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Biomass Production and GC-MS Analysis of *Selenastrum bibraianum* Reinsch

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Abstract

Alga *Selenastrum* has the high adaptability to changing environment; hence the biomass production is an easy task. In this study *Selenastrum bibraianum* reinsch was used. Alga was cultured in Bold's Basal medium and for biomass production the plastic bags were used. All the cultures were maintained in laboratory condition. The lipid was extracted using Bligh and dyer method and the extract was used for GC- MS profiling and the results are discussed.

Key words: *Selenastrum bibraianum*, Biomass Production, Isolation, GC – MS

Introduction

Biodiesel production using various materials, such as plants, microalgae and animal fat, has been attempting as an alternative energy source since the rise of global warming and the exhaustion of fossil fuels (Vasudevan & Briggs,2008). The industrialization and population increasing the need of energy continuously and in 2010 it is expected that the crude oil and natural gas may be depleted in 45.7 and 62.8 years, respectively and estimated indicates that the consumption rate will be tripled in 2025 year(BP Statistical Review of World Energy2010). Unfortunately greenhouse gas emissions from fossil fuel usage been credited as the major potential threat to the increasing global climate change (Ugarte DG *et al*. 2003) and in 2006 associated CO2 emissions were 29 Gtonnes (EIA 2006), whereas estimates shows that only about 12 Tones can be removed my natural processes, hence new strategies needed to neutralize the excess CO_2 (Bilanovic D *et al.* 2009).Policies focused on achievement of energy security, and mitigation of Green House Gases emissions has shown rapid global growth in the use of liquid biofuels in the transport sector (IEA.World energy outlook 2007). First generation biofuels from food and oil crops including rapeseed oil, sugarcane, sugar beet, and maize have been reached the economic level production and also vegetable oils and animal fats using conventional technology (FAO,2007 & 2008). While using food and oil crops or other sources of first generation biofuel for producing liquid biofuels the demands will be limited due to the competition with food and fibre production for the use of arable land, regionally controlled market structures, lack of well managed agricultural practices in developing economies, high water and fertilizer requirements, and a need for conservation of bio-diversity (FAO. Sustainable bioenergy & IEA technology essentials).Typically, first generation biofuels impact on global food markets and on food security, especially with regards to the most vulnerable regions of the world economy has raised pertinent questions on their potential to replace fossil fuels and sustainability of their production (Moore A 2008).

Algae are accepted as one of the primogenital life-forms (Falkowski PG and Raven JA 1997) and lack roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have chlorophyll-a as their primary photosynthetic pigment (Lee RE 1980). Algae organizations are predominantly for energy conversion without any development beyond cells, and their simple development allows them to adapt to most of the environmental conditions and flourish in the long term (Falkowski PG, and Raven JA. 1997).Microalgae are capable of all year round production (Schenk P et al. 2008) they grow in aqueous media, but need less water than terrestrial crops (Dismukes GC *et al*. 2008) can be cultivated in brackish water on non-arable land (Searchinger T *et al*. 2008) have a rapid growth potential and many species have oil content in the range of 20–50% dry weight of biomass, the exponential growth rates can double their biomass in periods as short as 3.5 h (Chisti Y2007, Metting FB1996 and Spolaore P *et al*. 2006), biomass production can effect bio fixation of waste CO_2 (Metting FB 1996), wastewater can be used as the nutrient medium (Cantrell KB et al. 2008) and they does not require any herbicides or pesticides application (Rodolfi L *et al*. 2008). They do have some byproducts like proteins and residual biomass after oil extraction, which may be used as feed or fertilizer (Spolaore P *et al*. 2006) in other hand the residual biomass can be fermented to produce ethanol or methane (Hirano A *et al*.1997).

Biodiesel can be defined as derivative of oil crops and biomass which can be used directly in conventional diesel engines (Clark J and Deswarte F 2008) and it is a mixture of mono alkyl esters of long chain fatty acids (FAME) (Demirbas A 2009). The extracted algal oil can be converted into biodiesel through a process called transesterification. It is a chemical reaction of triglycerides and alcohol in the presence of a catalyst which produce mono-esters which are called as biodiesel (Sharma YC and Singh B 2009). Algal biodiesel's properties must match or exceed the International Biodiesel Standard for Vehicles (EN14214) in order to be considered as the substitution fuel for fossil fuels (Song D *et al* 2008). The only limitation in use of algal oil , it has high degree of polyunsaturated fatty acids while compared to vegetable oils which makes it susceptible to oxidation in storage (ChistiY2007). However, algal biodiesel has parallel physical and chemical properties to petro-diesel, 1st generation biodiesel from oil crops and compares favorably with the international standard EN14214(Song D *et al* 2008). Algal biodiesel is renewable, biodegradable, and quasi-carbon neutral under sustainable production, non-toxic and contains reduced levels of particulates, carbon monoxide, soot, hydrocarbons and Sox (Sheehan J et al. 1998) and compared to 1st generation biodiesel, it is more suitable for use in the aviation industry due to their low freezing points and high energy density (NREL 2008). The major benefit of algal biodiesel is their reduced CO2 emissions of up to78% compared to emissions from petroleum diesel (Sheehan J et al. 1998).

Polyunsaturated fatty acids (PUFAs) are most essential for human development and physiology (Hu C et *al*. 2008) and they have been proven to reduce the risk of cardiovascular disease (FDA, 2004; Ruxton*et. al. 2007*). Presently, fish and fish oil remains as the sources of PUFA but application as a food additive are limited due to possible accumulation of toxins, fish odour, unpleasant taste, poor oxidative stability, and the presence of mixed fatty acids[31] and not suitable for vegetarian diets. Microalgae are a primary source of PUFA (Spolaore P *et al*. 2006), and supply whole food chains with these vital components while higher plants and animals lack the necessary enzymes to produce PUFA (Pulz and Gross W 2004). Microalgae PUFA also has many other applications and among that, using as additives for infant milk formula is one of the major utilization and chickens have been fed with special algae to produce omega-3 enriched eggs (Pulz and Gross W 2004).Presently, docosahexaenoic acid (DHA) is the commercially available in algal species(Spolaore P *et al* 2006).

Material and Methods

Collection Site

In the present study, sample was collected from different sites of a Pond in Kavalkinaru village, Thirunelveli district (Latitude – 8.279530, Longitude – 77.575012) (**Map 1).**The temperature (27°C -30°C), pH (8-8.5), and water depth (5 – 90 cm) were recorded at collection sites.

Biomass production and Lipid profile

Algal strains were isolated using streak plate method in BBM with the pH 7.8 and salinity zero. After successful isolation, the strain was developed into biomass and harvested using centrifugation (8000 rpm for 5 min, at 15˚C). Harvested biomass then freeze dried using lyophilizer(Christ Alpha 1-2/ LD Plus, Germany). Microalgal biomass then subjected to lipid extraction using Bligh and Dyer Method (1959).

Gas chromatographic analysis of fatty acid methyl esters

Extracted lipid produced from *Selenestrum bibraianum* Reinsch oil was analyzed by gas chromatography– mass spectrometry PerkinElmer Clarus 500 and analyzed using the software Turbomass ver 5.2.0 equipped with VF-5 MS Capillary Column Elite-5 (Crossbond 5%Phenyl 95% dimethyl polysiloxane (non polar, 30 mm length, 250µm diameter and 0.25 lm film thicknesses). The column temperature of each run was started at 70 ˚C for 3 min, then raised to 300 ˚C and maintained at 300 ˚C for 9 min. GC conditions were: column oven temperature, 70 ˚C; injector temperature, 280 ˚C; injection mode, split; split ratio, 10; flow control mode, linear velocity; column flow, 1.51 ml/min; carrier gas, helium (99.9995 % purity); and injection volume, 1 ll. MS conditions were: ion source temperature, 200 ˚C; interface temperature, 240 ˚C; scan range, 40–1,000 m/z; solvent cut time, 5 min; MS start time, 5 min; end time, 35 min; ionization, Electron Ionization (70 eV); and scan speed, 2,000.

Result and Discussion

The cultured strain was identified as *Selenastrum bibraianum* Reinsch **(Fig. 1)** and classification is given in **Table 1**. Alga was grown successfully in Bold's Basal medium at pH – 7.8. The biomass yielded 28.2% lipid in the bligh and dyer method. (McLarnon-Riches *et al*. 1998), was studied the effects of environmental factors and metals on *Selenastrum capricornutum* lipids. A high resolution mass spectrum equipped with a data system in combination

with Gas chromatography was used for the analysis of bioactive components present in the extract of *Selenastrum bibraianum* Reinsch. Based on spectral data it was found that the extract contained a mixture of volatile compounds. A total of 26 peaks were observed with retention times as presented in **Fig.2 and Table.2.**The NIST search brought up the structure of the compounds (**Table 3**). In this study the following fatty acids were found to be present as C10:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 . Fatty acids from various micro algae, Dodecanoic acid methyl ester, Tetradecanoic acid methyl ester, 7, 10-Hexadecdienoic acid methyl ester, 9-Hexadecenoic acid methyl ester, 11-Hexadecenoic acid methyl ester, Hexadecanoic acid methyl ester, 9(R),10(R)-Dihydroxyoctadecanoic acid methyl ester, 6,9,12-Octadecatrienoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, 9-Octadecenoic acid methyl ester, 11-Octadecenoic acid methyl ester, Octadecanoic acid methyl ester, 11-Methoxy octadecanoic acid methyl ester, 8,11-Eicosadienoic acid methyl ester, 11,14-Eicosadienoic acid methyl ester, 8,9-Dihydroxy docosanoic acid methyl ester, 10-Hydroxy octadecanoic acid methyl ester, 9,10-Epoxy octadecanoic acid methyl ester, 11-Eicosenoic acid methyl ester, Eicosanoic acid methyl ester, 13,16-Docosadienoic methyl ester, Docosanoic acid methyl ester, Tetracosanoic acid methyl ester and Hexacosanoic acid methyl ester (Syed Ghulam Musharraf *et al*. 2012), from which is almost similar to the current result.

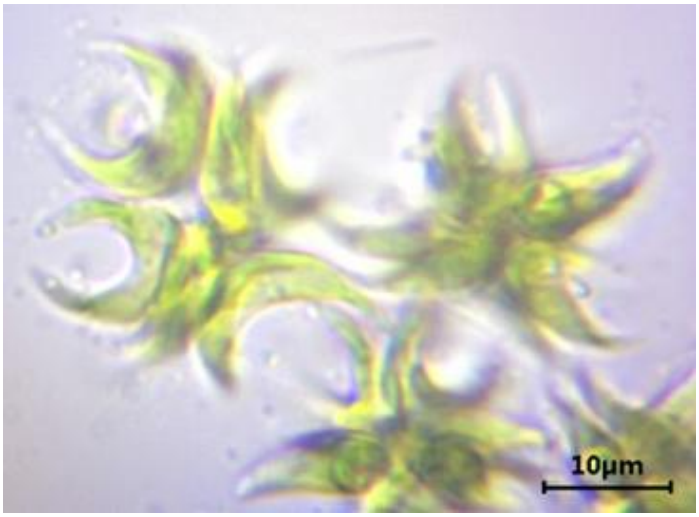


Fig. 1. Microscopic Image of *Selenastrum Bibraianum* Reinsch

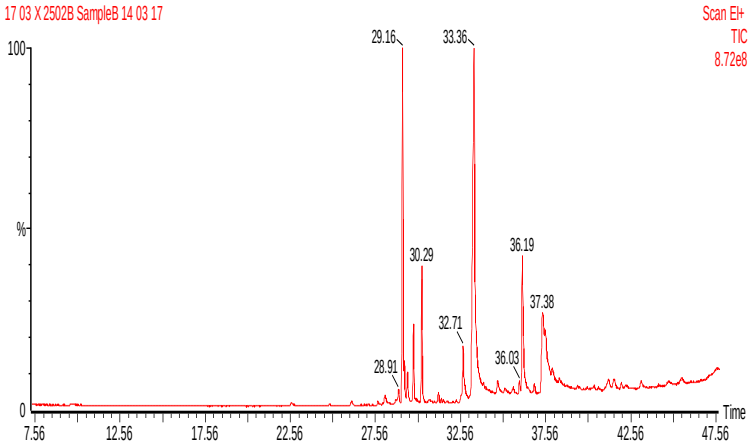


Fig. 2. GC MS Chromatogram of *Selenastrum Bibraianum Reinsch*

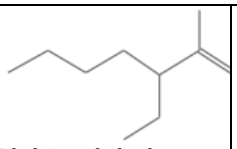
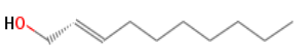
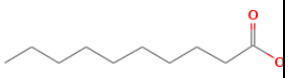
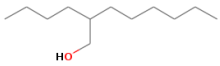
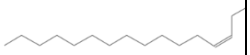
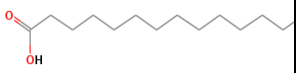
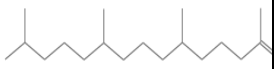
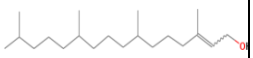
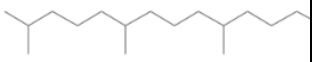
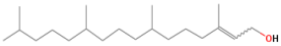
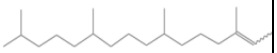
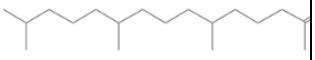
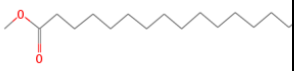
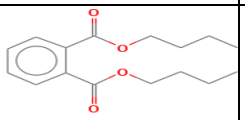
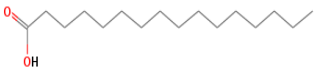
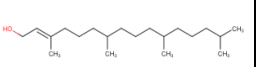
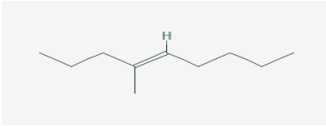
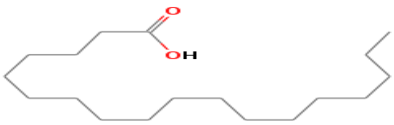
Table.1. Taxonomic Classification of the alga studied (Guiry & Guiry, 2017)

Empire	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Chlorophyta infrakingdom
Phylum	Chlorophyta
Subphylum	Chlorophytina
Class	Chlorophyceae
Order	Sphaeropleales
Family	Selenastraceae
Genus	*Selenastrum*

Table. 2. The GC-MS result showing the compound name, Formula, Retention time and Percentage of Peak area

S. No.	Peak Name	Formula	Molecular weight	Retention time	Peak area	% Peak area
1	3-Ethyl-2-methyl-1-heptene	$C_{10}H_{20}$	140	9.67	541896	0.1601
2	2-Decen-1-ol, (E)-	$C_{10}H_{20}O$	156	20.26	258762	0.0764
3	n-Decanoic acid	$C_{10}H_{20}O_2$	172	22.62	1423911	0.4206
4	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	24.86	447142	0.1321
5	3-Hexadecene, (Z)-	$C_{16}H_{32}$	224	26.16	1101018	0.3252
6	5-Octadecene, (E)-	$C_{18}H_{36}$	252	27.71	832925	0.2460
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	28.12	3200567	0.9454
8	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280	28.91	3065514	0.9055
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	29.16	64058632	18.9215
10	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	29.44	4337181	1.2811
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	29.79	12644279	3.7348
12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	30.29	19864552	5.8675
13	1-Hexadecanol	$C_{16}H_{34}O$	242	30.73	770509	0.2276
14	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	31.25	1824773	0.5390
15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	31.54	388759	0.1148
16	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	$C_{20}H_{40}O$	296	32.14	276706	0.0817
17	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	32.71	10273691	3.0346
18	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	33.36	154023712	45.4952
19	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	34.73	3676934	1.0861
20	3-Eicosene, (E)-	$C_{20}H_{40}$	280	36.03	1923078	0.5680
21	Phytol	$C_{20}H_{40}O$	296	36.19	34429444	10.1697
22	4-Methyl-4-nonadecene	$C_{20}H_{40}$	280	36.90	1609106	0.4753
23	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	37.38	13199062	3.8987
24	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	37.55	1691151	0.4995
25	Octadecanoic acid	$C_{18}H_{36}O_2$	284	37.94	1620822	0.4788
26	Octadecanamide	$C_{18}H_{37}NO$	283	38.37	1065424	0.3147

Table.3. Chemical Structure of the Compounds

 3-Ethyl-2-methyl-1-heptene	 2-Decen-1-ol, (E)-	 n-Decanoic acid	 1-Octanol, 2-butyl-
 3-Hexadecene, (Z)-	 5-Octadecene, (E)-	 Tetradecanoic acid	 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	 2-Pentadecanone, 6,10,14-trimethyl-	 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	 3,7,11,15-Tetramethyl-2-hexadecen-1-ol
 1-Hexadecanol	 2-Pentadecanone, 6,10,14-trimethyl-	 Hexadecanoic acid, methyl ester	 1-Hexadecen-3-ol, 3,5,11,15-tetramethyl
 Dibutyl phthalate	 n-Hexadecanoic acid	 Heptadecanoic acid	 3-Eicosene, (E)-
 Phytol	 4-Methyl-4-nonadecene	 9,12-Octadecadienoic acid, methyl ester	 9,17-Octadecadienal, (Z)-
 Octadecanoic acid		 Octadecanamide	

Map.1. Collection site of *Selenastrum Bibraianum* Reinsch Kavalkinaru Village, Thirunelveli District.

Conclusion

The isolated algal strain is *Selenastrum bibraianum* Reinsch, and which has the 28.2% lipid content in their cell content. The GC-MS analysis of lipid extract revealed the presence of 26 volatile compounds, which includes many of the common fatty acids. In this alga saturated, unsaturated and poly unsaturated fatty acids are presents. This proves that *Selenastrum bibraianum* Reinsch, is an ideal organism for biodiesel production.

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