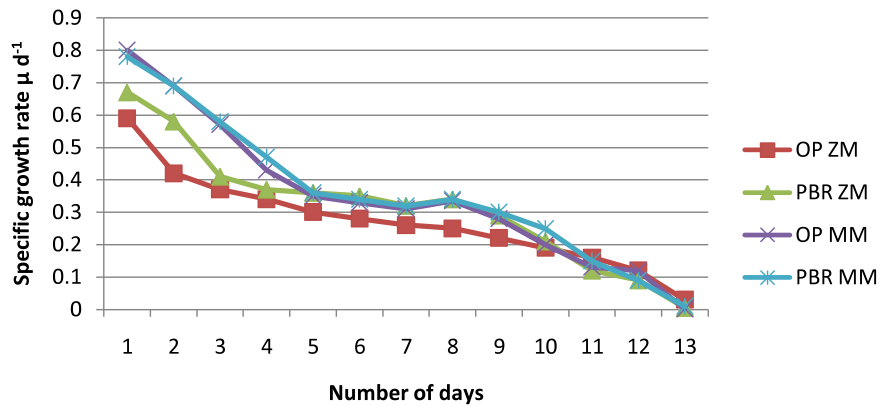

Fig. 5(b). Specific growth rate curve for open pond and closed reactor in Zarrouk's and modified media.

Total Chlorophyll mg/l $= 20.2 \times O.D\ 645 + 18.2 \times O.D\ 663$ (6)

3. Results and discussions

3.1. Effect of pH and temperature

Spirulina 's growth was better when grown at 30 °C compared to 25 and 35 °C. When it is below 25 °C and above 35 °C, minimal growth was observed. The maximum growth increase of 0.278 mg/ml occurs on day 8 to 9. On day 0–1, a minimum growth of 0.01 mg/ml was observed. The pH is one of the limiting parameters affecting the microalgae's metabolism. In the present study, the inoculation of Spirulina is done at pH 9. Gradually pH was increased, and it reached 10. Correspondingly, high biomass yield was obtained at pH 9.5 and a temperature of 32 °C. In our study, the optimum pH levels for this strain ranged between 8.5 to 10.5

in ambient conditions (30 °C), as concluded in similar studies at 30–35 ° C. The solubility of carbon dioxide and other mineral compounds are affected by pH. For the optimal growth of this strain, moderate alkalinity is required.

3.2. Effect of light intensity

Fig. 4 shows the variation of the optical density in open pond and closed reactor, and both coincide at wavelength 450 nm. All the readings were determined at wavelength 450 nm. These experiments were carried out at 1500 lux to 2500 lux light intensity. Spirulina cells have been reported to be able to regulate their photosynthetic efficiency by adjusting the nutrient media content (Kroon et al., 1989; Sukenik et al., 1991; Bernard, 2011). In fact, the continuous cultivation method with urea as the nitrogen source gives better biomass yield (Quinn et al., 2011; Bernard and Rémond, 2012; James et al., 2013; Béchet et al., 2013).
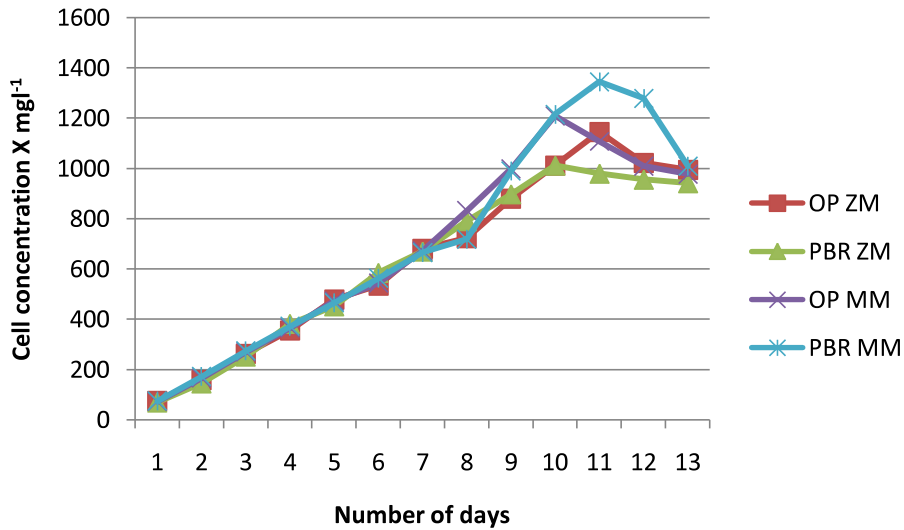
Fig. 6. Cell concentration of Spirulina platensis grown in an open pond and closed reactor with Zarrouk's and modified media.
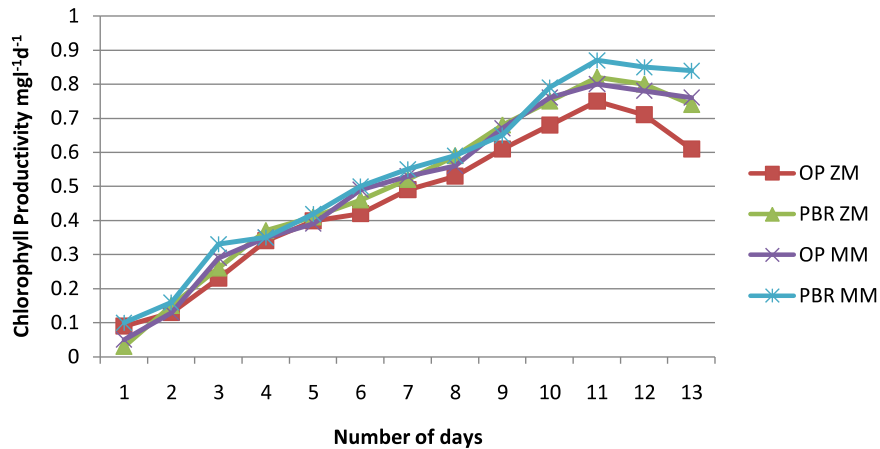

Fig. 7. Chlorophyll productivity in open pond and closed reactor with Zarrouk's and modified media.
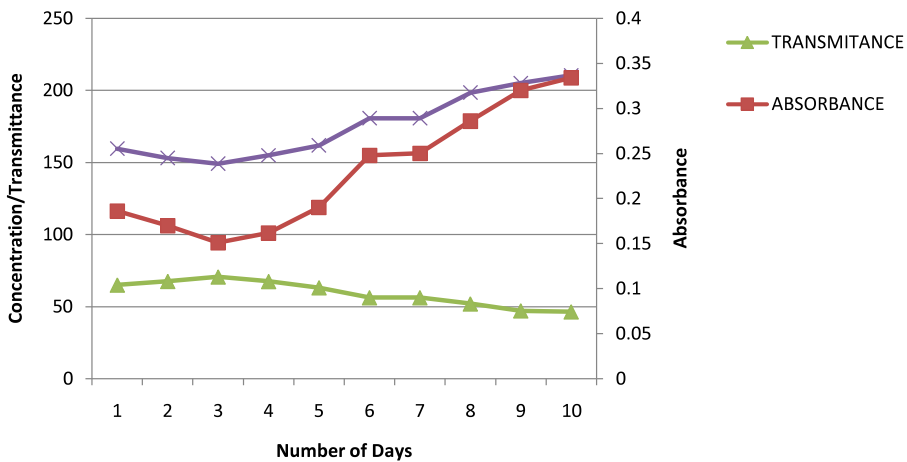

Fig. 8(a). Absorbance, Transmittance, and Concentration vs. days in an open pond with modified media.

The interaction between lighting and temperature on S.platensis growth has been studied extensively. The effect of photoinhibition is accentuated at low temperature and results in low cell concentration and productivity. These studies, however, carried out under natural lighting, where the temperature and light intensity varies with seasonal changes in climate and the photoperiod of the year. When grown in darkness or light intensity below 1000 lux, the algal cultures produced a very small amount of biomass. On the contrary, a large amount of biomass was produced with higher light intensities of 1500 to 3500 lux. With a light intensity of 2500 lux, the best growth rates were achieved.
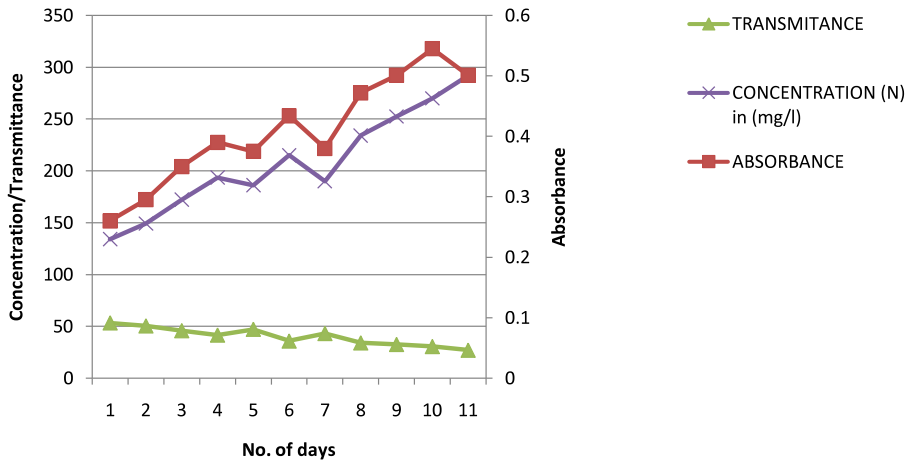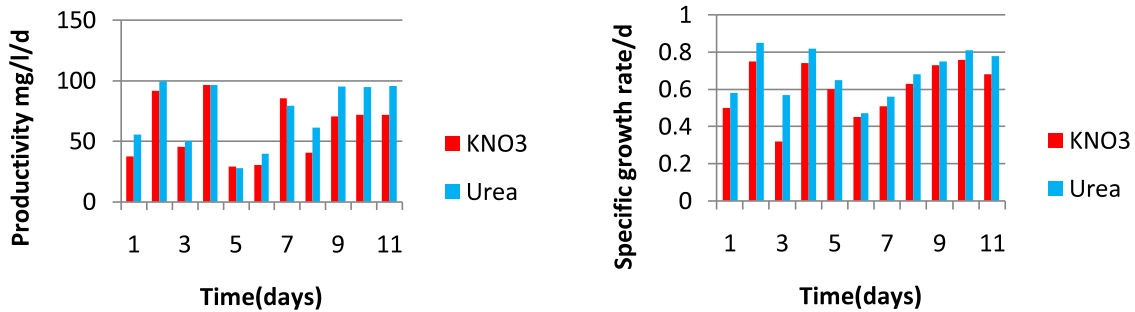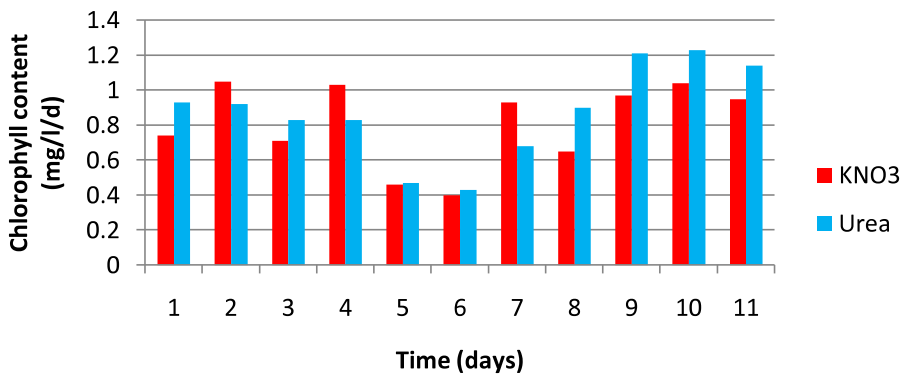
Fig. 8(b). Absorbance, Transmittance, and Concentration vs. days in a closed reactor with modified media.

(a) Variation in Productivity with time

(b) Variation in specific growth rate with time
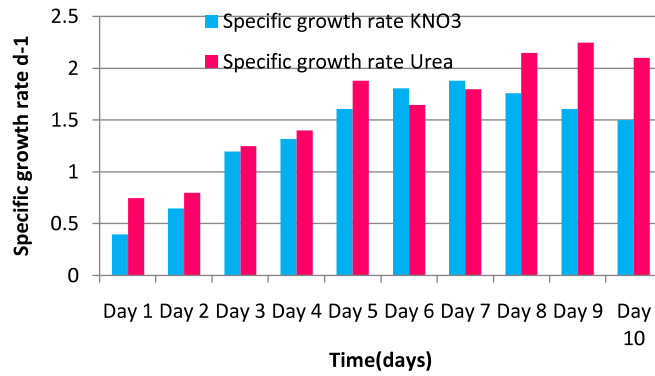

(c) Variation in chlorophyll concentration with time

Fig. 9. Variation in (a) productivity, (b) specific growth rate, (c) chlorophyll concentration.

Due to high variations in temperatures of Bhopal, the spirulina was grown at 17 °C to 37 °C under laboratory conditions. The best growth and biomass concentration were obtained at 32 °C with 12.28 g/l. However, growth rates and production of biomass were significantly lower when the temperature above 35 °C and below 25 °C. This can be due to the effect of temperature and light on photoinhibition. Therefore, the temperature between 25–35 °C was optimum for the cultivation of S.platensis strain.

Light intensity should be more than 1k; the water level should be more than 20 cm. In these lab experiments, S. platensis was successfully cultured at temperatures above 28 °C, and wet biomass was about 12.28 g/L in a closed reactor.

3.3. Effect of media

The growth and biomass yield of Spirulina depend on nutrients availability, pH, light, and temperature. Media composition and its cost are challenging factors for the viable mass cultivation of cyanobacteria. Two different growth media, such as Zarrouk media and modified media, were used to cultivate Spirulina. Zarrouk media served as standard media for cultivation of this microalga. Higher growth rates of Spirulina grown on both media were observed. In this investigation, the growth rates of spirulina under open pond and closed reactor were observed, and maximum growth was obtained at the end of cultivation in a closed reactor with modified media. The composition of modified

(a) Variation in specific growth rate with time
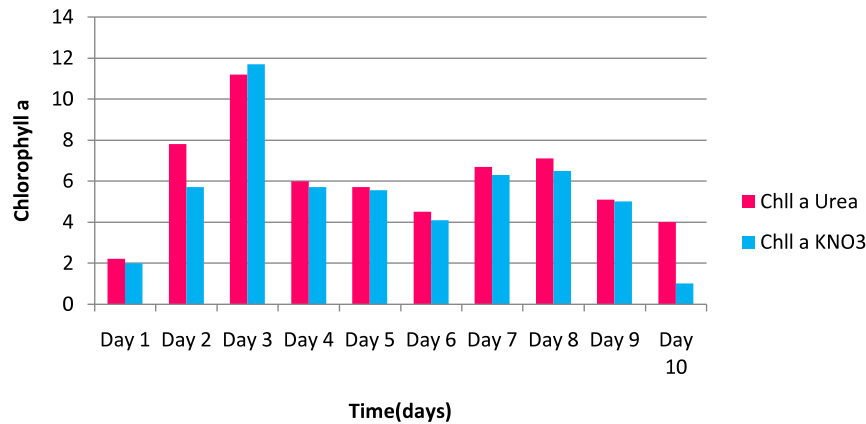
(b) Variation in chlorophyll concentration with time
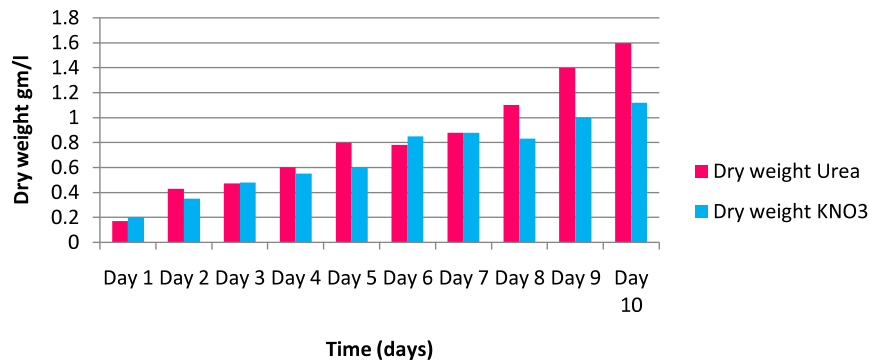
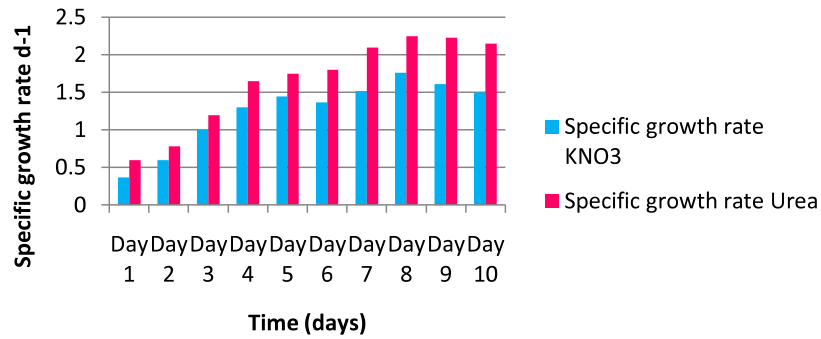(c) Variation in dry biomass weight with time

Fig. 10. Variation in (a) specific growth rate, (b) chlorophyll concentration, (c) dry biomass weight with manual aeration.

media was formulated based on systematic study and nutrient-wise permutations and combinations. The growth of Spirulina was evaluated in both the media. In the modified medium, $FeSO_4 \cdot 7H_2O$, EDTA was completely removed and the concentration of NaCl and $MgSO_4 \cdot 7H_2O$ concentrations were reduced, and more of urea was added when compared with Zarrouk media. The spirulina showed better growth performance with modified media. This investigation was carried out with the primary objective of providing a simple, organic and inexpensive media, and our results clearly show that the newly modified medium is better than Zarrouk 's medium in terms of the performance assessment criteria like productivity, specific growth rate, doubling time and biomass concentration. Cell productivity of 0.52 g/ L/day, was considered a relatively high value in open pond cultivation system. The maximum biomass productivities of Spirulina platensis is in relatively higher temperature habitat. 9 g dry biomass m^{-2} day^{-1} in summer and in the subtropical habitat 10 g dry biomass m^{-2} day^{-1} in autumn and 6 g dry biomass m^{-2} day^{-1} in winter in closed bioreactor.

Figs. 5 and 6 growth curves obtained for cell productivity and specific growth rate for open pond and closed reactor in Zarrouk and modified media shows that cellular concentration was practically constant on the 8th day the growth was steady and again it increased on the 11th day.

It was observed initially there is a net decrease in growth rate due to the low concentration of living spirulina in mother culture.

(a) Variation in specific growth rate with time
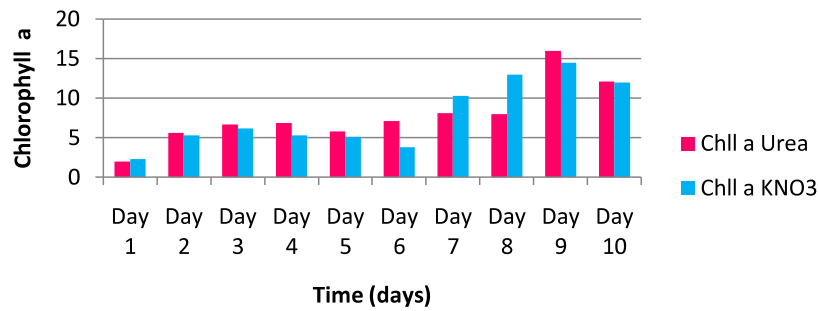
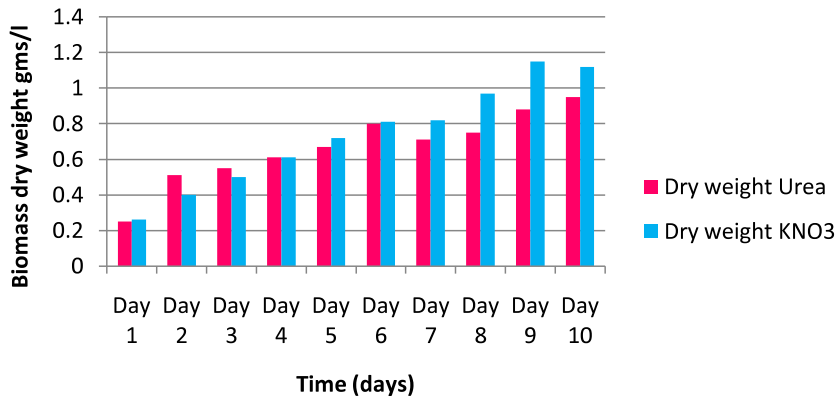(b) Variation in chlorophyll concentration with time

(c) Variation in dry biomass weight with time

Fig. 11. Variation in (a) specific growth rate, (b) chlorophyll concentration, (c) Dry biomass weight with Aeration Effect using Aquarium pump 6–8 h.

Upon mixing with media and change in nutrient concentration the growth increased, and after achieving peak value, it further decreased due to the low amount of light penetration because of the high density of spirulina. Fig. 7 shows the variation in Chlorophyll productivity in an open pond and a closed reactor. OP ZM-open pond with Zarrouk media, PBR ZM-closed reactor with Zarrouk media, OP MM-open pond with modified media and PBR MM denotes closed reactor with modified media. Doubling time calculated using the formula was five days for OP ZM, 5.5 days for PBR ZM, four days for OP MM and 2.8 days for PBR MM. This shows that a closed reactor with modified media provides the best results. Biomass for OP ZM was 8.568 g/l/day, and for PBR

ZM was 10.231 g/l/day, for OP MM was 11.34 g/l/day and for PBR MM it was maximum and was found to be 12.280 g/l/day.

Fig. 8 shows variations in absorbance, transmittance, and concentration with a number of days in an open pond and closed reactor for modified media with a temperature range of 28 to 35 °C and pH was maintained at 9. Aeration was done using an air pump, and the growth rate was highest in a closed reactor with modified media.

The best results for cell growth were observed when urea was substituted by the nitrogen source in the modified media. The chlorophyll content of the spirulina plantesis culture was 11.5 mg/g which was in accordance with the previous literature studies (Henrikson, 1989; Danesi et al., 2002). The chlorophyll

yield and growth rates of spirulina are also affected due to low aeration and bad weather conditions during the experiment period. Thus modified media can be considered as an economically superior source of nitrogen and can be substituted as a conventional nitrogen source.

3.4. Effect of nitrogen source as Urea and KNO_3

With regard to the nitrogen sources used in cultivation, the concentration of cells was higher in Urea than in KNO_3 (Salunke et al., 2016; Bernard and Rémond, 2012). The feasibility of using urea as a source of nitrogen in S Platensis culture was evident because the growth of microorganisms was higher with urea in all tested conditions, without significant influence on the chlorophyll content, leading to higher biomass yields. Fig. 9 shows the variation in biomass productivity, specific growth rate, chlorophyll concentration

3.5. Aeration effect on different nitrogen sources

This work examines the aeration effect on specific growth rate, chlorophyll concentration and dry biomass weight in different nitrogen sources. The spirulina species shows better results with maximum dry weight with urea when aeration is done manually. Maximum chlorophyll content in urea was seen in aeration when done with an aquarium pump. Hence aeration enhances the chlorophyll content in spirulina species. Aeration agitates the culture medium and gives the Spirulina filaments a homogeneous distribution throughout the cultivation system for adequate exposure to illumination. Fig. 10 shows the variation in specific growth rate, chlorophyll concentration and dry biomass weight when aeration was done manually. It also contributes to the uniform distribution of oxygen concentrations and eliminates certain inhibitory substances for example CO_2. Therefore, Aeration is a basic necessity for the Spirulina platensis cultivation. It should also be noted that constant mixing of the culture medium is important to avoid thermal stratification and settling of the cells. It is also essential to retain uniform distribution of nutrients and remove surplus oxygen. Fig. 11 shows the variation in specific growth rate, chlorophyll concentration and dry biomass weight when aeration was done with an air pump. If aeration is not sufficient, the production of biomass and efficiency of energy use is low.

4. Conclusions

Spirulina is claimed as a non-toxic, nutritious food, because of its richness in minerals, protein and necessary fatty acids. It is a healthy energy supplement that is particularly useful for low-calorie food. Spirulina growth in lab scale open pond and photobioreactor are studied for various growth conditions. The experimental study draws the following conclusions.

1. Modified media can be used for growing spirulina in both closed and open pond system.

2. The high concentration of sodium nitrate (2.5 g l^{-1}) in Zarrouk media can be substituted by low concentration of sodium nitrate (0.3 g l^{-1}) for the Spirulina growth.

3. The optimal growth yield of the Spirulina were obtained at pH levels between 8.5 to 10.5, temperature regimes of 25–30 °C and light intensities of 1500–3500 lux.

4. Spirulina can be cultivated in large scale for higher biomass production under outdoor and indoor conditions of Bhopal, M.P, India.

5. The experimental data will allow biomass productivity to be reliably predicted for the mass cultivation of spirulina.

Conflict of interest

All authors declare No conflict of interest to this manuscript.

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