Research Article

Growth rate, Biochemical and Biomass Analysis of *Scenedesmus obliquus* Algae in Shahpura Lake Bhopal (MP)

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ABSTRACT

In the present study, the algae *Scenedesmus obliquus* has shown excellent growth in the water of Shahpura Lake in Bhopal (M.P.). Besides this, when CO_2 concentration has increased in the water, the growth rate of this algae was much better. This alga could be use to decrease CO_2 concentration present in the environment. Results showed that the growth rate of algae *S. obliquus* in 4 liter water of different lakes at 680 nm and it was observed that maximum growth rate 1.43 nm was seen in Shahpura lake water on 7th day which was followed by lower lake 1.21 nm on day 8th and on the 7th day 1.17 nm in distilled water and lowest growth was seen in Upper lake on 6th day. When, growth rate of algae *Scenedesmus obliquus* was seen in 8 liter water of Upper Lake at 680 nm with CO_2 was found to be highest 1.21 nm on day 8th and without CO_2 it was noticed to be lowest 0.94 nm on day 9th. Hence, production of algae can be a good source for Bio diesel production because of high oil content present in it.

Keywords: *Scenedesmus obliquus,* Biodiesel, Biomass, Microalgae, Biofuel.

INTRODUCTION

The diverse group of eukaryotic organisms that belong to the phylum Protista is called algae. Algae use light energy to convert CO_2 and H_2O into carbohydrates and other cellular products, during this process oxygen is released. Algae contain chlorophyll a that is required for photosynthesis. Many algae contain other pigments that extend the range of light that can be used by these organisms for photosynthesis (Nester et al. 2004). Organisms that are classified as algae include both microscopic unicellular and macroscopic multi cellular. As one of the primary producers of carbohydrates and other cellular products, algae are essential in food chains of the entire world. A large portion of the oxygen in the atmosphere is produced by algae (Nester et al. 2004). All algae are primarily made up of proteins, carbohydrates, fats and nucleic acids in varying proportion while percentages can vary with the type of algae. Some types of algae are made up of up to 40% fatty acids based on their overall mass. It is the fatty acid that can be extracted and converted in to bio fuel. Algae have the ability to convert carbon dioxide to biomass that can further processed downstream to produce biodiesel, fertilizer and other useful products. Photosynthetic growth of algae requires carbon dioxide, water and sunlight. Temperature must be in the range of 20–30°C in order to have good growing conditions. Algae also need other inorganic nutrients like phosphorus and nitrogen in order to grow. It is well known fact that micro algae grow in aqueous suspensions, allows for more efficient access to H_2O, CO_2 and other nutrients which explain the potential for the production of more oil per unit area than other crops currently used. The chemical composition of algae differs based on species. Algae have several characteristics that cause them to be a runner biodiesel feedstock that deserves serious investigation. The advantages of using algae for biodiesel production including, no competition for land with crops, no competition with the food market and ability to grow in water with high levels of salt so there is no additional demand of fresh water. Moreover, areas with saline ground water that has no other useful applications can be targeted. Overall it uses less water than oil seeds. High oil yield algae of the aquatic species require less land for growth than biodiesel feedstock from terrestrial plants because they are capable of producing more oil per hectare. Furthermore, the oil content in algae (per dry weight) can reach as high as 80%. It is worth noting that the oil from microalgae can be extracted with yields up to

80-90% (Grima et al.1994**,** Fajardo et al. 2007, Belarbi et al. 2000). CO_2 is a common industrial pollutant, thus microalgae can contribute in reducing atmospheric CO_2 by consuming CO_2 wastes from industrial sources such as power plants. In light of the fact that micro algae are the most efficient primary producers of biomass, it is very likely that they will eventually become one of the most important alternative energy sources as biodiesel (Li et al., 2008). In the present study, microalgae *Scenedesmus obliquus* was selected for observing their growth rate, biochemical and biomass study, through carbon capture in fresh water bodies for utilizing the atmospheric CO_2.

Experimental

In the present study, gratis sample of micro algae *Scenedesmus obliquus* (Strain No. 276-10) was obtained from the Albert Von Haller Institute, George August University Gottingen, Germany and brought into the, Rajiv Gandhi Prodhyogiki, Vishwavidyalaya, Bhopal, which was stored in the Tissue Culture Lab., at 15-18°C under low light intensity and multiplication of cultures was done by transferring in to new culture media. In the present study, for cultivation of this alga, the media which was used known as Basal media (SAG, 2007) which was used for the maintenance of culture of algae in test tubes. Media was usually prepared from stock solutions of macronutrients, trace metals and vitamins which were added to a large proportion of the final volume of water in order to avoid precipitation. Media was used as liquid or solidified by 1.0-1.5% agar. The composition of Basal medium is as under:

Composition	Stock solution [gm/100ml]	Nutrient solution [ml]
KNO_3	1	20
K_2HPO_4	0.1	20
$MgSO_4.7H_2O$	0.1	20
Soil extract*	-	30
Micronutrient solution**	-	5
de-ionized or distilled water	-	905

***Preparation of soil extract**

In the present study, a 6 liter flask was filled one third with garden soil of medium then added de-ionized water until it stands 5 cm above the soil and sterilized by heating in a steamer for one hour twice in a 24 hrs interval. Then it was separated with the decanted extract from particles by centrifugation. It was filled into small containers of stock solution each of a size appropriate to making a batch of media, then autoclaved for 20 minute at 121°C and stored in the refrigerator.

Preparation of a micronutrient solution- It was prepared by the following manner:

Composition	Stock solution [gm/100ml]	Applied solution
$ZnSO_4.7H_2O$	0.1	1 ml
$MnSO_4.4H_2O$	0.1	2 ml
H_3BO_3	0.2	5 ml
$CO(NO_3)_2.6H_2O$	0.02	5 ml
$Na_2MoO_4.2H_2O$	0.02	5 ml
$CuSO_4.5H_2O$	0.0005	1 ml
de-ionized	-	981
$FeSO_4.7H_2O$	-	0.7 gm
EDTA	-	0.8 gm

After mixing in above mentioned composition components of micronutrient were autoclaved separately in two solutions which were united after cooling.

Solution I

881 ml distilled water+ stock solution of salts without $FeSO_4$+0.4 gm EDTA.

Solution II

100 ml distilled water+ 0.7 gm $FeSO_4$ + 0.4 gm EDTA.

In the present study, basal medium (Medium 1) was prepared with 0.1% proteose-peptone. Before sterilization agar was dissolved in the medium in a steamer. After this the test tube can be filled with 10 ml of the hot medium, closed with cotton plugs. Then sterilized usually by autoclaving at 121°C for 15 minutes and was stored for several weeks after cooling in a refrigerator.

Serial dilution method for algae culture

In the present study, 20 screw capped test tubes was used which was filled with 9 ml of

algal culture medium i.e. basal medium. Then numbered the test tube from 1-10. Then put the test tube containing the strain to be diluted in the biological safety cabinet by removing the cap and flaming its neck and by using a sterilized 1 ml pipette take 1 ml sample from the initial contaminated strain of culture prepared in the previous laboratory and added it to the tube No.1, then stir gently. Follow up the same procedure with the contaminated algal sample by instructor. By using a new sterile 1 ml pipette repeated the previous step by taking 1 ml inoculums from tube No. 1 and inoculated it in to the tube No. 2. Then repeated the same procedure with the remaining tube from 3-10. Each tube pipetting 1 ml from the previous tube (gently stirred) in to the next one, then flamed neck and capped them. Finally keep it, under controlled environmental conditions. When cell growth reappeared, checked sample of the tube under microscope and get rid of the tubes that were still contaminated, typically the initial ones and keep only the purified culture, usually in the more diluted tubes. Repeat the process using last dilution if necessary. For present study, before inoculation the p^H of the each flask was adjusted to 7.6 with 0.1N HCl and 0.1 N NaOH. About 100 ml of media were distributed to each of the flasks and all are inoculated with 10 ml of inoculums. This process was done in to laminar air flow chamber.

Determination of specific growth rate of algae

There are several factors responsible to determine the growth rate of algae. However, some important factors that determine the growth rate of algae viz. Light that is needed for the photosynthesis, Temperature that is required for algae to grow, Medium/Nutrients - Composition of the water is an important consideration (including salinity), pH between 7- 9 needed to have an optimum growth rate of algae, Aeration - The algae need to have contact with air, for its CO_2 requirements, Mixing prevents sedimentation of algae and makes sure all cells are equally exposed to light and a photoperiod: Light and dark cycles. Optical density of microalgae cultures was measured at a regular interval of time (24Hrs) by checking absorbance at 680 nm with the help of Perkin Elmer Spectrophotometer and growth rate of algae was observed with the help of spectrophotometer at 680 nm. For the present experiment fluorescent lamps was used as a source of light and intensity was adjusted to 2500~3500 lux for all the culture flasks and 16 hrs of light and 8 hrs of dark cycles were repeated for growth of all the cultures. The temperature was adjusted to 25°C for all the flasks. Aeration/Mixing is necessary to prevent sedimentation of the algae. Depending on the scale of the culture system, mixing was achieved by stirring daily by hand or using aerating jet pumps. After culturing the algae, it was filtered with muslin cloth, algae rinsed with distilled water, dried with paper tissue, frozen at −20°C and freeze-dried. The dried material was powdered manually with the use of mortar and pestle and kept in desiccators containing silica gel and protected from light at room temperature until chemical analysis.

Biochemical analysis

Ash content

The ash content of the sample is the inorganic residue left after complete removal of the organic residue by muffling at about 550-650°C in the muffle furnace. Most of the chemical constants were determined according to the standard procedure of AOAC (2000).

Moisture content

Moisture content was determined by complete drying of the sample at 100±5°C. Moisture content of algae was determined according to the method described by AOAC (2000) with slight modifications. One gm of sample of algae was put in a crucible and dried in a universal oven at 105°C until constants weight was obtained.

Carbohydrate analysis

Carbohydrate was analyzed by Anthron method (Hedge and Hofreiter, 1962) which was first hydrolyzed into simple sugars by using dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxyl methyl furfural. This compound forms with Anthrone a green colored product with an absorption maximum at 630 nm. For this experiment the required materials was 2.5 N HCl, Anthrone reagent and then dissolved 200 mg anthrone in 100 ml of ice-cold 95% H_2SO_4. Standard glucose: Stock solution was dissolved 100 mg in 100 ml water. Then stored refrigerated after adding a few drops of toluene. Then, weighed 100 mg of the sample into a boiling tube and hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cooled at room temperature. Neutralised it with solid sodium carbonate until the effervescence cease. Made up the volume to 100 ml and centrifuged. Collected the supernatant and took 0.5 and 1 ml aliquots for analysis. Prepared the standard by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the

working standard and 0'served as blank. Make up volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Then, added 4 ml of anthrone reagent. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green color at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube. Calculated amount of carbohydrate present in 100 mg of the sample

$$= \frac{\text{Mg of glucose}}{\text{Volume of test sample}} \text{X100}$$

Protein analysis

It was done by Biuret method in which the –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a purple color which can be measured at 540 nm. For this experiment, required materials were Biuret Reagent, Protein Standard: 5 mg BSA/ml. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes. Then, pipetted out 1 ml of the given sample in another test tube. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank. Now, added 3 ml of Biuret reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'. Mixed the contents of the tubes by vortexing/shaking the tubes and warm at 37 ºC for 10 min. Now cooled the contents and record the absorbance at 540 nm against blank. Then plotted a standard curve by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis and calculated the concentration of protein in the given sample by standard curve.

Lipid analysis

Micro algal lipid extraction was done by Bligh and Dyer method (1959). Micro algal biomass was collected by centrifuging the cells at 4000Xg for 10 minutes. The cells were washed with distilled water, lyophilized and weighed. The known amount of biomass (100mg) was then homogenized with chloroform: methanol 1:2 at 35.C. Extract was centrifuged for 7 minutes at 10000Xg and supernatant was collected in a separating funnel. The residue was further homogenized with chloroform and again centrifuged (10000Xg) to collect the supernatant. Now 0.9% NaCl solution was added to the filtrate and washed, lower layer of chloroform was separated and treated with anhydrous Na_2SO_4 to remove the traces of water. The lipid content was determined gravimetrically and expressed as dry weight % after evaporating the chloroform. This analysis was completed without CO_2. Then after, the algae culture was done in 20 liter jar and media was put in 8 lit with CO_2 and aeration because the growing of algae for energy reduces the threat of global warming, as it contributes to the limitation of consumption of fossil fuels and uses large amounts of CO_2 for its production. The carbon comes from carbon dioxide necessary for algal growth (Sanchez et al. 1999). To produce 100 mg of biomass, algae need approximately 183 mg of CO_2 (Frac et al., 2010). Proper carbon source is very essential for growth of microalgae along with other nutrients. In the present study, CO_2 gas was tried to blown in the media but, there is loss of CO_2 in the air because of its poor solubility. For that, carbon dioxide gas is supplied periodically to the cultures by maintaining a steady pH of the cultures. A flow rate of CO_2 was maintained at approximately 1.5 %/min/hour for a week by using a digital gas flow Rota meter.

RESULTS AND DISCUSSION

Results mentioned in Table (1) described about the growth rate of algae *S. obliquus* in 4 liter waters of different lakes at 680 nm and it was observed that maximum growth rate 1.43 nm was seen in Shahpura lake water on 7^{th} day which was followed by lower lake 1.21 nm on day 8^{th}, and on the 7^{th} day 1.17 nm in distilled water and lowest growth was seen in Upper lake on 6^{th} day. Results mentioned in Table (2) described about the growth rate of algae *Scenedesmus obliquus* in 8 Liter water of Shahpura Lake at 680 nm with CO_2 was found to be highest 1.21 nm on day 8^{th} and without CO_2 it was noticed to be lowest 0.94 on day 9^{th}. Very recently, Aikins et al. (2010) have described that the growth rate of algae requires carbon dioxide as one of the main nutrients. Results mentioned in Table (3) described about the biochemical analysis of the fresh water algae Scenedesmus obliquus and it was found that before supplying CO2 the moisture content was 83.10%, crude protein 4.30%, Fat/lipid 3.45%, ash 4.02% and carbohydrate was found 5.13% whereas after supplying CO2, the moisture content was 78.05%, crude protein 5.95%, Fat/lipid 5.08%, ash 4.54% and carbohydrate was found 6.38%. From the results it is quite obvious that lipid content in algae after supplying CO_2 got increased. Similarly, Chisti (2008a) has reported that CO_2 is needed for growth and for

increasing the lipid content in it. From the results it is quite obvious that lipid content in algae after supplying CO_2 got increased. Results mentioned in Table (3) depicted the dry biomass of algae *Scenedesmus obliquus* after culturing in 8 liter water of Shahpura Lake which was found to be 5.241 gm before supplying CO_2 and after supplying CO_2 it got increased three times more i.e. 14.224 gm very rapidly. From the results it is quite clear that CO_2 increased biomass rate three times more. In this context, Dismukes et al. (2008) have described micro alga as an important type of aquatic biomass. Finally it can concluded that CO_2 is a common industrial pollutant, thus microalgae *Scenedesmus obliquus* could be a contributor in reducing atmospheric CO_2 by consuming CO_2 wastes from industrial sources. Very recently, Mandal and Mallick (2011) have described waste utilization and biodiesel production by the green micro alga *Scenedesmus obliquus.*

Table 1: Growth rate of algae *S. obliquus* in 4 liter water of different lakes at 680 nm

Estimation Dates	Distilled water	Shahpura Lake	Upper Lake	Lower Lake
25-11-2013	0.66	0.61	0.57	0.41
26-11-2013	0.75	0.65	0.59	0.60
27-11-2013	0.95	0.80	0.71	0.71
28-11-2013	0.99	0.92	0.86	0.84
29-11-2013	1.00	1.14	0.87	0.96
30-11-2013	1.10	1.18	0.91	0.99
02-12-2013	1.17	1.43	0.86	1.08
04-12-2013	0.96	0.96	0.82	1.21
05-12-2013	0.63	0.87	0.76	0.91
06-12-2013	0.68	0.79	0.61	0.74

Table 2: Growth rate of algae *S. bliquus* in 8 Liter water of Shahpura Lake at 680 nm

Estimation Dates	Shahpura Lake With CO_2	Shahpura Lake Without CO_2
16-12-2013	0.52	0.32
17-12-2013	0.60	0.43
18-12-2013	0.67	0.49
19-12-2013	0.84	0.52
20-12-2013	0.94	0.60
23-12-2013	0.99	0.64
24-12-2013	1.01	0.74
26-12-2013	1.21	0.81
27-12-2013	0.87	0.94
28-12-2013	0.81	0.77
29-12-2013	0.64	0.65
30-12-2013	0.49	0.42

Table 3: Biochemical analysis of the fresh water algae *Scenedesmus obliquus*

Parameters	Before supplying CO_2	After supplying CO_2
Moisture %	83.10	78.05
Crude Protein %	04.30	05.95
Fat %	03.45	05.08
Ash %	04.02	04.54
Carbohydrate %	05.13	06.38

Table 4: Dry Biomass of algae *Scenedesmus obliquus* after culturing in 8 liter water of Shahpura Lake

Before CO_2 weight of *Scenedesmus obliquus* algae (gm)	After CO_2 weight of *Scenedesmus obliquus* algae (gm)
5.241	14.224

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