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EFFECT OF ALTERED CONCENTRATION OF NITROGEN ON LIPID AND BIOMASS PRODUCTION IN *CHLORELLA VULGARIS* (BEYERINCK)

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Effect of different concentrations of nitrogen (N) source ($NaNO_3$) of BG-11 medium on growth of freshwater microalgae *Chlorella vulgaris* is studied. Different nitrogen step down concentrations of $NaNO_3$ in per litre of medium were used like 17.6 mM (control), 8.8 mM and 4.4 mM. Optimum lipid was obtained with treatment of 8.8 mM $NaNO_3$ concentration that showed 29.30 µg lipid per 100 µg of biomass. However, biomass in term of protein was higher (602.64±0.97 µg/ml) at 17.6 mM of $NaNO_3$ concentration. Treatment of 4.4 mM $NaNO_3$ concentration resulted in 20.03 µg of lipid per 100 µg biomass, which was higher than control, but with poor yield of biomass. Result indicates that changing the N concentration in BG-11 medium result in enhanced lipid production in *Chlorella vulgaris*.

Key words: BG-11, Biodiesel, Biomass, *Chlorella vulgaris*, Lipid, Microalgae

Introduction

Conventional fuels are on the edge of their depletion because the rate of their consumption is far more than the rate of formation. Therefore, to fulfil the growing need of transportation fuels, microalgae appeared as potential raw material for biodiesel production. Microalgae have some advantages over customary oil crops that they can grow on wasteland and there is no competition with edible crops[1]. Biodiesel has equivalent capabilities to conventional fuel like there is no need make changes in engine for using it in blending ratio and it is less volatile[2,3,4]. There are many ways which enhanced the lipid in microalgae like nutrient limitation, genetic modifications and variation in temperature and pH ranges[5]. Concentration of nitrogen and phosphorus decide the production of neutral lipid in microalgae[6]. Microalgae triggered for more production of lipid and carbohydrate by applying nitrogen limiting conditions, upon these conditions photosynthetic machinery of microalgae increases the availability of carbon towards lipid synthesis pathway instead of protein synthesis[7,8]. However this N deprivation leads to lower the biomass [9]. In this study effect of nitrogen deficient conditions is studies on lipid and biomass production in fresh water microalgae *Chlorella vulgaris* Beyerinck

Material and Methods

Microalgae *Chlorella vulgaris* was previously collected from lake Fatehsagar, Udaipur, isolated and identified in our laboratory. Microalgae cultures maintain at 28°C under cool fluorescent light with photoperiod of 16:8 in BG-11 medium (pH 7.4) in 250 ml Erlenmeyer flasks (Macro-

elements: $NaNO_3$ (1.5g/L), K_2HPO_4 (0.04g/L), $MgSO_4.7H_2O$ (0.075g/L), $CaCl_2.2H_2O$ (0.036g/L), Na_2CO_3 (0.02g/L), Citric acid (0.006g/L), Ferric ammonium citrate (0.006g/L), EDTA (Na_2 Salt) (0.001g/L). Microelements: H_3BO_3 (2.86g/L), $MnCl_2.4H_2O$ (1.81g/L), $ZnSO_4.7H_2O$ (0.22g/L), $Na_2MoO_4.2H_2O$ (0.39g/L), $CuSO_4.5H_2O$ (0.079g/L), $Co(NO_3)_2.6H_2O$ (0.049g/L)[10]. All the chemicals used during work were research and analytical grade.

Experimental setup:

BG-11 medium prepared with different concentration of $NaNO_3$ viz. 17.6 mM is the concentration of $NaNO_3$ in standard BG-11 medium and 8.8 mM (half of the standard concentration) and 4.4 mM (one fourth of the standard concentration). Erlenmeyer flask of 250 ml capacity was used with 100 ml of working medium. 100 µg of protein in term of biomass was inoculated in flasks and culture conditions are same as mention above. All experiments were performed in triplicates to reduce the errors.

Protein estimation:

Protein estimation was carried out at every fifth day using Bovine Serum Albumin (BSA) as standard protein solution of 1 mg/ml to plot standard curve. For protein estimation 1 ml of sample in test tube is taken and 1 ml of NaOH is added and boiled for five minutes in water bath and then allowed to cool at room temperature. In second step 5 ml of the a chemical mixture is added. Chemical mixture is prepared by mixing of 50 ml Na_2CO_3 (sodium carbonate) (10%), 1 ml of 4% $KNaC_4H_4O_6 \cdot 4H_2O$ (potassium sodium tarterate tetrahydrate) solution and 1ml of 2% $CuSO_4$ (Copper (II)sulphate) with final volume made up to 100 ml with distilled water (DW) and incubated for 10 minutes. In the last step, 1 ml of folin-phenol (1N) reagent is added and allowed to set for 15 minutes. Then O.D. was taken at 650 nm using UV- Vis spectrophotometer[11]. Growth biomass is calculated as per given formula:

Total biomass productivity = Biomass at final day – Biomass at first day / days

Lipid quantification:

Canola oil was used as standard lipid (2 mg/ml in chloroform), for standard curve preparation after making a series of 10 µg to 100 µg canola oil. For estimation of lipid 100 µg dried microalgal biomass is harvested and suspended with 100 µl of DW in test tube. 2 ml of concentrated sulphuric acid is mixed and allowed to boil in water bath for ten minutes. After cooling at room temperature, 5 ml of phospho-vanillin reagent (400 ml of 85% phosphoric acid + 10 ml absolute alcohol + 90 ml DW + 600 mg vanillin) is added and incubated into incubator shaker (at 37°C, 200 rpm for 15 minutes) and OD was taken at 530 nm in UV- Vis spectrophotometer[12].

Total lipid productivity = weight of total lipid / weight of biomass × 100

Results and Discussion

Graphical representation (Fig. 1) of the growth under different N concentration was showing that N has direct impact on the growth of microalgae. When N decreased from 17.6 mM to 8.8 mM and 4.4 mM, the concentration of biomass was also reduced by 1.20 times and 1.40 times, respectively, in comparison to control. At all the three ranges maximum biomass produced on 20^{th} day of culture after that a sharp decline in growth was observed. Lipid was measured at 20^{th} day because higher growth rate was observed on that day. As shown in Table 1, that at 8.8 mM concentration of N yield of lipid was 1.8 folds and at 4.4 mM produced 1.25 times more lipid than control. Under 8.8 mM $NaNO_3$ lipid kept on increasing along with biomass, however 4.4 mM N yielded significant amount of lipid but inferred itself as a poor treatment for lipid

production due to low biomass productivity. There is a study which states that accumulation of lipid can be increased via down regulating N assimilating pathway by applying N limiting conditions[13]. This was also suggested that higher $NaNO_3$ in medium reduced the uptake of glucose by *Chlorella sorokiniana* which directly slowdown glycolysis, HMP and TCA results in lower yield of fatty acid[14]. Mainly triacylglycerides and free fatty acids are responsible for quality of biodiesel and these involve C12-24 saturated and unsaturated fatty acids which are identical to those of vegetable fatty acids [15].

This study concluded that N is the sole source of microalgal growth and it has great influence on growth and lipid production. $NaNO_3$ at 8.8 mM enhance the neutral lipid accumulation in *Chlorella vulgaris*, which is key constituent of biodiesel and this concentration also maintain equilibrium between biomass and lipid production. Lipid production also found desirable at 4.4 mM $NaNO_3$ but this is subjected to low biomass production. Nitrogen limited in association with phosphorus deficient and sufficient conditions also greatly increased the desirable fatty acids.

Conc.of N in mM	Biomass (µg/ml)	Biomass productivity (µg/ml/d)	Lipid content (µg/100 µg Dry Weight)
17.6 (control)	602.64 ±0.97	25.13	16.13
8.8	499.94 ±0.48	19.99	29.30
4.4	421.88 ±0.48	16.09	20.03

Table 1: Estimation of protein and lipid at 20th day of experiment, along biomass and lipid productivity.

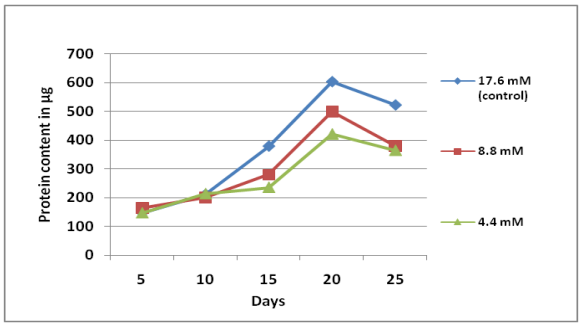

Figure 1: Growth rate of Chlorella vulgaris at different concentration of $NaNO_3$ source in BG-11 medium

Conclusion

Our experiment revealed that with reducing the standard sodium nitrate concentrations (17.6 mM) by half (8.8 mM) and one fourth (4.4 mM) in BG 11 medium can enhanced the lipid content in *Chlorella vulgaris*. Nitrogen step down can be a useful method to produce biodiesel from algae.

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References

1. Francisco EC, Neves DB, Jacob-Lopes E, and Franco TT 2010, Microalgae as feedstock for biodiesel production: carbon dioxide sequestration, lipid production and biofuel quality. *J. of Chem Technol & Biotech.* **85**(3) 395-403.
2. Meher LC, Vidya SD, Naik SN 2006, Technical aspects of biodiesel production by transesterification—a review. Renew Sust Energy Rev. **10** 48–268
3. Wang WG, Lyons DW, Clark NN, Gautam M, Norton PM 2000, Emissions from nine heavy trucks fueled by diesel and biodiesel blend without engine

modification. Environ Sci Technol. **34** 933–939
4. Tyson KS 2001, Biodiesel Handling and Use Guidelines. Report NREL/TP 580–30004. *National Renewable Energy Laboratory, Golden, CO*.
5. Bharte S, and Desai K 2019, The enhanced lipid productivity of *Chlorella minutissima* and *Chlorella pyrenoidosa* by carbon coupling nitrogen manipulation for biodiesel production. *Environmental Science and Pollution Research*. **26**(4), 3492-3500.
6. Converti A, Casazza AA, 2009, Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Process*. **48** 1146–1151.
7. Chen C, Zh ao X, Yen H, 2013, Microalgae-based carbohydrates for biofuels production. *Biochem. Eng. J.* **78** 1–10.
8. Hu, Q, Sommerfeld, M, Jarvis, E, 2008, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J*. **54** 621–639.
9. Eroglu, E, Melis, A, 2016, Microalgal hydrogen production research. *Int J. Hydrog. Energy* **41** 12772–12798.
10. Herdman, M, Janvier, M, Rippka, R, and Stanier, R Y 1979, Genome size of cyanobacteria. *Microbiology*, **111**(1) 73-85.
11. Herbert D, Phipps P J, and Strange RE 1971, Chapter III chemical analysis of microbial cells. In *Methods in microbiology Academic Press*. **5** 209-344
12. Mishra, SK, Suh WI, Farooq W, Moon M, Shrivastav A, Park M S, and Yang JW 2014, Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. *Bioresource technol*, **155** 330-333.
13. Gupta N, Khare, P, and Singh D P 2019, Nitrogen-dependent metabolic regulation of lipid production in microalga *Scenedesmus vacuolatus*. *Ecotoxicology and environmental safety* **174** 706-713.
14. Zhu Y, and Huang Y 2017. Use of flux balance analysis to promote lipid productivity in *Chlorella sorokiniana*. *Journal of Applied Phycology*. **29** (2) 889-902.
15. Vadivel M, Arvindnarayan S, Kumar G, Shobana S, Dharmaraja J, Nguyen DD and Prabhu KKS 2019. Biodiesel Potentiality of Microalgae Species: evaluation Using Various Nitrogen Sources. *Waste and Biomass Valorization.* 1-9.