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Influence of various Carbon and Nitrogen sources on Lipid productivity of *Chlorella minutissima* and *Scenedesmus* sp. and their FAME analysis

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Abstract

Green fuels are getting greater attention day by day due to increase in energy demands and environmental problems. In the current study, *Chlorella minutissima* and *Scenedesmus sp*., were cultivated in BBM medium. The impact of various nitrogen (Sodium Nitrate, Potassium Nitrate, Yeast Extract, Glycine and Urea) and carbon (Glucose, Glycerol, Fructose, Maltose, Starch, Sodium acetate and Sucrose) sources was seen on lipid productivity and FAME analysis. The lipid content was analyzed utilizing Folch strategy by changing the solvent system and amongst the six solvent systems used, Chloroform: Methanol (2:1) was seen demonstrating the best outcomes and was further utilized for extraction. Maximum lipid content and productivity was found in Potassium nitrate nitrogen source (50.08%, 1350.96mg/L/day) for *Chlorella minutissima* and Urea as a nitrogen source (79.05 %, 4027.31 mg/L/day) for *Scenedesmus sp*. Among organic carbon sources, the maximum lipid content and productivity was found in Glucose (36.79% and 2577.27mg/L/day respectively) for *Chlorella minutissima* and Maltose as a carbon source (27.4%, 1690.18 mg/L/day respectively) for *Scenedesmus* sp. Further, it was observed both the algae contained fatty acids from C: 16 to C: 18 which are essential for biodiesel production.

Keywords: Biodiesel, Biomass growth,; *Chlorella minutissima; Scenedesmus sp*.; Lipid extraction; FAME analysis

Introduction

Microalgae utilises sunlight and fix CO2 during photosynthesis and produces biomass more efficiently and rapidly than terrestrial plants. They have been considered for biomass to energy production, based on their fast growth rate, biomass productivity and compatibility for the various kinds of biofuels. (Kumar L, et al., 2017). Power is backbone of each United States's financial improvement and prosperity. India is the sector's 5th largest primary energy patron and fourth largest petroleum consumer after U.S.A, China and Japan (Sharma P. et al., 2015). With an outlook for slight to robust monetary boom and a growing population, growing infrastructural and socio-financial improvement will stimulate an boom in power intake throughout all main sectors of the Indian economic system (Muhit I.B, et al.,2014).Transportation is one of the fastest growing sectors the usage of 27% of the primary electricity (Antoni D,et al.,2007). At the present notable rates of utilization, the world fossil oil holds are probably going to be depleted in below forty five years (BP Statistical overview of the world vitality, 2008). In this way, the growing energy (gas) emergency and ecological debasement have represented an intense problem to our doable improvement and survival. So attention has now moved to the choice powers like biodiesel, bio-ethanol and biogas. Biodiesel is synthesis of unsaturated fat of ethyl or methyl ester produced the usage of virgin or utilized vegetable oil (either palatable or non-consumable).

The most of the biodiesel manufacturing are primarily based on fit to be eaten oil like soybean oil, rapeseed oil, canola oil or sunflower oil in developed countries (Sharma P. et al., 2015). In India, the palatable oil request is better than its actual technology. So there's no chance of occupying this oil for generation of bio diesel. The precept item warm spots for bio diesel can be non-consumable oils obtained from vegetation that may supplant the neighborhood oil makers. There are numerous species, which bear seeds wealthy in oil content and out of those some encouraging tree species had been distinguished. Those are *Jatropha curcas* (Ratanjyot), *Pongamia pinnata* (Karanja), *Calophyllum inophyllum* (Nagchampa or Polanga), Mahua, Castor, Seemarouba and etc (Sharma P. et al., 2015).

Utilizing the ebb and waft yields, mammoth measures of land and crisp water might be expected to create sufficient oil to absolutely supplant petroleum spinoff use. It'd require double the land vicinity of America to be dedicated to soybean technology, or sixty six% to be devoted to rapeseed advent, to meet modern US warming and transportation needs. Microalgae are an emerging source for biodiesel manufacturing in recent years as because of higher biomass and lipid productiveness, and the dearth of opposition with meals plants for agriculture lands and sparkling water resources as they may be develop on non-arable land using saline or waste water (Abdelazi A.E. et al., 2013; Amaro H.M. et al., 2011; Leite G.B. et al., 2013). Microalgae

are photoautotrophic sunlight-driven mobile factories which can convert carbon dioxide to diverse merchandise along with lipids, carbohydrates, proteins, fatty acids, nutrients, antibiotics, and antioxidants (Chisti Y. 2007).

The lipid content has been elevated in many microalgae as a response to excessive way of life conditions which include CO2, nitrogen awareness and mild depth (Yoo et al., 2010). For the fast accumulation of lipid, microalgae had been cultured in increase-restricting environment such as nitrogen depletion, (Illman AM.et al., 2000; Takagi M. et al., 2000; Li Y. et al., 2008), high mild depth (Khotimchenko et al., 2005), low temperature (Renaud SM. et al., 2002), excessive salt awareness (Takagi M. et al., 2006) and high iron awareness (Liu ZY. et al., 2008). It has been seen that carbon and nitrogen source changes highly influence the biomass and lipid technology of microalgae.

Various carbon sources including Glucose, Sucrose, Fructose, Starch, Maltose, Sodium Acetate and Glycerol and nitrogen sources such as Urea, Yeast Extract, Glycine, Potassium Nitrate, Sodium Nitrate, and Malt Extract had been used. The growth of these resources was recorded by taking absorbance at 680nm on alternate days and additionally through recording dry cell weight on alternate days. The biomass content, lipid content and productivity were additionally calculated for each source. Eventually FAME evaluation confirmed how much percent of fatty acids are present in each alga using various unique carbon and nitrogen sources.

Materials and methods

1. Micro algal strain and culture condition

Pure cultures of *Chlorella minutissima*, *Scenedesmu*s sp., were collected from IARI, New Delhi and The Energy and Resources Institute (TERI), Delhi (India) respectively. These species after collection were sub cultured on BBM agar media plates under laboratory conditions (with initial pH of 6.8) and at 25 ˚Celsius under (~ 1935 lux) light intensity and 12/12 light dark cycle. All the strains were transferred from agar plates to the medium and incubated under the same conditions of temperature and light in 5L culture flasks. Liquid cultures were used as the inoculums for the designed experiments. *Chlorella minutissima* showed better biomass production in 24 hours of illumination of white light of 20 W tube light and shaking periods of 2 hours in Erlenmeyer flasks (Saxena G, 2016)

2. Carbon sources and concentration

The effect at single carbon concentration (0.5g/L) was seen using several sources of carbon in culturing *Chlorella minutissima* and *Scenedesmus sp*. The carbon sources used were Glucose, Fructose, Sucrose, Maltose, Glycerol, Sodium acetate, and Starch. This was done in order to investigate their effect on lipid content and productivity because the inclusion of carbon sources in BBM media showed an increase in lipid production. As there is no carbon sources present in the BBM media the addition of these sources is an added benefit for higher biomass and thus lipid production. The growth and lipid productivity of these species under different carbon sources was recorded and compared.

3. Nitrogen sources and concentration

The effect at single nitrogen concentration (0.25g/L) was seen with several sources of nitrogen such as Yeast extract, Malt extract, Urea, Glycine, Sodium nitrate and Potassium nitrate along with Glucose (0.5g/L) in order to investigate the effect of nitrogen sources on the lipid production of Chlorella and Scenedesmus sp. The lipid content and productivity of these species under different nitrogen sources was recorded and compared

4. Assessment of combination of solvent systems using Folch method

Lipid was extracted by using Folch method (Folch et al. 1957). As per this method, a weighed amount of biomass i.e. around 250 mg was taken and 5 ml chloroform/methanol (2:1 v/v) was added and vortex for 30 s. This was followed by keeping the mixture for 15–20 min at room temperature. The mixture was then centrifuged at 4000 rpm for 10 min to isolate cell trash from supernatant. This supernatant was washed by 0.9 % NaCl arrangement/water and vortex for few moments. Then this mixture was centrifuged at 3000 rpm for 5 min. Lower chloroform layer with lipid was evacuated deliberately and gathered in 20 ml pre-weighted glass vial. The biomass was re-extricated with 2.5 ml chloroform/methanol (1:1 v/v) until biomass became white. The supernatant was gathered in same vial. The supernatant was then air dried until consistent weight of lipid was attained. The lipid content was calculated gravimetrically. The standard solvent system was changed with different solvent systems such as: Dichloromethane: Methanol (Bligh and Dyer Method); Chloroform: Methanol (Folch method); Hexane: Isopropanol; Cyclohexane: Isopropanol; Hexane: Ethanol; Petroleum Ether.

5. Kinetic parameter :

The relationship developed between OD 680 and biomass (g/l) is given as follows:

The maximum specific growth rate (μmax day^{-1}) was calculated as follows:

μ_{max} (day^{-1})= $(\ln X_2 - \ln X_1) / (t_2 - t_1)$

Where X_1 and X_2 were the dry biomass weight (g/l) at time t1 and t2 respectively.

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

TD (days) = $\ln(_2)/\mu_{max}$

Lipid content (C_{lipid}) = (Weight of lipid/ Weight of sample) x 100

Lipid productivity (mg/l/day) = (C_{lipid} x DCW)/ t

Lipid yield (mg/L) = [Weight of lipid/Volume of sample] x 1000

Biomass yield or Dry Cell Weight (mg/ L) = [weight of dry sample (mg)/ weight of culture (ml)] x 1000

6. Sudan test

After completion of extraction of lipid, it was also confirmed whether the extracted material is actually the desired lipid or not. For this conformation, Sudan test which uses Sudan IV dye was performed. This dye is not soluble in water however it is soluble in lipids. 100 ml stock solution of 1mg/ml concentration of Sudan dye was prepared. Lipid sample was prepared by dissolving the extracted lipid in ethanol for each case of above mentioned experiments. For confirmation of lipid, 5ml water is added in a test tube and then the lipid sample dissolved in ethanol was added in to it very slowly. Due to difference in chemical nature, two different phases were formed. Upper phase was lipid phase and lower phase was water. The 20 drops of Sudan IV dye were added slowly with the help of micropipette. The dye was absorbed by the lipid available in upper phase and rest of it was settled in the bottom of the test tubes. This retention or absorbance of dye by the upper phase confirmed that the extracted material was lipid.

7. Fatty Acid Methyl Ester (FAME) analysis of potential lipid yielding algal biomass

Based on the results of potential lipid yielding culture conditions experiment was set for FAME production and analysis.
5-10ml of algae grown under various conditions is taken and centrifuged in order to obtain the biomass and remove all the media from it. 5-10ml of algae was harvested and was collected in screw cap glass tubes in accordance to the density of the material. 2% of Methanolic HCl was added to this algae and the tube caps were tightened properly. These glass tubes are incubated at 80 degree Celcius for 1 hr and 1 ml of 0.9% NaCl in water is added to it. Followed by vortexing the mixture 2 ml of Hexane was added to it. After vortexing the tube is spun at 2000 rpm for 2 minutes for phase separation. After phase separation the upper phase (hexane) is pipetted out in new tube. It is further dried under nitrogen flow and 50 µl of Hexane is added to it. For FAME analysis only 1 µL of sample is injected in the GC.
Once the analysis is done all the fatty acids composition is calculated and tabulated in order to find out the percentage of MUFA, PUFA and Saturated fatty acids.

Result and discussion

Growth curve

Potential of microalgae for the production of high and low value commercial products is very huge but effect of environmental conditions on productivity is a great hurdle. The environmental stress affects the biomass productivity as well as final productivity of the commercial product. So identification of potential culture conditions is primary requirement of algal cultivation. All the microalgae species *Chlorella minutissima* and *Scenedesmus sp* were cultured in BBM media and nitrogen source (NaNO3) was replaced with other nitrogen sources and also different carbon sources were added externally. The cultures in different sources were grown in 250 ml flasks for 2 weeks and the results revealed that in some sources the algae had reached the exponential phase between 7-11 days and in some sources the algae reached exponential phase after that.

Chlorella minutissima

Carbon Sources

In case of carbon sources used in *Chlorella minutiisma* Maltose, Glucose, Sucrose and Fructose show a better growth than the control (no carbon source added to it) in which Maltose and Glucose show the best growth curve. Sodium acetate did show good results up-to 7 days but after that it started to decline.

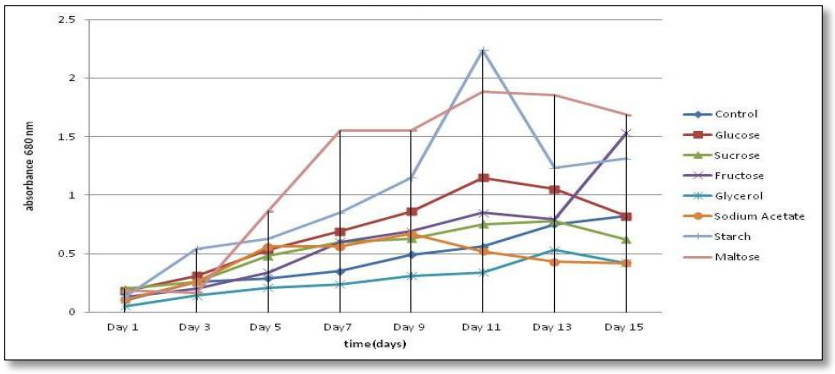

Fig 1. Growth curve of *Chlorella minutissima* under Carbon sources

Nitrogen sources

In case of nitrogen sources, every component showed a better and increased growth than the control but Urea and Yeast extract continued to grow even after 14 days which indicate that they might prove to be better nitrogen source for the growth of *Chlorella minutissima*. Malt extract was also considered as a nitrogen source but it showed no growth after 3 days, i.e the algae grew only for 3 days and after that it started to go in death phase. The experiment using Malt extract was repeated three times and still the algae did not grow after 3 days.

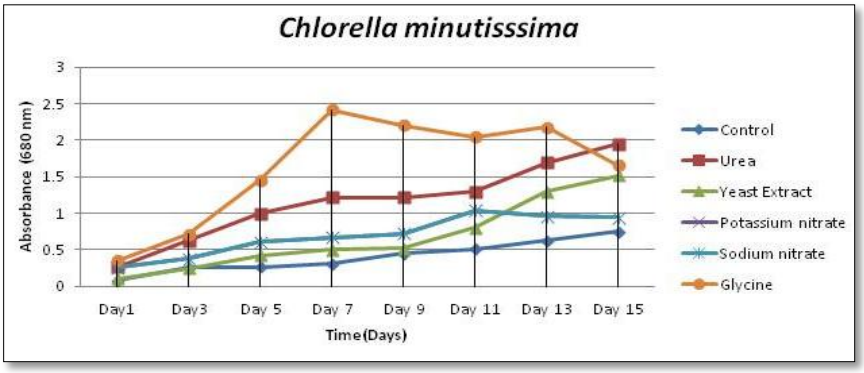

Fig 2. Growth of *Chlorella minutissima* under various nitrogen sources

Growth curve of *Scenedesmus sp.*

Carbon Sources

In case of carbon sources, all except Sodium acetate, Glycerol and Fructose showed a better and increased growth in *Scenedesmus sp.* than control (having no carbon source). But best growth was observed in Maltose, Glucose and Sucrose

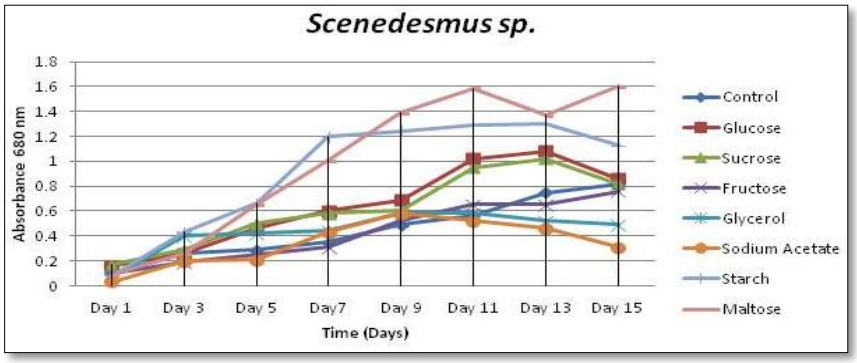

Fig 3. Growth of Scenedesmus sp. under various carbon sources

.Nitrogen sources

In case of nitrogen sources Urea and Yeast extract showed an increasing growth and tend to increase even after a time frame of 7 days. Malt extract was also considered as a nitrogen source but it showed no growth after 3 days, i.e the algae grew only for 3 days and after that it started to go in death phase. The experiment using Malt extract was repeated three times and still the algae did not grow after 3 days

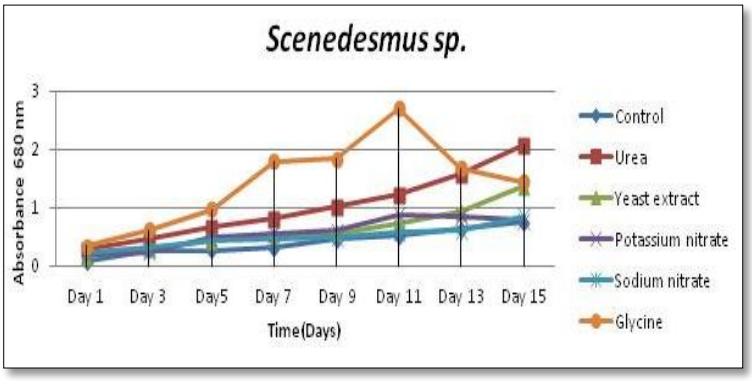

Fig 4. Growth of Scenedesmus sp. under various nitrogen species

Lipid yield and productivity

Firstly the lipid productivity for *Chlorella minutissima* and *Scenedesmus sp*. was checked for the best solvent and the further lipid extraction was done accordingly.

Table 1: lipid productivity for *Chlorella minutissima* and *Scenedesmus sp*.(Dry biomass)

Solvent System	Lipid Content (%) of *Chlorella sp.*	Lipid Content,(%) of *Scenedesmus sp.*
S1	8.42	9.8
S2	9.42	11.58
S3	8.5	4.66
S4	8.65	8.65
S5	8.038	7.03
S6	1.64	3.6

Table 2: lipid productivity for *Chlorella minutissima* and *Scenedesmus sp*.(Wetbiomass)

Solvent System	Lipid Content (%) of *Chlorella sp.*	Lipid Content,(%) of *Scenedesmus sp.*
S1	7.4	8.9
S2	12.08	12.8
S3	11.28	7.6
S4	12.01	11.2
S5	11.09	10.4
S6	1.8	3.8

Looking at this data it can be seen that Chloroform:Methanol (2:1) is the most appropriate solvent system for lipid extraction both using dry and wet biomass . Though wet biomass shows better results but it poses problem of having high moisture content and the results are not accurate and due to this reason dry biomass is used for lipid extraction.

Chlorella minutissima

Carbon Sources

Glucose shows the highest amount of lipid yield and productivity followed by Fructose and Sucrose for *Chlorella minutissima*. The lipid content calculated was 36.79% and lipid productivity was 2577.27mg/L/day.

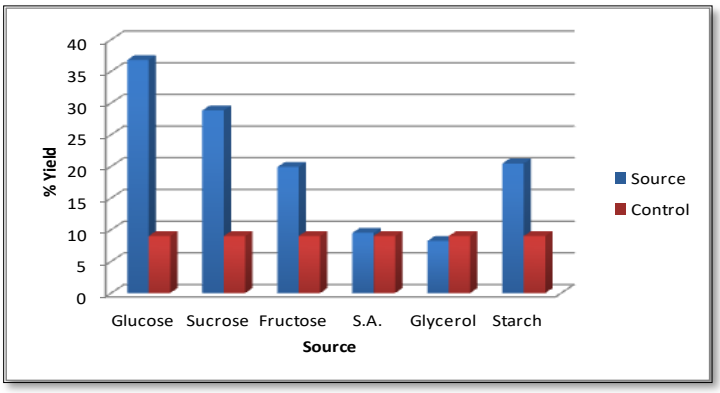

Fig5. Lipid yield for *Chlorella minutissima* under various carbon sources

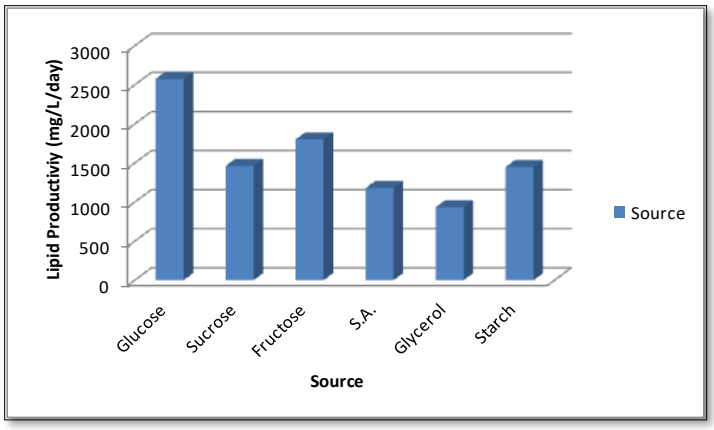

Fig 6 Lipid productivity for *Chlorella minutissima* under various carbon sources

Nitrogen sources

Urea showed best lipid productivity followed by Potassium nitrate and Sodium nitrate for *Chlorella minutissima*. The lipid productivity was 381.5 mg/L/day and potassium nitrate showed maximum lipid content 50.08 %.

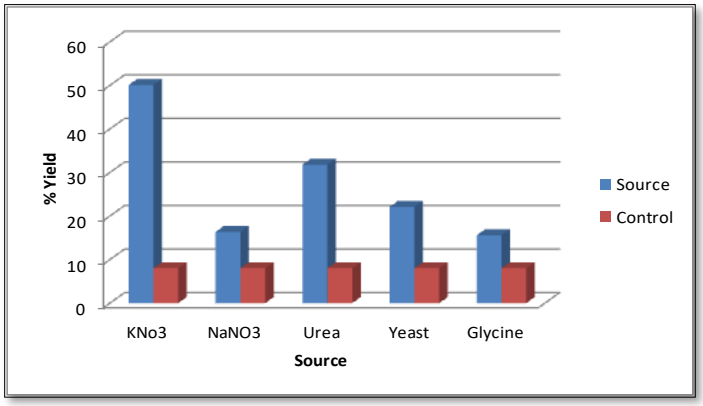

Fig 7 Lipid yield for *Chlorella minutissima* under various nitrogen sources

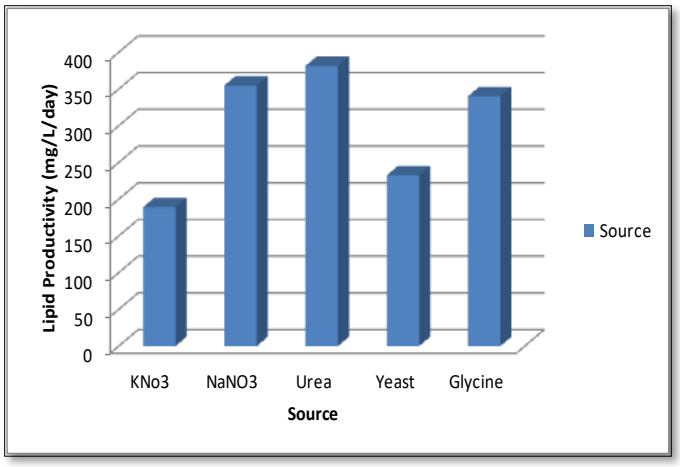

Fig 8 Lipid productivity for *Chlorella minutissima* under various nitrogen sources

Scenedesmus sp.

Carbon Sources
Glucose and sucrose showed better lipid content and productivity for carbon sources in *Scenedesmus sp.*

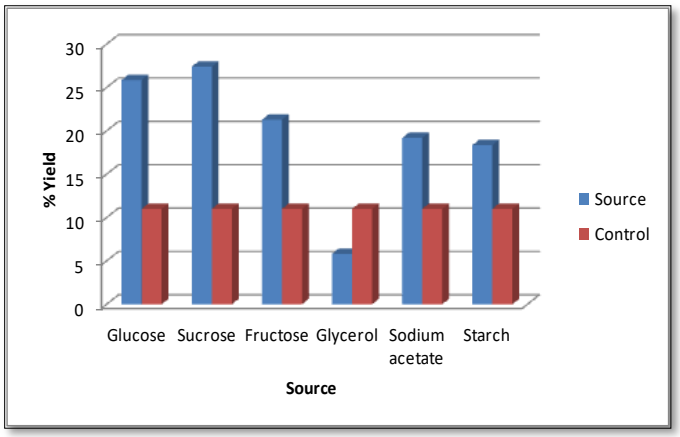

Fig 9 Lipid yield for *Scenedesmus sp*. under various carbon sources

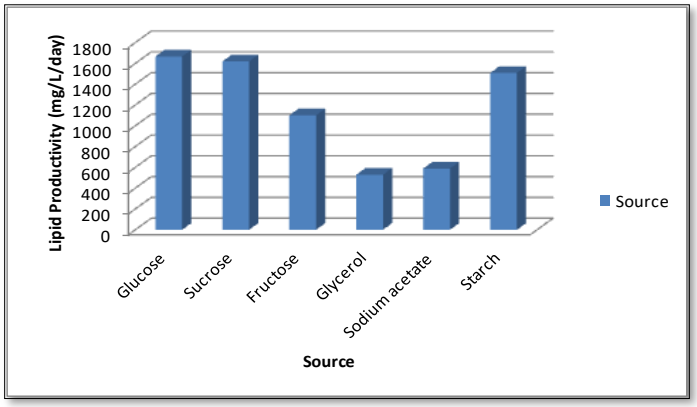

Fig 10 Lipid productivity for *Scenedesmus sp*. under various carbon sources

Nitrogen sources

Urea showed maximum lipid productivity (4027.31 mg/L/day) and lipid content (79.05%).

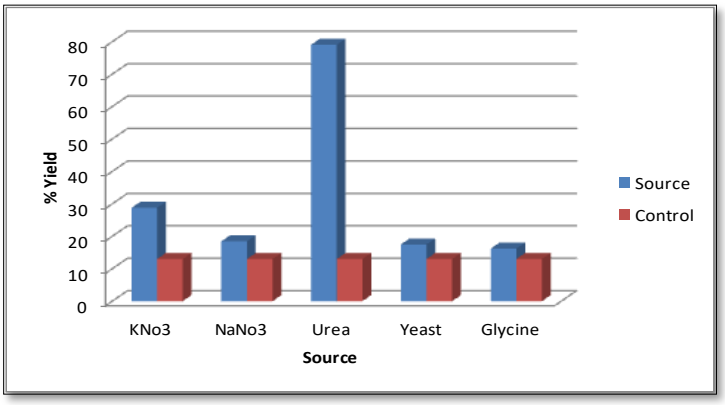

Fig 11 Lipid yield for *Scenedesmus sp*. under various nitrogen sources

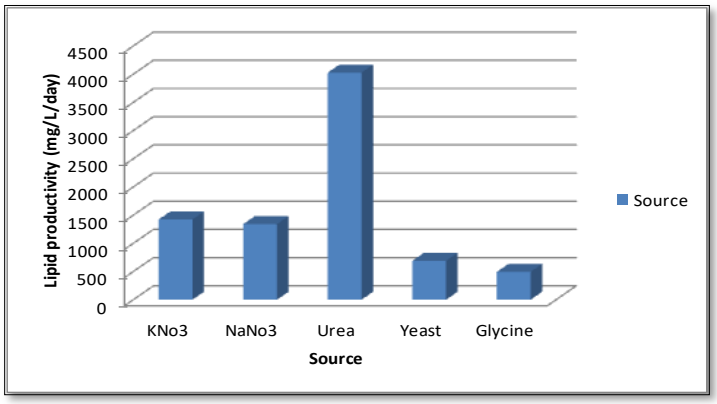

Fig 12 Lipid productivity for *Scenedesmus sp*. under various nitrogen sources

Extraction Process

Among the various combinations of solvent tested, combination of Chloroform: Methanol in the ratio of 2:1 was found most suitable for lipid extraction from *Chlorella minutissima* and *Scenedesmus* sp microalgae. The procedure of extracting lipid utilizes different volume of organic solvents. So for easy comparison, the results were projected on using 5 ml of organic solvents. This extraction process via organic solvents was also combined with the best method of cell disruption i.e. sonication which helped in increasing the lipid yield. These data are supported by the fact that nitrogen starvation condition results in more lipid accumulation (Converti et al. 2009; Chen et al. 2011; Feng et al. 2011; Kumari et al. 2011; Li et al. 2012). The selection of carbon and nitrogen sources depends upon various factors such as the target product, growth rate, and medium cost. However, nitrogen is the key factor in growth medium and also a limiting nutrient affecting the lipid productivity of various microalgae (Griffiths and Harrison 2009). Qiang Lin examined the effects of nitrogen source ((NH4)2CO3, urea, NaNO3, urea and NaNO3 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 NaNO3-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 (NH4)2CO3-N increased continuously for 17 days and reached 42.94 ± 2.05% in the indoor photo-bioreactors (Qiang Lina, b et al., 2011). 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 (Arumugam, Muthu et al., 2013)

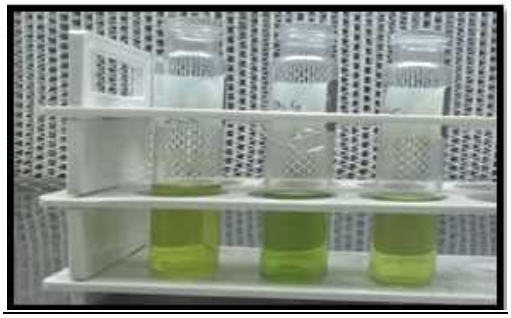

Fig 13 Extraction of lipids

Sudan Test

The presence of brown and red color on the upper phase of the test tube showed the presence of lipids in the sample.

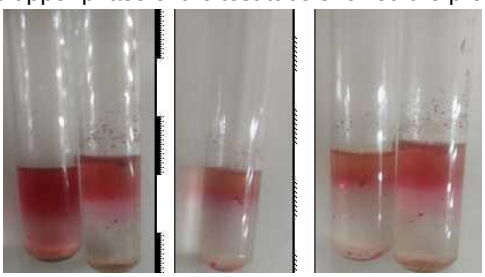

Fig 14 Sudan Test for lipids

Kinetic Parameters

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 (Dittamart, Doungpen et al., 2014). Gim et. al. (2014) also cultivated chlorella vulgaris in different organic carbon sources and observed glucose as better source for growth enhancement (Geun Ho Gim et al., 2013). 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 (Geun Ho Gim et al., 2013; Droop MR et al., 1974; Neilson AH et al., 1974).

Table 3. Kinetic Parameters of *Chlorella minutissima*

Media	Specific Growth Rate (day-1)	Doubling Time (day)	Lipid Content (%)	Lipid Yield (mg/L)	Volumetric Lipid Productivity (mg/L/d)
Glucose	0.2801	2.47	36.79	360.83	2577.27
Sucrose	0.2990	2.31	28.82	205	1463.98
Fructose	0.2939	2.35	19.89	255.66	1802.29
Glycerol	0.2995	2.31	8.21	130.75	933.25
Sodium acetate	0.2911	2.38	9.49	165.25	1179.30
Starch	0.2769	2.50	20.45	261.33	1447.93
KNo3	0.3022	2.29	50.08	189.16	1350.96
NaNo3	0.2942	2.35	16.34	354.75	2532.50
Urea	10.2761	2.51	31.78	381.5	2726.83
Yeast	0.2983	2.32	22.08	232.41	1659.41
Glycine	0.3220	2.15	15.56	340.15	1001.95

Table 4. Kinetic Parameters of *Scenedesmus sp.*

Media	Specific Growth Rate (day -1)	Doubling Time (Days)	Lipid Content (%)	Lipid Yield (mg/L)	Volumetric Lipid Productivity (mg/L/d)
Glucose	0.2943	3.35	25.86	233.33	1666.58
Sucrose	0.3183	2.17	27.4	226.66	1619.04
Fructose	0.3152	2.19	21.28	154.16	1100.98
Glycerol	0.2918	2.37	5.82	74.25	529.89
Sodium acetate	0.2910	2.38	19.18	82.5	589.1
Starch	0.2794	2.48	18.34	78.24	1508.24
KNo3	0.3203	2.16	28.78	199.16	1422.55
NaNo3	0.2843	2.43	18.44	187.75	1340.41
Urea	0.3158	2.19	79.05	566	4027.31
Yeast	0.3259	2.12	17.48	96.58	689.62
Glycine	0.2987	2.32	16.21	92.45	493.44

Fame analysis

The FAMEs were not measured from the samples of days 8 and 10 because within 6 days, all cultures reached the late exponential growth period or stationary growth period (Hsieh, C.-H et al., 2009) which is well known as the maximum lipid production period in the cells.

The analysis of fatty acid from different Carbon and Nitrogen sources in algae species: *Chlorella minutissima* and *Scenedesmus* sp. by GC-MS showed that they contain various fatty acid methyl esters including Pentadecanoic acid, Octadecadienoic acid, Hexadecanoic acid, Octadecatrienoic acid in major concentration. Heptadecanoic acid and Methyl stearate are present in less concentration. Unsaturated fatty acid Octadecadienoic and Octadecatrienoic acid are the most important essential fatty acids as our body cannot synthesize these fatty acids.

The GC-MS result of FAME sample of both algae are given in below figures.

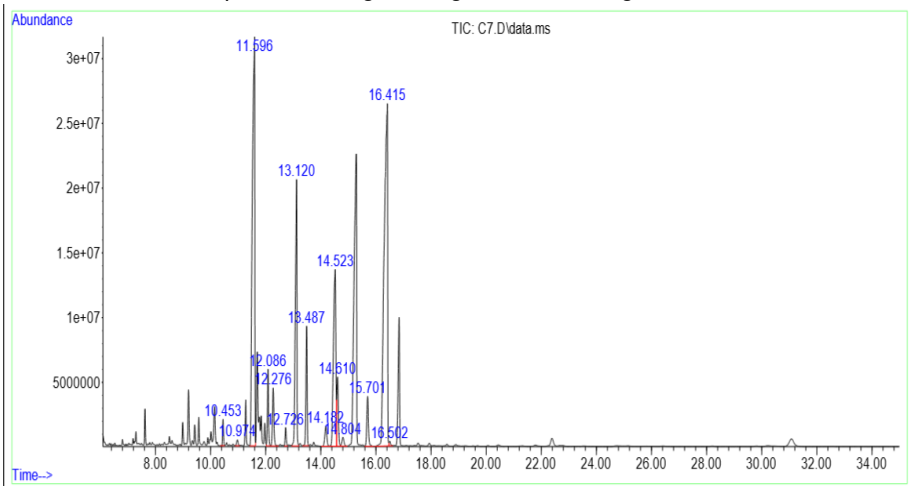

Fig 15 FAME analysis for *Chlorella minutissima*

Table 5: Percentage of Methylated fatty acids in C7 sample *: Chlorella minutissima*

Fatty acid	Common name of Fatty Acid	Carbon number and bonds	Relative %age Content of Fatty acid
Pentadecanoic acid	Pentadecylic acid	C15:0	0.657
Hexadecanoic acid	Palmitic acid	C16:0	27.285
9-Hexadecanoic acid	-	C16:1	2.351
7,10 hexadecadienoic acid	-	C16:2	2.301
Heptadecanoic acid	Margaric acid	C17:0	0.592
7,10,13-Hexadecatrienoic acid	-	C17:3	11.490
Methyl stearate	-	C19:0	1.105
9-Octadecenoic acid, methyl ester	Oleic acid	C18:1	11.886
gamma.-Linolenic acid	-	C18:3	2.269
9,12,15-Octadecatrienoic acid	Alpha linolenic acid	C18:3	32.238
Total			92.174
Saturated Fatty Acid Total			29.639
Monounsaturated Fatty Acid (MUFA) Total			14.237
Polyunsaturated Fatty Acid (PUFA) Total			48.298

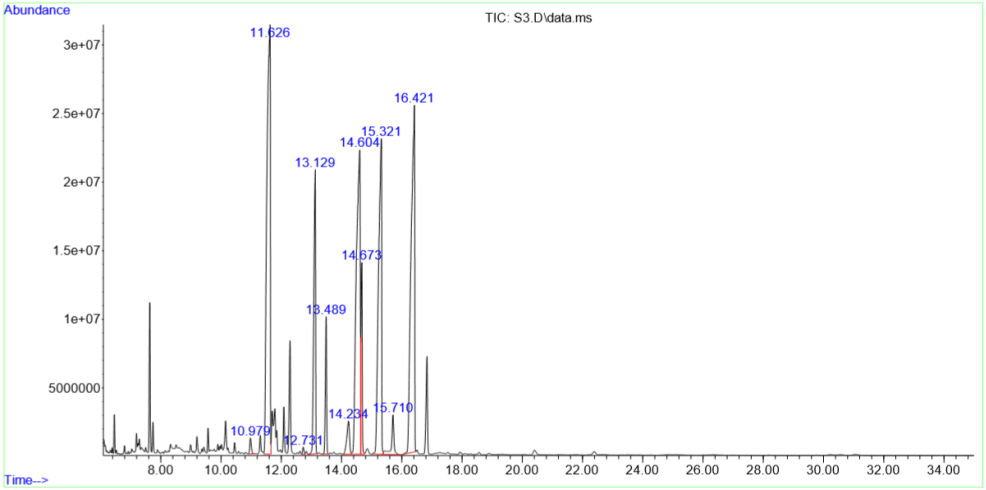

Fig 16. FAME analysis for *Scenedesmus sp.*

Table 6 : Percentage of Methylated fatty acids in S3 sample *: Scenedesmus sp.*

Fatty acid	Common name of Fatty acid	Carbon number and bonds	Relative %age Content of Fatty acid
Pentadecanoic acid	-	C15:0	0.421
Hexadecanoic acid	Palmitic acid	C16:0	24.330
9,12-Octadecadienoic acid	Linolenic acid	C18:2	16.267
Heptadecanoic acid	Margaric acid	C17:0	0.174
7,10,13-Hexadecatrienoic acid	Roughanic acid	C16:3	8.627
Methyl 4,7,10,13-hexadecatetraenoate	-	C17:3	2.830
Methyl stearate	-	C19:0	1.484
9-Octadecenoic acid, methyl ester	Oleic acid	C18:1	19.469
gamma.-Linolenic acid	-	C18:3	1.556
9,12,15-Octadecatrienoic acid	Alpha-linolenic acid	C18:3	20.791
Total			95.949
Saturated Fatty Acid Total			26.409
Monounsaturated Fatty Acid Total			19.469
Polyunsaturated Fatty Acid Total			50.071

Conclusion

Several studies reviewed the effect of C/N ratios in alga-based biodiesel productivity (Xu, H et al., 2006; Yoo, C., et al., 2010). However, the increase of oleic acid content to total FAMEs produced by microalgae in media having high C/N ratios has never been reported before this study, even though it was referred to indirectly by Piorreck and Pohl (Piorreck, M. and P. Pohl. 1984). On the other hand, the increase of oleic acid to total FAMEs in *Chlorella vulgaris* has already been reported as the response of temperature increase from 25˚C to 38˚C (Converti, A., A et al., 2009). It has been previously reported that the fatty acid composition produced by microalgae varies with their physiological status and culture conditions, even with extraction methods for recovery of fatty acids (Converti, A., et al., 2009; Hu, Q., et al., 2008; Mulbry, W et al., 2008; Piorreck, M. et al., 1984; Tran, H. L et al., 2009).

Chloroform: Methanol (2:1) was seen to be the best extracting solvent for lipid extraction. Using this maximum lipid content and productivity was found in Potassium nitrate nitrogen source (50.08%, 1350.96mg/L/day) for *Chlorella minutissima* and Urea as a nitrogen source (79.05 %, 4027.31 mg/L/day) for *Scenedesmus sp*. Among organic carbon sources, the maximum lipid content and productivity was found in Glucose (36.79% and 2577.27mg/L/day respectively) for *Chlorella minutissima* and Maltose as a carbon source (27.4%, 1690.18 mg/L/day respectively) for *Scenedesmus* sp. Further it was observed both the algae contain Fatty acid from C: 16 to C: 18 which are essential for biodiesel production.

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