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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Nitrogen for Increasing Carbohydrate Content in Microalgae

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ABSTRACT

The biggest problem of the world with industrial revolution is consumption of non- renewal source of energy which is limit. If human race is not going to divert toward renewal sources of energy, then the day is not far when all non-renewal sources of energy get exhausted and complete industrial revolution will be shutdown. This force us to move towards exploring alternate source of energy. Microalgae is one of the most promising sources due to its availability and fast growth, efficient carbon dioxide fixation and there is not competing for arable lands and potable water, and potentially accumulating high amounts of lipids and carbohydrates in these micro-algae. Therefore, the aim of this study is to observed production of carbohydrates at different concentration of nitrogen in Chlorella vulgaris. After analyzing the data, the maximum carbohydrate concentration was recorded in Chlorella vulgaris is 312mgL^{-1}at 14th day of growth at 0.05mM concentration of Nitrogen. It means the maximum concentration of Carbohydrate present in Chlorella vulgaris may be used for bioethanol production.

Keywords: *Biofuels, Carbohydrate, Chlorella vulgaris, Microalgae, Non-renewal source of energy.*

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INTRODUCTION

The continuous growth in global population and the ongoing development of countries such as China and India have contributed to a rapid increase in worldwide energy demand, which is projected to increase by 50% or more by 2030 [11, 24]. Fossil fuels such as oil and gas are finite resources, and their current rate of consumption cannot be sustained. With the depletion of resources and the growing emphasis on mitigating climate change and greenhouse gas emission, an urgent need for industrial and scientific community to introduce new alternative biofuels on the global energy market [7, 16].

To confront energy shortage, global warming and climate change, biofuels derived from biomass have received increasing attention from the industry, academia and governments [27, 26]. We need to develop the technology to utilize hidden energy of bio product intern as biofuels as it is renewal and available in abundant in our nature. Biofuel can be developed by biomass conversion which is derived from living organism majorly from plant and plant derivatives through carbon fixation, and it is most commonly seen in plant and microalgae. [5].

There are various kind of biofuel are available which are developed from natural sources like biodiesel which can be used as alternate for diesel oil, another is bioethanol can be used as alternate for gasoline. [2, 9]. Bioethanol receives more attention as different generation biofuel in the sustainable energy industry [16].

Lately microalgae (green algae and cyanobacteria) biomass has also remarkable potential as a feedstock for the bioethanol production due to their high carbohydrate content. Microalgae cultivation with wastewater treatment can reduce CO_2 emissions and the cost of microalgae bioethanol production [4]. The advantages to produce bioethanol from microalgae include easy adaption to environmental condition, high photosynthesis efficiency, high carbohydrate content and noncompetition for farmlands [26].

Another way by which microalgae help in reducing global warming as it consumes carbon dioxide (Greenhouse gas) and recycle it else these gases will be emitted in the atmosphere.Octane rating in

bioethanol is higher than gasoline, which help in increasing engine compression ration which intern increase thermal efficiency and non-harming renewal liquid [17]. In addition, for every gallon of ethanol being used; a gallon of fossil-fuel gasoline is displaced. To fulfill the need to future and keep growth moving using microalgae is a perfect source of bioethanol. With these details about microalgae the aim of present study was to observe the effects of different nitrogen concentration on growth and carbohydrate contents of the *Chlorella vulgaris* for ethanol production.

MATERIAL AND METHODS

Source of organism

The microalgae *Chlorella vulgaris* used in the experiment was provided by Prof.Rangaswami, from Department of Botany, University of madras, (Tamil Nadu). The microalgae were grown in BBM medium for 15 days and after that the algae were transfer in slant and preserved at low temperature for further use. [21]. For the preparation of solid medium 2-3% agar was used.

Concentration of nitrogen on media:

To study the effect of nitrogen on microalgae, different concentration of nitrogen were used to stimulate bioethanol production. The original nitrogen source concentration in the medium wa 2mM $NaNO_3$.the experiment was performed in – 0, ½, ¼ and double of the original nitrogen source concentration. The effect of initial nitrogen concentration on the chlorella vulgaris protein and carbohydrate content was investigated. All the flasks were autoclaved at 121°C for 15 minutes.

Inoculation of algal culture:

After sterilization of the broth medium the algal culture *Chlorella vulgaris* was inoculated in the broth and incubated at 22^{o}C for 25 days in culture room. The biomass estimation of algae was observed at regular interval of 24 hours.

Biomass estimation:

For the measurement of culture density Known amount of culture was taken out aseptically in a glass cuvette and the optical density was taken at 688nm.The dry and wet weight of algal biomass was obtained by according to method given by Zhu and Lee [25].

Estimation of Protein

The total proteins contents present in the test organism was determined by Lowry *et al*., [15] and Herbert *et al*., [12] method.

Determination of Carbohydrate:

For determination of carbohydrates contents the algal culture was centrifuged in cooling centrifuge at 5000 rpm/min for 30 min. After centrifugation two layers was developed namely supernatant and pellet. The supernatant was collected in another sterilized tubes and pellet was discarded. The total carbohydrate content present in the supernatant was detected according to phenol sulphuric acid method given by Dubios *et al*., [6].

Cell bound Polysaccharide:

In detection of cell bound polysaccharides 0.5ml of culture was centrifuged at 10,000rpm for 10min.After centrifugation supernatant was discarded and pellet was used for estimation of cell bound polysaccharide.

Extracellular Polysaccharide:

For determination of extracellular polysaccharides 1ml culture was centrifuge at 10,000 rpm for 10min.After centrifugation pellet was discarded and supernatant was used for estimation of extracellular polysaccharide.

RESULT

Effect of nitrogen concentration on Protein value: Nitrogen is one of the most important nutrients for microorganism to synthesize amino acids, proteins and nucleic acids and the concentration of nitrogen provided may have a major impact on the efficiency of cell growth and metabolite formation. Therefore, the culture medium contains different concentration of nitrate the value of protein is decreases with respect to nitrogen concentration.

Figure1 show the time course biomass profile of *Chlorella vulgaris* grown autotrophically in BBM medium with different concentration of $NaNO_3$ in the medium. The original nitrogen source concentration in the medium was 12.5gL^{-1} (2mM) $NaNO_3$. Different sets of nitrogen concentration – 0, ½, ¼ and double of control was used to investigate the effect of nitrogen on growth and carbohydrate content of the organism. Growth of alga increased after a short lag phase of 2 days followed by a logarithmic phase and attained stationary phase at about 12 days. By increasing the $NaNO_3$ concentration, growth and protein concentration also increased. In the absence of a nitrogen source (0g L^{-1}), no growth was observed and

the cells appeared bleached. At day 10th maximum protein concentration of 130mgL-1was recorded in the culture with double the concentration of nitrogen (4mM $NaNO_3$). Protein of 108mgL-1 at 8th day, 98mgL-1 at 8th day and 93mgL-1at 8th day was observed in cultures with 2mM $NaNO_3$ (control), 1mM $NaNO_3$ (1/2 of control) and .05mM $NaNO_3$ (1/4 th of control) respectively (figure1).

Effect of nitrogen on Carbohydrate concentration: In this experiment it is noted that the value of carbohydrate is increases with inversely proportional to nitrogen concentration. Nitrogen depletion caused relevant changes on the cell chemical composition. Protein cell content decline when nitrogen exhausted.

When the nitrogen source was decreased from 2mM $NaNO_3$ to .05mM $NaNO_3$ (1/4 of control), the total carbohydrate content increased. A sharp rise in carbohydrate accumulation 312mgL-1at 14th day was recorded when the cultures were grown in initial nitrogen concentration of .05mM $NaNO_3$ (1/4th of original concentration). Total carbohydrate accumulation 280mgL-1at 16th day, 236mgL-1 at 14th day and 142 mgL-1 at 10th Day was observed in culture with 1mM $NaNO_3$ (1/2 of control), 2mM $NaNO_3$ (control) and 4mM $NaNO^3$(Double of control) respectively (Figure2).

The extracellular and intracellular carbohydrate concentration was maximum at 14th day was 130mgL-1 and 182mgL-1 respectively (Figure3 and figure4). The intracellular and extracellular carbohydrate concentration is inversely proportional to nitrogen concentration.

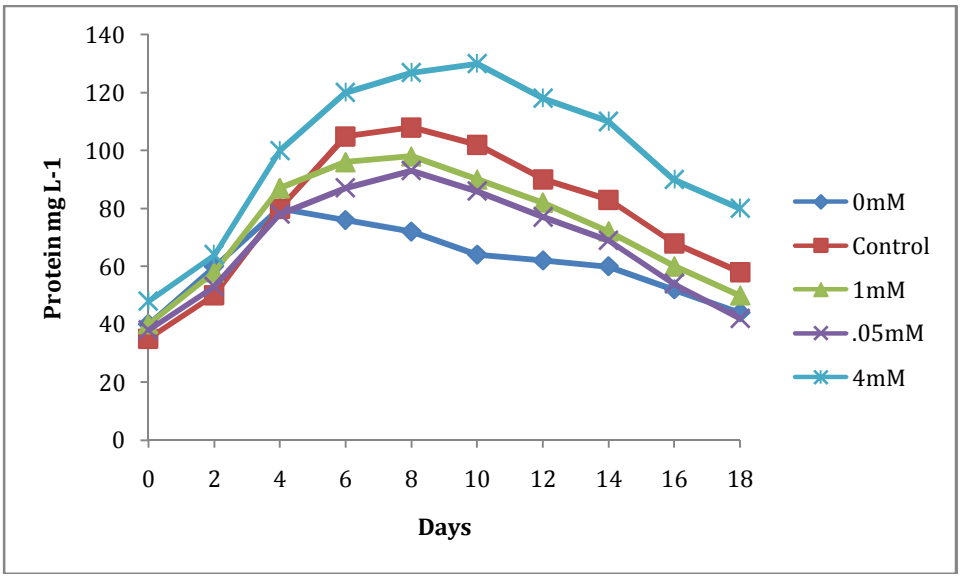

Figure 1: Protein concentration on growth of *Chlorella vulgaris* with different initial $NaNO_3$ concentration (.05-4 mM).

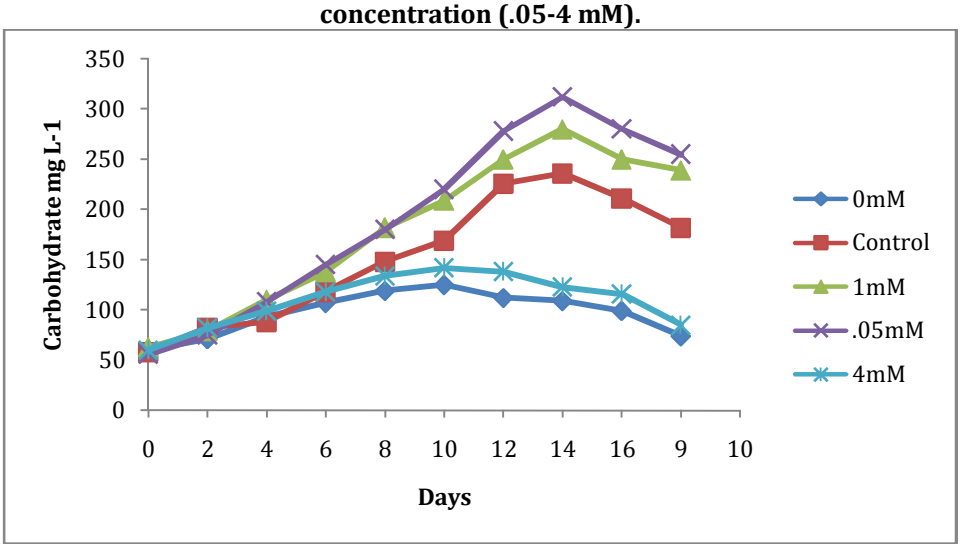

Figure2: Total Carbohydrate Concentration on growth of *Chlorella vulgaris* with different initial $NaNO_3$ concentration (.05-4 mM).

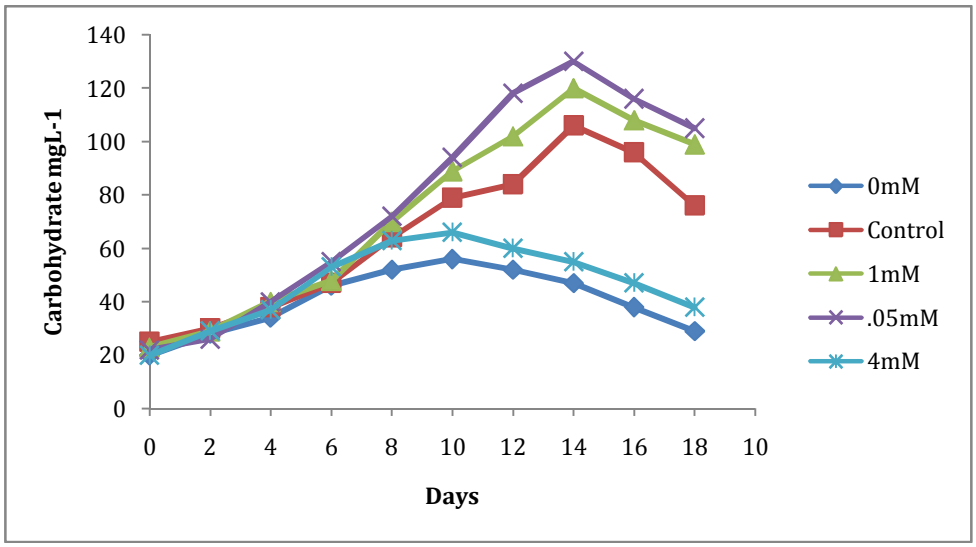

Figure 3: Extracellular Carbohydrate Concentration on growth of *Chlorella vulgaris* with different initial $NaNO_3$ concentration (.05-4 mM).

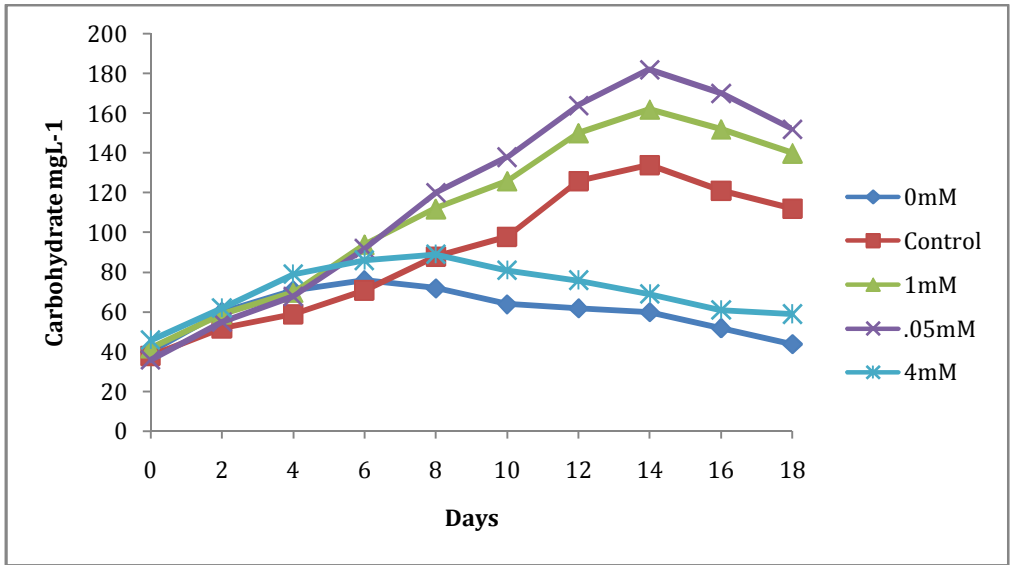

Figure 4: Cell bound Carbohydrate Concentration on growth of *Chlorella vulgaris* with different initial $NaNO_3$ concentration (.05-4 mM).

DISCUSSION

For economical production of bioethanol from microalgae, biomass as well as carbohydrate carbohydrate content plays an important role. In the present study, the results mentioned above indicate that high concentration of nitrogen source supported the protein concentration in contrast to the carbohydrate content. The alga, *chlorella vulgaris* cannot grow without nitrogen source and its growth is directly proportional to the concentration of nitrate in the medium. As nitrogen source increase in the medium, enhancement in protein concentration was recorded (Figure1).A number of studies [13, 23] have demonstrated that nitrogen depletion leads to a sharp increase in the carbohydrate content of microalgae, because this forces them to transform protein or peptides to lipids or carbohydrates. The protein content of *Chlorella vulgaris*decreased in contrast, the carbohydrate content is increased (Figure1 and figure2). The result show that strategy of nitrogen depletion can dramatically promote the carbohydrate content of the microalgae. The decline of protein and chlorophyll content is well described as general response to nitrogen deprivation in green microalgae [19, 22, 23, 27]. Accumulation of starch and lipids in microalgae subjected to nitrogen restriction has been described in previous work, although the storage patterns appear to be different between species [1, 10, 8]. These results showed when the nitrate concentrations decrease the carbohydrates content increase, so the bioethanol yield increase too,

because the carbohydrates is considered as a substrate for bioethanol production by fermentation [18, 4, 20, 14].

CONCLUSION

Carbohydrate content of algae is an important parameter that determines the economy of bioethanol production from microalgae. The carbohydrate content of strain is reversely proportional to the Nitrogen concentration. The most effective approach to enhance carbohydrate in *Chlorella vulgaris* is grow it autotrophically in growth medium with initial concentration of .05mM $NaNO^3$. This gives a hike in carbohydrate content over control and cell growth also.

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