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Preet Sagar SinghCollege of Agricultural
Engineering and Technology,
Punjab Agricultural University,
Ludhiana India**Manpreet Singh**School of Renewable Energy
Engineering, College of
Agricultural Engineering and
Technology, Punjab Agricultural
University, Ludhiana 141004,
India**Monica Sachdeva Taggar**School of Renewable Energy
Engineering, College of
Agricultural Engineering and
Technology, Punjab Agricultural
University, Ludhiana 141004,
India**Correspondence****Preet Sagar Singh**College of Agricultural
Engineering and Technology,
Punjab Agricultural University,
Ludhiana India

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Mass cultivation and harvesting of microalgae (*Chlorella Sorokiniana*) for biomass and lipid production

Preet Sagar Singh, Manpreet Singh and Monica Sachdeva Taggar

Abstract

Mass accumulation of microalgae was studied and different parameters such as pH and absorbance (OD at 600nm) were observed during the growth studies. At 32°C, the absorbance varied from 0.011 to a maximum value of 0.985 on the 7th day while in the experiment at 36°C the absorbance varied from 0.082 to a maximum value of 0.935 on the 7th day. The pH values varied from 7.85-8.55 for 32°C and 7.59-8.01 for 36°C. During harvesting the centrifugation efficiency was found out to be 98.4% at 10,000rpm after 10min of centrifugation at 4°C. Maximum flocculation efficiency (97.23%) was achieved for 200ppm sample followed by 95.42% for 100ppm sample followed by 77.42% for 80 ppm sample after 30min of flocculation. For centrifugation, the biomass yield was 0.4923 gL⁻¹ at 36°C as compared to 0.4665 gL⁻¹ at 32°C. For flocculation, the biomass yield was 0.4683 gL⁻¹ at 36°C as compared to 0.4425 gL⁻¹ at 32°C. Calorific value for dried biomass after centrifugation was found out to be 23.13MJ/kg as compared to flocculated sample which was found out to be 15.16MJ/kg. Total lipids from dried biomass harvested using centrifugation was found out to be 12.25% as compared to flocculated sample which was found out to be 11.51%.

Keywords: *Chlorella Sorokiniana*, Growth, Harvesting, Biomass Yield, Properties

Introduction

Microalgae are one of the earth's most important natural resources. They contribute to approximately 50 percent of global photo-synthetic activity. For autotrophic algae, photosynthesis is a key process for their survival as they convert solar radiations and CO₂ absorbed by chloroplasts into carbohydrates which are subsequently utilized during respiration to produce energy and support growth [1]. The growth rate of the algae is very high; they nearly double themselves in the period of 24 h. During the peak growth phase, some microalgae can double every 3.5 h [2]. The major constituents of the algal biomass are; proteins, carbohydrates and lipids/natural oils. They also produce anti-oxidants such as β-carotene. Omega-3 fatty acids which are believed to be obtained from fish are actually produced by microalgae; the fish consume microalgae and become a source of these polyunsaturated fatty acids. Recent researches in the field of biotechnology have revealed that certain species of micro-algae have pharmaceutical applications and can be used to synthesize anticarcinogenic substances.

One of the other major utilization of algae is for production of biodiesel from microalgal oils. Oil content of microalgae is usually between 20 per cent and 50 per cent, while some strains can reach as high as 80 per cent [3, 4]. Biodiesel produced from algae can be used as an alternative to the diminishing conventional energy sources such as fossil fuels. Moreover, it is a carbon neutral energy source i.e. it releases the same amount of carbon in the atmosphere on combustion as it takes from the atmosphere during photosynthesis. The main source of biofuel energy in algae comes from its lipid content. Microalgae potentially provide several advantages for their use for biodiesel production i.e. i) High biomass productivity ii) utilize carbon dioxide and thus, mitigate greenhouse gases iii) have ability to grow on non-agricultural land thus overcoming the food versus fuel issue iv) Much higher efficiency in converting solar energy into chemical energy v) require much less water than plant crops [5]. major factors that affect the growth of microalgae are light, temperature and pH other factors includes salinity, mixing, nitrogen and phosphorus content, etc. Light is very important for the growth of microalgae since these are autotrophic species and hence they need light to produce

food via photosynthesis. Avoiding photo-inhibition can help to increase the daily growth rate of microalgae biomass [2]. The effect of temperature on the growth rate is not direct but rather indirect as it affects the process of photosynthesis which further determines the growth rate of these species. Temperature determines the activity and reaction rates of intracellular enzyme, which will have an influence on algal photosynthesis, respiration intensity, affect the growth of microalgae and to limit its distribution [6]. pH also affects the growth rate of microalgae and Generally, alkaline environment is conducive for the growth of microalgae. According to a study, the content of chlorophyll of microalgae will decrease when the pH value goes from 8.5 to 9.5 [7].

Harvesting of microalgae from the medium in which it grows can be done with the help of various methods. Algae are typically in a dilute concentration in water and can be harvested using membrane separation, centrifugation, flocculation, sedimentation and flotation. Centrifugation is a method of separating algae from the medium by using a centrifuge to cause the algae to settle to the bottom of a flask. Centrifugation is energy intensive and currently considered too expensive for personal use, though may prove useful on a commercial and industrial scale. Flocculation is a method where something is added to the mixture of water and algae that causes the algae to "clump" together (or aggregate) and form colloids. Chemical flocculants include alum and ferric chloride. Chitosan is a linear polysaccharide composed of randomly distributed β -linked D-glucosamine and N-acetyl-D-glucosamine. It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide. Also, the extensive use of chemical flocculants has been restricted because of their neurotoxic and carcinogenic properties. Chitosan not only has been proved highly effective for water treatment and environmental protection, but also shows interesting properties in removing both freshwater algae and marine algae. Chitosan has a net positive charge because of the high charge density of the chitosan. As the overall charge of microalgae cells is negative, the positively charged chitosan is strongly adsorbed on microalgae cells. Chitosan first neutralizes charges on the microalgae cells, weakens the electrostatic repulsion between the microalgae cells, and then reduces the interparticle repulsion [8]. Keeping this in view, a study was planned with the objectives to study the growth behavior of microalgae during mass cultivation, harvesting of algal biomass using different techniques and properties of algal biomass.

Material and Methods

Procurement of culture

A pure culture of *Chlorella Sorokiniana* NCIM 5561 was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, and Maharashtra, India.

Media composition

The composition of culture medium used for growth of microalgal culture is as follows: KH_2PO_4 : 0.50 g L^{-1} , K_2HPO_4 : 0.20g L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.20g L^{-1} , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 3mg L^{-1} , Glycine: 5.00g L^{-1} , Vitamin B1: 0.05mg L^{-1} , and A5 trace mineral solution: 1 ml L^{-1} . The nutrient media was prepared with double distilled water and pH was maintained in the range of 6-7. The culture media was sterilized by autoclaving at 121°C and 15 psi for 15 min. The microalgae was repeatedly sub-cultured and maintained on solidified basal agar media.

Cultivation of microalgae

A loopful of the microalgal culture was transferred into 250 mL conical flask and was kept in an orbital shaking incubator at 30°C. The prepared inoculum (100mL) was then, transferred to culture medium (5 Liters) and kept in BOD Incubator at 32°C with light intensity of 7000 Lux. The same experiment was conducted at 36°C and 7000 Lux light intensity.

Microalgae growth measurement

The growth of microalgal culture was monitored by measuring absorbance at 600nm with uv-visible spectrophotometer at regular intervals of 24 h for 7 days. The growth of the microalgae was compared to two different temperatures i.e. 32 and 36°C

Harvesting of microalga

Microalgae was harvested from the culture medium by the process of centrifugation as well as flocculation method.

Centrifugation

The culture was transferred into the 30mL centrifuge tubes and the centrifugation was done at 10,000 rpm for 10 minutes at 4°C using a cold centrifuge. The initial microalgal biomass concentration was estimated in terms of absorbance at 600nm and was used as control. The absorbance of the supernatant thus, obtained after centrifugation of control culture was measured. Centrifugation efficiency was calculated using following formula:

$$\text{Centrifugation Efficiency (\%)} = \left(\frac{B - A}{B} \right) \times 100$$

Where, A= Absorbance at 600nm of supernatant and B= Absorbance at 600nm of control

Flocculation

Flocculation of microalgae was standardized using chitosan as flocculent. One hundred milligrams of chitosan powder was accurately weighed into a glass beaker and mixed with 10 mL of 0.1 M HCl solution and kept aside for about an hour to dissolve completely. It was then diluted to 100 mL with tap water to obtain a solution containing 1.0 mg chitosan per mL of solution. The freshly grown microalgal culture (100mL each) was taken in four 250mL beakers and different concentrations of chitosan viz. 40, 80, 100 and 200 ppm were added separately in the microalgal cultures. After addition of chitosan solution, each beaker was kept in an orbital shaker incubator and stirring speed was maintained at 100 rpm. The flocculation was then, observed visually with time. Flocculation efficiency was calculated at different time intervals by using the same formula as described above.

Analytical procedures

Dry biomass yield

The harvested algal biomass was then dried in oven at 50°C. The dry biomass was weighed after different intervals till constant weight was obtained and expressed as total dry biomass produced per litre of micro algal culture (g L^{-1}).

Calorific Value

Dried sample of microalgae (1g) was used to determine the calorific value of biomass using digital bomb calorimeter. The heat released in combustion of biomass will be absorbed by the known amount of water. The temperature reading is taken at regular intervals until three successive readings were found to be constant which was recorded as final temperature. The

heat of combustion was computed from initial and final temperature of water. The following formula was used to calculate the calorific value of the sample

$$\text{HCV (MJkg}^{-1}\text{)} = \frac{\text{Rise in Temperature (}^\circ\text{C)} \times \text{Water Equivalent (} \frac{\text{MJ}}{^\circ\text{C}} \text{)}}{\text{Sample Weight (g)}} \times 1000$$

Total Lipids

Total lipids were extracted according to standard AOAC method with some modifications using soxhlet apparatus [9]. One g of dry algal sample and 150 ml petroleum ether were used for extraction by Soxhlet apparatus for 6 hours. The solvent was evaporated at 35°C using a rotary evaporator. Finally, the lipid content of samples was determined gravimetrically.

Results and Discussion

The microalgae was grown in a glass vessel (5L) covered with the polyethylene sheet and a hole of 2.5cm diameter was made in the sheet to insert a cotton plug for air circulation (Fig 1). This avoided the evaporation of culture media during the 7 days experiment. The cotton plugging was done to prevent significant loss of water from culture medium due to evaporation.



Fig 1: Tank covered with polythene sheet and cotton plug inserted in the hole

Effect of temperature on growth of microalgae

The studies on growth of microalgae in terms of absorbance revealed that absorbance of the microalgal culture increased from 0.011 to a maximum value of 0.985 on the 7th day in a culture medium incubated at 32°C while the absorbance increased from 0.082 to a maximum value of 0.935 on the 7th day in a culture medium incubated at 36°C. The growth was reported to be less during the first 3 days of cultivation, when microalgae was grown at 32°C but slightly higher microalgal growth was reported at 32°C during remaining days of cultivation (Fig. 2).

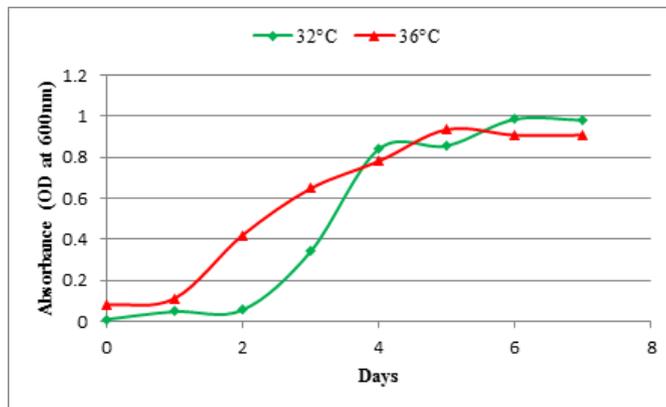


Fig 2: Effect of temperature on growth of microalgae at different days after culturing pH of culture medium

The pH values of the microalgal culture medium varied from 7.85-8.55, when microalgae was grown at 32°C and 7.59-8.01 for 36°C. Maximum value of pH was reported to be 8.55 (Day 2) at 32°C and 8.01 (Day 3) at 36°C (Fig. 3).

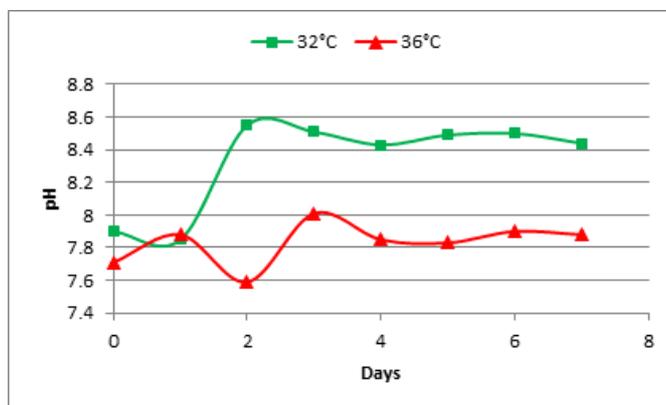


Fig 3: Effect of temperature on pH of the culture medium at different days after culturing

Harvesting of Microalgae

Harvesting of microalgae was carried out using chitosan as flocculant. The concentration of chitosan required for efficient flocculation was standardized. The algal culture was flocculated using different concentrations of chitosan viz. 40, 80, 100 and 200ppm. Flocculation efficiencies of chitosan at different time intervals have been presented in Table 1 and Fig. 4. The absorbance of microalgal culture decreased from initial value of 0.939 at zero day of culturing to 0.830 at 7DAC with 40ppm chitosan concentration and flocculation efficiency of 11.61 percent was observed at 7 DAC. With 80 and 100ppm chitosan concentration, absorbance of microalgal culture decreased from initial value of 0.939 to 0.212 and 0.043 at 7DAC with flocculation efficiencies of 77.42 and 95.42 per cent, respectively. The absorbance of microalgal culture decreased from initial value 0.939 to 0.026 at 7DAC with flocculation efficiency of 97.23 per cent. The sharp decline in absorbance from 0.939 to 0.344 was seen after 2min of flocculation with 200ppm chitosan concentration, however, decline in absorbance from 0.939 to 0.290 was observed after 5min of flocculation with 100ppm chitosan concentration. A gradual decrease in absorbance from 0.939 to 0.212 was observed with 80ppm chitosan concentration after 30min of flocculation. Based on above results, maximum flocculation efficiency (97.23%) was achieved with 200ppm chitosan concentration followed by 95.42 per cent with 100ppm chitosan after 30min of flocculation. The flocculation

efficiency of more than 90 per cent was achieved after 13 min with 200ppm chitosan while same efficiency was observed after 20min with 100ppm chitosan concentration. The visible difference in flocculation using different chitosan concentrations after one hour and 24 hours can be made from Fig. 5. From the above studies, 100 ppm concentration of

chitosan was considered to be most suitable and was used in subsequent experiments for harvesting of microalgae. Separation of microalgae by centrifugation at 7DAC revealed that centrifugation efficiency was found out to be 98.4 per cent when microalgal sample was centrifuged at 10,000rpm for 10min at 4°C in a cold centrifuge.

Table 1: Absorbance and flocculation efficiencies (%) of microalgae at different chitosan concentrations and time intervals

S. No.	Time (mins)	40ppm		80ppm		100ppm		200ppm	
		OD	Efficiency (%)	OD	Efficiency (%)	OD	Efficiency (%)	OD	Efficiency (%)
1	0	0.939	-	0.939	-	0.939	-	0.939	-
2	2	0.863	8.09	0.695	25.98	0.665	29.18	0.344	63.36
3	5	0.851	9.37	0.630	32.91	0.290	69.12	0.207	77.95
4	10	0.842	10.33	0.455	51.54	0.220	76.57	0.110	88.28
5	15	0.842	10.33	0.348	62.94	0.122	87.0	0.070	92.54
6	20	0.841	10.44	0.295	68.58	0.086	90.84	0.048	94.88
7	30	0.830	11.61	0.212	77.42	0.043	95.42	0.026	97.23

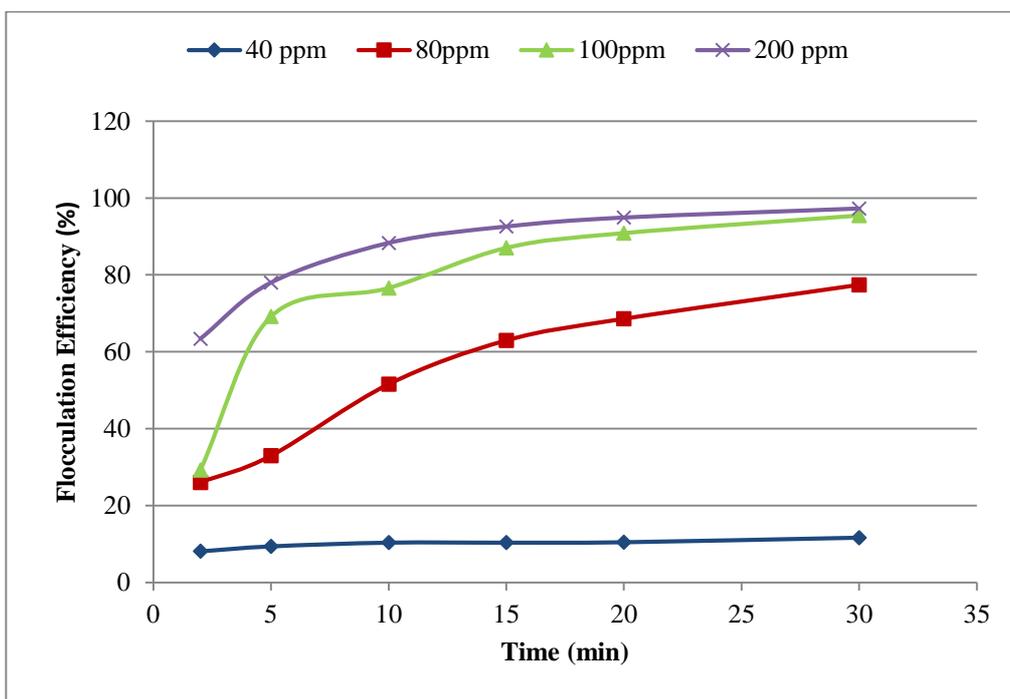


Fig 4: Microalgae flocculation efficiencies at different chitosan concentrations and time intervals



a) After 1 hour



b) After 24 hours

Fig.5 Flocculation of microalgae at different chitosan concentrations

Dry biomass yield: The dry biomass of microalgal culture grown at different temperatures and harvested by two different methods has been given in Fig. 6. Higher biomass was obtained at 36°C as compared to 32°C irrespective of the harvesting method used. Total algal biomass was 0.492 gL⁻¹ at 36°C as compared to 0.467 gL⁻¹ at 32°C, when centrifugation method was used for separation of microalgal biomass from the culture media. With flocculation process using chitosan as flocculant, the biomass recorded was 0.468 gL⁻¹ at 36°C as compared to 0.443 gL⁻¹ at 32°C. The per cent increase in algal biomass with centrifugation and flocculation process was 5.53 and 5.83, respectively as the temperature was increased from 32°C to 36°C.

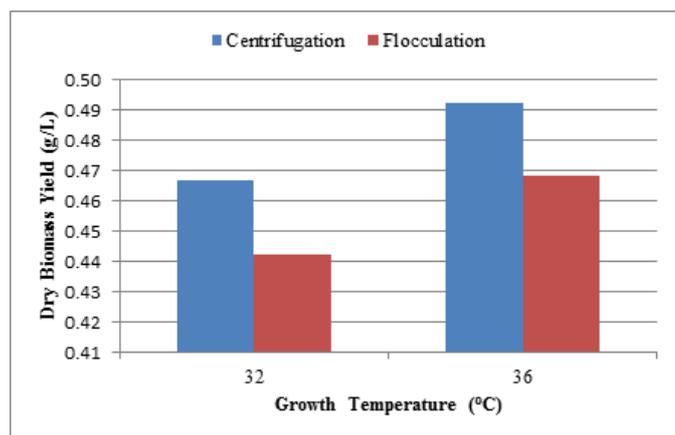


Fig 6: Effect of growth temperature and harvesting method on dry biomass yield (g/L)

Calorific Value

Calorific values of the dry biomass obtained after centrifugation and flocculation with chitosan were determined as per methodology discussed. Calorific value of dried biomass after centrifugation was observed to be 23.13MJ/kg as compared to flocculated microalgal sample with calorific value of 15.16MJ/kg. This reduction in calorific value may be due to the uncombustible material present in the biomass which came along the dried biomass during flocculation.

Total Lipids

Total lipids of dry microalgal biomass was determined after separation of biomass from culture medium by centrifugation and flocculation with chitosan. The total lipids of dried biomass harvested using centrifugation was found out to be 12.25 per cent as compared to flocculated sample which was found out to be 11.51 percent. This reduction in calorific value may be due to the flocculant material present in the biomass which came along the dried biomass during flocculation.

Conclusion

Chlorella sorokinina culture was used for mass cultivation of microalgae at 5L scale. The maximum microalgal biomass achieved was 0.4923 g/l when microalgae were grown at 36°C. Flocculation efficiency of chitosan was maximum 97.23% after 30min of flocculation when chitosan was used at 200ppm concentration. However, centrifugal efficiency was found out to be better as compared to flocculation efficiency achieved using chitosan. The dried microalgal biomass exhibited high calorific value and total lipid content of 23.13MJ/kg and 12.25 per cent. Thus, *Chlorella sorokinina* microalgal strain can be potentially used for biofuel production.

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