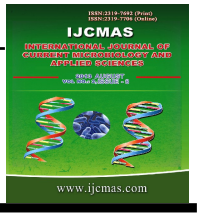
Int.J.Curr.Microbiol.App.Sci (2013) 2(8): 389-393

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 8 (2013) pp. 389-393**
http://www.ijcmas.com



Short Communications

Activity of microalgal lipid accumulating key enzyme isocitrate dehydrogenase on nutrient feeding modulation

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Keywords

Microalgal; bio-fuel; Nutrient feeding; biomass production.

A B S T R A C T

Enhancing microalgal lipid production is the recent attraction because of its application in bio-fuel production, there are various methods developed on targeting the improvement of lipid yield in microalgae. In this study two different nutrient feeding methods was used to modulate the algal nutrients. Supplying all of the nutrients required for the entire growth phase in one initial feeding; feeding the optimal quantity of nutrients at a regular interval as it was consumed by the algae. The results revealed; method -B found suitable for biomass production, whereas method -A found ideal for activating the lipid accumulating system with less biomass yield.

Introduction

The bio-fuel production from photosynthetic microorganisms is considered as a process to produce renewable energy for global warming mitigation. In recent times microalgae have received much attention as a renewable energy resource because its photoautotrophic mechanism which can convert atmospheric carbon dioxide into biomass, fatty acids, and lipids (Hsieh and Wu, 2009). Microalgae have the ability to grow faster and accumulate high amounts of lipids. Lipid biosynthesis and accumulation has been studied extensively to modulate/enhance its production by several biotic and abiotic factors. This process had drawn the attention of using microalgae for biodiesel production as a potential renewable resource and for essential fatty acids. The lipid content of microalgae is the most important aspect for biofuel production (Ratha et al., 2013). In this study we have modulated the nutrient feeding method in order to enhance the lipid metabolic system.

When nitrogen is limiting in the medium, a chain reaction starts via increased adenosine monophosphate (AMP)-deaminase activity (Ratledge and Wynn,

2002). This leads to a decrease in cellular AMP levels, which in turn reduces isocitrate dehydrogenase activity-a unique effect found only in oleaginous species. Subsequently, aconitase equilibrates isocitrate with citrate, and the accumulated citrate is transferred to the cytosol by citrate/malate translocase (CMT). Here, ATP citrate lyase cleaves citrate into oxaloacetate and acetyl-CoA (acetyl-CoA converts it to malonyl CoA as a starting step of fatty acid synthesis) (Ratledge and Wynn, 2002).

Materials and Methods

Experimental organism and culture conditions

The microalgae *Desmodesmus communis* LUCC 002 was obtained from the culture collection of Center for Bioenergy, Lincoln University in Missouri, USA. The organism was maintained and grown in 500 ml Erlenmeyer flasks containing 200 ml of synthetic BG 11 medium (Stanier *et al.,* 1971). Experimental cultures were incubated at 25 ± 2 °C, in a shaking platform with 120 rpm, 14:10 h light–dark cycle, with illumination of 27 µE m^{-2} s^{-1} under cool white fluorescent lights.

Experimental setup

Mid log phase culture grown in the laboratory at the above mentioned conditions was harvested by centrifugation at 4000rpm. The pellet was washed with distilled water and resuspended in 40 liters aquarium tanks. The initial inoculum was adjusted evenly (0.1 OD) in all the replicate tanks by dissolving the harvested thick slurry of biomass, and the final volume of the tanks was made to 10 liters. For agitation, aquarium tank (water circulation) pump - Aqueon, Italy, with the flow rate of 500 gallons $h^{-1}$was used. Commercial fertilizer with the chemical composition as represented in table 1, was used as a nutrient source and the feeding strategy was maintained as follows - in two replicate tanks of each system.

Feeding method-A supplying nutrients altogether required for the entire growth phase, i.e., 7.4 grams of fertilizer in 10 liters.

Feeding method-B feeding 740mg for 10 liters culture at the interval every three days.

The feeding concentration (74mg/L) was selected based on the optimal growth requirement of *Desmodesmus communis* LUCC 002 (data not shown). The aquarium tanks were maintained in green house provided with controlled temperature (25°C ± 2°C).

Biomass estimation and nutrient feeding

Every three days water level was maintained against evaporation and 15 ml of triplicate samples were obtained for growth estimation from each replicate tank. Once after sample collection 740 mg of fertilizer were added to the (method-B) tanks. The samples were filtered using 4.7 cm Whatman GF/C filters and muffled at 475 °C for 1 hr, and the mass of the dried biomass was measured gravimetrically. The results presented are the means of three independent experiments. Sample variability is given as the standard deviation of the mean.

Whole Cell Protein Preparation, native PAGE and activity staining

To study the response of isocitrate dehydrogenase, cultures from the tanks

were harvested by centrifugation at 6000 rpm for 10 minutes. Then the thoroughly washed algal pellets were homogenized with an extraction buffer (62.5 mM Tris–Cl, pH 6.8) and the whole cell protein was extracted using 6800 freezer mill. Total soluble proteins, which served as the enzyme source, were obtained after three centrifugations, each for 20 minutes at 12000 rpm at 4°C. All the protein preparations were made inside an ice bath. Electrophoresis was carried out at 4 ± 1°C with 1.5 mm polyacrylamide gels in a Tris–glycine buffer (pH 8.3) under standard native conditions (Laemmli, 1970). A uniform amount (75 µg) of protein, estimated by Lowry et al., (Lowry *et al.,* 1951) was loaded with the sample buffer, devoid of sodium dodecyl sulfate and b-mercaptoethanol. Samples were then electrophoresed at 50 V through the stacking gel (5%) and at 100 V through the resolving gel (8%). Gel images were scanned by a Canon scanner (Canoscan LIDE210), and the enzyme activity profiles were analyzed by software provided with the gel documentation system (GeneTools, Syngene). The electrophoresed gel was stained using the procedure (Gennedy, 2003).

Results and Discussion

Method-B showed 24.4% increased growth comparing to method-A (Figure 1). While suppliying all the nutrients required for the entire growth phase (method –A); the organism consumed the whole nutrients in a short time and made the media deprived of nitrate. Whereas feeding nutrients as it was consumed by the algae (method –B) left residual nitrate in the medium, presence of this trace nitrate might not allowed to increase the adenosine monophosphate (AMP)-deaminase activity (Ratledge and Wynn, 2002). As a consequence, the presence of nitrate in the medium has not permitted triggering the lipid accumulating system, which was evident form figure 2. Isocitrate dehydrogenase is the key enzyme for lipid accumulation in the cells of oleaginous microorganisms, which was apparent as noticed in Figure 2, showed less activity - representing the active phase of lipid accumulating system. Though the biomass production is higher on method-B, less lipid content is not suitable for applying the biomass for bio-energy. Both the feeding methods has its own characteristics and suitability for different applications. On concerning the overall bio-energy production cost, less lipid biomass is not appropriate. Thought the method-B produces higher biomass, the time duration required for lipid accumulation will negatively influence on the cost effectiveness. Three times biomass harvest can be done if following method-A for cultivation, when comparing the two times biomass yield at the stipulated time if followed with method-B. In case of using the algal biomass as feed or pigment producing source (Spolaore *et al.,* 2006) method-B will be suitable for cultivation.

From our previous study it was proved that the concentration of fertilizer used for the cultivation has not created toxic effect to the algae {Formatting Citation}. Isoenzymes are sensitive to metals, it was proved that metal ions regulates the expression of isozyme activity on microalgae (Palanisami and Lakshmanan, 2010). Based on the nutrient feeding it was clear that the activity of isocitrate dehydrogenase got masked due to the nitrogen deprived condition. Interference or modulation in the activity of isocitrate denhdrogenase in reason of metal content has to be studied further on

Table.1 Chemical composition of the fertilizer used as algal nutrients – representing the concentrations of individual elements when 74mg dissolved in 1 L.

Chemical composition	74 mg/L of fertilizer holds (mg)	740 mg/L of fertilizer hold (mg)
Nitrate nitrogen	2.13	21.33
Ammonical nitrogen	0.74	7.4
Urea nitrogen	14.9	149.03
Available Phosphate[P2O5]	5.92	59.25
Soluble potash	11.85	118.51
sulfur[combined sulfur]	0.74	7.4
Boron	0.01	0.14
Copper [Chelated copper]	0.037	0.37
Iron [Chelated iron]	0.07	0.74
Manganese [chelated manganese]	0.03	0.37
Molybdenum	0.0007	0.007
Zinc [Chelated zinc]	0.037	0.37

Figure.1 Growth difference representing while growing *D. communis* on different nutrient feeding conditions. B = feeding the optimal quantity of nutrients at a regular interval as it was consumed by the algae; A = Supplying all of the nutrients required for the entire growth phase in one initial feeding.

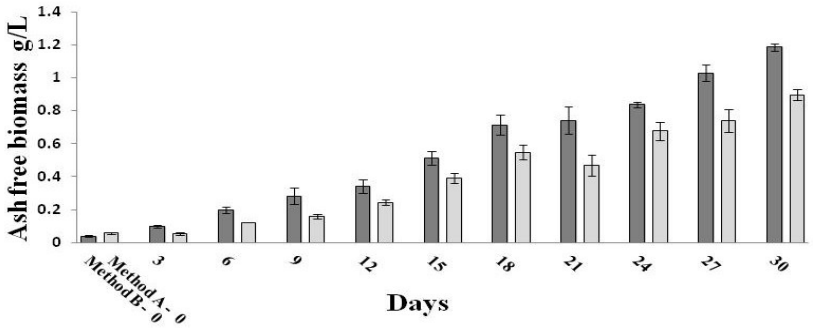
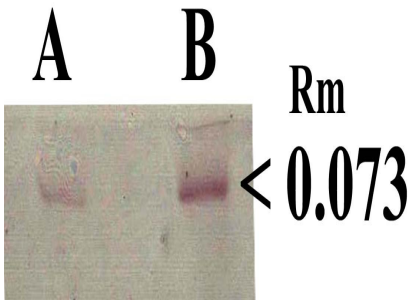


Figure.2 Activity staining of isocitrate dehydrogenase while growing *D. communis* on different nutrient feeding conditions. A = Supplying all of the nutrients required for the entire growth phase in one initial feeding; B = feeding the optimal quantity of nutrients at a regular interval as it was consumed by the algae.



D.communis LUCC 002 for unambiguously understanding the physiology. Modulations in nutrient feeding have hope on driving the microalgal cellular machinery towards the product of desire. There are various processes like lipid accumulation, enhancing the pigment and polysaccharide production can be regulated by altering the nutrient supply, both in varying the concentration and feeding duration. Identifying a suitable ideal condition for the simultaneous production of two or more bio-products from the same harvest will be energy saving and cost effective means.

Acknowledgement

This study was financially supported by the United States Department of Energy. The authors are indebted to Mr. Jon Haslag for proofreading the manuscript.

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