Extraction and Characterization of Biodiesel from Sargassum Whitti Brown Algae from Karwar region

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Abstract

Biodiesel, which can also be known as fatty acid methyl ester (FAME), is produced from transesterification of vegetable oils or animal fats with the addition of methanol. Biodiesel is quite similar to petroleum-derived diesel in its main characteristics such as cetane number, energy content, viscosity and phase changes. Biodiesel contains no petroleum products, but it is compatible with conventional diesel and can be blended in any proportion with fossil-based diesel to create a stable biodiesel blend. Therefore, biodiesel has become one of the most common biofuels in the world .This paper describes an approach to extract oil from marine algae Sargassum whitti collected from the coastal region of Karwar, Karnataka, India. and to convert it into biodiesel by using Bling and Dyer(1959). The amount of oil from this algal source varies from 0.5 to 5.0 wt % depending its types. This process yields 15-20% oil from this algae. Characterization of biodiesel shows that unsaturated fatty acid is more compared to saturated fatty acid

Key words: Biodiesel, Sargassum whitti, Transessterfication,

Inroduction:

Benthic marine macroalgae, commonly known as seaweeds are multicellular photosynthetic organisms with considerable potentials for using as a source of bioactive compounds of immense pharmaceutical and nutraceutical importance. They are rich sources of nutritionally beneficial components such as proteins, carbohydrates, polyunsaturated fatty acids (PUFAs), antioxidants, minerals, dietary fibers and vitamins (Chandini et al., 2008; Mohamed et al., 2011) and are thus consumed as functional foods. There are 250 macroalgal species commercially utilized

worldwide, of which 150 are consumed as human food (Barrow, 2007). The macroalgal species, in general, are low in lipids and contain 1–5% on dry wt. basis. Methods to convert biomass to competitive biofuels are increasingly attractive as fossil hydrocarbons are likely to become scarce and costly. Interest has now been diverted to the third-generation biomass like algae, since the first-generation feedstock (edible crops, sugars and starches) are under serious controversy considering the competition between food and fuel and the second-generation biomass (lignocellulosic biomass) are limited by the high cost for lignin removal. Algae is a very promising source for renewable energy, Nevertheless despite their obvious potential, there are no economically-viable commercial-scale quantities of fuel from either micro- or macroalgae .

Nevertheless, the nutritionally important C18and C20 PUFAs including n-3 PUFAs are present in substantially high amounts with anti-inflammatory, anti-thrombotic and antiarrhythmic responses (Kumari et al., 2010; Gillies et al., 2011). The n-3 PUFAs are of particular importance as they cannot be synthesized by humans and are thus obtained only through dietary sources. Furthermore, FA compositions of numerous macroalgae have been reported world-wide for their nutritional potential but their chemotaxonomic implications gained importance only in the last decade. Recently, Galloway et al. (2012) reported FA signatures of 40 temperate macrophytes (both seaweeds and sea grasses) from San Juan Archipelago, USA. But the diversity of macroalgal species investigated for FA composition is still low, approximated to be <200 (Gosch et al., 2012) which is minuscule against total estimated species of 9255, to date (Guiry and Guiry, 2012).

Moreover, it is evident from this study that macroalgae contained lipids as high as 5% on fresh weight basis that diminishes their prospects for biodiesel production. On the contrary, microalgae especially Chlorella, Botryococcus, Chaetoceros and Phaeodactylum are promising sources of biodiesel as they contain lipids more than 40% on dry weight basis (Becker, 2007; Ryckebosch et al., 2012; Yang et al., 2012). However, recently Gosch et al. (2012) studied the macroalgae of genus Dictyota, Spatoglossum, Derbesia and Caulerpa for lipids and reported a range from 10% to 12% on dry wt. basis that is quite comparable with those reported for several microalgal species such as Tetraselmis, Rhodomonas, Scendesmus and a few strains of Skeletonema and Isochrysis (Huerlimann et al., 2010; Mata et al., 2010), and thus emphasized the need for considering macroalgae as a promising resource for production of oil-based bioproducts. So here

we tried to extract biodiesel from Sargassum macro algae collected from karwar region, Karnataka, India.

Materials & Methods

Materials Required: Sargassum was collected from Karwar region of Karnataka, India, chosen on the basis of higher accessibility. It is found throughout the year but higher amount found from October to November. Total lipid extraction from algae performed by chemical extraction methods, relying on the chloroform–methanol solvent system, based on the Bligh and Dyer method (Bligh and Dyer 1959). Sodium chloride solution was added to facilitate oil extraction from algae (Axelsson and Gentili 2014). A slight modification of this process was done to facilitate the lipid extraction from Sargassum. Lipid found from this step was then used for the transesterification reaction to produce biodiesel.

Chemicals required

Chloroform (Loba Chem, Mumbai, India), Methanol (Merck chem Mumbai) and Sodium hydroxide (Merck, Mumbai, India) and Sodium chloride (Merck, Mumbai, India) used for the oil extraction and transesterification process.

Oil extraction:

Oil extraction and fatty acid methyl ester preparation were done following the method as described by Bligh and Dyer method (Bligh and Dyer 1959). 20g Algal Samples were crushed from their dried form using a glass homogenizer. The samples were placed into different small screw-cap test tubes. The Chloroform: Methanol (2:1 Ratio) solution was added to the tubes using the disposable glass pipettes. The test tube was capped and vigorously stirred for 5 minutes followed by a 2 minute stop and then stirred vigorously for 5 minutes. The test tube was kept in the test tube rack at room temperature overnight where the supernatant (lipids) would separate from the remaining residue of the algae.The residue once again re extracted Using chloroform and water in the ratio of 2:1. 2ml of sodium chloride is added to facilitate to complete extraction of lipid.

Biodiesel production: Alkaline catalyst sodium hydroxide, at an amount of 0.8 % of algal oil was dissolved in the methanol at an amount of 25 % of algal oil by hand shaking and whirling. Resulting sodium methoxide was then added to the preheated (at 60 °C) algal oil and air tight the reaction system to avoid the loss of methanol. Temperature was kept constant at 55–65 °C and heated for 3 h to complete the reaction. Once the reaction was completed, two major products exist-biodiesel and glycerin. Biodiesel was separated from glycerin by gravity settling as the glycerin was much denser than biodiesel, it settled down at the bottom. Separated biodiesel contained some soap and methanol. The methanol was removed by vaporization. After the methanol had been removed, the biodiesel was washed with distilled water by liquid–liquid extraction process to remove the soap, and catalyst. The washing procedure was repeated for 3–4 times until the soap totally removed. Remaining water present in the biodiesel was removed by heating it at 100 °C for 10 min. Finally usable form of biodiesel was found (Mamun et al. 2013).

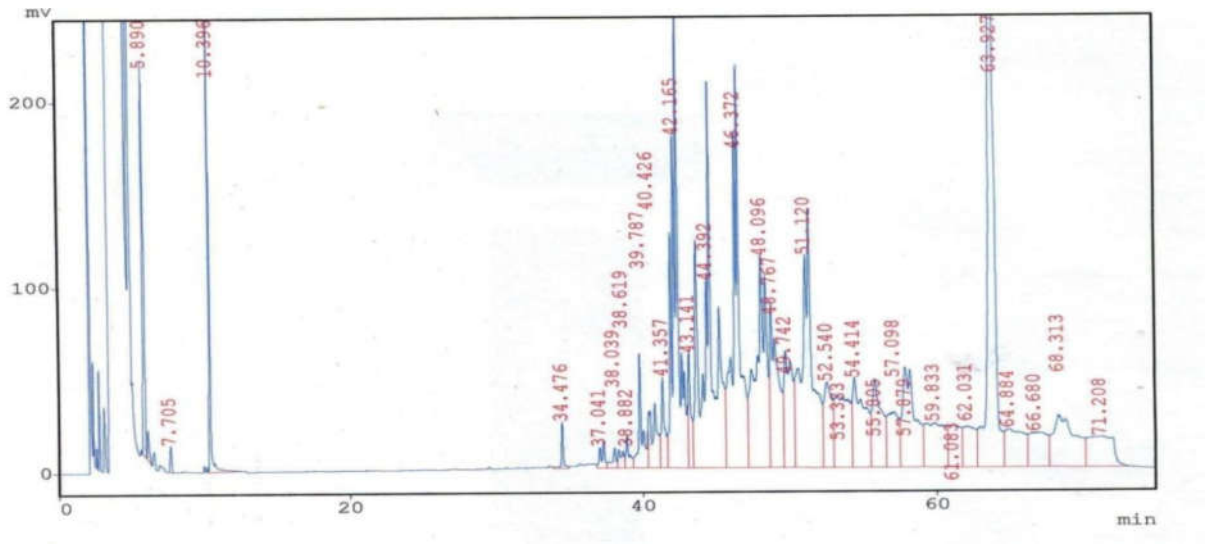
GC-MS of extracted lipid

The GC–MS analysis of FAME samples was carried out on a QP-2010 gas chromatography–mass spectrometer (GC-2010 coupled with GC–MS QP-2010) equipped with an auto sampler (AOC-5000) from Shimadzu (Japan) using a RTX-5 fused silica capillary column, 30 m _ 0.25 mm _ 0.25 lm (Rastek). Helium (99.9% purity)was used as the carrier gas with the column flow rate of1 ml/min and the pre-column pressure of 49.7 kPa. The column temperature regime was 40 _C for 3 min, followed by a 5 _C/min ramp up to 230 _C followed by 40 min at 230 _C. The injection volume and temperature was 0.2 ll and 240 _C and the split ratio was 1/30. The mass spectrometer was operated in electron compact mode with electron energy of 70 eV. Both the ion source temperature and the interface temperature were set at 200 _C. FAME peaks were identified by comparison of their retention times with authentic standards by GC–MS Post run analysis and quantified by area normalization.

Result and discussion

Lipid from SargassumWhitti was extracted by using chloroform, methanol and sodium hydroxide solvent. Total lipid content of Sargassum is 7.6 mg/g of dry weight. Fatty acids composition of algae lipids has varied widely with species, environmental condition, salinity, light, wave current and nutritional regime Fatty acids are most important components of lipids. The most frequent saturated fatty acid in plant and algal cells is the palmitic acid (16:0)

constituting 21−42% of total fatty acids (Anderson & Beardall 1991; Nelson *et al*. 2002; Harwood & Guschina 2009). In contrast to red and brown algae, low levels of C20 and C22 polyunsaturated fatty acids (PUFAs) are typical for green algae (Fleurence *et al*. 1994; Thompson 1996), which contain high levels of C16 and C18 PUFAs (Khotimchenko *et al*. 2002). In the brown alga *Saccharina japonica* the amount of PUFAs in different parts of the blade is approximately the same, but the level of n-6 PUFAs increases and n-3 PUFAs decreases towards the base of the blade (Khotimchenko & Kulikova 2000.) The fatty acids compositions of investigated algae Consistes of Saturated Fatty Acids (SAFs) and unsaturated fatty acids.The total sum of Unsaturated Fatty Acids (UFAs) range from 17.27- 37.70%. Palmitic acid (C16:0) is the major fatty acid which accounted nearly 45%.The saturated fatty acid content was higher than the unsaturated fatty acid content which is comparable with previous studies.For biofuel production, algae with a high proportion of saturated fatty acids are preferred as this leads to higher oxidative stability and higher ignition quality (cetane number), and produces an overall higher quality product (Hu et al. 2008; Knothe 2008).The amount of Monounsaturated Fatty Acids (MUFAs) also varied. The total MUFAs content for *S. wightii* was 18.36%. Debbarma reported that Oleic acid was the most dominant monounsaturated fatty acid (MUFA) in the seaweeds where as in this study Eladic Acid (2.34 and 4.66 %) was dominated as MUFAs.The important long chain Polyunsaturated Fatty Acids (PUFAs) such as docosahexanoic acid (DHA, C 22:6,n-3), linoleic acid (LA, C18:2, n-6) and arachidonic acid. Linoleic acid was the dominant PUFAs of this algae account for 6.22%.



Properties of biodiesel: The calorific value of algae biodiesel was determined by 'oxygen bomb calorimeter'. Kinematic viscosity and flash point were determined by 'SAYBOLT/REDWOOD viscometer bath' and 'flashpoint tester, type-00-ESR' respectively. Density was determined by 'mass/ volume' equation.

Important physical properties of algae biodiesel produced from Sargassum spp were determined and it was found that values were resided within the ASTM range of the physical properties of biodiesel presented in Table 2. The value of viscosity of algae biodiesel was 5.003 centistokes that reside within the American Society for Testing and Materials (ASTM) standard of biodiesel. Higher flash point and calorific value indicate the high quality biodiesel; flash point and calorific value were found 133 °C and 8655.106 kcal/kg correspondingly exist within the (ASTM) standard. Density value was also within the (ASTM) standard of biodiesel.

Table 2 Physical properties of algae biodiesel produced from Sargassum whitti

Properties	Algae biodiesel	Biodiesel (ASTM)
Viscosity	4.7 cst	3.5–5.5 cst
Flashpoint	125 °C	>130 °C
Calorific value	8855.106 kcal/kg	8860–9,400 kcal/kg
Density	0.75 g/ml	0.75 to 0.80 g/ml

Conclusion

Biodiesel was produced from macroalgae Sargassum to predict the best performing conditions for calorific value and yield of algae biodiesel. The production condition were chloroform, sodium chloride concentration and temperature. The Optimal condition of biodiesel production parameter was recognized to be 198 ml Chloroform with 0.75 % sodium chloride at 65 °C temperature where the calorific value of biodiesel was 8855.106 kcal/kg and yield 7.5 ml. This study signifies that the biodiesel produce from Sargassum could be used as a possible alternative fuel and further study will make it suitable for large scale production.

Acknowledgments
The authors express genius thanks to the department of Applied Botany Kuvempu university India for providing many inspiring and illuminating discussions and also grateful to Dr. T, Parameshwara naik. for his valuable comments and suggestions.

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