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Experimental Investigation on Production of Biodiesel from *Padina boergesenii* sp. Macro Algae

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Abstract

An alternative energy source to replace the use of non-sustainable and environmentally harmful fossil fuel which considered as main energy source worldwide and especially in the Middle East region. Therefore use of biofuels which extracted from different sources such as algae, corn oil, sunflowers seeds, palm oil and coconuts oil as energy source has been studied to use as an alternative of fossil fuel but the cost of biomass and availability are major concern. However, among sources of clean energy algae has been selected to be biodiesel is largest yield compared to other biofuel sources. The present study focused on *Padina boergesenii* macro algae to extract its oil content by using n-hexane as a solvent with biomass to solvent ratio of 1:4 and the oil yield was 65 from 70gm algae. The transesterification reaction for biodiesel by reacting extracted oil with methanol with use of KOH as a catalyst at a ratio of 1:4 oil to methanol ration, contact time of 30 minutes at 61oC about 18.7 ml biodiesel produced from 20 ml oil used by 93.5% conversion. The fuel properties of biodiesel was characterized through FTIR analysis and found to be similar chemical composition as to petroleum diesel. Also fuel properties of the synthesized biodiesel was studied through ASTM D6751 standard test and evaluation made was comparable to that of conventional fuel indicates that feasible production of commercial standard biodiesel was possible through methodology adopted in present study.

Keywords: *Padina boergesenii*; Biofuels; Macro Algae; FTIR; Transesterification

Introduction

In twenty-first century the environmental concerns and alarming energy crises are the major issues. To tackle with environmental concerns, the creation of a pollution-free green environment is the need of the hour. Simultaneously, energy is inevitable in today's global scenario as almost all activities are driven by energy [1]. Many sources and methods of energy generation have been/are being explored, and to date, it can be obtained from thermal, tidal, hydro, solar, mechanical, and nuclear power, or from fossil fuels. In fact, 85% of the energy, which we use, is obtained from fossil fuels, i.e., in the form of oil, coal, and natural gas, whereas renewable energy sources and nuclear power contributed only 13.5 and 6.5%, respectively, to the total energy needs in 2007 (Asif and Muneer, 2007). This situation has led us to solely depend on fossil fuels to sustain the energy requirements. However, an alarming concern is that the natural source of fossil fuel is finite and it is depleting very rapidly due to uncontrolled consumption, indicating the non-renewable nature of fossil fuels as energy sources [2].

Energy security has become a nationwide as well as a global issue, and a serious attempt is needed to search for viable alternatives in the form of renewable energy sources to meet the futuristic demand. Although prices are escalating, fossil fuels are a major source for use in transport and other sectors, and aside from this, they emit large amounts of carbon and hence have become a major cause of global warming [3]. Against this backdrop, there is the pressing need to search for non-edible and eco-friendly alternative sources that paved the path for the emergence of the so-called second and third-generation biofuels. Second generation biofuels are derived from any renewable feedstock other than edible feedstock sources, and the third-generation biofuels particularly emphasize the use of microorganisms. The prime scope of this present work is to find an alternate of fossil fuel by producing a biodiesel from algae and study best-operating conditions which give maximum yield fuel. This study concerned in finding cost-effective alternate biofuel which could be achieved by producing environmentally friendly biodiesel from algae which are not exploited in Oman thus this study aimed to get use of widely existing algae and find clean energy source which could replace the non-sustainable fossil fuel.

Experimental Procedure

Collection and preparation of the raw material and classification of seaweeds

The experiments were carried out in different places which are, the laboratory of ministry of defence (MOD), the Chemical laboratory of Caledonian College for Engineering, in the laboratory Marine Sciences & Fisheries/Centre Ministry of Agriculture & Fisheries and as well as Petroleum Development Oman is the main oil exploration and production company in the Sultanate of Oman [4].

Padina boergesenii macro algae species of selected macro algae were collected from several places in Oman and the pre-treatment process has been carried out by cleaning, washing with double distilled water, dried under sunlight for 48hrs and grinding into 0.2mm pieces

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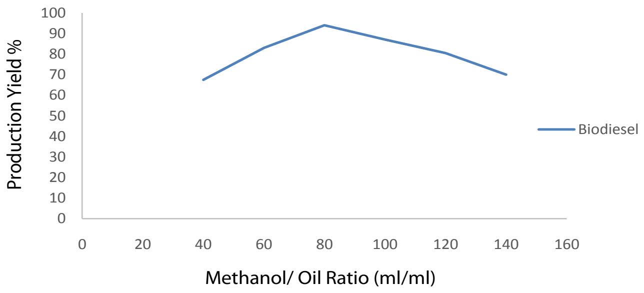

Figure 1: Effect of methanol to oil ratio changes on the production yield.

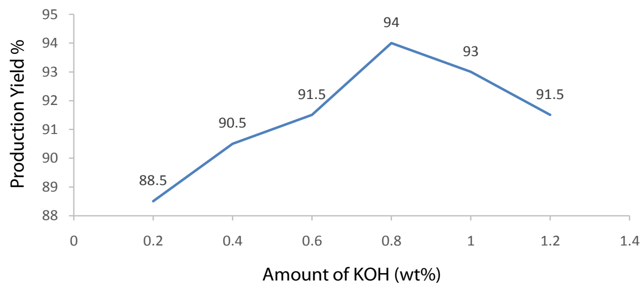

Figure 2: Effect of KOH percentage changes on the production yield.

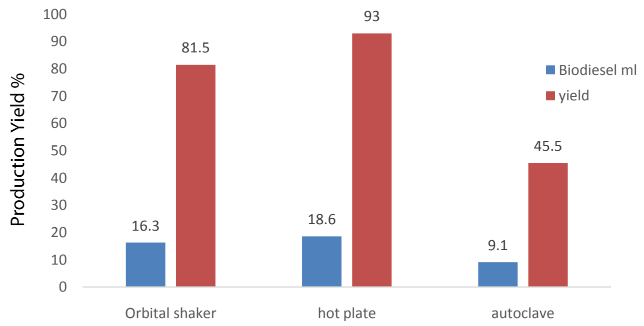

Figure 3: Effect of different process on the biodiesel production yield.

for the experimental work. The following stages has been carried out during the experimentation

- The collected fresh macro algal *Padina boergesenii* macro algae are brought to the laboratory of Ministry of Defence, Oman; first washed with seawater carefully, and then the epiphytes were taken away from the algae biomass manually by gloves separately. The fresh algal biomass has a lot of impurities stick in it, also sand particles, and other microbes that in the seawater.
- Therefore, biomass were intensely washed with running water and followed by distilled water to take out the contaminants from it. Further, the washed seaweed biomass was dried under the sun in open place for 48 hours.
- Then dried macro algae samples were grinded finely for a short period of 15s, then the fine powder was passed through a 500-µm sieve to remove the large particles.
- To get very small size particles which will have a physical contact with the solvent used for extraction? However, the reaction and the contact between the solvent and the powder algae will be faster to extract the oil from the algae as the size of the algae is very small.

Chemical treatment of algae with n-hexane using soxhlet extraction

The method carried out for oil extraction from algae species was Soxhlet apparatus using n-hexane as solvent. The weight of extracted oils was gravimetrically determined and used to calculate the extraction yield, as shown in Equation (1).

$$\%yield = \frac{(extract\ weight\ of\ oil)}{(sample\ dry\ weight)} * 100 \quad \text{---------------------- (1)}$$

70gm of powder algae of *Padina boergesenii* macro algae was placed in thimble of Soxhlet and this thimble made from filter paper. 400ml of n-hexane solvent was taken to extract the oil in the bottom flask and maintained at the boiling point of n-hexane at 68^{0}C. Then hexane was vaporized and it reaches the top layer of the thimble through the sidearm. The condensation arrangement liquefies the n-hexane vapor and reacts with the algae powder rupturing the cell wall. The expelled liquid mixes with n-hexane forming a colloidal solution and then is brought down to the conical flask at the bottom. And the way was repeated 10 to 15 times which collected the oil from the powder algae into the conical flask (H. Venkatesan, 2017). At the end of extraction the oil dissolved in n-hexane was separated by distillation column at boiling point of n-hexane at 68^{o}C [5-8].

Preparation of catalyst sample for biodiesel production

The extracted Algae oil from *Padina boergesenii* macro algae was collected and converted into biodiesel and approximately 20mg of oil was measured and was taken in a beaker. Methanol (CH_3OH) and potassium hydroxide (KOH) were taken in another beaker; it was measured with proper ratio and dissolved by heating with a stirrer to produced methoxide. Then the methoxide was added with the oil that which extracted from the previous step and followed three different processes- heating with stirring, Orbital shaker and Auto Clave. After the reaction completed the solution was kept in separating funnel for 24h to settle down the biodiesel and glycerin into different layers as shown in figure (a). After 24 hour was completed a heavy layer of glycerin was observed at the bottom and the second layer was biodiesel. The glycerin layer was poured into a small beaker and biodiesel layer remained in separating funnel for purification. The remaining biodiesel layer was washed with about 5% or little more of hot water repeatedly until it becomes clean to remove the unreacted components with hot water repeatedly until it becomes clean and pH about 8.0 as shown in figure(c). The pure biodiesel was dried by heating & measured and stored in a glass bottle for the test.

Optimization of biodiesel production

In this process the parameters such as reaction temperature, reaction time, catalyst concentration, changing methanol to oil ratio, changing the percentage of KOH were studied to optimize the maximum yield quantity of algae [9].

Fourier transforms infrared spectroscopy

FTIR Analysis is an infrared spectroscopy method utilized to identify organic, polymeric, and inorganic materials and test was studied in PDO laboratory in Oman. The FTIR test utilizes infrared light to scan test samples and observe and monitor chemical properties [10]. In According to a Fourier Transform Infrared Spectroscopy (FTIR), 81001 Spectrophotometer the infrared spectra of the biodiesel samples were recorded. The transmittance range of scan was 370 to 4000 cm^{-1}.

Gas chromatography and mass spectrometry (GC-MS)

The Gas chromatography-mass spectrometry (GC-MS) is an analytical chemistry method in which combines the features of gas-chromatography with mass spectrometry to determine various substances within a test sample and analysis has been carried out at Ministry of Defence laboratory at Oman. Applications of GC-MS include fire investigation, environmental analysis, explosives investigation, and as well as identify the unknown samples (O. David Sparkman, 2011) [11].

Characterization of product composition

The present investigation cannot be stated viable unless the final product formed is compatible to that of commercial biodiesel available. Therefore, the final analysis that would be carried out for this experimental study would be to study the quality standard of the biodiesel formed and compare the results obtained to that of the conventional ones. In this concern, the American Society of Testing and Materials (ASTM D6751) standard test has been conducted to meet a set of standards mentioned primly in terms of density, kinematic viscosity, calorific values, flash point and acid value. All those properties were studied in the Chemical laboratory of Caledonian College for Engineering and Ministry of Defence laboratory [12].

Results and Discussion

Chemical treatment for algae with n-hexane using soxhlet extractor

In order to investigate the optimal quantity of algae oil, the first step in this study was to study the extracted oil from *Padina boergesenii* macro algae have high quantity of oil after extraction by Soxhlet extractor using hexane as solvent and separated by distillation column. The maximum optimal quantity oil was is *Padina boergesenii* macro algae and it was selected for conducting experiments of present study.

Optimization of experiment parameters

The yield of the product obtained from the experiment is a function of a number of factors such as change quantity of methanol used in reaction, amount of catalyst, time of contact and temperature [13].

The effect of quantity of methanol used in the reaction: In order to investigate the optimal quantity of methanol for the formation of biodiesel of the extracted oil from species of algae as an effect on the efficiency of the biodiesel as the variable volumes of methanol used in the reaction, the reaction was carried out, in the first step, for each 20ml of algal oil, amount of methanol changed such as 40ml, 60ml, 80ml, 100ml, 120ml, 140ml and constant volume of catalyst (KOH) 0.2gm whereas, the reaction time was 30minutes, 150rpm and pH between 7 to 8 were maintained. The transesterification reaction was carried out at 55^{0}C for each amount of methanol changed. It was observed that 94% biodiesel with methanol to oil ratio at 4: 1 and

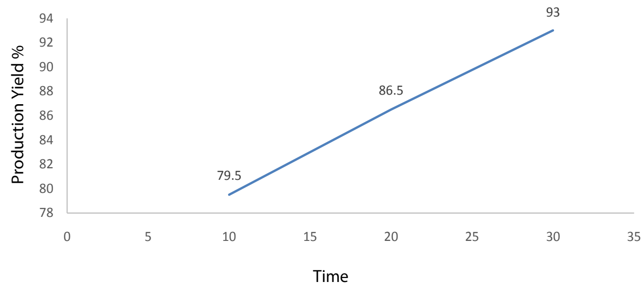

Figure 4: Effect of time variation on the biodiesel production yield.

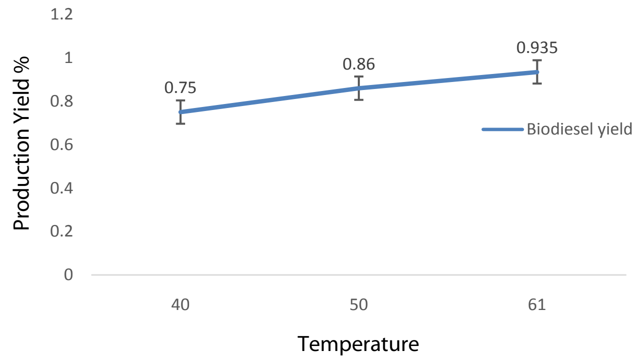

Figure 5: Effect of temperature change on the biodiesel production yield.

other parameters constant has shown in figure 1.It was observed that the maximum oil yield was obtained in the 80ml of the methanol volume has shown in figure 1. It was shown that maximum biodiesel 18.8gm that is 94% produced in methanol to oil ratio at 4: 1 and other parameters are constant [14]. Whereas, increasing the amount of methanol 140 to the weight of the algae oil shown in a reduction of the reaction rate. This effect can be summarized by the concept that as methanol concentration or as the quantity of methanol increases from 60ml to 120ml both the transesterification rate as well as the yield of final product increases from 83% to 94%, while further increasing the methanol volume to 140 results in a decrease of both the reaction rate and oil yield. This effect was explained as the transesterification being an equilibrium reaction requires high methanol quantity to shift the equilibrium towards the direction of the product whereas increasing the methanol quantity beyond optimal creates a dilution effect which disturbs the triglyceride structure resulting in formation of monoglyceride from triglyceride of algae oil which increases the solubility of glycerol in final product forming soap effect. Thus the separation of biodiesel from glycerol becomes difficult in presence of excess methanol and hence methanol quantity 80ml was considered to be optimal yield for the biodiesel yield [15].

The effect of concentration of catalyst (KOH) on the production yield of biodiesel: In order to find out the direction of possible difference on the production of biodiesel yield formed as an effect of the catalyst amount used, reaction was carried out using the catalyst (KOH) with methanol at 55^{o}C for 30min. 20ml of algae oil and 80ml of methanol were taken for all samples and the transesterification reaction was carried out at 55^{o}C for six different amounts of catalyst (KOH) such as 0.2, 0.4, 0.6, 0.8,1 and 1.2ml taken for the reaction respectively [16]. The similar procedure of this stage

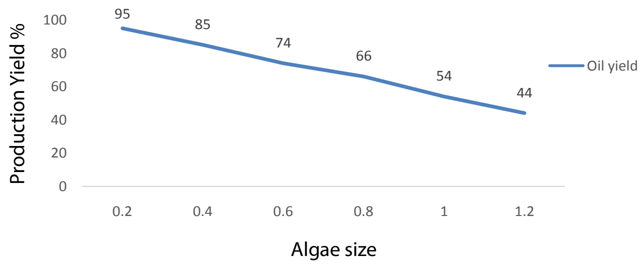

Figure 6: Effect of the algal biomass size variation on the biodiesel yield.

of the experiment was accordance to Mohd. A et al. 2015 and the rate of reaction represented in figure 2.

From the figure 2 it was noticed that the optimum oil yield was 94% and also shown the effect of concentration of KOH on the production yield of biodiesel at CH_3OH to Oil ratio of 4:1 and other parameter remains same [17]. The reaction yields were optimum, when the KOH ranges from 0.6wt% to 1.2wt%.

Effect of different production process on the production yield of biodiesel: From figure 3, it was studied that the production of biodiesel in three methods such as an orbital shaker, hot plate and autoclave. The reaction was carried out for each 20ml of algae oil using of constant amount of the catalyst (KOH) which was 0.2gm as well as a constant amount of methanol 80ml at different temperatures such as 25, 55, 130°C in 30min for the orbital shaker, hot plate, and autoclave respectively. It was observed that the maximum oil yield was obtained in the hot plate was 93% and this is because of temperature and continuous stirring [18]. Whilst in the autoclave had the lowest yield of 45.5% due to the evaporation of methanol at 130°C.

Effect of reaction time on the production yield of biodiesel: From figure 4 it was observed that the higher the reaction time resulted in increasing the yield of biodiesel. The reaction time for present study was varied between 10 to 30 minutes [19]. It was observed that the yield increases as the reactants spend more time in the reacting vessel. The higher yield (93%) was obtained at 30 minutes of reaction time.

Effect of temperature on the production of the biodiesel: The temperature is the important factor which plays a significant role in biodiesel conversion process. Therefore, in this study different temperature such as 40, 50, 61°C were investigated to improve the extraction. It was observed that reaction temperature was one of the factors effects on amount of biodiesel production. The temperature varied from 40 to 61°C and the reaction was carried out near the boiling point of methanol under atmospheric conditions with the condition of other parameters constant. From the figure 5 it was observed that higher temperature favors the biodiesel production. The present study focused the maximum biodiesel produced 93.5 % at 61°C and similar study has been reported by Denery et al. 2004 at 55° C gave good extraction yield at high temperature [20-23].

However, solvent based lipid extraction helps to reduce high temperature and it gives better yield at low temperature ranges 40 to 61°C. It was also observed that the temperature will enhance the solvents to dissolve in to lipid and supports to release the lipid the content from the biomass which is in agreement with study has done by Denery et al., 2004.

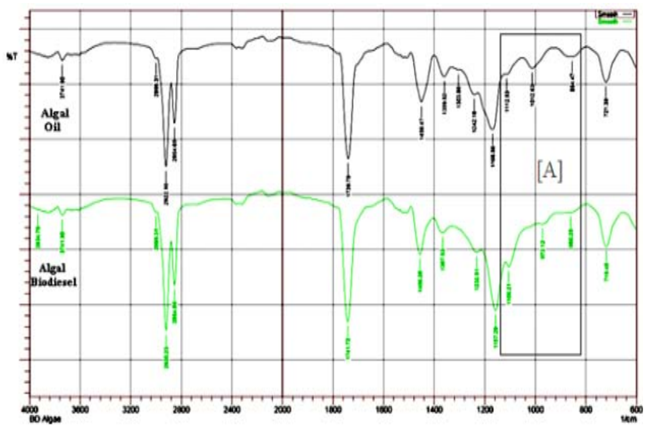

Figure 7: FTIR spectrum of algae oil and algae biodiesel.

Effect of the algal biomass size on the production of the biodiesel: Another known factor lead to affects the amount of oil extracted and that was the size of algal biomass affects the amount of oil extracted. In this experiment, All parameters were constant other procedures were carried out in similar manner as written before the only changed was the size of the algae such as 0.2, 0.4, 0.6, 0.8, 1, 1.2 at 55°C and optimum yield was in 0.2mm of the size of algae. From the figure 6 it was observed that the relationship between the size of biomass and oil yield inversely proportional which was mean that, as the size of biomass decreases, the oil yield increases and when the size of algae increases, the oil yield decreases. This can be justified by the improved interaction between the algae species and solvent, due to the larger surface area of smaller algal species. The smaller sized particles have a good interaction with solvent as compared to large particles and thus enhance the yield [24-27]. This similar study has been reported by Ihsanullah et al. 2015.

Fourier transform infrared spectroscopy (FTIR): In order to figure out the chemical composites of biodiesel algae product, FTIR test was performed and results are shown in Figure 7. The FTIR spectrum of both conventional diesel and biodiesel obviously shows the similar absorption band in the area of 2850~3000 cm^{-1} and 1350~1480 cm^{-1} due to C-H stretching vibration, which indicates the identical functional group of alkane in their molecular structures. However, FTIR spectrum of biodiesel shows new two absorption bands in region of 1670~1820 cm^{-1} and 1000~1300 cm^{-1}. These absorption bands are due to the C=O and C-O stretching vibration in ester which led to prove the presence of oxygen in biodiesel [28-30].

However, there was no absorption peaks appeared in these regions for petroleum diesel. This result gave further evidence of oxygen molecule in the biodiesel product. This result is in line with the experimental data obtained by Dumas and Miller, 2003. The macro algae sample *Padina boergesenii* contain different functional groups such as alcohols, Phenols, Carboxylic acid, alkanes, α, β- unsaturated aldehydes, ketones, Nitro compounds, alkanes, alcohols, carboxylic acids, esters, ethers, alkenes, alkyl halides. These are depends on the stretch bonds orders and frequencies and characteristic functional groups contributing to the formation of absorption bands at specific wave-numbers. In response to Cd stress, it was general decrease in

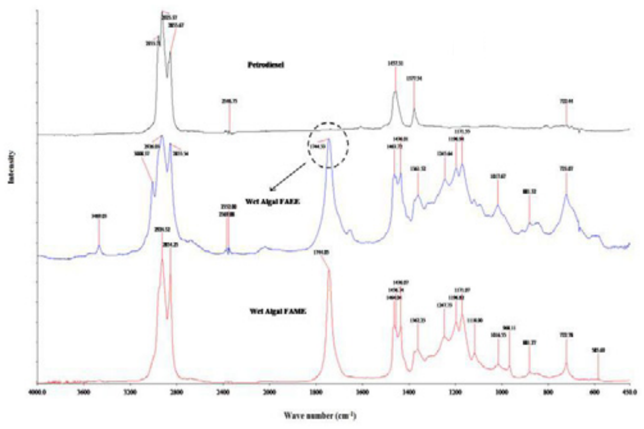

Figure 8: FTIR spectra of petroleum diesel, algal FAEE and algal FAME.

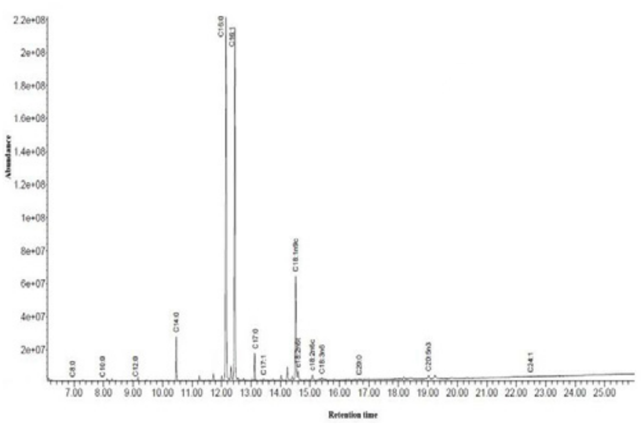

Figure 9: GC chromatogram of crude biodiesel result of Padina boergesenii biodiesel in POD, Oman.

the protein and carbohydrate content indicated by a decrease in the intensity of absorption bands especially in the 1800 to 800 cm^{-1} region [31-32].

From the figure 8 it was noticed that the absorbance spectra of FAEE are shown along with petroleum diesel and algal FAME. The intense C=O stretching band of ester appears around 1743 cm^{-1} for algal FAEE and algal FAME which is not observed in petroleum diesel spectra. The detailed functional group of *Padina boergesenii* was shown in Table 1.

Analysis of fatty acid methyl esters (FAME) by gas chromatography and mass spectrometry: The GC-MS analysis of the macro algae sample *Padina boergesenii* showed the presence of acid Eicosapentaenoic acid and Docosahexaenoic acid which confirms the presence of omega 3 fatty acids in these compounds and also other common fatty acids found, such as cis-9, 12-Octadecadienoic acid and hexadecanoic acid was shown in Table 2.

From figure 9 it was noticed that GC chromatogram data of experimental run carried at 265°C for 20 minutes with 1:9 algae/methanol reveals the presence of a major proportion of saturated and mono unsaturated fatty acid ethyl esters. Depending on reaction conditions the major constituents found were tetradecanoic acid ethyl ester (C14:0) 2-5%, Hexadecanoic acid ethyl ester (C16:0) 26-45%, Hexadecenoic acid ethyl ester (C16:1) 25-38%, octadecanoic acid ethyl ester (C18:0) 1-2%, oleic acid ethyl ester (C18:1) 9-13%, Eicosapentaenoic acid ethyl ester (C20:5) 1.2-5.1%. As the temperature increased it was observed that the percentage of C20:5 present in the crude biodiesel was decreased nearly to 1.2% at 265°C which is a good indication for fuel properties [33].

Table 1: FTIR functional group of *Padina boergesenii*.

Frequency	Bond	Functional Group
1480	N-O asymmetric stretch	Nitro compounds
3424	O-H stretch, H- bonded	Alcohols, Phenols
1032	C-O stretch	Alcohols, carboxylic acids, esters, ethers
3173	O-H stretch	Carboxylic acid
653	C-H bend	Alkenes
2920	2C-H	Stretch Alkanes
1694	C-O stretch	α, β- unsaturated aldehydes, ketones
1351	C-H rock	Alkanes
1101	C-O stretch	Alcohols, carboxylic acids, esters, ethers
602	C-Br stretch	Alkyl halides

Table 2: GC-MS analysis of Fatty acid compounds of *Padina boergesenii* sp.

Retention Time	Fatty Acids	Molecular formula	Molecular weight
14.12	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302.451
16.34	cis-9,12-Octadecadienoic acid	$C_{18}H_32O_2$	280.446
17.45	Docosahexaenoic acid	$C_{22}H_{32}O_2$	328.488
19.78	hexadecanoic acid	C16H32O2	256.424

Table 3: Comparison of fuel properties of algal biodiesel and conventional diesel.

Fuel property	Biodiesel	Diesel
Heating Value (MJ/Kg)	38.88	43.37
Density, kg/m^3	0.879	0.88
TAN (mg/gm KOH)	0.218	0.12
Viscosity (cSt)	4.2	~4.0
Flash point °C	144	60~80
Sulphur content, wt %	0	0.05 Max
Cetane Number	66	40~55

ASTM standard test of synthesized biodiesel: The results obtained through subjecting the product biodiesel to ASTM D6751 standard test and it was compared with conventional diesel and the methodology explained in the experimental section were shown in Table 3. Calorific Value for the biodiesel and Acid value were 44.75(kJ/kg) and 0.31 respectively [34-36].

The heating value of biodiesel was measured and shown in Table 3. The heating value of biodiesel blend was decreases with the increase of biodiesel percentage. However, TAN, Viscosity and specific gravity were compatible to petroleum diesel. The higher cetane will increase the fuel combustion efficiency. The Sulphur content was zero that is good sign for environmental concern. The higher flash point 144°C is safe uses of biodiesel and the similar study was carried out by Ashok Kumar et al. 2017.

Conclusion

The detailed investigation on an alternative energy source to replace of non-sustainable and environmentally harmful fossil fuel has been carried out during the experimental study. The *Padina* sp. proves that a better choice for biodiesel production than other species such as *Codium* sp. The use of various equipment to enhance the conversion process has also led to variation in conversion percentage as that hot plate at 55°C gave 93% conversion, orbital shaker at 25°C gave 81.5% conversion and autoclave at 130°C gave 45.5% conversion only. Furthermore, variation of time affect the reaction and increased conversion from 79.5% to 93% when time changed from 10 min to 30 min. Similarly, the increase in temperature led to increase in conversion from 75% to 93.5% when temperature changed from 40°C to 61°C respectively. The GC chromatogram data of experimental run carried at 265°C for 20 minutes with 1:9 algae/methanol reveals the presence of a major proportion of saturated and mono unsaturated fatty acid ethyl esters. Depending upon the reaction conditions the major constituents found were tetradecanoic acid ethyl ester (C14:0) 2-5%, Hexadecanoic acid ethyl ester (C16:0) 26-45%, Hexadecenoic acid ethyl ester (C16:1) 25-38%, octadecanoic acid ethyl ester (C18:0) 1-2%, oleic acid ethyl ester (C18:1) 9-13%, Eicosapentaenoic acid ethyl ester (C20:5) 1.2-5.1%. As temperature increased it was observed that percentage of C20:5 present in the crude biodiesel was decreased nearly to 1.2% at 265°C which is a good indication for fuel properties. The FTIR spectra of petroleum diesel of algal FAEE and algal FAME were performed. The intense C=O stretching band of ester appears around 1743 cm^{-1} for algal FAEE and algal FAME which is not observed in petroleum diesel spectra. The produced oil was subjected to ASTM D6751 standard test and its properties were accessed and found to be 879 kg/m^3 of density, 4.2 (mm^2/sec) of kinematic viscosity, 4.75(kJ/kg) of calorific value, 144°C flash point and acid value of 0.31. The evaluated values of all of the parameters were compared with conventional diesel and found to be within the standard range of values set for conventional biodiesel. The cetane number for biodiesel was 66 and for conventional diesel was from 40 to 55 so as per the literatures review the higher cetane will increase the fuel combustion efficiency. The Sulphur content was zero its good for environment while for conventional diesel was 0.05. Thus algae were used as a feedstock of renewable source of energy to replace the existing energy sources of fossil fuel and also to reduce pollution.

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