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A COMPARATIVE STUDY ON BIODIESEL PRODUCTION FROM BIOMASS OF THREE DIFFERENT SPECIES OF ALGAE

FAROOQ AHMAD[1*], TAYYABA MUNAWAR[2], SAHAR AZHAR[3], SEHRISH IMRAN[2] AND NASEEM HIJAZI[3]

[1]*GC University, Sustainable Development Study Centre, Lahore, Pakistan*
[2]*PMAS Arid Agriculture University, Rawalpindi, Pakistan*
[3]*Superior College University Campus Mandi Bahauddin, Pakistan*
[*]*Corresponding author's email: fagondal82@yahoo.com*

Abstract

This study was aimed to compare the efficiency of biodiesel production by *Chlorella emersonii, Cladophora fracta* and *Spirogyra.* Algae were grown in artificial media consisting of wastewater for further growth and preservation. Harvested biomass of algae was placed in an oven at 60°C for drying and powdered to size of 1.0mm by grinding mill. Extraction of oils rom biomass of algae were carried out by hexane solvent in a Soxhlet extractor for 18 h and analyzed for various parameters such as fatty acid composition, iodine value, water contents, saponification value iodine value and acid value. Growth rate, lipid content, protein and carbohydrate contents of above mentioned species were measured in dried biomass o algae. Sodium methoxide prepared rom methanol and sodium metal was used as a catalyst or transesterification o extracted oil to methyl ester. Biodiesel yield was higher in *Chlorella emersonii* than *Cladophora fracta* and *Spirogyra*. Analysis of biodiesels produced through transesterification was carried out for various properties such as iodine values (49, 53 & 47), flash point (155, 156 & 160°C), Specific gravity (0.912, 0.914 & 0.91), kinematic viscosity (4.7, 5.0 & 4.9), water content (17, 23 & 15 mg/kg), sulphur (0.01, 0.012 & 0.013 wt%), sulfated ash (0.004, 0.003 & 0.007 mass%), carbon residue (0.01, 0.02 & 0.01 mass%) cetain number (47, 49 & 51 min) and acid number (0.46, 0.5 & 0.49 mg.KOH/g) for *Spirogyra, Cladophora fracta* and *Chlorella emersonii* respectively. Additionally biodiesel properties of all samples were in accordance with ASTM standards.

Key words: Spirogyra, Cladophora, Chlorella, Biodiesel, Oil extraction, Algae.

Introduction

The rate of worldwide population is getting high so the demands for energy, food, water and other natural resources are also rising. But this increased population is leading toward the decline of fossil fuels due to limited fossil deposits. The main natural sources for energy needs are coal, methane gas and nuclear energy. The use of petroleum for energy is major cause of environmental pollution, as it raises greenhouse gases and other pollutants like Carbon monoxide (CO), Nitrous Oxide (NO) and Volatile Organic Compounds (VOCs) in environment, which is dangerous for human in various perspectives (Rahpeyma & Raheb, 2019). To cope with the energy demands, scientists are approaching to biofuels. Biofuels are produced from biomass resources (Saladini *et al*., 2016).

Biofuel has been made from three generations. First generation involves the production of biofuel by edible sources like soybean, sugarcane, sunflower and rapeseed. But this method raised the shortage of food affecting the economic values (Yuvarani *et al*., 2017). Hence biofuel production was done through second generation which involves the biofuel production from non-edible resources like rice bran, jatropha and rubber plant etc. This is advantageous to first generation in one aspect but it is not cost friendly method because it requires several steps for biofuel production(Vinoth *et al*., 2012).Third generation is also there having advantage on rest of two because it does not require much land for cultivation and also is cost friendly. Third generation involves the formation of biofuel by microalgae or macroalgae (Chen *et al*., 2015). Microalgae are single celled phytoplanktons (Lemões *et al*., 2016) and are rich in proteins, 30-40% lipids, polysaccharides content and can be converted to bio oil by pyrolysis (Gao *et al*., 2017). Microalgae also help in removing toxic substances from waste water acting as bioremediation (Khan *et al*., 2017). Microalgae are attracting scientists due to their great potential in the production of methyl ester (biodiesel). The synthesis of methyl ester from microalgae is an emerging technology considered best for synthesis of methyl ester because of their ability to grow rapidly on less cropland with no care. Biodiesel is the most studied biofuel used as alternative to natural diesel due to less CO and pollutants emission. It is good lubricant, sulphur free and have no toxic compounds; hence is preferred to fossil diesel (Aransiola *et al*., 2014). Biodiesel produced from microalgae is tested by several vehicles and is preferred over fossil diesel. Several companies like Euglena, Sapphire Energy, and Solazyme used microalgal biodiesel for running cars and jets with its different compositions and marked it good for usage (de Jesus *et al*., 2020). It was proved in 2008 that biodiesel derived from microalgae has highest potential for fulfilling the energy needs at global level (Chisti, 2008).

Oil produced from microalgae is 30 times more in comparison to other plants (Fuentes-Grünewald *et al*., 2009). Biodiesel can be produced from microalgae by preparing dry algal powder of microalgae, lipid extraction and mono alcholic transesterification of oil to biodiesel. Dry powder of microalgae was used to extract lipids because water content in biomass can decrease the efficiency (Yang *et al*., 2014). Extraction can be done by using both polar and nonpolar solvents. Previous literature showed that mostly hexane has been used for biodiesel production (Mubarak *et al*., 2015). This research was aimed to find out the efficiency for biodiesel production among *Chlorella emersonii*, *Cladophora fracta* and *Spirogyra.*

Material and Methods

The moisture content was determination by drying the constant weight of 3g to 5g sample at 378K (Demirbaş, 1999). Then ashing was done for 2 hours at 1025 K (Demirbaş, 2001). Protein and lipid content was analyzed by blocked digestion and solvent extraction method, respectively (Boccard *et al*., 1981). Algal sample was extracted with hexane for 18 hours in a Soxhlet extractor for obtaining oils. The method of (Kusdiana & Saka, 2001; Demirbas, 2002) was followed for transesterification process. It was done by using supercritical methanol in 100-mL cylinder. A thin layer chromatography was prepared by coating glass slide with 0.25m polyethanol succinate and used for fractionation of fatty acids into saturated, monounsaturated, polyunsaturated, and free forms.

Algal sampling and identification: Samples of selected algal species were collected from laboratory of fisheries department where these species were grown as fish feed. Pure culture were obtained and re-identified in phycology laboratory of GC University, Lahore following methods reported by Zarina *et al*., (2005a, b).

Experimental design: Artificial ponds were designed of various dimensions having different water holding capacity. Three types of pond (P1, P2 & P3) were designed for lab scale experiments with varying width, length and height of 0.15 x 0.15 x 0.3m for P1, 0.3 x 0.3 x 0.15m for P2 and 0.9 x 0.15 x 0.45 m for P3 having water carrying capacity of 6.75 L, 13.5 L and 60.75 L respectively. A large pond was selected in the field for pilot scale study or growth of algal culture. Dimensions of that pilot scale pond were 8 x 2 x 1 m (P4) and it was designed to carry sixteen thousand liter in it (Hammouda *et al*., 1995).

Inoculation of algal species: Algal species were inoculated in different culture media including wastewater collected from municipal drain, solution prepared from nutrient agar and artificially synthesized media. Rate of growth of selected algal species were determined after every 24 hours. Fresh biomass were harvested from each pond and kept in an oven for 48 hours at 60°C. Growth rate was measured as increase in biomass per liter in a day.

Biodiesel Processing

Oil extraction from algal biomass: Solvent extraction method was used to extract oil from dried algal biomass by chemical method. Hexane was used as solvent to extract oil following procedure as under.

Methods of extraction of oil: In methods of oil extraction two methods are commonly used in which mechanical method includes ultrasonic assisted extraction and expeller/expression press while chemical method includes supercritical fluid extraction, Soxhlet extraction and hexane solvent method. In this research work hexane solvent extraction method was used.

Solvent extraction method: Depending on algal species 40-60g of algal biomass was used in 300ml o n-Hexane solvent for extraction procedure. Algal oil content was measured by extracting oil using Soxhlet extractor with retention time of 4 hours (UNE-EN 734-1, 2006). Oil was extracted in round bottom flask with 0.5 liter capacity and distillation procedure was used to separate component from extracted solution. Recovered solvent was used again or next batch of extraction in order to minimize operational cost.

Characterization of oil: Fatty acid composition of algal oils extracted from various species of algae was analyzed by Gas chromatography and mass spectrometry (GC-MS, Shimadzu GC-14-A). Oil was characterized for various parameters such as free fatty acids; saponification value, acid value and iodine value were measured following the protocols reported by Raie (2008).

Sodium methoxide preparation: A catalyst was prepared by reacting with pure ethanol and sodium hydroxide. Sodium hydroxide and methanol were taken with 1%wt and 1:6.1 with extracted oil respectively (Charoenchaitrakool & Thienmethangkoon, 2011). Both of these reagents were mixed and stirred for 30 minutes to prepare sodium methoxide.

Trans esterification procedure: Methyl ester i.e. biodiesel was prepared from algal oil using sodium methoxide as a catalyst. Algal biomass was also directly transesterified to biodiesel in order to avoid extraction step. In this procedure 100 grams of oven dried biomass of algae was added in 300ml of solvent i.e., n-hexane, then it was placed in a bioreactor having thermostat. The resultant solution was thoroughly mixed and heated at 62^{o}C before addition of appropriate catalyst. After mixing catalyst i.e., sodium methoxide, reaction was sustained at same temperature for 4 hours by constantly stirring at 110rpm. On completion of reaction the product was cooled to room temperature, solid and liquid phase of reaction product were separated with the help of separating funnel. Glycerin accumulated at bottom was separated from upper biodiesel layer. Liquid phase was rinsed with water to free it from remains of catalyst and solvent "(Karaosmanoglu *et al*., 1996; Lang *et al*., 2001)."Distillation process was used to remove remaining solvent to obtain crude biodiesel. Solvent separated by this method was reused in next batch of experiment. Efficiency of both methods was similar in all respects.

Biodiesel identification: Thin layer chromatography was used to identify and check purity of final product (Methyl ester) of transesterification process.

Thin layer chromatography: Water and Silica gel were used to prepare thin layer chromatogram of 20cm length and width of 0.25cm thickness which is used in thin layer chromatography for analysis of biodiesel. After one hour, heating and drying, it was placed in an oven for further process at 105^{o}C. Two solvents such as hexane and diethyle ether were used in ratio of 80:20 to dissolve biodiesel. Ultra violet light of 366nm wavelength were

used to visualize color bands of purple-yellow coloration with the help of nondestructive locating agent 2,7 dichloroflourescein.

Results and Discussions

Growth rate measurement: Culture media selected for algal growth includes wastewater collected from municipal drain, solution prepared from nutrient agar and artificially synthesized media which were used to grow *Chlorella emersonii, Cladophora fracta* and *Spirogyra* and it was found that synthetic media was proved to be an excellent media for growth as all species showed maximum growth in this media. Reason for higher growth in artificial media was due to presence of all nutrients necessary for growth of these species Rate of growth of *Cladophora fracta* was 1.45 $g.L^{-1}.day^{-1}$ and 0.75 $g.L^{-1}.day^{-1}$ in synthetic medium and agar solution respectively. *Chlorella emersonii* and *Spirogyra* showed less growth in these media but in wastewater growth rate of Chlorella *emersonii* was comparatively higher (Table 1). Growth rate of all species decreased with the passage of time from 1 to 6 days with decrease in nutrients in all media if fresh media was not added. Similar findings were reported by Ruiz-Ruiz-Marin *et al*., (2010) that growth rate of microalgae was higher in initial days but decreased with time when grown in batch culture conditions.

Algal biomass analysis: Biomass harvested from algae was dried and its carbohydrate, protein and lipid contents were analyzed. It was found that lipid content of *Chlorella emersonii* was higher (38%) than *Cladophora fracta* (18%) and *Spirogyra* (27%). Proteins were calculated to be higher in *Cladophora fracta* (49%) while carbohydrates (37%) were more in *Spirogyra* (Table 2). *Chlorella protothecoides* was grown in bioreactors of different sizes ranging from few liters to 11000 liters and its dried biomass was analyzed for lipid content which was obtained between 44.3-48.7% (Li *et al*., 2007).

Characterization of extracted oil: Oil after extraction from selected species of algae was analysed for various parameters such as species acid number, saponification value, water contents, iodine value, and free fatty acids. Oil extracted from all species showed similar characters except minor differences (Table 3).Water content in oil of all species was less than 1% making it feasible for its conversion to methyl ester through transesterification. This process was negatively affected if water content was increased above 10% of oil quantity. Water content of extracted oil was major limiting factor it should not be more than 10% but in current study it was maintained at less than 1% to make transesterification feasible. Value of saponification or saponification value and acid value given in Table 3 was more or less similar to the values reported by Li *et al*., (2007) i. e., 189.3 mg KOH g^{-1} and 8.97 mg KOH g^{-1} respectively.

Algal oil was also analysed for saturated and unsaturated fatty acids (14:0, 16:0, 16:1, 16:2, 16:3, 16:4, 18:0, 18:1, 18:2, 18:3, 18:4, 20:0, 20:1, 20:2, 20:3 and 20:4) and it was observed that unsaturated fatty acid content of *Chlorella emersonii* (78.75%) was higher than those of *Cladophora fracta* (76.68%) and *Spirogyra* (77.38%) (Table 4). Oil composition of microalgae was analysed with Gas Chromatography by Chinnasamy *et al*. (2010) for fatty acid profile i.e. 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:2, 20:3, 20:4, 20:5, 22:5 and 22:6. The results of this study were similar to study conducted by Gouveia and Oliveira (2009). They also reported results of unsaturated fatty acids (50-65%) in their experiment on various species of algae (*Chlorella vulgaris, Scenedesmus maxima, Nannochloropsis oleabundans, Scenedesmus obliquus* and *Dunaliella tertiolecta*) which were in conformity with the current study.

Table 1. Measuring growth rate of all three species of algae in selected media.

Rate of growth of algae	Culture media		
	Wastewater	Agar solution	Synthetic medium
Chlorella emersonii ($g.L^{-1}.day^{-1}$)	0.55 ± 0.2	0.65 ± 0.9	1.3 ± 0.5
Cladophora fracta ($g.L^{-1}.day^{-1}$)	0.75 ± 0.15	0.58 ± 0.34	1.45 ± 0.8
Spirogyra ($g.L^{-1}.day^{-1}$)	0.6 ± 0.23	0.47 ± 0.09	1.39 ± 0.75

Table 2. Analysis of algal biomass for protein, carbohydrate and lipid contents.

Sample species	Protein	Carbohydrates	Lipids	Others
Chlorella emersonii	44 ± 3	17 ± 1.5	38 ± 1.9	4 ± 0.4
Cladophora fracta	49 ± 2.1	22 ± 1.8	18 ± 0.9	11 ± 0.7
Spirogyra	28 ± 1.6	37 ± 3.4	27 ± 2.8	8 ± 1.3

Table 3. Properties of oils obtained from selected species of algae.

Analytical parameters	*Chlorella emersonii*	*Cladophora fracta*	*Spirogyra*
Water content (%)	0.07 ± 0.01	0.08 ± 0.04	0.06 ± 0.012
Iodine value (mg/g)	64 ± 2	74 ± 1.5	70 ± 2.3
Saponification value (mg/g)	171.12 ± 3.4	167.9 ± 3.5	181.8 ± 1.2
Free fatty acid (%)	2.8 ± 0.3	3.4 ± 0.6	3.2 ± 0.2
Acid number (mg KOH/g)	32.46 ± 2.1	35.1 ± 1.4	36.5 ± 2.3

Table 4. Fatty acids content of oil extracted from *Chlorella emersonii*, *Cladophora fracta* and *Spirogyra.*

Composition of fatty acid	Oil profile of *Chlorella emersonii* (%)	Oil profile of *Cladophora fracta* (%)	Oil profile of *Spirogyra* (%)
C14:0	2.96	0.34	2.0
C16:0	17.9	21.79	18.93
C16:1	10.53	12.18	14.95
C16:2	0.04	n.d.	0.02
C16:3	6.79	5.29	4.4
C16:4	7.62	10.01	9.0
C18:0	0.63	1.19	2.2
C18:1	18.08	14.48	13.98
C18:2	11.15	10.2	14.9
C18:3	22.17	22.97	19.17
C18:4	n.d.	0.08	0.03
C20:0	0.12	n.d.	0.16
C20:1	0.91	0.69	0.58
C20:2	0.79	0.44	0.68
C20:3	n.d.	0.04	n.d.
C20:4	0.01	n.d.	n.d.
Saturated	21.25	23.32	22.62
Unsaturated	78.75	76.68	77.38

n.d stands for "not detected"

Table 5. Percent yield of product and residual material obtained after transesterification process.

Products parameters	*Chlorella emersonii*	*Cladophora fracta*	*Spirogyra*
Total biomass used (g)	100	100	100
Residual biomass (g)	60 ± 2	84 ± 3.5	74 ± 2.6
Quantity of oil extracted (ml)	42 ± 0.9	25 ± 0.6	31 ± 1.1
Yield of biodiesel (%)	95.1	91.6	91.9
Glycerin and other byproducts (%)	4.9	8.4	8.1

Table 6. Analytical efficiency of biodiesels and its comparison with international standards.

Properties	Units	*Chlorella emersonii's* Biodiesel	*Cladophora fracta's* Biodiesel	*Spirogyra's* Biodiesel	ASTM D-6751~02 Standards
Kinematic viscosity at 40°C	mm^2/s	4.9	5.0	4.7	1.9–6.0
Flash point	°C	160	156	155	>130
Specific gravity at 28°C	g/ml	0.91	0.914	0.912	0.88
Cetain number	Min	51	49	47	>47
Iodine value	(mg/g)	47	53	49	<120
Acid number	mg.KOH/g	0.49	0.5	0.46	0.8 max.
Carbon residue	mass %	0.01	0.02	0.01	0.05 max.
Sulfated Ash	mass %	0.007	0.003	0.004	0.02 max.
Sulphur	Wt %	0.013	0.012	0.01	0.05 max.
Water contents	mg/kg	15	23	17	<300

Yield of biodiesel: Quantity of oil and yield percentage of biodiesel produced from dried biomass was analysed for various parameters. Oil extracted from *Chlorella emersonii* (42ml/100g) was higher than those of *Cladophora fracta* (25ml/100g) and *Spirogyra* (31ml/100g). Yield of biodiesel from transesterification of extracted oil was also higher in *Chlorella emersonii* (95.1%) than *Cladophora fracta* (91.6%) and *Spirogyra* (91.9%) (Table 5). Biodiesel production efficiency of microalgae was measured to be 98.15% by Li *et al*., (2007) in 12 hours of transesterification process. A recent study revealed that Chlorella protothecoides showed an oil yield of 55% in a process that combined bioengineering and transesterification for biodiesel production (Adewuyi, 2022).

Chromatographical technique for testing of biodiesel: Methyl ester produced from selected species of algae was compared with international standards and it was observed that biodiesel produced from oil of *Chlorella emersonii*, *Cladophora fracta* and *Spirogyra* was in accordance with pure biodiesel as there were no glycerides found in all three samples.

Biodiesel analysis: Biodiesel was also characterized for various parameters and then compared with ASTM D-6751~02 standards, values of all parameters obtained by this analysis were in accordance with international standards mentioned in (Table 6). Biodiesel produced from microalgae oil has exhibited properties similar to those of petro-diesel fuels (Adewuyi, 2022). Similar findings of properties of biodiesel produces from algal oil were reported by Li *et al*., (2007) and found that algal biodiesel was similar to commercial diesel and met the US Standard (ASTM 6751). Biodiesel use leads to reduction in noxious pollutants like carbon monoxide, unburnt hydrocarbon and some particulate matter with an obvious enhance in fuel consumption and nitrogenous oxides emission (Sakthivel *et al*., 2018).

References

Adewuyi, A. 2022. Production of Biodiesel from Underutilized Algae Oil: Prospects and Current Challenges Encountered in Developing Countries. *Biology*, 11(10): 1418.

Aransiola, E.F., T.V. Ojumu, O.O. Oyekola, T.F. Madzimbamuto and D.I.O. Ikhu-Omoregbe. 2014. A review of current technology for biodiesel production: state of the art. *Biomas Bioen*., 61(C): 276-97.

Boccard, R., L. Buchter, E. Casteels, E. Cosentino, E. Dransfield, D.E. Hood and C. Touraille. 1981. Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the Commission of the European Communities'(CEC) beef production research programme. *Livest. Prod. Sci.*, 8(5): 385-397.

Charoenchaitrakool, M. and J. Thienmethangkoon. 2011. Statistical optimization for biodiesel production from waste frying oil through two-step catalyzed process. *Fuel Proces. Technol.*, 92: 112-118.

Chen, H., D. Zhou, G. Luo, S. Zhang and J. Chen. 2015. Macroalgae for biofuels production: Progress and perspectives. *Renew & Sustain. Ener. Reviews*, 47: 427-437.

Chisti, Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends Biotechnol.*, 26(3): 126-131.

de Jesus, S.S., G.F. Ferreira, L.S. Moreira and R. Maciel Filho. 2020. Biodiesel production from microalgae by direct transesterification using green solvents. *Renew. Ener.*, 160: 1283-1294.

Demirbaş, A. 1999. Proximate and heavy metal composition in chicken meat and tissues. *Food Chem.*, 67(1): 27-31.

Demirbas, A. 2001. Mineral, protein, and fatty acids contents of hazelnut kernels. *Ener. Edu. Sci. Technol.*, 7: 37-43.

Demirbas, A. 2002. Biodiesel from vegetable oils via transesterification in supercritical methanol. *Ener. Convers. Manag*., 43: 2349-2356.

Fuentes-Grünewald, C., E. Garcés, S. Rossi and J. Camp. 2009. Use of the dinoflagellate Karlodinium veneficum as a sustainable source of biodiesel production. *J. Ind. Microbiol. Biotechnol.,* 36(9): 1215-1224.

Gao, W., K. Chen, J. Zeng, J. Xu and B. Wang. 2017. Thermal pyrolysis characteristics of macroalgae Cladophora glomerata. *Biores. Technol.*, 243: 212-217.

Gouveia, L. and A.C. Oliveira. 2009. Microalgae as a raw material for biofuels production. *J. Ind. Microbiol. Biotechnol.*, 36: 269-274.

Hammouda, O., A. Gaber and N. Abdelraouf. 1995. Microalgae and wastewater treatment. *Ecotox. & Env. Safety*, 31(3): 205-210.

Karaosmanoglu, F., K.B. Cigizoglu, M. Tuter and S. Ertekin. 1996. Investigation of the refining step of biodiesel production. *Ener. Fuels.,* 10: 890-895.

Khan, S., R. Siddique, W. Sajjad, G. Nabi, K.M. Hayat, P. Duan and L. Yao. 2017. Biodiesel production from algae to overcome the energy crisis. *HAYATI J. Biosci*., 24(4): 163-167.

Lang, X., A.K. Dalai, N.N. Bakhshi, M.J. Reaney and P.B. Hertz. 2001. Preparation and characterisation of biodiesels from various bio oils. *Biores. Technol.*, 80: 53-62.

Lemões, J.S., R.C.A. Sobrinho, S.P. Farias, R.R. de Moura, E.G. Primel, P.C. Abreu and M.G. M. D'Oca[a]. 2016. Sustainable production of biodiesel from microalgae by direct transesterification. *Sustain. Chem. & Pharm.,* 3: 33-38.

Li, X., H. Xu and Q. Wu. 2007. Large-scale biodiesel production from microalga chlorella protothecoides through heterotrophic cultivation in bioreactors. *Wiley Inter. Sci*. (www.interscience.wiley.com). DOI 10.1002/bit.21489.

Mubarak, M., A. Shaija and T.V. Suchithra. 2015. A review on the extraction of lipid from microalgae for biodiesel production. *Algal Res.*, 7: 117-123.

Rahpeyma, S.S. and J. Raheb. 2019. Microalgae biodiesel as a valuable alternative to fossil fuels. *BioEner. Res.*, 12(4): 958-965.

Raie, M.Y. 2008. Oil, Fats and Waxes. Edition 1, National Book Foundation Islamabad.

Ruiz-Marin, A., L.G. Mendoza-Espinosa and T. Stephenson. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi continuous cultures treating real wastewater. *Bioresour. Technol*., 101: 58-64.

Saka, S. and D. Kusdiana. 2001. Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. *Fuel*, 80(2): 225-231.

Sakthivel, R., K. Ramesh, R. Purnachandran and P.M. Shameer. 2018. A review on the properties, performance and emission aspects of the third generation biodiesels. *Renewable and Sustainable Energy Reviews*, 82: 2970-2992.

Saladini F., N. Patrizi, F,M. Pulselli, N. Marchettini and S. Bastianoni. 2016. Guidelines for energy evaluation of first, second and third generation biofuels. *Renew Sustain. Energy Rev.*, 66(C): 221-227.

Vinoth, V., S. Subash, M.H. Kumar and S. Sivanesan. 2012. Removal of free fatty acids in Pongamia pinnata (Karanja) oil using divinylbenzene-styrene copolymer resins for biodiesel production. *Biomas & Bioen*., 37: 335-41.

Yang, F., W. Xiang, X. Sun, H. Wu, T. Li and L. Long. 2014. A novel lipid extraction method from wet microalga *Picochlorum* sp. at room temperature. *Mar. Drugs*, 12(3): 1258-1270.

Yuvarani, M., D. Kubendran, A.R. Salma Aathika, P. Karthik, M.P. Premkumar, V. Karthikeyan and S. Sivanesan. 2017. Extraction and characterization of oil from macroalgae *Cladophora glomerata*. Energy Sources, Part A: Recovery, *Utiliz. & Env. Effects*, 39(23): 2133-2139.

Zarina, A., Masud-ul-Hasan and M. Shameel. 2005a. Taxonomic study of Vaucheriophyta Shameel from certain areas of the Punjab and NWFP, Pakistan. *Int. J. Phycol. Phycochem.*, 1: 159-166.

Zarina, A., Masud-ul-Hasan and M. Shameel. 2005b. Taxonomic study of the order Ulotrichales (Chlorophyta) from north-eastern areas of Pakistan. *Pak. J. Bot.*, 37: 797-806.

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