Synthesis and Characterization of Biodieselfrom Micro Algae (Oscillotoria Formosa)

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Abstract
Synthesis and characterization of biodiesel from algae oil (*Oscillotoria formosa*) were done to ascertain its potentials as an alternative to fossil fuel. The algae strain was identified cultured in an open pond system, harvested with the aid of a flocculant, sun dried and ground prior to the oil extraction. Solvent extraction (n-hexane) method was used to extract the oil from the algae biomass. The quality of the biodiesel was accessed by the following fuel parameters; flash point, specific gravity, pour point, cloud point, kinematic viscosity, cetane number, and pH value. The algae biodiesel had viscosity 5.87mm$^2/_s$, the specific gravity 0.884, flash point 118^{o}C, cloud point -6 ^{o}C, pour point -10 ^{o}C, cetane number 49, and pH 6.7. These values of the oscillotoria Formosa biodiesel fell within both European and American standards for 100% biodiesel except the viscosity which was slightly higher than the European standard but was within the American standard for 100% biodiesel. These showed that biodiesel from oscillotoria Formosa oil could serve as a good alternative to fossil fuel.
Keywords: biodiesel, algae oil, *Oscillotoria Formosa* and fuel.

1.0 INTRODUCTION

With the present energy consumption level, it is inculpable that fossil oil and gas will be exhausted in the next 40 to 60 years (Sayad, Ghatnekar, and Kavion, 2011). Over the past century, estimated levels of heat trapping gases mainly from green house gases have substantially elevated one to burning of fossil fuel with integration of deforestation.

Renewable energy plays a critical role in addressing issues of energy instability and security and climate change globally. (Jia, Xu,Zhang, Hu, Sommerfield, and Chen. 2010).

Biodiesel as an alternative fuel is increasingly attracting attention because its resource is infinite and its use is environmental friendly. (Michael 2009).

Currently, biodiesel is made from a variety of feed stocks, including vegetable oils, waste cooking oils and animal fats. However, the limited supply of these feed stocks impedes the further expansion of biodiesel production.

Algae are unicellular or multicellular organisms that photosynthesize, but lack the features such as leaves, roots, seeds and flowers of higher vascular plants: (Nabors, 2004). Generally, they have relatively simple structure, which may be a single cell, a filament of cells, a plate of cells or a solid body that may began to approach the complexity of a plant body. (Helena 1983). Micro algae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO_2, N, P, & K) that can produce lipids, protein, and carbohydrates in large amount over a short period of time. These products can be processed into both biofuel and valuable co-products (Brennan and Owende, 2010).

Micro algae have long been recognized as potentially good source for biodiesel production because of their high oil content and rapid biomass production. (Michael 2009).

Oscillotoria is a genus of filamentous cyanobacterium which is named for the oscillation in its movement. Filaments in the colonies can slide back forth against each other until the whole mass in reoriented to its light source, (Oscillotoria, Wikipedia, 2014). It is commonly found in watering-troughs waters and is mainly blue green or brown green. As an organism, it reproduces by fragmentation. It forms long filament of cells which can break into fragments called hormogonia; which subsequently grow in a new longer filament. (Oscillotoria, Wikipedia, 2014). Oscillotoria Formosa produces its food through photosynthesis. Each of the filaments is made up of rows of cells. Buba and Wum, 2008 reported that oscillotoria Sp. is the subject of research into the natural production of butylated hydroxy toluene (BHT) an antioxidant, food additive and industrial chemical.

First generation liquid biofuel such as corn based ethanol in US and sugar cane ethanol in Brazil have already been widely produced and published (Sims, Mabee, Saddler, Taylor, 2010). However, the mass production of first generation biofuel has resulted in series of problems, related to hike food prices, food shortage and land use. (Sims et al, 2010).

Much interest is focused on research based on production of energy from renewable sources like hydrocarbon by biomass (Sheelan, 1998). Most renewable sources that are utilized in biomass pyrolysis are being obtained via higher plants rather than micro algae.

Second generation biofuel derived from ligno cellulosic agricultural forest residues and from non-food crop feed stocks addresses some of the problems encountered in the first generation biofuel, however there is concern over competing land use or required land use changes. (Brennan et al 2010).

Third generation biofuel derived from micro algae are devoid of problems encountered in first and the second generations of biofuel. Micro algae can be grown in land that would not be considered suitable for the growth of the regularly used crops (Mata et al, 2009). Again, waste water that would normally hinder plant growth has been shown to be very effective in growing algae (Demirbas, 2011)

Biofuels are not only the best and reliably available fuels obtained from renewable sources, but they are pollution free and are abundantly available in locals, easily accessible and highly sustainable. (Demirbas 2008).

This paper reports the synthesis and characterization of biodiesel produced from micro algae (*Oscillotoria formosa*).

2.0 METHODS

Samples were collected from a garden in Onitsha, Anambra state, Nigeria, by carefully removing the hold fast from the substrate with fingers and were placed gently into containers with sufficient habitant water in order to maintain natural environment for the algae.

2.1 Identification of Algae Sample

The algae sample was shaken to suspend the sediment and then triplicate aliquots were removed and diluted with 100ml of sterile distilled water and was filtered using Millipore filters. The filters were placed into the petriplate containing BG-II medium. The plates were incubated for 15 days and were microscopically examined for the growth of the cultures.

2.2 Algae Culture

The algae biomass was cultured in an open pound in a medium of the following: NaCl (58.5g) $MgCl_2.6H_2O$ (1.5g), KNO_3 (1.0g), $Mg_8O_4.7H_2O$ (0.5g), KCl (0.2g), $CaCl_2.2H_2O$ (0.2g), $NaHCO_3$ (43mg), KH_2PO_4 (40.8mg), K_2HPO_4 (0.495g), $FeCl_3$ solution (1.0ml) and metal solution (1.0ml). The $FeCl_3$ solution consisted of the following (per litre), $FeCl_3$ (0.03g), EDTA.2Na (5.84g). The metal solution consisted of the following (per litre), H_3BO_4 (0.61g), $MnCl_2.4H_2O$ (23mg), $SO_4.7H_2O$ (87mg), $CuSO_4.7H_2O$ (0.06g), $(NH_4)_6$ $MO_7O_{24}.4H_2O$ (21mg), $COCl_2.5H_2O$ (15mg), EDTA.2NaCl (89g). The culture medium was carried out at 27^oC and pH of 7 and the algae was cultured for 2 months.

2.3 Harvesting/Drying/Oil Extraction

The algae biomass was harvested with the aid of a chemical flocculant; alum. The algal biomass was filtered with a clean white cloth, dried under sunlight for four days and was ground prior to oil extraction. The oil was extracted by soxhlet extractor using n-hexane as a solvent.

2.4 Methyl Ester (Biodiesel) Preparation

Methyl ester was prepared by a two-step transesterification reaction; acid esterification followed by base esterification as described by Hanny and Shizuko 2008.

2.5 Sample Analysis

The extracted oil and the methyl ester were analyzed for their physical and chemical fuel properties such as flash point, relative density, cetane number, fire point, cloud point, free fatty acids (FFA), pour point, acid value, iodine value, peroxide value, saponification value, viscosity colour, odour, solubility, and physical state at room temperature.

The iodine value, acid value, free fatty acid value, peroxide value, and saponification value were determined methods described by Usoro, et al 1992. The relative density, cetane number, flash point, fire point and viscosity were determined by methods described by Clark 1998 and ASTM 1985.

3.0 Results and Discussion

The analytical results of the algae oil and its methyl ester (biodiesel) are given in tables I and II respectively.

The colour of the algae (*Oscillotoria formosa*) oil was dark green (probably due to the pigment in algae biomass), the algae oil has a non irritating odor and was immiscible in water. The oil percentage yield of 28% compared favorably with some seed oil used as feed stock for biodiesel production, example soybean (14-25%) (Ajiwe et al 2000).

The results of the chemical parameters analyzed showed that alternative diesel oil could be obtained from algae (*Oscillotoria formosa*) oil. Compared with petroleum diesel, the biodiesel obtained from the algae had similar physio-chemical characteristics.

The relative density (0.884) *Oscillotoria formosa* biodiesel was in conformity with both ASTM and

European Union requirement. This showed that the biodiesel was not of aromatic or asphaltic fuel rather possessed good ignition property (Ajiwe et al 2006).

Cetane number is a measure of fuel delay of ignition time. The biodiesel had cetane number of 49 which was within the standard for 100% biodiesel by both American and European Union Standard. This implied a shorter delay time and complete combustion of fuel.

The flash point and fire points are often used to ascertain the flammability of a liquid fuel and the risk of fire outbreak during storage. Both values obtained were within acceptable limits, thus the use of the biodiesel from *Oscillotoria formosa* does not pose any fire risk.

The viscosity value showed that the biodiesel was sufficiently vicious and would be properly with mixed air in the combustion chamber of diesel engine (ASTM 1985).

The oxidative stability of the biodiesel was determined by its peroxide value, iodine value, acid value, and saponification values. Results obtained were all within the acceptable limits of European Union (EN14214) and American (ASTM) standards for 100% biodiesel.

4.0 Conclusion

The physiochemical parameters had shown that biodiesel produced from micro algae (*Oscillotoria formosa.*) could serve as an alternative fuel to petroleum diesel and its production could as well be commercialized.

Table I: Physical and chemical characteristics of *Oscillotoria Formosa* oil.

Properties	Values
Color	Green
Odor	Pleasant
Solubility	Immiscible with water
State at room temperature	Liquid
Acid value(mgNaOH/g)	7.580
Free fatty acid (%)	3.790
Iodine value (g/100g)	95.000
Saponification value(mgKOH)	123.000
Peroxide value (meq/kg)	3.200
Oil yield (%)	28.000

Table II. Comparative analysis of characteristics of *Oscillotoria Formosa* biodiesel (OFB) with American (ASTM) and European (EN) Standards.

Properties	OFB	Petroleum diesel	EN14214	ASTMD6751
Viscosity(mm^2/s)	5.870	1.200 to 4.000	3.500 to5.000	1.900 to 6.000
Specific gravity	0.884	0.840	0.870 to 0.900	0.870 to 0.900
Flash point(^oC)	118.000	60.000 to 80.000	>110.000	130.000
Fire point (^oC)	128.000	87.000	>120.000	-
Cloud point (^oC)	-6.000	-15.000to15.000	-3.000 to10.000	-3.000 to 12.000
Pour point (^oC)	-10.000	-35.000 to -15.000	-10.000 to 15.000	-15.000to 20.000
Free fatty acid (%)	0.230	-	0.500	0.500
Acid value(mgNaOH/g)	0.460	-	0.500	0.600
Cetane number	49.000	51.000	47.000 to 52.000	45.000 to 55.000
Peroxide value meq/kg	2.800.	-	5.000max	1.000 to 5.000

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