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MICROBIOLOGY

Evaluation of Fatty Acid Profile and Biodiesel Characterization Obtained from Novel Algae *Scenedesmus vacuolatus X56104*

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ABSTRACT

Microalgae are the rapidly growing photosynthetic microorganisms and can be used as a source of renewable biofuels. The present investigation focuses on the perspectives regarding the use of newly isolated microalgae as a better biomass and lipids producer. The algal strain was isolated from local Godavari River and identified as *Scenedesmus vacuolatus* X56104 on the basis of 18s rRNA sequence. Microalgae were cultivated under controlled environment in the laboratory. Early stationary grown microalgae revealed 2.3 mg/ml biomass. Algae oil was extracted using soxhlet apparatus and found to be 26.7% of total biomass. Physiochemical properties of oil were recorded as density (0.85gm/cc), viscosity (4.2 mm^2), moisture (1.8%), acid value%(0.5 mg of KOH/gm), flash point (130°C), calorific value (9110Kcal/Kg) and Cetane number (54). The fatty acid profile was evaluated by GCMS and showed Palmitic acid (5.81%), stearic acid (1.86%), Oleic acid (65.83%), Linoleic acid (20.10%), Linolenic acid (4.66%), Arachidic acid (0.52%) and Eicosenoic acid (1.22%). The total fatty acids were subjected to lipase based trans esterification so as to obtained FAME and finally the biodiesel. The biodiesel was characterized using FTIR and Mass spectroscopy. Spectroscopic data were compared with spectra of standard diesel which revealed 98% similarity. The values of physicochemical properties of biodiesel were compared with the standard diesel showing 80-90% similarities. The resulting experimental data proved that the microalgae *Scenedesmus vacuolatus* X56104 oil could be a potential source of biodiesel and therefore this alga can be used as a source of renewable biofuels.

Highlights

- Isolation of oil-producing microalgae from local river Godawari and species identified by 18s r RNA gene sequencing method.
- Optimization of microalgae for biomass production under a controlled condition at 25°C (±1) and 1.2 to 0.2 Klux- lights irradiated for 16:8 hr light and dark cycle for 15 days.
- Microalgae biomass was analyzed with FTIR to detect the fatty acid content in novel strain
- Microalgal lipid was extracted using ether and ethanol solvent and evaluated by GCMS spectroscopy and revealed that it contains Palmitic acid (5.81%), stearic acid (1.86%), Oleic acid (65.83%), Linoleic acid (20.10%), Linolenic acid (4.66%), Arachidic acid (0.52%) and Eicosenoic acid (1.22%).
- The total fatty acids were subjected to lipase based trans esterification to obtained FAME and finally the bio diesel.

Keywords: Microalgae, Biodiesel, Transesterification, spectral characterization, fatty acids

Microalgae are photosynthetic, mostly aquatic microorganisms and are the abundant biomass of the aquatic ecosystem. Economically an alga has importance; algae can be a source of human and animal food (Raja *et al*. 2014). They are now considered as the third generation energy biomass because the algal cell is rich in lipids and cellulose, (Huber and Dale 2009). A constant rising worldwide demand for fossil fuel to transportation and power generation is increasing day by day. Petroleum-based fuels leads to causes global warming, M. Garilescu and Chesty (2005), Eman M Fakhary (2013), Vandana Pathak (2015). The uncertainty of the availability of mineral fuels considered to the important trigger for researcher to search for alternative source of energy, which can supplements with the fossil fuel, Harun, Rsingh, Mfordy (2010), Mata. T.M, Martin A.A. (2010), Mohd Hafiz Mat Yasin (2012), Gulab Chand Shah (2012). Microalgae are the alternative players for bioenergy source. Biofuel gaining a significant value as an attractive fuel. Microalgae can provide substantially more biodiesel than the existing oil seed crop with simultaneous less water and land demands (Ali Bahader 2014; Tomas Zavrel 2015), some algal species contain 30 to 60% lipids (dry weight basis), (Rrichmond 1990; Mahavir Yadav 2012; Rathinam Raja 2014). Currently, crude algal oil is extracted from algal biomass with the organic solvent like n-hexane or ether. Algae oil is a source of many kinds of essential fatty acids, they contain unsaturated and saturated fatty acids, and some algae are reported to contain omega fatty acid (Jagannathan N* Amutha. K and Anand N 2010) Algal oil can be trans esterified into biodiesel using several alkali based catalyst. These chemical conversion processes require a high temperature (60–80 ˚C) in the presence of alcohols (Li *et al*. 2011; Kiman Silas, Highina Bitrus Kwaji 2015). Alkali-based transesterification is infeasible because this reaction carried at the higher temperature and produce soap and another by-product; to overcome these problem lipases based transesterification can be used because this reaction carried at 30-40 ˚C, soap and by-product will not form (Włodzimierz Bednarsk 2010; Ghaly and Dave 2010.) The objective of this investigation is to determine the significance of novel algae *Scenedesmus sp YACCYB70,* as a prospective source of fatty acid and the potential future biodiesel using bacterial lipases. Fatty acid profile and biodiesel were analysed with FTIR and GCMS and the comparatives physiochemical studies was carried by using standard biodiesel and mineral diesel.

MATERIALS AND METHODS

Reagents

Peptone, sodium chloride, hexane, ether, methanol, BG 11 media, NaOH, HCl, KOH are purchased from (Hi-media) store. All the chemicals were high purity grade reagents.

Isolation and identification of algal culture

Algae sample was collected from the Godavari River at Gangakhed during the winter season in the year 2013. One ml water sample was added to BG11 growth medium and was enriched at 25^{o}C (+-1) under 1.2 to 0.2 Klux- lights irradiated for 16:8 hr light and dark cycle for 15 days. Pure culture was isolated using pour plate method as described by R.C, Dubey (2004). Microalgae culture was identified using 18S r RNA sequencing method as described by A. Ravishanker *et al.* (2012).

Cultivation of microalgae

The pure culture of isolated species grown in the one litre Erlenmeyer flask containing 500ml sterilized BG 11 25°C (+-1) under 1.2 to 0.2 Klux- lights irradiated for 16: 8 hr light and dark photoperiod with manual shaking twice per day for 16 days, to obtain maximum growth. Growth was collected by sedimentation using a centrifuge and allowed to dry it in the oven at 50°C to get powder. Typical IR band assignment from the literature revealed the peak (cm^{-1}) wavelength number range (cm^{-1}) between 2809-3012 conformed Lipid-Carbohydrates in algal biomass.

Biomass estimation

The known volume of culture was harvested by centrifuge at 5000rpm for 5 minutes and the pellet was washed at least twice with deionised water and freeze-dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in term of dry weigh gram per litre.

Lipids extraction, detection and analysis by FTIR, AOAC and GCMS

Algae biomass was analysed using FTIR. As described by Gulab Chand Shaha *et al.* (2012). Lipids were extracted by transferring 100 gm. of algae powder to soxhlet apparatus and 100ml of hexane was added to rupture cell wall of algae, after some time algae oil was collected from the collecting flask and it is considered as crude algal oil this was carried according to Suseela *et al.* (2011). The physico chemical parameter such as density, moisture, flash point, acid value and the calorific value was determined by the standard method of analysis such (AOAC, 1995) and GCMS (FAME) were prepared by following the procedure of Christie (1982).

Transesterification reaction optimization and production of biodiesel

The enzymatic transesterification reactions were carried as suggested by Liu Meng and Jaillani (2011). The reaction was optimized with respect to temperature and the molar ratio of methanol to algae oil. Standard methods were used and studies were carried out in triplicate. For this experiment 6ml algae oil, 3 ml methanol, and 10% volume of crude lipase from optimized culture were used and reaction conditions were maintained at 40 ˚C at 150 rpm for 5 hours. Biodiesel was analysed using FTIR.

Gas chromatography analysis

The fatty acid methyl ester (FAME) was prepared by following the procedure of Christie (1982) the sample was esterified in1% sulphuric acid in absolute methanol. The mixture was left overnight at 50 ˚C, water containing sodium chloride is added and the required esters were extracted from the hexane to separate the layers. The hexane layer was washed with water contained potassium bicarbonate, then dried over anhydrous sodium sulphate, the solvent evaporated and the composition of FAME was quantified. FAME was identified with Shimadzu gas-liquid chromatography equipped with FID packing column material HP5. The carrier gas was nitrogen and the short speed was 5mm/min. The fatty acid methyl ester was deduced by comparing their retention time for those of authentic standards.

Statistical Analysis

All analysis was carried out in triplicate, and the standard deviation was determined.

Physicochemical properties of algal biodiesel

Physicochemical properties of micro algal biodiesel such as Cetane number, viscosity, flash point, density were studied by standards ASTM and compared with the standard biodiesel.

RESULTS AND DISCUSSION

Isolation, Identification and cultivation of microalgae

Colony characteristic and morphological characteristics of Indian isolate have demonstrated its close similarity with the genus *Scendemus sp*. The individual cells are in the range 5-15μm in diameter shown in Fig. 1, the photography of algae was done with Labo made trinocular LX 400 D.G using 10X ocular and 100X objectives lens.

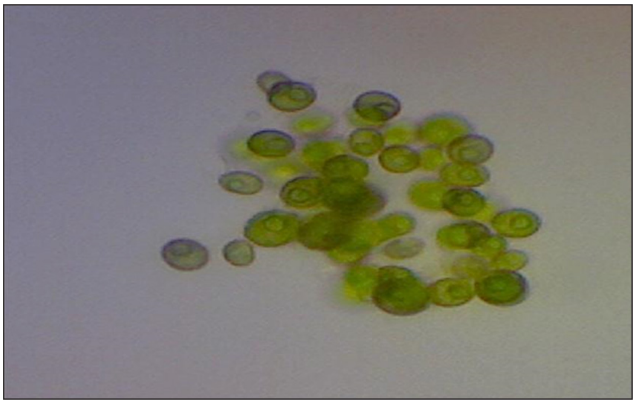

Fig. 1: Microscopic image of *Scenedesmus vacuolatus* X56104

Isolated algae were subjected to 18s-rRNA sequence analysis, this study reveals the taxonomic relation to order Chlorococcales and further, the sequence has shown more than 95% similarity with the reported 18s RNA sequence *Scenedesmus* sp. The Indian isolates are found to be *Scenedesmus vacuolatus* X56104. Growth studies of algal were determined after the 11^{th} day, the number of the cell reaches a maximum value of 7.0×10^{6} cell/ml, this value corresponded to 2.3mg/L dry weight and relatively high Oil contains 26.7% (Table 1) lipid was detected in algae biomass using FTIR Fig. 3.

Table 1: Cell density and oil content of *Scenedesmus spYACCYB70*, at stationary phase

Sl. No.	Cell number (10^{6} cell/ml)	Dry weight (mg/ml)	Crude oil (% DW)
1	7.0	2.5	26.7

(Data expressed as mean (+ -) SD (n=3)

The lipid spectra were characterised by two sets of strong vibration, the C-H at 2926 cm^{2} and the - C=O- mode of side chain from the ester carbonyl group at frequency cm^{-1} 1710-1666, this bands revealed that the biomass of *Scenedesmus species* contain lipids, our finding is in accordance with the *Scenedesmus obliquus* (Turpin) Kutzing 1833. Novel algae contain 26.70% of oil by its dry weight of the cell biomass.

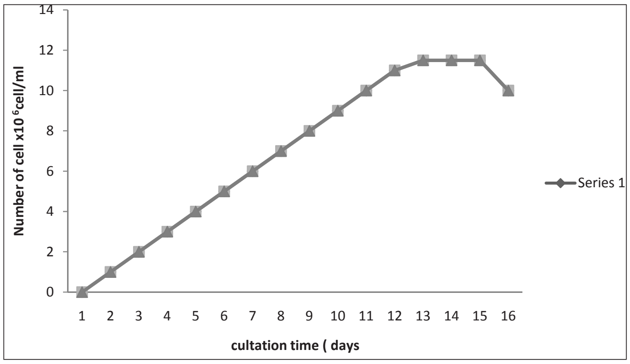

Fig. 2: The number of cells of *Scenedesmus vacuolatus* X56104 over 16-day cultivation

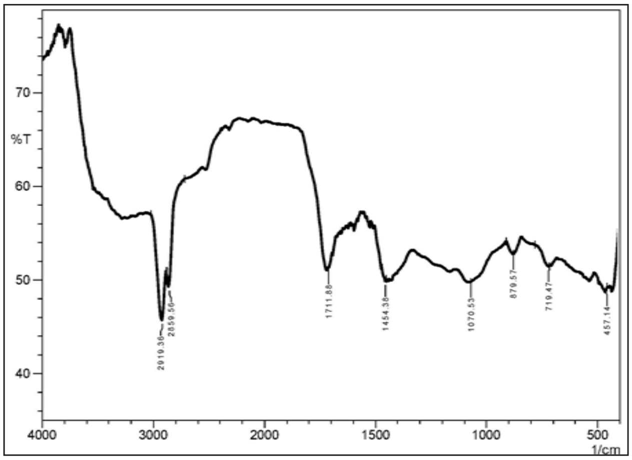

Fig. 3: FTIR chromatogram of *Scenedesmus vacuolatus* X56104 biomass

Fatty acid compositional profiles

A preliminary study of the fatty acid composition was carried out by GCMS analysis and the dominant were found to be in the following percent, palmitic acid, (5.81), steric acid (1.86%) oleic acid (65.83%) linoleic acid (20.10%) linolenic (4.66%) Arachidic (0.52%) shown in the peak 1, 2, 3, 4 (Fig. 4). The extracted microalgal oil consist of major fatty acid which is similar to the *Chaetoceros sp*. fatty acid composition reported by Shaleesha *et al.* and were recorded as palmitic acid (2.9%), linoleic acid (12.07%), Palmitic acid (5.97%) oleic acid 0.7% This novel algae contained major fatty acid as oleic acid (65.83%).

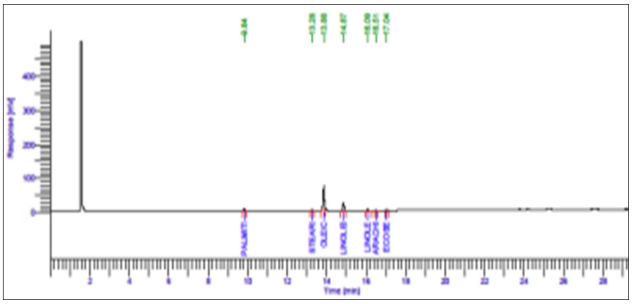

Fig. 4: Chromatogram of *Scenedesmus vacuolatus* X56104 oil

Enzyme catalysed transesterification

The result of crude enzyme based transesterification of algae oil showed the production of biodiesel, the FTIR study revealed the production of biodiesel. In Fig. 3. The peak 7 at the frequency, 1750-1735 cm^{-1}, C=O stretch showed the esters groups. The similar peak found in the reference biodiesel. Fig. 5 and 6. Further GCMS analysis also revealed the peaks of methyl ester Fig. 7, which confirmed methyl ester in transesterification product. Liu Meng and Jailani (2011) conducted a similar study using palm oil with crude enzyme and confirmed by GCMS spectral studies, our result is in accordance with their findings.

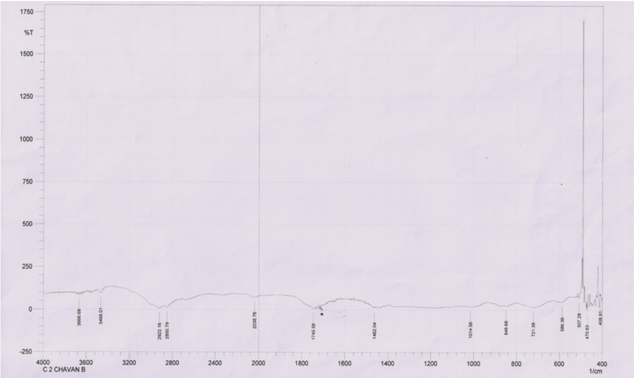

Fig. 5: FTIR spectrogram of standard biodiesel

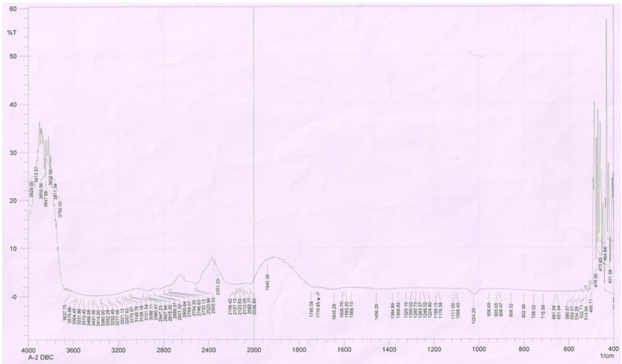

Fig. 6: FTIR spectrogram of sample biodiesel

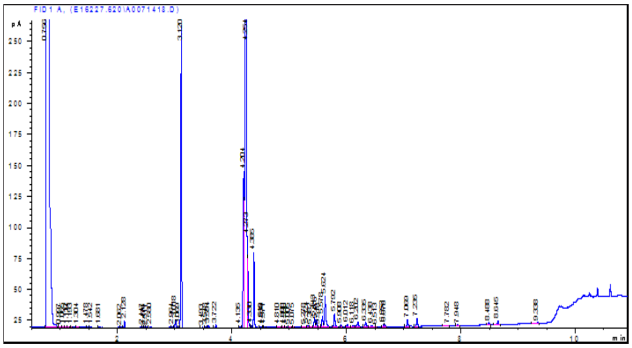

Fig. 7: GCMS Chromatogram of biodiesel from *Scenedesmus vacuolatus* X56104 oil

Comparative study of Physiochemical properties of algae oil, Algal biodiesel and mineral diesel

Table 2 summarise the physicochemical properties of algae biodiesel and mineral diesel. The result showed that the properties of biodiesel are 80-90% similar to the mineral diesel.

Table 2: Comparisons between algal biodiesel and mineral biodiesel

Sl. No.	Physiochemical parameter	Algal biodiesel	Petroleum based diesel
1	Density (g/cm^3)	0.84	0.829
2	Viscosity Mm^2/s	4.2	4.24
3	Flash point $^\circ$C	120	70
4	Cetane number	65	71.6

Microalgae production is a gaining importance of its application for bioenergy and feed stock in the world. Number of researchers documented algal media for its cultivation. The potential for microalgae as a sustainable source of bioenergy is very promising because of their higher growth rates and the capability to accumulate large amount of lipids (Pascal Schagermann *et al.* (2012) The growth of *Dunaliella Salina* algae in growth medium was reaching up 7.1×10^6 cell/ml after 12 days reported by E.M Fakhery *et al.* (2013), Growth rate of *Chaetoceros* species reached to a maximum of up to 3.5×10^6 cell at the 8th day. Ananadhi P. *et al.* (2012) the productivity of biomass of *Scenedesmus diamorphus* was reported on BG11 media was 22.742 cell gm/L reported by Al-shatril *et al.* (2014). In our study, the growth of novel scenedesmus spYACCYB70 showed the maximum 7.0×106 cell/ml on the 11th day this value corresponds to 2.3mg/L dry weight, these findings are matching with Paschal Schagermann (2012).

Algae stores a large number of lipids in their cell as compared with the oil seed crop (Ali Bahadar 2014), algal oil can be a source of saturated fatty acid and unsaturated fatty acids. Most of the algae contain oleic acid, linoleic acid, linoleic acid etc., these acids are the raw material for biodiesel production (Chisti Y., 2007). The fatty acid composition of *Chaetoceros* sp., reported by Ananadhi *et al.* (2012) showed the presence of oleic acid, pentadecanoic acid, methyl palmitate, palmitic acids as a major fatty acid, the similar finding was also reported by Eamn M Fakhry 2013). In the light of above data when we compare fatty acid of *Scenedesmus* sp YACCYB70, we found the approximately similar proportion of fatty acid such as palmitic acid, (5.81), steric acid (1.86%), oleic acid (65.83%), linoleic acid (20.10%), linoleic (4.66%), Arachidic (0.52%. The present data is in a close agreement with other data available in the in the research studies on *Scenedesmus sp,* and *Chlorella species* (Shaleesha A Stanley 2010). The reporting novel strain has oleic acid as its major fatty acid. Unsaturated and saturated fatty acids were also present in the novel algae.

Researchers have investigated lipase based transesterification and supercritical methanolysis (Nguyen 2011) lipase based transesterification is more suitable as compared with alkali and acid-based transesterification because lipase based transesterification does not form soap and glycerol, this reaction can be carried out at 30 to 45°C *Cesarini et al.* (2014) Lipase from microorganism *Mucor miehei*, *Rhizopus oryzae*, *Candida antarctica* and *Pseudomonas cepcia* are the most commonly used enzymes. Hanifa Taher *et al.* (2011)and some researchers showed that lipase activities are deactivated by methanol and only traces of biodiesel ware produced. A. Nag (2008), R.D.A. Bigor (2009), H. Noureddini, X. Gao *et al.* (2005) but Soumnou and Borscher (2003) reported that some *Pseudomonas* strain develop resistance to methanol,

Palm oil was successfully trans esterified into biodiesel using crude Enzyme by Liu Meng and Jailani Salihon (2011) by *Pseudomonas sp.* Enzymatic Biodiesel Production from Microalgae was carried out by Xiong *et al.* (2008) and produced biodiesel with 98% conversion to *C. protothecoides* lipase with 30wt% of *Candida* sp. Lipase. A similar conversion was obtained by Li *et al*. (2007). In our study biodiesel was produced form novel algae oil using menthol resistant *Aneurinibacillus aneurinilyticus strain LP-II* bacterial crude lipase for transesterification reaction, the maximum conversion has been achieved at 40 ˚C and considered optimum temperature for product formation. Our data are in close agreement with those finding of other researchers (Meng L. Salihon 2011).

Fuel properties of oil like viscosity, Cetane number, density, flash point. The viscosity of algae oil is 5.2 mm^2, viscosity increase with increasing length of fatty acid chain and degree of saturation. The increasing in viscosity affect the fluidity of fuel that produces several failures of the engines beside it may lead to less accurate operation of the fuel injector, (Balat 2008; Hoekman 2012).

To resolve this problem bacterial lipase transesterification was designed to reduced viscosity, in our study after transesterification viscosity oil was reduced from 5.3 mm^2 to 4.2mm^{-2}. This range corresponds to the range provided by ASTM (minimum 1.9 and maximum 6.0 mm^{-1}/s). our finding are in close range with Mohd Hafizil Mat Yashin (2012) and De-gange Li (2004).

Flash point: The flash point is the lowest temperature at which a fuel will ignite when exposed to an ignition source. The flash point of biodiesel is in the range of 130 -140 (Ali Bahadur 2014) the Flash point of B100 biodiesel is 180 reported by Mohd Hafizil Mat Yashin (2012) The flash point of algae oil was 210 and the after transesterification it is reduced to 130 hence novel algal biodiesel can be verified.

Cetane number: The cetane number is a commonly used indicator for the determination of diesel fuel quality, especially the ignition quality. It measures the readiness of the fuel to auto-ignite when injected into the engine M. Balat and H. Balat (2010), The shorter the ignition delay time, the higher the CN, and *vice-versa* Gopinath *et al.* (2009) Cetane of *Scenedesmus obliquus* 65.5, *Chlorella vulgaris* 63.8, *Botryococcus terribilis* 61.7 was reported by Muhammad Aminul Islam, Marie Magnusson (2013). Our algal oil showed cetane numbers 54, after transesterification the number increase to 65, this data is in closed range of the previous studies (Leon Schumachar 1995; S.J. Ojolo 2012; Muhammad Aminul Islam, Marie Magnusson (2013)).

Density: Density is an important biodiesel parameter, with impact on fuel quality. The density of algal biodiesel 0.801 kg/L from Freshwater Algae reported by Krishnan Vijayaraghavan and K. Hemanathan (2009) The density of biodiesel from *Dunaliella salina* 9.43 g/cm^3 was reported by Eman M. Fakhry, Dahlia M. El Maghraby (2013).We are reporting the density 0.84 g/cm^3 and it was reduced to a significant extent when compared with the density of algal oil (0.92g/cm^3) Comparing the result gotten with that of ASTM D standard for biodiesel and ASTM D standard for diesel fuel, it falls within the acceptable range (0.86.-0.90g/cm^3). The density of biodiesel depends on the raw material used for the production of biodiesel. The present study assumed that the density depends on the raw material used for biodiesel production and FAME profile. The density of fatty acid is inversely proportional to their number of a carbon atom, (Gouw and Vlugter 1964). The increase in the number of carbon may cause the decrease in density and also the density can be estimated form parameter related to the chemical composition of FAME in particularly MW and the number of double bonds (Ramirez-verduzco *et al.* 2005).

CONCLUSION

The novel *Scenedesmus* sp YACCYB70 is more promising feedstocks to their widespread availability and more oil yield. Based on the fatty acid profile this alga was evaluated as an oil producer. The alga was cultivated for biomass production on batch-wise under optimal laboratory condition. The cell density of 7.0X 10^6 cell/ml was recorded. This alga produces 26.70 % of oil equivalents of dry weight. Fatty acids profile were recorded in the following percentage as, palmitic acid,(5.81), steric acid (1.86%) oleic acid (65.83%) linoleic acid (20.10%) linolenic (4.66%) Arachidic (0.52%) Ecosenic (1.22%). The properties of methyl ester (biodiesel) described by its of flash point, viscosity, density, cetane number showed about 90% similarity with mineral diesel from this we conclude that *Scenedesmus* sp YACCYB70 is a suitable for biodiesel production and lipase of *Aneurinibacillus aneurinilyticus strain LP-II* can be used for transesterification, however cultivation, optimization, and exploring the modification before commercial production. Novel bacterial Enzyme purification, characterization need to be improved

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REFERENCES

Abubakar, L.U, Mutie, A.M. and Kenya, S. 2012. *Journal of Applied Phytotechnology in Environmental Sanitation*, **1**(4): 147-153.

Ali Bahadar, 2014. *Production of biodiesel using Hetrogeneouscatylyst and supercritical fluid extraction.* PhD Thesis 2014, school of chemical and material engineering (SCME) National University of science & technology (NUST).

Ananadhi Padmanabhan M.R, Shaleesha A. Stanley 2012. *Microalgae as an oil producer for biofuel application, of recent science* **1**(3): 57-62.

Barnwal, B.K. and Sharma, M.P. 2005. Prospects of biodiesel production from vegetable oils from India. *Renew Sustain Energy Rev.*, **9**: 363-378.

Becker E.W. 1994. Microalgae. Biotechnology and Microbiology, Cambridge University Press, Cambridge UK.

Benemann, J. and Tillett, D. 1987. E*ffects of Fluctuating Environments on the Selection of High Yielding Microalgae.* Solar Energy Research Institute Report, February 27, 1987.

Chavan, D.B. and Khobragade, C.N. 2017. Fungal Fermentation of Novel *Scenedesmus sp* YACCYB70 Biomass for Bioethanol Production, *Trends in Biosciences*, **10**(13).

Chisti, Y. 2007. Biodiesel from microalgae. *Science Direct, Biotechnology Advances* **25**: 294-306.

Collyer, D.M. and Fogg, G.E. 1955. Studies of fat accumulation by algae. *J. Exp. Bot.*, **6**: 256–275.

Dayananda, C., Sarada, R., Usha Rani, M., Shamal, T.R. and Ravishankar, G.A., 2007. Autotrophic cultivation of *Botryococcus braunii* for the production of hydrocarbon exosaccharides in various media. *Biomass Bioenergy*, **31**: 87-93.

De-gang, Huang Zhen 2005. physicochemical properties of ethanol-diesel blend fuel and its effect on performance and emission of diesel engines, Renewable energy,www.sciencedirect.com. doi:10.1016/j.renene.2004.07.010

Dildar Ahmed and Shahid Rehman Khan *et al.* 2012. Physicochemical, thin layer and gas-liquid chromatographic analysis of engrafted desi mango flower oil and mineral estimation in its flowers. *African Journal of Biotechnology*, **11**(41): 9844-9848.

Eman, M. Fakhry*, Dahlia M. El Maghraby 2013. Fatty Acids Composition and Biodiesel Characterization of *Dunaliella salina. Journal of Water Resource and Protection*, **5**: 894-899.

Fan, J., Cui, Y., Wan, M., Wang, W. and Li, Y. 2014. Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. *Biotechnol Biofuels.* **7**(1): 17.

Felizardo P., Correia M.J.N. *et al.* 2006. Production of biodiesel from waste frying oil. *Waste Management*, **26**(5): 487-494.

Gopinath, A., Puhan, S. and Nagarajan, G. 2009. Relating the cetane number of biodiesel fuels to their fatty acid composition: A critical study. *Proc. Inst. Mech. Eng. Part D J. Automob. Eng.*, **223**: 565–583.

Greenspan, P., Mayer, E.P. and Fowler, S.D. 1985. Nile red: a selective fluorescent stain for intracellular lipid droplets. *J. Cell Biol.*, **100**: 965–973.

Gulab Chand Shah and Mahavir Yadav 2012. Analysis and Characterization of Algal Oil using Different Chromatographic Techniques for the Higher Production of Biodiesel from Scenedesmus Diamorphus Algal species. Open Access Scientific Report dx.,doi.org/10.4172/scientificreports.350.

Kiman Silas and Highina Bitrus Kwaji 2015. Lipid extraction and transesterification techniques of microalgae –A Review, *International Journal of Recent Research in Physics and Chemical Sciences (IJRRPCS)*, **2**(1): 26-37.

Krishnan Vijayaraghavan and Hemanathan, K. 2009. Biodiesel Production from Freshwater Algae. *Energy Fuels*, **23**(11): 5448–5453.

Kulkarni, M.G. and Dalai, A. K. 2006. "Waste cooking oil—An Economical Source for Biodiesel: A Review," *Industrial & Engineering Chemistry Research*, **45**(9): 2901-2913.

Lam, M.K. and Lee, K.T. 2012. Microalgae biofuels: A critical review of issues, Problems and the way forward. *Biotechnol. Adv.*, **30**: 673-690.

Lee, Y.K. 1997. Commercial production of microalgae in the Asia Pacific rim. *J. Appl. Phycol.*, **9**: 4.3-411.

Li, X., Xu, H. and Wu, Q. 2007. "Large-scale biodiesel production from microalga Chlorella protothecoides throughheterotrophic cultivation in bioreactors," *Biotechnology and Bioengineering*, **98**(4): 764–771.

Liu Meng and Jailani Salihon 2011. conversion of palm oil to methyl ester using a crude enzyme. *J. Biotechnol, Biomaterials*, **1**: 110.

Lundquist, T.J., Woertz, I.C., Quinn, N.W.T. and Benemann, J.R. 2010. A Realistic Technology and Engineering Assessment of Algae Biofuel Production. Energy Biosciences Institute, University of California, Berkeley, California, USA.

Mamoru, I.S.O., Baoxue, C. and Masashi, E. 2001. Production of biodiesel fuel from triglycerides and alcohol using Immobilized lipase, *J. Mol. Catal.*, **16**: 53-58.

Mbatia, B. and Aldlercreutz, D. *et al.* 2010. Enzymatic oil extraction and positional analysis of ω-3 fatty acids in Nile perch and Salmon head. *Process Biochemistry*, **45**: 815-819.

Metzger, P. and Largeau, C. 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.*, **66**: 486-496.

Mohamed, M.S. and Uwe, B. 2003. Improvement in lipase-catalysed the synthesis of fatty acid methyl esters from sunflower oil, *Enzyme Microb. Technol.*, **33**: 97-103.

Mohd Hafizil, Mat Yasin and Rizalman Mamat 2012. Fuel physical characteristics of biodiesel Blend Fuel with Alcohol as additives, Malaysian Technical University conference on Engineering and Technology MUCET.

Muhammad Aminul Islam and Marie Magnusson. 2013. *Microalgal Species Selection for Biodiesel Production Based on Fuel Properties Derived from Fatty Acid Profiles Energies*, **6**: 5676-5702.

Mulberry, W., Konrad, S. and Buyer, J. 2008. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates". *Journal of Applied Phycology*, 9314-9318.

Noureddini, H., Gao, X. and Philkana, R. S. 2004. "Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil," *Bioresource Technology*, **96**(7): 769–777.

Pascal Schlagermann and Gerold Gottlicher *et al.* 2012. The composition of Algae oil and its potential as Biofuel, Hindawi Publishing Corporation, *Journal of Combustion, volume* 2012, Article ID 285185,doi:10.1155/2012/285185.P

Raja, R., Shanmugam, H., Ganesan, V. and Carvalho, I.S. 2014. Biomass from Microalgae: An Overview. *Oceanography*, **2**: 118.

Sandip Kumar Haldar and Ahindra Nag, 2008. utilization of three Non-Edible vegetable oil for the production of biodiesel catalysed by the enzyme. *The Open Chemical-engineering Journal*, **2**: 79-83.

Sudip Shah, Prakash and Lokesh 2015. Evaluation of biodiesel production from microalgae collected from freshwater habitat. *Int. J. Fund. Appl. Sci.*, **4**, 60 No. 3: 56.

Xin, L., Hong-Ying, H. and Jia, Y. 2010. Lipids accumulation and nutrient removal properties of a newly isolated freshwater microalga *Scenedesmus* sp. LX1, growing in secondary effluent. *New Biotechnol*., **27**: 59-63.

Xiong, W., Li, X., Xiang, J and Wu, Q. 2008. "High-density fermentation of microalga Chlorella protothecoides in a bioreactor for microbial-diesel production," *Applied Microbiology and Biotechnology*, **78**(1): 29–36.

Yen, H.W. and Brune, D.E. 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource. Technol.*, **98**: 130-134.

Yong, W. and Jieyu Nie 2010. Production of Biodiesel from Waste Cooking Oil via a Two-Step Catalyzed Process and Molecular Distillation. *Energy Fuels*, **24**(3): 2104–2108.

Zamalloa, C., Vulsteke, E., Albrecht, J. and Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalga, *Bioresour. Technol.*, **102**: 1149-1158.