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Effect of Two Different Nitrogen Sources on Lipid Accumulation in Microalgae *Chlorella Pyrenoidosa*

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Abstract: In present scenario, microalgae have been used as a third generation promising source for production of biofuel. So it is necessary to screen microalgal strains rich in oil and adapt to variable environment conditions. The present study deals with cultivation of *Chlorella pyrenoidosa* in Fogg's media using urea and KNO_3 as nitrogen source. The effect of two different nitrogen sources at different concentration (0-2g/L) on biomass and lipid content was studied. The result showed that with increase of N-source in media biomass increased but lipid content decreases and vice versa. Moreover, at same concentration of urea and KNO_3, biomass as well as lipid content was more in microalgal cell growing in urea. Urea at concentration of 0.1g/L gives optimum conditions for biomass production and lipid content in cells. At high concentration of urea due to ammonium toxicity growth rate decreased in algal culture. The optimum concentration of urea proves better than other nitrate source to culture *Chlorella pyrenoidosa* in order to increase lipid content and to use it as biofuel feed stock.

Keywords: *Biofuel, Microalgae, Dry Weight, Biomass, Lipids, Fatty Acids, Bioenergy*

I. INTRODUCTION

These days concern on global warming and climate changes has drawn the attention of researchers to look for alternatives of fossil fuels. Bioenergy produced from algal biomass can be used as potential source of energy. Microalgae are tiny, sunlight driven biofuel producing factories. Algae are also called as 3rd generation biofuel. It has advantage over 1st generation fuel i.e. food crop as it does not require arable land and valuable fresh water. Microalgal require less space for mass cultivation and easy to cultivate as it require CO_2 and sunlight.Microalgae convert the raw material in high energy producing organic compound which can be used to produce biodiesel. Microalgae have high rate of growth and lipid productivity which could produce 40-50 times more biomass compared to higher plants [20, 29]. Thousands of microalgal strains have been screened out which have capability to store large quantities of lipid in cells in form of storage lipids. A number of microalgal stains have been screened which are capable to produce large quantities of energy stored in form of TAGs (Triacylglycerides) which can be extracted with help of solvent and can be easily converted to biofuel through trans-esterification process. Microalgae can be grown in extreme and diverse conditions. Usually a large amount of algal biomass is produced in favorable culture conditions but lipid content is low. Under stress condition usually biomass production is reduced but it enhances neutral lipid accumulation in microalgal cell in form of TGA as it helps to overcome stress conditions [15, 19].Nitrogen is essential for growth and regulation of metabolism of algae. Under N-stress condition lipid productivity increases [1, 3, 23, 24, 26]. Hence the growth rate and lipid content in microalgal cell is highly affected by the nitrogen source used. Urea is cheaper than other nitrogen sources and does not affect the chlorophyll content of microalgal cell [7]. In previous study use of urea for *Spirulina* and *Isochrysis* has tremendously increased the lipid content as compared to nitrate and nitrite[7, 18]. In present study the effect of different source of nitrogen at different concentrations on biomass and lipid productivity of *Chlorella pyrenoidosa* was studied. Urea and KNO_3 at different concentrations has been used as sole source of nitrogenin media. An attempt focused to identify the most suitable N-source to improve biomass production as well lipid productivity in *Chlorella pyrenoidosa.*

II. MATERIALS & METHODS

A. Sample collection

Samples of sewage water were collected from Waste Water Treatment Plant Kholriwal, Jalandhar. From Sewage water the algae were isolated by step dilution method. Each dilution was poured on agar solidified Fogg's media by streaking method [11]. Different types of algae were observed with the help of Compound microscope. For further study *Chlorella pyrenoidosa* was identified and selected.

B. Culture of algae

Starter culture of *Chlorella pyrenoidosa* was prepared by increasing the number of microalgae cells before treatment.It was cultured in the flask with 250 mL of Fogg's medium under controlled temperature at 25^0C, providing 16:8 light/dark conditionsthe cultures were grown in an incubator. This was taken as control culture.In order to prevent sticking of algal cells with the glass walls, the culture flasks were shaken manually with hand three to four times daily. The media and all glass wares were sterilized before starting the inoculation.

C. Culture of Chlorella pyrenoidosa in different N-sources (Urea and KNO_3)

50 ml isolate of *Chlorella* sp. was inoculated in 250 ml of Fogg's media [11]. Different concentration of urea and KNO_3 as nitrogen source is used. The different concentrations were 2.0g/L, 1.5g/L, 1.0g/L, 0.5g/L, 0.1g/L, and without nitrogen.Biomass growth was observed by taking O.D. at 640nm.

D. Dry Cell Weight analysis

In order to determine dry weight of culture, 3ml of culture sample was taken in centrifuge tube, which was centrifugated at 2500 rpm for 10min. The supernatant was discarded and centrifuge tube with wet algal pellet was dried in oven at temp 80^0C for 2hours to get constant weight. After getting the constant weight the weight of centrifuge tube at end was subtracted from weight before drying. DCW was measured only 0, 2, 5, 9, 14, 17 and 21 day of cultivation i.e. upto stationary phase.

$DCW= W_2-W_1$

W_2= weight of wet algal biomass before drying

W_1= weight of dry algal biomass after drying.

E. Lipid content analysis

Analysis of lipid content in microalgae was done using Bligh and Dyer method [28]. A sample of algal suspension was centrifugated at 3800 rpm for 10 minutes. A concentrated algae pallet was obtained and the wet weight estimation was done gravimetrically. This algal paste was dried at temp 80^0C for 2hours or till we get the constant weight. Then for 1g of algal biomass 2mL of chloroform and 1mL methanol was added. This suspension was left undisturbed for 24 hours at 18^oC. After 24 hours this solution was mixed for 1 min on vortex after adding 1 ml of chloroform. Than 2 ml of water was added and agitated again for 2 min. Then there was layer separation and these layers were separated by centrifugation at 2000 rpm for 10min. Thelower layer with lipids was extracted with help of glass syringe and transferred to pre weighed vial (W_1). By Using water bath solvent was completely evaporated and vial was again weighed (W_2). Lipid content was calculated as W_1 -W_2 and expressed as % dry cell weight.

III. RESULT & DISCUSSION

A. Effect of different source of nitrogen on algal growth

Nitrogen plays an important role in algal growth. Our previous study showed that with decreasing conc. of nitrate, fatty acids/lipid production increases [24]. Many studies also have reported that different nitrogen sources have different effect on growth of algae**[**14, 16]. In our present study two nitrogen sources i.e. KNO_3 and urea has been used. Growth was observed in both nitrogen sources.

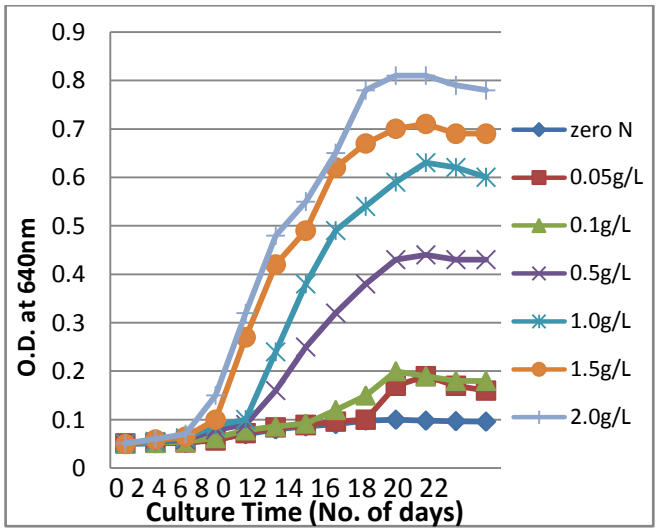

Fig. 1 Growth curve of *Chlorella pyrenoidosa* grown on Fogg's medium with different concentrations of KNO_3 (0-2.0 g/ L)

Fig. 1 shows growth curve of *Chlorella pyrenoidosa* in different concentration of KNO_3 which depicts that initial growth of was microalgae is almost similar even at different concentration of KNO_3. Then a sharp increase in biomass conc. was noticed at conc. (>0.5g/L) around 6-7 day which is considered to be as exponential phase of algal growth. In subsequent study period (6-17 days) as concentration of KNO_3 increased in medium, algal biomass also increased which could be depicted from O.D of culture media. In case of C.pyrenoidosa the growth rate is directly proportional to nitrate concentration as nitrogen plays an important role in metabolism. In case of KNO_3 as concentration increases biomass production also increases. Our result agrees withearlier studies performed [26, 30, 15, 17].

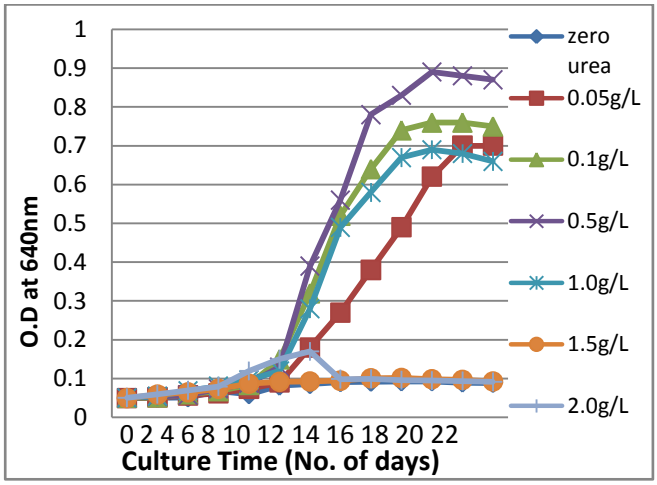

Fig. 2 Growth curve of Chlorella *pyrenoidosa* grown on Fogg's medium with different concentrations of Urea (0-2.0 g/ L)

Fig. 2 illustrates effect of different concentration of urea on biomass growth of *Chlorella pyrenoidosa*. Microalgal growth is strongly influenced by concentration of urea in culture media as concentration of urea directly affects cell division. In lag phase of growth, biomass production is quite same even at different concentration of urea. There was increase in biomass content with increase in urea concentration upto $0.5gL^{-1}$. From Fig. 2 it is obvious that higher urea concentration ($>0.5gL^{-1}$) led to decrease in biomass. This could be attributed to fact that there is ammonium toxicity in culture media with high concentration of urea. Urea quickly breaks into ammonium & CO_2. Excess of ammonium in culture media causes the ammonium toxicity which causes drop in pH of media also. There both factors inhibit the synthesis of ATP in chloroplast which ultimately leads to inhibition of algal growth [21, 22, 23]. On another hand if we compare microalgal growth rate in KNO_3 and urea. Urea proved to be best nitrogen source at low concentration which gives biomass content (on dry weight basis)of 6.7g/L at concentration $0.5gL^{-1}$. So urea at concentration<0.5g/L is good for algal growth which is 23% more to biomass obtained in culture medium with KNO_3 at concentration of 2g/L. The reason behind this increment in biomass is due to that urea dissociates to form ammonium & CO_2. The additional CO_2 in culture media increases the rate of photosynthesis and ammonium is used by algal cell to form amino acids which are used in chlorophyll [3, 7, 16].

B. Effect of different source of nitrogen on lipid production in algal cells.

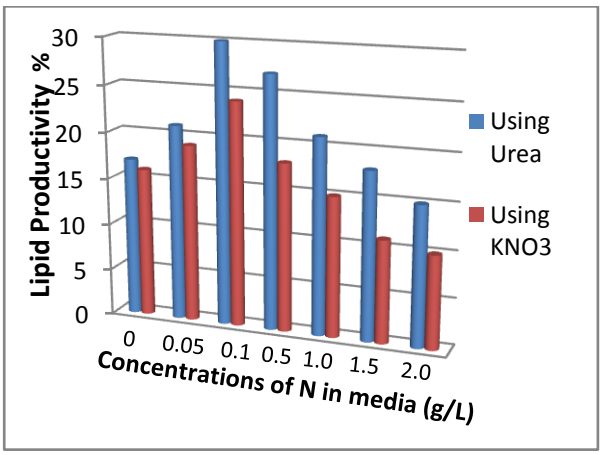

Fig 3: Effect of different sources of nitrogen at different concentrations on lipid productivity of *Chlorella* sp.

Fig. 3 illustrates effect of different source of nitrogen at different concentration on lipid production in *Chlorella pyrenoidosa*.

It has been observed that with the increase in N-concentration in media lipid content decreased. In media with KNO_3 as nitrogen source, maximum lipid content obtained was 24% of dry cell weight at concentration of 0.5g/L. While in case of media using urea as nitrogen source maximum lipid content recorded was 30% of dry cell weight at concentration of 0.1g/L. In N-deficient media, lipid content in algal cells is more as compared to N-rich media. In nitrogen starvation conditions rate of photosynthesis is decreased due to which glucose content in microalgal cell get lower. A decrease in glucose affected the acetyl Co-A synthesis which directly lowers rate of synthesis of Mallyl Co-A so in nitrogen deficient conditions microalgal cells start accumulate carbon metabolites in lipids [19, 20, 29]. If we compare lipid content in algal cell growing in media using KNO_3 and urea as N-source, it has been observed that urea favors lipid accumulation more as compared to KNO_3. Urea splits into CO_2& ammonium by urease enzyme present in algal cell. This additional CO_2 in medium not only enhance algal growth but also provides excess carbon flux for lipid production [8, 9].

The results suggest that decrease in KNO_3 concentration leads to decrease in biomass and an increase in nitrogen source concentration brings decline in lipid content in algal cells [7, 24, 26, 30].In present study the critical urea concentration at which biomass grew with high lipid content was 0.1g/L. our result is in agreement with earlier studies performed[1, 5, 7, 14, 16]. Moreover urea was reported best nitrogen source for culturing of chlorella consequently deficiency of KNO_3 and an optimized concentration of urea is considered to be best cultivation strategy to enhance lipid production in microalgal cells [27, 30].

CONCLUSION

The present study suggests that nitrogen starvation triggers lipid accumulation in microalga *Chlorella pyrenoidosa*. Urea proves to be better than other nitrate sources as it is cheap and even at low concentration it can enhance biomass content as well as lipid productivity in Chlorella sp. The most effective method for enhancement in lipid production is to grow in microalgae using urea at concentration of 0.1g/l which gives 20% increment in lipid content than using KNO_3 at same concentration.

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References

[1] A.A. Converti, E.Y., Casazza, P. Ortiz, M. Perego and Del Borghi, “Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsisoculata and Chlorellavulgaris for biodiesel production,” Chemical Engineering Process Intensification. vol. 48, pp. 1146–1151, (2009).

[2] A.M. Illman, A.H. Scragg and S.W. hale, “Increase in Chlorella Strainscalorific values when grown in low nitrogen medium,” Enzyme MicrobiologicalTechnology, vol. 27, pp. 631–635, (2000).

[3] B. Cheirsilp and C. Yeesang, “Effect of nitrogen, salt, and iron content in thegrowth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand,” Bioresource Technol., vol. 102, pp. 3034-3040,(2011).

[4] C. Zhu, Y. Lee and T. Chao, “Effects of temperature and growth phase on lipidand biochemical composition, of Isochrysisgalbana,” Journal of Applied Phycology, vol. 9, pp. 451–457, (1997).

[5] C.H. Hsieh and W.T. Wu, “Cultivation of microalgae for oil production with acultivation strategy of urea limitation,” Bioresource Technol,vol. 100, pp. 3921 – 3926, (2009).

[6] D. Der-Vartanian, F.J. Espardellier, and C. Astier, "Contributions of respiratoryand photosynthetic Pathways of a facultative photoautotrophic cyanobacterium, Aphanocapsa6714,” Plant Physiology vol. 68, pp. 974–978, (1981).

[7] E.D.G Danesi, C.O Rangel, J.C.M Carvalho, and S. Sato, "An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulinaplatensis,” Biomass and Bioenergy, vol. 23, pp. 261 – 269, (2002).

[8] F. Chen, and M. Johns, “Effect of C/N ratio and aeration on fatty acid composition ofheterotrophic Chlorella sorokiniana," Journal of Applied. Phycology, vol. 3, pp. 203 – 209, (1991).

[9] F. Martinez, and M.I. Orus, “Interactions between glucose and inorganic carbon metabolism in Chlorella vulgarisstrain UAM101,” Plant Physiology, vol. 95, pp. 1150–1155, (1991).

[10] G. Hu, M. Sommerfeld, E. Javis, M. ghiradi, M. Posewitz and M. Seibert, “Microalgal triglycerols as feedstock for biofuel production: prospective and Advances,” Plant journal, vol. 54, pp. 621-639, (2008).

[11] G.E. Fogg, “Growth and heterocyst production in Anabaenacylindrical in relation to carbon and nitrogen Metabolism,” Ann. Bot., vol. 13, pp. 41-59, (1949).

[12] H. Xu, X. Miao and Q. Wu, “High quality biodiesel production from a microalga Chlorellaprotothecoides by heterotrophic growth in fermentors.” Journal Biotechnology, vol. 126, pp. 499-507, (2006).

[13] I.A. Guschina and J.L. Harwood, “Lipids and lipid metabolism in eukaryotic algae,” Journal of Progress in Lipid Research, vol. 45, pp. 160–186, (2006).

[14] J. Jeanfils, M.F. Canisius and N. Burlion, “Effect of Highnitrate concentrations on growth and nitrate uptake by free living and immobilized Chlorella vulgariscells,” Journal of Applied Phycology, vol. 5, pp. 369 – 374,(1993).

[15] J. Singh and S. Gu, “Commercialization potential of Microalgae for biofuels production,” Renewable and Sustainable Energy Reviews. vol. 14(9), pp. 2596-2610, (2010).

[16] J.P. Fidalgo, A. Cid, E. Torres, A. Suken and C. Herrero , “Effect of nitrogen source and growth on proximate biochemical composition, lipid classes and fatty acid profile of marine microalga Isochrysis galbana,” Aquaculture, vol.166 (1-2), pp. 105 – 116, (1998).

[17] K. Shameera, R. Praveenkumar, G. Mahalakshmi, M.A. Akbarsha, and N. Thajuddin, “Influence of nutrient Deprivationson lipid accumulation in a dominant indigenous microalga Chlorella sp. Evaluation for biodiesel production,” Biomass Bioenery, vol. 37, pp. 60–66,(2012).

[18] K. Sudhakar, M. Premalatha , “Theoretical Assessment of Algal BiomassPotential for Carbon Mitigation and Biofuel Production,”Iranica Journal of Energy and Environment, vol. 3, pp. 232-240, (2012).

[19] K.L. Yeh and J.S. Chang, “Nitrogen starvation strategies and photobioreactor design for enhancing lipid production of a newly isolated microalga Chlorella vulgaris.esp-31: Implications for biofuels.” Journal Biotechnology. vol. 6, pp. 1358–1366, (2011).

[20] L. Gouveia and A.C. Oliveira, “Microalgae as a raw material for biofuelsproduction,” Journal Industrial Microbiology and Biotechnology, vol. 36, pp. 269-274,(2009).

[21] L. Xin, H. Hong-ying, G. Ke and S. Ying-xue, “Effects of different nitrogen and phosphorus concentrations on the growth, nutrientuptake and lipidaccumulation of a freshwater microalga Scenedesmus sp.,”Bioresource Technology, vol. 14, pp. 5494-500, (2010).

[22] L.Y. Zhu, X.C. Zhang, L. Ji, X.J. Song, X.J. and C.H. Kuang, “Changes of lipid content and fatty acid composition of Schizochytriumlimacinuminin response to different temperatures and salinities,” Process Biochemistry, vol. 42,pp. 210–214, (2007).

[23] M. Takagi, K. Watanabe, K. Yamaberi and T. Yoshida, “Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of Nannochloris sp. UTEX LB1999,” Applied Microbiology and Biotechnology, vol. 54, pp. 112–117, (2000).

[24] R. Kaur, A. Mahajan and A. Bhatia, “Estimation of lipid accumulation inChlorella pyrenoidosa culturing in Different Concentrations of KNO3,”International Journal for Research in Applied Science & Engineering Technology, vol. 4 (10), pp. 621-624, (2016).

[25] S. Mandal and N. Mallick, “Microalga Scenedesmus obliqusas a potential source for biodiesel production,” Applied Microbiology and Biotechnology, vol. 84, pp. 281-291, (2009).

[26] S. Nigam and M. Rai, “Effect of nitrogen on growth and lipid content ofChlorellapyrenoidosa, ”American Journal of Biochemistry and Biotechnology, vol. 7 (3), pp. 124-129, (2011).

[27] T. Berman and S. Chava, “Algal growth on organic compounds as nitrogen sources,” Journal of Planktonic Research,vol. 21 (8), 1423-1437, (1999).

[28] W.J. Dyer and E.G. Bligh, “A rapid method of total lipid extraction and purification,”Canadian Journal of Biochemistry and Physiology, vol. 37(8), pp. 911-917,(1959).

[29] Y. Li, M. Horsman, B. Wang, N. Wu and C.Q. Lan, "Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochlorisoleoabundans," Applied Microbiology and Biotechnology, vol. 81, pp. 629–636,(2001).
[30] Y. Rajasri, C.S. Rao, R.D. Chandrakanth, K.S.R. Sivasai and R.S. Ramgopal, "Lipid Productivity Of Chlorellapyrenoidosain a customized lab scale photobioreactor under stress conditions,"International Journal of ChemTech Research, vol. 5,pp. 719-726, (2013).
[31] Z.Y. Liu, G.C. Wang and B.C. Zhou, "Effect of iron on growth and lipid accumulation inChlorellavulgaris," Bioresource Technology, vol. 99, pp. 4717–4722, (2008).