IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 1 Ver. 1 (Jan -Feb. 2015), PP 48-53
www.iosrjournals.org

Influence Of Different Nitrogen And Organic Carbon Sources On Microalgae Growth And Lipid Production

[1] Amit Kumar Sharma, [2] Pradeepta Kumar Sahoo, [3] Shailey Singhal
(The Alternate Energy Research Institute, University of Petroleum and Energy Studies, Bidholi, Dehradun, India)

Abstract: *Microalgae based biofuels are getting attention due to energy crisis and enviromental protection. In the present study, the Chlorella sp. was cultivated in BG-11 medium at batch mode. The effect of different nitrogen (sodium nitrate, potassium nitrate and urea) and organic carbon (glucose, glycerol and sucrose) sources were analyzed on growth and lipid accumulation on this species. The highest biomass growth and biomass productivity of chlorella sp. was found 1.29±0.04 g/l, 76.96±4.5mgl^{-1}d^{-}1 in urea. However in case of organic sources, the biomass growth and productivity was found maximum in glucose (1.43±0.075 g/l 86.04±3.2 mgl^{-1}d^{-1}). The lipid content was examined using folch method and found better in potassium nitrate nitrogen source (11.84%) . Among organic carbon sources, the maximum lipid content (13.22% and lipid yield 189.94 mg/l were found in case of glucose, followed by glycerol and sucrose. Various properties of biodiesel obtained from chlorella sp. such as Cetane number, Saponification value, Iodine value and Degree of unsaturation were followed standerds set by the national petroleum agency (ANP255), ASTMD6751 and EN14214.*
Keywords: *Biodiesel, Biomass growth, Chlorella sp., Lipid extraction, Transesterification*

I. Introduction

Energy is backbone of every country's economic development and prosperity. India is the world's fifth largest primary energy consumer and fourth largest petroleum consumer after United States, China and Japan.. India is the world's fifth largest primary energy consumer and fourth largest petroleum consumer after United States, China and Japan. With an outlook for moderate to strong economic growth and a rising population, growing infrastructural and socio-economic development will stimulate an increase in energy consumption across all major sectors of the Indian economy [1]. Transportation is one of the fastest growing sectors using 27% of the primary energy [2]. At the present staggering rates of consumption, the world fossil oil reserves are likely to be exhausted in less than 45 years [3]. Thus, the rising energy (fuel) crisis and environmental degradation have posed a very serious problem to our sustainable growth and survival. So attention has now shifted towards alternative fuels like biodiesel, bio-ethanol and biogas. Biodiesel is fatty acids of ethyl or methyl ester made from virgin or used vegetable oil (either edible or non-edible). The most of the biodiesel production are based on edible oil like soybean oil, rapeseed oil, canola oil or sunflower oil in developed countries. In India the edible oil demand is higher than its domestic production. So there is no possibility of diverting this oil for production of biodiesel. The main commodity sources for biodiesel can be non-edible oils obtained from plant species. There are many species, which bear seeds rich in oil content. Out of these some promising tree species have been identified. These are Jatropha Curcas (Ratanjyot), Pongamia Pinnata (Karanja), Calophyllum Inophyllum (Nagchampa or Polanga), Mahua, Castor, Seemarouba etc. Using the current yields, vast amounts of land and fresh water would be needed to produce enough oil to completely replace fossil fuel usage. It would require twice the land area of the US to be devoted to soybean production, or two-thirds to be devoted to rapeseed production, to meet current US heating and transportation needs.

Microalgae are an emerging source for biodiesel production in recent years as due to higher biomass and lipid productivity, and the lack of competition with food crops for agriculture lands and fresh water sources as they can be grow on non-arable land using saline or waste water [4, 5, and 6]. Microalgae are photoautotrophic sunlight-driven cell factories that can convert carbon dioxide to various products such as lipids, carbohydrates, proteins, fatty acids, vitamins, antibiotics, and antioxidants [7]. The lipid content has been increased in many microalgae as a response to severe culture conditions such as CO2, nitrogen concentration and light intensity [8]. For the rapid accumulation of lipid, microalgae have been cultured ingrowth-limiting environment such as nitrogen depletion, [9–11], high light intensity [12], low temperature [13], high salt concentration [14] and high iron concentration [15]. Carbon and nitrogen source changes will greatly affect the biomass and lipid production of microalgae. Dittamart et. al., cultured the Scenedesmus sp. AARL G022 under different organic carbon sources such as glucose, glycerol and sodium acetate and found glucose most suitable for biomass growh [16]. Carbon and nitrogen source changes will greatly affect the biomass and lipid production of microalgae. However, to the best of our knowledge, very little attempts have been made on such a

work. Hence, as the pioneering attempts in this direction, the present study focus on microalgae growth and lipid accumulation under different nitrogen and carbon sources

II. Material And Methods

1.1 Microalgae and growth condition

Pure strain of chlorella sp was Yogi Vemana University, Vemanapuram, Kadapa,, Andhra.Pradesh., India and maintained in BBM medium in 250 ml Erlenmeyer flasks containing 100 ml liquid with initial pH 6.8 and incubated under cool florescence light (~2500 lux) at 24^{o}C (±1 ^{o}C) with 16 :8 Light. Chlorella sp. was cultured in sterile BG-11 medium at room temperature (~22-36 ^{o}C) under cool white, fluorescent lights (with fluorescent illumination of ~3000 lux) for 14 days. One liter bottle was used as a lab scale photobioreator. Working volume of photobioreactor was kept 550 ml with 10% (v/v) inoculum. The photoperiod was set 16:8 light: dark period. The culture was aerated (100 – 200 ml/min) by aquarium pump to avoid settling of algal biomass. Microalgae are cultivated in different nitrogen sources ($NaNO_3$, KNO3, Urea). But nitrogen content remained same as in BG-11 medium.

Without considering CO_2 in air, the effect of different organic carbon sources (Glucose, Glycerol and Sucrose) were tested with selected optimal Nitrogen Sources. But the carbon content of different organic sources was remained same ($0.5gL^{-1}$).

Growth analysis and lipid extraction

Microalgae growth was observed by measuring the optical density at 680nm (OD_{680}) using UV-visible spectrophotometer (Thermo Scientific) daily and related to algal biomass (g/l). For biomass estimation, 10 ml sample containing algae is filtered through pre-weighted Whatman GF/C glass fiber filter and dry wt. of algae is determined gravimetrically. The relationship developed between OD_{680} and biomass (g/l) is given as follows:

$$\mathbf{y=0.3942\times OD_{680} +0.0188 \qquad (R^2=0.997)} \qquad \mathbf{(1)}$$

Where y is algal biomass in g/l and OD_{680} is optical density of culture at 680 nm
The maximum specific growth rate (μ_{max} day^{-1}) at exponential stage was calculated as follows:

$$\mathbf{\mu_{max}\ (day^{-1}) = (lnX_2\text{-}lnX_1)/\ (t_2\text{-}t_1)} \qquad \mathbf{(2)}$$

Where X_1 and X_2 were the dry biomass weight (g/l) at time t_1 and t_2 respectively.

The doubling time (T_D,days) was calculated as follows:

$$\mathbf{T_D(days)= ln(2)/\ \mu_{max}} \qquad \mathbf{(3)}$$

The rate of biomass production (P, mg $L^{-1}day^{-1}$) was calculated by following equation:

$$\mathbf{P\ (\ mg\ L^{-1}day^{-1}) =(X_2\text{-}X_1)/t_x} \qquad \mathbf{(4)}$$

Where X_1 and 2_2 were the dry biomass weight (g/l) at time t_x
Lipid was extracted by applying folch extraction method [17].

1.2 Fatty acid analysis

The extracted fatty acid is converted in to their methyl esters by transesterification of lipid using 1 ml of 1% NaOH in MeOH followed by heating for 15 minute at 55 ^{o}C, adding 2ml of 5% methanolic HCl and again heated for 15 minute at 55 ^{o}C, washed by 1ml distilled water. FAME is extracted with hexane (3×1) and evaporated to dryness (18). The fame was re-dissolved in 200µl hexane and analysed using a GC (gas chromatograph) Nucon 5700 series with EOX column (serial no 5061; 30 m length, 0.25 mm ID and 0.25 mm outer dia). Pure Nitrogen (99.9%) used as carrier gas with a flow rate of 1 ml/min and pre-column pressure of 49.7kPa. the initial temperature was set to be 120º C for 2 min, followed by a 4º C/min ramp up to 240º C and maintained for 30 min. the injector and FID detector temperature was set 240º C and 230º C respectively. Fame peaks are identified by comparison of their retention time with authentic standard by GC and quantified by normalization.

1.3 Analysis of biodiesel quality

The key qualities of biodiesel the Saponification value (SV), Iodine value (IV), Cetane number (CN) and (DU) degree of unsaturation values were calculated by using empirical Eq. 5-8 (19 20).

$$SV = \Sigma (560 \times N) / MW \quad (5)$$

$$IV = \Sigma (254 \times N \times D) / MW \quad (6)$$

$$CN = (46.3 + 5458 / SV) \_ (0.225 \times IV) \quad (7)$$

$$DU = (MUFA, wt\ \%) + (2 \times PUFA, wt\ \%) \quad (8)$$

Where N is the percentage of each fatty acid, MW is the molecular mass of fatty acid, D is the number of double bonds, MUFA is monounsaturated fatty acids and PUFA is polyunsaturated fatty acid by wt. %.

During the entire experiment, the measurements of the values were done in triplicates and the mean and ± standard deviation (SD) was calculated using GraphPad Prism 6 statistical software.

III. Results & Discussion

1.4 Biomass and Lipid accumulation of chlorella sp. at different nitrogen sources

In this study, Chlorella sp. was grown in Bg-11 for 14 days and source of nitrogen sodium nitrate is replaced by KNO_3, and Urea. The effect of different nitrogen sources on Chlorella sp. is shown in fig 1. It was observed that Chlorella sp. shows higher biomass content (1.29±0.04 g/l) in Urea, followed by sodium nitrate (1.28 ±0.11 g/l and KNO3 (1.26±0.05 g/l). The specific growth, doubling time, biomass productivity and lipid yield at stationary phase are shown in table 1. Highest specific growth and biomass productivity was observed 0.19369±0.0058 day^{-1} and 76.96±4.5 mg/l/day in urea while lowest was 0.192457±0.0044 day^{-1} and 74.92±3.3 mg/l/day in KNO_3. The doubling time of microalgae biomass was minimum in urea (3.58±.105 days), followed by KNO_3 (3.60±.088 days) and NaNO3 (3.62±.169 days). Maximum lipid content was recorded 11.83±1.8% for KNO3 while minimum lipid content was found in case of urea (8.49%). However, the lipid yield was found better for KNO3 (149.65±4.8 mg/ml), followed by $NaNO_3$ (141.21±10.63 mg/ml) and urea (109.01±10.85 mg /ml). Qiang Lin examined the effects of nitrogen source ($(NH_4)_2CO_3$, urea, $NaNO_3$, urea and $NaNO_3$ mixture) and concentration on the ash free dry biomass (AFDB) and oil accumulation and productivity of a Scenedesmus rubescens and found that the microalgae nurtured with the mixture of urea-N and $NaNO_3$-N had the highest AFDB productivity of 0.539 ± 0.040 g/L/d and the content of fatty acid methyl esters (FAME) (%) fed with $(NH_4)_2CO_3$-N increased continuously for 17 days and reached 42.94 ± 2.05% in the indoor photo-bioreactors (21). Muthu Arumugam also investigated the influence of different nitrogen source (potassium nitrate, sodium nitrate, urea, calcium nitrate, ammonium nitrate and ammonium chloride) of varying concentrations on biomass production of green algae Scenedesmus and found nitrate was the promising source for growth of Scenedesmus at low concentration (22).

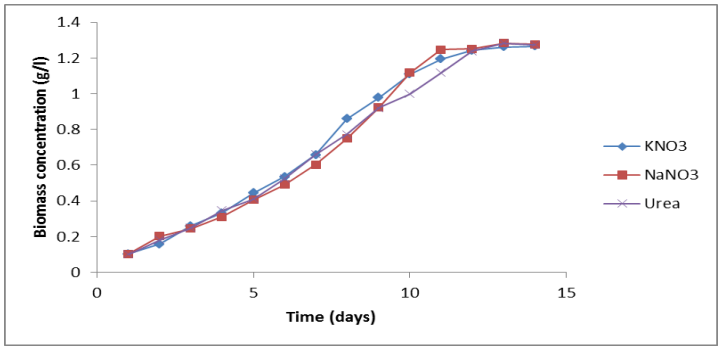

Fig.1. Dry weight of Chlorella sp. under different nitrogen sources

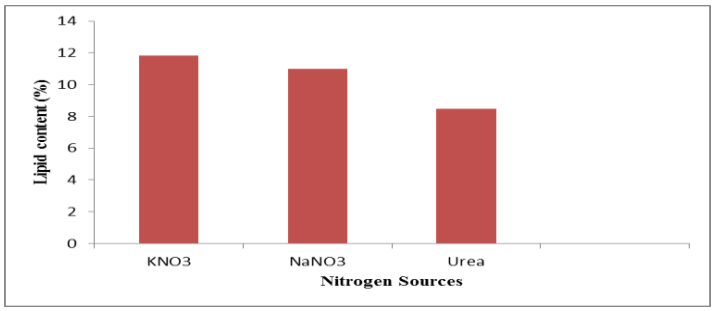

Fig.2. Lipid content of Chlorella sp. under different nitrogen sources

Table 1. Growth characterization of Chlorella sp. under different nitrogen sources

Nitrogen sources	Specific growth (μ_{max}) Day^{-1}	Doubling time	Rate of biomass (mg $l^{-1}day^{-1}$)	Lipid yield at the end of experiment (mg/l)
NaNO3	0.192±0.0066	3.62±0.169	75.98±7.7	141.21±10.63
KNO3	0.192457±0.0044	3.60±0.082	74.92±3.3	149.654.8
Urea	0.19369±0.0058	3.58±.105	76.96±4.5	109.01±10.85

3.2 Biomass and Lipid accumulation of chlorella sp. and chlorella pyronidisa at different Carbon sources

After achieving optimal nitrogen sources, Chlorella species were grown in different organic carbon sources such as Glucose, Glycerol and Sucrose (shown in Fig.3). At stationary phase, it was observed that addition of glucose resulted in maximum biomass productivity of 86.04±3.2 mg $l^{-1}day^{-1}$ while addition of sucrose and glycerol results in 69.38±9.2 and 80.39±3.9 mg $l^{-1}day^{-1}$ respectively. The specific growth and doubling time was found better in glucose (0.197±0.002 day^{-1} and 3.50±0.04 day), followed by glycerol (0.196±0.0026 day^{-1} and 3.53±0.04 day) and sucrose (0.186±0.0016 day^{-1} and 3.73±0.21day).The lipid content and lipid yield of Chlorella sp. also found maximum 13.22± 0.86%, 189.94 ±8.95 mg/l in glucose in comparesion to glycerol (11.28667±1.15% and 163.72±7.6 mg/l) and sucrose (12.18 ± 0.87 and 109.04±10.8 mg/l). In the study of Dittamart et. al. (2014) the most suitable carbon source was found to be 0.05M glucose, giving a yield of 2.78 ± 0.86 g./l of biomass and 233.68 ± 35.34 mg.L-1 of crude lipid (16). Gim et. al. (2013) also cultivated chlorella vulgaris in different organic carbon sources and observed glucose as better source for growth enhancement (23). This is mainly due to that the glucose is a simple hexose monosaccharide, which is first catabolized glucose-6-phosphate (important intermediate product for various metabolic precursors) and subsequently to pyruvate through anaerobic glycolysis process, and then entered into TCA cycle followed by mitochondrial oxidative phosphorylation for ATPs production (23, 24, 25).

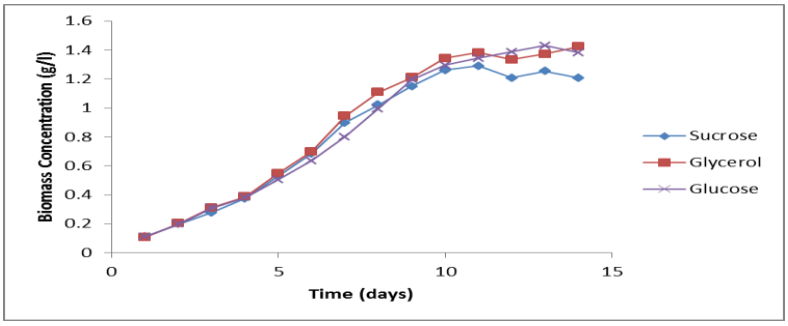

Fig.3. Dry weight of Chlorella sp. under different organic carbon sources

3.3 Fatty acid composition and Fuel properties of chlorella sp.

Fig 5 shows fatty acid composition of chlorella sp. and it was observed that Chlorella sp. contain 45.21% saturated FAME, 22.56% mono saturated FAME and 22.56% poly saturated FAME. According to European standards EN14214, for an ideal biodiesel the percentage of linolenic acid (C18:3) and polyunsaturated FA (>4 double bond) should not increase 12% and 1% respectively. In the present study C18:3 for Chlorella sp. are 4.54% and C18:4 is absent which makes it stable for fuel application.. Different Physico chemical properties of fuel obtained from this species were shown in table 3.

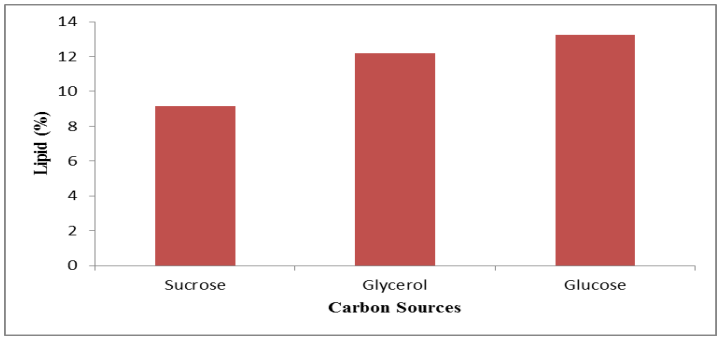

Fig.4. Lipid content of Chlorella sp. under different carbon sources

Table 2 Growth characterization of Chlorella sp. under different Carbon sources

Carbon sources	Specific growth (μ_{max}) Day^{-1}	Doubling time	Rate of biomass $mg\ l^{-1}day^{-1}$	Lipid yield at the end of experiment (mg/l)
Sucrose	0.186±0.0016	3.73±0.21	69.38±9.2	109.04±10.8
Glycerol	0.196±0.0026	3.53±0.04	80.39±3.9	163.72±7.6
Glucose	0.197±0.002	3.50±0.04	86.04±3.2	189.94±8.15

Table 3 Physico-chemical characterization of Chlorella sp

Properties	SV	IV	CN	DU	References
Chlorella sp.	00.43	26.59	5.04	45.26	This study
Chlorella sp.	217.8	65	56.7	74.1	(28)
Sunflower	-	-	50	152.2	(29)

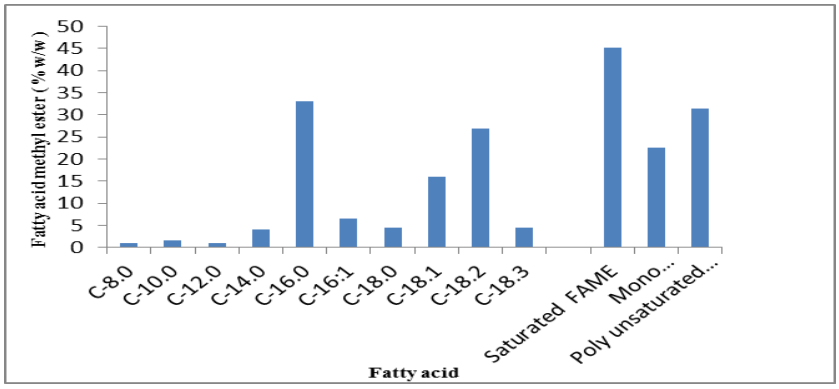

Fig.5 Fatty acid composition of Chlorella sp.

IV. Conclusion

In the present study, the growth and total lipid contents of Chlorella sp. was compared in different nitrogen and organic carbon sources. Comparing to sodium nitrate, higher lipid yield was observed in potassium nitrate. The addition of organic carbon sources in control medium results in higher biomass growth and lipid medium. Among the various carbon sources tested, glucose was the best organic carbon source for Chlorella sp. and lipid yield was found maximum (189.94±8.15) in comparison to other organic carbon sources. The presence of more than 60 % saturated and mono saturated fatty acid in Chlorella sp. prove that it is a good candidate for biodiesel production. The quality and properties of biodiesel met the criteria of the national petroleum agency (ANP255), Standard ASTMD6751 and European standards (EN 14214).

Acknowledgements

Authors are highly thankful to our Chancellor Prof. S. J. Chopra and Vice Chancellor Dr. Parag Diwan University of Petroleum and Energy Studies, Dehradun for their continuous encouragement and valuable mentorship.

References

[1]. I.B. Muhit, D. Baidya, Nurangir Nahid, Prospect of Algal Biodiesel Production in Bangladesh: Overview from Developed Countries, IOSR-JMCE, 11(1), 2014, 49-54.
[2]. Antoni. D.; Zverlov, V.V.: Schwarz, H. Biofuels from Microbes. Appl. Microbiol. Biotechnol. 2007, 77, 23-35.
[3]. BP Statistical Review of the world energy; June 2008.
[4]. Abdelaziz, A.E., Leite, G.B., Hallenbeck, P.C.,. Addressing the challenges for sustainable production of algal biofuels: I. Algal strains and nutrient supply. Environ. Technol. 34, 2013a, 1783–1805.
[5]. Amaro, H.M., Guedes, A., Malcata, F.X.,. Advances and perspectives in using microalgae to produce biodiesel. Applied energy, 88, 2011, 3402–3410.
[6]. Leite, G.B., M.Abdelaziz, A.E., Hallenbeck, P.C.,. Algal biofuels; challenges and opportunities. Bioresource Technology. 145, 2013, 134–141.
[7]. Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 2007, 294–306
[8]. Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM () Selection of microalgae for lipid production under high levels carbon dioxide. Bioresour Technol 101, 2010, 71–74
[9]. Illman AM, Scragg AH, Shales SW (2000) Increase in Chlorella strains calorific values when grown in low nitrogen medium.Enzyme Microb Technol 27, 200, 631–635.
[10]. Takagi M, Watanabe K, Yamaberi K, Yoshida T (2000) Limited feeding of potassium nitrate for intracellular lipid and triglycerideaccumulation of Nannochloris sp. UTEX LB1999. Appl Microbiol Biotechnol 54, 200, 112–117.
[11]. Li Y, Horsman M, Wang B, Wu N, Lan CQ Effects of nitrogen sources on cell growth and lipid accumulation of greenalga Neochloris oleoabundans. Appl Microbiol Biotechnol 81, 2008, 629–636.

[12]. Khotimchenko SV, Yakovleva IM Lipid composition of the red alga Tichocarpus crinitus exposed to different levels ofphoton irradiance. Phytochemistry 66, 2005, 73–79.
[13]. Renaud SM, Thinh LV, Lambrinidis G, Parry DL, Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures, Aquaculture 211, 2002, 195–214
[14]. Takagi M, Karseno, Yoshida T , Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride inmarine microalgae Dunaliella cells,. J Biosci Bioeng101, 2006, 223–226
[15]. Liu ZY, Wang GC, Zhou BC , Effect of iron on growth and lipid accumulation in Chlorella vulgaris, Bioresour Technol 99, 2008, 4717–4722.
[16]. Doungpen Dittamart, Chayakorn Pumas, Jeeraporn Pekkoh and Yuwadee Peerapornpisal, Effects of organic carbon source and light-dark period on growth and lipid accumulation of Scenedesmus sp. AARL G022, Maejo Int. J. Sci. Technol. 8(02), 2014, 198-206
[17]. Folch, J., Lees, M., Sloane-Stanley, G.H.,. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 1957, 497-509.
[18]. J.P. Carreau, J.P. Dubacq, Adaptation of macroscale method to the microscale for fatty acid methyl transesterification of biological lipid extracts, J.Chromatogr. 151, 1978, 384–390.
[19]. Francisco, E.C., Neves, D.B., Jacob-Lopes, E., Franco,T.F, Microalgae as feedstock for biodiesel production: carbon dioxide sequestration, lipid production and biofuel quality. J. Chem. Technol. Biotechnol. 85, 2010, 395–403.
[20]. Ramos, M.J., Feranandez, G.M., Casas, A., Rodriguez, L., Perez, A., Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour. Technol. 100, 2009, 261–268.
[21]. Qiang Lina, b and Junda Lin, Effects of nitrogen source and concentration on biomass and oil production of a Scenedesmus rubescens like microalga, , Bioresour Technol 102(2), 2011, 1615-1621.
[22]. Muthu Arumugam, Ankur Agarwal , Mahesh Chandra Aryaa, Zakwan Ahmed, Influence of nitrogen sources on biomass productivity of microalgae Scenedesmus bijugatus, Bioresour Technol, 131 (2013) 246–249
[23]. Geun Ho Gim , Jung Kon Kim , Hyeon Seok Kim, Mathur Nadarajan Kathiravan , Hetong Yang , Sang-Hwa Jeong , Si Wouk Kim, Comparison of biomass production and total lipid content of freshwater green microalgae cultivated under various culture conditions, Bioprocess Biosyst Eng (2014) 37:99–106.
[24]. Droop MR . Heterotrophy of carbon. In: Stewart WDP (ed).Algal physiology biochemistry. Blackwell Scientific Oxford, UK 1974, pp 530–559.
[25]. Neilson AH, Lewin RA , The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry.Phycologia 13, 1974, 227–264.