Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Mapping Algae of Sundarban Origin as Lipid Feedstock for Potential Biodiesel Application

Nirupam Barman[1], Gour Gopal Satpati[1], Sudeshna SenRoy[1], Nilofer Khatoon [1], Ramkrishna Sen[2], Sanjit Kanjilal[3], RBN Prasad[3] and Ruma Pal[1*].

[1]Department of Botany, University of Calcutta. 35, Ballygunge Circular Road, Kolkata- 700019. India
[2]Indian Institute of Technology, Kharagpur (IIT-KGP), India
[3]Indian Institute of Chemical Technology (IICT), Tarnaka, Hyderabad-500007, India
*Author for correspondence: rpalcu@rediffmail.com

Abstract

Six Cyanobacterial and fifteen other algal taxa from Sundarban mangrove forest of South East Asia have been analyzed for total lipid estimation and fatty acid profiling to search for a suitable feedstock for algal biodiesel production. Total lipid content varied from 7 – 23 % for the studied genera. GCMS analysis revealed that Palmitic (16:0) and Oleic (18:1) as major fatty acids from the taxa collected from freshwater, brackish water and marine habitat of Sundarban with variable salinity (0-23psu). A few microalgae from cyanophyceae like, *Lyngbya majuscula, Phormidium valderianum, Synechocystis pevalekii* and chlorophycean genera viz. *Rhizoclonium riparium, Rhizoclonium africanum, Pithophora cleveana, Spirogyra orientalis and Cladophora crystallina*, having suitable fatty acid composition were identified for biodiesel production. Fatty acid profile showed more diversification in unsaturated fatty acid in fresh water region, indicating more variable environment in upstream fresh water regions of Sundarban.

Keywords: Biodiesel, fatty acid, GCMS, Indian Sundarban, lipid, Microalgae.

Introduction

Microalgae are among the few photosynthetic organisms, which can directly produce and accumulate lipid in great quantities. Algal biomass is considered as one of the emerging sources of sustainable energy. Several algal genera have already been tested and screening of many others are under practices. In earlier study fatty acid profiling of several algal genera including cyanobacteria have been reported by various authors (Berge *et al.* 1995; Sallal *et al*. 1990, Renaud *et al*. 1994). Feedstock selection for algae biodiesel production in the past has focused only on the quantity of lipid produced by a species (Hu *et al.* 2008). Therefore, till date a very few microalgal strains with high oil content have been commercialized as source of biodiesel due to the either lack of suitable fatty acid profile or high biomass production (Knothe, 2005; Widjaya, 2009). Moreover biodiesel produced from most of the microalgae have poor oxidative property and the octane number is also out of specification with poor cold flow property (Stansell *et al.* 2011). Different countries have already published the guidelines of biodiesel standards (Knothe 2006; Meher *et al.* 2006; Mittelbach, 1996). As the fuel properties of microalgal biodiesel are predicted from fatty acid profile of lipid feed stock, therefore further search for promising algal genera is needed to be continued for cheap and sustainable biodiesel production.

Sundarban (21°13'-22°40' North and 88°05'-89°06'East) is the world's largest tiger inhabiting mangrove forest with an area of 4,000 sq .Km. in Indian subcontinent of South East Asia. This area is exposed to diurnal tidal cycle showing a prominent salinity gradient and is most suitable for various algal growth in fresh water, brackish water and marine habitat. Thick algal vegetations in swamps and mangrove forest beds are available together with unicellular planktonic form in fresh water rivers and marine coastal water. Lipid content and fatty acid composition of microalgal biomass varies significantly in different environmental condition [20, 21]. Therefore algal biomass collected from different salinity range of sundarbans mangrove forest may be a good source of lipid feed stock for algal biodiesel production. So far only a few reports are available regarding biochemical composition of the algal flora of this area including lipid analysis (Sen and Naskar, 2003; Chakraborty and Santra, 2008). A thorough investigation of fatty acid analysis of Sundarban algae including cyanobacteria have been taken by the present group in search for new potential taxa as lipid feed stock for algal biodiesel production. In present communication estimation of total lipid and their corresponding fatty acid profiling of twenty one algal strains from Indian Sundarban have been reported in relation to habitat water.

Materials and Methods

Algae culture

Twenty one algal taxa were isolated from various parts of Sundarban of variable salinity (1-23 psu) and

cultured in laboratory condition. Among them six were from Cyanophyceae, ten from Chlorophyceae one genus from Bacillariophyceae and four Rhodophycean taxa were brought to laboratory for lipid analysis. Unialgal cultures were set up in 500 ml culture flasks at 23°C in 16:8 light dark cycles in exposure to cool fluorescent light of 36×10^{10} lux for most of the microalgae. For marine taxa ASN III medium (containing 25gms NaCl, 2g $MgCl_2$, 0.5g KCl, 0.75g $NaNO_3$, 0.02g K_2HPO_4,$3H_2O$, 3.5g $MgSO_4$,$7H_2O$, 0.5 $CaCl_2$, 0.0005g EDTA, 0.02g Na_2CO_3 in 1L of glass dist. water), for freshwater cyanobacteria BGII medium (Ripka *et al.* 1979) and for brackish water taxa ASW (Goldman and McCarthy, 1978) media were used. Green algal genera were grown in BBM (Bold, 1942). Healthy growing biomass was used for fatty acid analysis. For a few taxa like, *Rhizoclonium*, *Cladophora*, *Catenella*, *Gelidium* etc. natural biomass were used after proper washing.

Lipid Extraction

For each species triplicate samples of freeze dried cells were analyzed for total lipid and fatty acid analysis. The biomass (5.0 g) was taken in a Velp Soxhlet extractor and extracted with 75 ml of 2:1, v/v of chloroform: methanol for 4 hrs. The organic phase was removed and the left over biomass was extracted further with 75 ml of 2:1, v/v of chloroform: methanol for 4 hrs. The combined organic phase was evaporated in a rotary evaporator, dried under vacuum and weighed to get the crude extract. The neutral lipid was extracted from this extract using hexane. About 100 ml of hexane and 100 ml of water were added to the crude extract and the content was transferred to the separating funnel. The hexane phase was separated and the aqueous phase was further extracted with 100 ml of hexane. The combined hexane phase was evaporated in rotary evaporator, dried under vacuum and weighed. The experiment was performed in duplicate and the values given are average of two extractions.

Transesterification

Direct transesterification of the biomass was also conducted for the analysis of fatty acid composition of lipids present in it. Briefly, the biomass (5.0 g) was taken in 100 ml round bottom flask followed by the addition of 20 ml of 2% H_2SO_4 in methanol and the resulting reaction mixture was refluxed for 4 hrs. After completion of reaction, the content was filtered and methanol was removed. The fatty acid methyl ester (FAME) was extracted with ethyl acetate and washed with water until neutral. The organic phase was dried over anhydrous Na_2SO_4, concentrated in rotary evaporator, dried under vacuum and weighed to get FAME, which was subsequently analyzed by GC and GC-MS.

GC and GC-MS analysis

The fatty acid composition of algal oil was analyzed qualitatively using GC-MS and quantitatively using GC. The GC-MS detection was performed with an Agilent 6890N Gas Chromatograph connected to an Agilent 5973 Mass Selective Detector at 70 eV (*m*/*z* 50-550; source at 230 °C and quadruple at 150 °C) in the electron impact mode with a HP-5 ms capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The oven temperature was programmed for 2 min at 160 °C and raised to 300 °C at 5 °C/min and maintained for 20 min at 300 °C. The carrier gas, helium, was used at a flow rate of 1.0 mL/min. The inlet temp was maintained at 300 °C, and the split ratio was 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation patterns of authentic compounds. GC analysis was performed on HP 6850 Series gas chromatograph equipped with a FID detector and DB-225 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injector and detector temperatures were maintained at 300 and 325 °C, respectively. The oven temperature was programmed for 2 min at 160 °C and raised to 300 °C at 5 °C/ min and maintained for 20 min at 300 °C. The carrier gas, nitrogen, was used at a flow rate of 1.5 mL/min. The injection volume was 1 μL, with a split ratio of 50:1. The identification of individual fatty acids was done on the basis of retention time of authentic fatty acids.

Results

Total lipid content and fatty acid profile of freshwater, brackish water and marine algal taxa, collected from Sundarban area are presented in Table 1, 2, and 3 respectively. Table 1 depicts that among the 6 fresh water taxa studied, chlorophycean taxa *Spirogyra orientalis* (21%) showed maximum amount of total lipid content followed by *Chlorococcum infusionum* (11.3%). *Synechocystis pevalekii* showed maximum amount of total lipid content (9%) among other freshwater cyanophycean taxa. *Nostoc ellipsosporum*, *Spirulina platensis*, *Rhizoclonium fontinale* showed 7-8% of lipid. Brackish water taxa *Cladophora cystallina* contained maximum amount of total lipid content (23%). *Pithophora cleveana and Chaetomorpha gracilis* showed 19% and 16% of lipid respectively. Only one brackish water cyanophycean genera *Lyngbya birgei* was recorded which showed 12% of total lipid content (Table2). Marine diatom genus *Navicula minima* (16.2%) showed maximum lipid content than that of other recorded marine algal genera followed by chlorophycean genera *Ulva lactuca* (11%) (Table3). Cyanophycean genera *Phormidium valderianum* and *P. tenue*, chlorophycean genera *Rhizoclonium africanum*

Rhodophycean taxa *Gelidium pusillum* and *Ceramium manorensis* showed 7-9 % of total lipid content.

Table 1: Total lipid content and fatty acid profile of fresh water microalgae

Genus		*Synechocystis pevalekii*	*Nostoc ellipsosporum*	*Spirulina platensis*	*Spirogyra orientalis*	*Chlorococcum Infusionum*	*Rhizoclonium fontinale*
Total Lipid (%)		9±2	7±1.5	8.5±2	21±2.5	11.34±1	7.5±1.4
Fatty Acid							
Saturated Fatty Acid	C12:0	-	0.3	9.34	0.3	3.77	4.18
	C14:0	1.0	12.0	13.27	0.8	8.5	6.49
	C15:0	0.2	0.4	-	0.5	-	-
	C16:0	34.2	22.05	21.1	34.08	25.62	29.36
	C17:0	-	-	-	-	-	-
	C18:0	1.4	4.5	7.22	1.5	4.54	0.23
	C20:0	-	-	0.1	-		0.57
	C21:0	-	-	-	-		-
	C22:0	-	-	-	1.1		-
	C23:0		-	-	-		-
	C24:0	-	-	-	0.7		-
Mono unaturated fatty acid	C16:1	3.8	6.6	9.32	-	5.46	2.26
	C18:1	29.8	17.85	11.27	6.4	15.66	22.13
	C20:1	-	-	-	0.32		3.62
	C22:1	-	5.7	0.53	1.5		-
Poly unaturated fatty acid	C16:2	4.9	9.5	5.31	3.4	-	-
	C18:2	14.9	2.5	0.24	6.7	7.5	-
	C20:2	0.9	13.5	20.9	20.4	18.6	21.65
	C16:3	2.6	-	0.3	-	0.43	1.54
	C18:3(n3)GLA	6.3	-	0.7	19.0	0.31	6.26
	C18:3(n3)ALA	-	0.2	-	-		-
	C20:3(n-3)	-	0.3		1.9	0.23	-
	C20:3(n-6)	-	3.6		-	9.38	-
	C20:4	-	0.6	0.1	-	-	-
	C20:5	-	0.4	0.3	1.4	-	1.71

Table 2: Total lipid content and fatty acid profile of brackish water microalgae

Genus		*Lyngbya birgei*	*Rhizoclonium riparium*	*Pithophora cleveana*	*Cladophora crystallina*	*Cheatomorpha gracilis*	*Enteromorpha intestinalis*	*Polysiphonia mollis*
Total Lipid (%)		12±2.8	8.65±1	19±2	23±1.8	16±0.5	12±1	7.8±1.6
Fatty Acid								
Saturated Fatty Acid	C12:0	1.4	7.0	0.7	-	0.4	4.32	0.3
	C14:0	3.2	7.8	9.4	8.8	13.0	9.96	7.5
	C15:0	0.7	0.5	0.2	-	0.8	-	0.5
	C16:0	59.6	37.4	37.0	39.1	52.4	22.57	44.7
	C17:0	0.3	0.3	0.3		0.3	-	1.1
	C18:0	3.9	2.0	2.7	1.1	4.9	4.94	2.3
	C20:0	0.5	0.3	0.5	0.2	0.2	0.67	-
	C21:0			1.2		0.3	6.25	0.7
	C22:0	0.3	0.9		0.6		0.58	0.6
	C23:0	0.1						-
	C24:0	0.3	2.7		0.1			-
Mono UnSaturated Fatty acid	C16:1	2.3	3.1	3.1	2.8	6.0	9.7	3.4
	C18:1	13.1	17.3	34.3	31.8	15.9	18.49	10.3
	C20:1	0.3		0.9	0.8			-
	C22:1	0.1						-
Poly unsaturated Fatty acid	C16:2	0.7		6.8			-	-
	C18:2	9.1	17.2		11.6	3.6	14.83	-
	C20:2	0.1			0.5		1.42	2.1
	C16:3						1.57	-
	C18:3(n3)GLA	0.6		0.8	0.3		-	-
	C18:3(n3)ALA	3.4	1.1	1.1	0.7	0.8		1.2
	C20:3(n-3)		2.4	1.0	1.6	0.7	4.7	2.5
	C20:3(n-6)					0.7		10.7
	C20:4							
	C20:5							12.1

Table 3: Total lipid content and fatty acid profile of marine water microalgae

Genus		*Phormidium valderianum*	*Phormidium tenue*	*Rhizoclonium africanum*	*Ulva lactuca*	*Navicula minima*	*Catenella repens*	*Geledium pusillum*	*Ceramium manorensis*
Total Lipid (%)		7.6±2.8	8.01±2.2	7.2±2.7	11±1	16.23±0.59	8±1.5	9.7±2.8	8±1.9
Fatty Acid									
Saturated Fatty Acid	C12:0	-	0.9	3.4	3.21	0.8	0.6		0.6
	C14:0	1.9	4.8	6.5	0.9	2.7	5.3	2.0	2.4
	C15:0	1.3	1.4	2.8	0.8	2.2	0.5	0.5	0.4
	C16:0	35.8	32.9	30.2	45.2	26.4	56.2	36.2	60.6
	C17:0		1.7	-	-	2.6	-	2.3	-
	C18:0	8.5	3.5	1.4	1.3	5.0	3.5	3.3	-
	C20:0	-	0.1	-	8.8	0.9	0.2	0.7	-
	C21:0	-	-	-	-	5.1		-	1.8
	C22:0	0.2	0.4	-	2.6	0.6		-	0.8
	C23:0	-	-	-	-	-	0.4	-	-
	C24:0	-	0.5	11.2	-	-	3.5	-	1.0
Mono Saturated	C16:1	16.1	24.3	9.4	2.0	18.7	7.6	9.2	2.0
	C18:1	23.8	15.2	20.0	10.8	25.3	10.4	25.6	17.4
	C20:1	-	-	-	0.9	0.5	-	-	-
	C22:1	-	-	-	-	-		-	1.3
Poly Saturated	C16:2	8.3	2.3	-	-	3.6	-	-	-
	C18:2	2.5	2.9	5.3	4.89	2.2	7.0	7.1	9.6
	C20:2	-	-	-	-	-	0.8	-	-
	C16:3	-	1.4	-	-	1.3	1.5		-
	C18:3(n3)GLA	-	0.6	-	-	-	-		-
	C18:3(n3)ALA	-	3.1	7.4	15.0	1.6	-	8.3	0.2
	C20:3(n-3)	-	-	2.4	0.9	-	0.3	2.4	-
	C20:3(n-6)	-	-	-	1.4	-	0.8	-	-
	C20:4	1.6	2.3	-	1.3	-		-	0.9
	C20:5	-	1.7	-	-	0.5	1.4	2.4	1.0

The fatty acid profile of all the taxa studied (Table 1, 2, 3) indicated the presence of palmitic (C16:0) acid as major fatty acid showing a variation of 21% (*Spirulina platensis*) to 60% (*Ceramium manorensis*) of total fatty acid. Among cyanophycean taxa *Lyngbya birgei* showed highest amount of Palmitic acid (C16:0). It showed 59.6% Palmitic acid (C16:0) of total fatty acid followed by other cyanophycean genera *Phormidium valderianum* (34%), *Synechocystis pevalekii* (34%), *P. tenue* (32%). Chlorophycean genera *Cheatomorpha gracilis* showed 52.4% Palmitic acid (C16:0) of total fatty acid. 37% - 39%

Palmitic acid (C16:0) was recorded in *Cladophora crystallina, Pithophora cleveana, Rhizoclonium riparium.* Only in *Pithophora* and *Cladophora* 18:1 PUFA was recorded as the dominant one (31%-34%). Comparatively high amount of linoleic acid (18:2) was recorded from *Synechocystis pevalekii Rhizoclonium riparium,* and *Cladophora crystalline* (14 to 17% of total fatty acid). Among all the genera, tricosanoic acid (23:0) was found only in cyanophycean genera *Lyngbya birgei* and rhodophycean genera *Catenella repens*. Maximum diversification in fatty acid profile was recorded in *Lyngbya birgei* (19 types). *Nostoc ellipsosporum, Spirogyra orientalis* sdowed 16 types of fatty acid followed by *Spirulina platensis* and *Pithophora cleveana* (15 types) and least variation in fatty acids was recorded in *Phormidium valderianum* (10 types).

Discussion

Lipids are the esters of fatty acids and alcohols that comprise a large group of structurally distinct organic compounds including fats, waxes, phospholipids, glycol-lipids etc. Several authors studied and recorded algal lipid and FA composition to determine the quality of algal biomass as lipid feed stock for algal biodiesel production (Knothe, 2005, 2008, 2009). Different algal genera contain significant quantities of lipids (fats and oil) with similar compositions to those of vegetable oils (Singh *et al.* 2002). The lipids of some cyanobacterial taxa are rich in essential fatty acids such as C18 linoleic (18:2w6) and y-linolenic (18:3w3) acids and their C20 derivatives - eicosapentaenoic acids (20:5w3) and arachidonic acid (20:4w6) and are used as essential components of important feed additives in aquaculture[5]. Some of the filamentous cyanobacteria tend to have large quantities (25 to 60 % of the total) of polyunsaturated fatty acids (Parker *et al.* 1967; Holton and Blecker 1972; Kenyon *et al.*1972).

Several algal genera have already been identified as good source for biodiesel production. Among which, genera like *Prymnesium* (22-38%), *Scenedesmus dimorphus* (16-40%), *Euglena* sp (14-20%) are remarkable (Becker, 1994). Among the members of Sundarban flora chlorohycean genera *Cladophora crystallina* showed more lipid (23%). *Lyngbya birgei* and *Synechocystis pevalleki* showed maximum amount of lipid among other Cyanophycean genera

Patil *et al.* (2007) studied fatty acid composition of several unicellular algae including *Isochrysis, Pavlova, Phaeodactylum, Porphyridium, Chroococcus, Synechococcus* and *Tribonema* and also recorded C16:0, C18:1, C18:2x6 and C18:3x3 as the dominant fatty acids. Among these C16:0 and C18:1 accounted for 50% of the total fatty acids, which is typical of freshwater green algae as reported by other author also (Volkman *et al*. 1989; Cranwell *et al*. 1990; *Renaud et al*. 1994). From the present study it was recorded that in *Synechocystis pevelekii* C16:0 and C18:0 comprises of ~ 64% of total fatty acids followed by other fresh water algae *Chlorococcum infusionum* (~42%), *Spirogyra orientalis* (~40%), *Nostoc ellipsosporum* (~40%). Among brackish water algae *Chaetomorpha gracilis* C16:0 and C18:1 accounted for 68% and marine red algae *Ceramium manorensis* comprises highest amount of C16:0 and C18:0 (~78%). Cyanophycean genera *Phormidium valderianum* also showed high amount of C16:0 and C18:0 (~60%)

It is known that feed stocks rich in monosaturated fatty acids (MUFAS) are desirable for biodiesel production but the composition of saturated fatty acids (SFAs) is also shown to be of great importance (Stansell *et.al* 2011). The critical fuel properties are dependent on the fatty acid composition of the feed stock (Knothe, 2006; Meher *et al*. 2006; Millelbech. 1996). The most significant challenge facing algal biodiesel is poor oxidative stability because most microalgae contain very high concentrations of FAs with more than four double bonds (Stansell *et al*. 2011). The ideal biodiesel feedstock would be composed entirely of C16:1and C18:1 MUFAs (Knothe, 2008), so in a practice a biodiesel feed stock should have high concentrations of C16:1 and C18:1 with less variation in fatty acid profile. It is also known that algal FA composition within a species varies due to environmental variables (Alvarez–Cobelas, 1989). Therefore there is potential to modify algal FA compositions using environmental variables. Overall more unsaturation in fatty acid bonds were observed in algae from fresh water zone of Sundarban, showing 4-7 saturated FA and 7-11 unsaturated FA. This indicates more variable environmental condition in upstream riverine region of Sundarban. On the other hand brackish water and marine taxa showed more variation in saturated fatty acid like, *Lyngbya birgei, Rhizoclonium riparium, Pithophora cleveana, Cladophora crystallina, Chaetomorpha gracilis, Enteromorpha intestinalis and Polysiphonia mollis.* But most of the rhodophycean member like, *Gelidium pusillum, Ceramium manorensis* and *Catenella repens* together with cyanobacterial taxa, *Phormidium valderianum* and *P. tenue* showed equal number of saturated and unsaturated FA.

From the present study it revealed that among the twenty one studied taxa of Sundarban area, chlorophycean genera like *Rhizoclonium riparium, R. africanum, Pithophora cleveana, Spirogyra orientalis, Cladophora crystallina* containing more MUFAs (16:1, 18:1) with a support of 16:0 FA, would be more suitable for biodiesel production. Among the cyanobacterial taxa, fatty acid composition of freshwater genus *Synechocystis*, together with brackish water species *Lyngbya majuscula*

and marine taxa *Phormidium valderianum* and *Phormidium tenue* with favorable fatty acid composition have also been projected as good feed stock for biodiesel in the present study. Therefore filamentous cyanobacteria with high growth rate can be a suitable alternate for biodiesel production in Indian scenario.

Acknowledgement
The authors are thankful to Dr. Puspito Ghosh, Director CSMCRI as co-ordinatior of the project and CSIR for financial support under NIMTLI program.

References

Alvarez-Cobelas, M. 1989. Lipids in microalgae A review II Environment. Grasas y Aceites 40:213–223.

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

Berge, J.P., Gouygou J.P, Dubacq J.P, Durand P. 1995. Reassessment of lipid-composition of the diatom *Skeletonema costatum*. Phytochemistry 39:1017–1021.

Bold, H.C. 1942. The cultivation of algae. The Botanical Review 8, pp 90–96.

Borowitzka, M.A. 1988. Fats, oils and hydrocarbons. In *Micro-algal Biotechnology* (ed. Borowitzka, M. A. and Borowitzka, L.J., Cambridge University Press, Cambridge, pp.257-287.

Chakraborty, S. and Santra, S.C. 2008. Biochemical composition of eight benthic algae collected from Sundarban. Indian J. of Marine Sci. 37(3):329-332.

Cranwell, P.A., Jaworski, G.H.M. and Bidey, H.M. 1990. Hydrocarbons, sterols, esters and fatty acids in six freshwater chlorophytes. Phytochem. 29:145–151.

Goldman, J.C. and McCarthy, J.J. 1978. Steady-state growth and ammonium uptake of a fast-growing marine diatom. Limnology & Oceanography 23:695–703.

Holton, R.W. and Blecker, H.H. 1972. Fatty acids in blue-green algae. In Properties and Products of Algae (ed. Zaick, J.E.) Plenum, New York, 115-127 pp.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., and Darzins, A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production perspectives and advances. Plant. 54: 621–639.

Kenyon, C.N., Rippka, R. and Stanier, R.Y. 1972. Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch. Microbiol. 83:216–236.

Knothe, G. 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technology 86:1059-1070.

Knothe, G. 2006. Analyzing biodiesel: standards and other methods. J. Am. Oil Chem. Soc. 83:823–833.

Knothe, G. 2007. Some aspects of biodiesel oxidative stability. Fuel Process Technol. 88:677–699.

Knothe, G. 2008. "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuels 22:1358–1364.

Knothem, G. 2009. Improving biodiesel fuel properties by modifying fatty ester composition. Energy Environ. Sci. 2:759–766.

Knothe, G. and Dunn, R.O. 2009. A comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. J. Am. Oil Chem. Soc. 86:843–856.

Meher, L.C., VidyaSagar, D. and Naik, S.N. 2006. Technical aspects of biodiesel production by transesterification— a review. Renew Sust. Energ. Rev. 10: 248–268.

Mittelbach, M. 1996. Diesel fuel derived from vegetable oils, VI: specifications and quality control of biodiesel. Biores. Technol. 56:7–11.

Olie, J.J., Potts, M. 1986. Purification and biochemical analysis of the cytoplasmic membrane from the desiccation-tolerant cyanobacterium *Nostoc commune* UTEX 584. Appl. Environ. Microb. 52:706-711.

Ritter, D. and Yopp, J.H. 1993. Plasma membrane lipid composition of the halophilic cyanobacterium Aphanothece halophytica. Arch. Microbiol. 159:435 – 439.

Parker, P.L., VanBaalen, C. and Maurer, L. 1967. Fatty acids in eleven species of blue-green algae geochemical significance. Science 155: 707-708.

Patil, V., Ka¨llqvist, T., Olsen. E., Vogt. G., Gislerød, H.R. 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacul. Int. 15:1–9.

Renaud, S.M., Parry, D.L. and Tinh, L. 1994. Microalgae for use in tropical aquaculture: I. Gross chemical and fatty acid compositions of twelve species of microalgae from Northern Territory, Australia. J. Appl. Phycol. 6:337–345.

Renaud, S.M., Parry, L. and Luong-Van, T. 1994. Microalgae for use in tropical aquaculture. Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. J. Appl. Phycol. 6:337-345.

Rippka, R., Deruelles, J., Waterbury, J., Herdman, M. and Stanier, R. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111:1-61.

Sallal, A.K., Nimer, N.A. and Radwan, S.S. 1990. Lipid and fatty acid composition of freshwater cyanobacteria. J. Gen. Microbiol. 136:2043–2048.

Sen, N. and Naskar, K. 2003. Algal flora of Sundarbans Mangal. Daya publishing house New Delhi, India.

Sen Roy, S., Barman, N. and Pal, R. 2009. Stress induced changes in total lipid and fatty acid composition of *Navicula minima*. J. Bot. Soc. Bengal 63(1):47-51.

Singh, S.C., Sinha, R.P. and Häder, D.P. 2002. Role of lipids and fatty acids in stress tolerance in cyanobacteria. Acta Protozoa 41:297-308.

Stansell, G., Gray, V.M. and Sym, S. In press 2011. Microalgal Fatty Acid Composition: Implications for Biodiesel Quality. J. Appl. Phycol. DOI: 10.1007/s10811-011-9696-x.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rodgers, G.I. and Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128:219–240.

Widjaja, A. 2009. Lipid production from microalgae as promising candidate for biodiesel production. Makara Teknologi 13(1):47-51.