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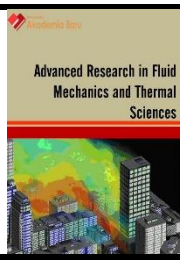

A Brief Study on Bio-Diesel Production using Oleaginous Hydrophytes and Waste Cooking Oil with Physico-Chemical Characterization

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ABSTRACT

Salvinia Molesta is commonly found aquatic weed (hydrophyte) which is low in lignin and contains high amounts of cellulose and hemicellulose with lipid content on the surface of the leaves to achieve non-wetting phenomenon. The current work looks at assessing the feasibility of an aquatic plant *Salvinia Molesta* in the production of bio-diesel. The study consisted of carrying out the culturing of the plant under different aqueous conditions for maximum yield, extraction of the leaf, petioles, and stalks for lipid extraction followed by the lipid analysis through FFA (Free Fatty Acid). The culturing of the plant provided insights into the space requirement, salinity, and exposure to sunlight. The lipid yield from the dried plant was low and showed an FFA value of 4.2% which necessitated the blending of the lipid extract with waste vegetable oils (Plant lipid content 5%-10% by volume) during the transesterification reaction to yield biodiesel. The thermo-physical characterization of this bio-diesel and benchmarking with High-Speed Diesel has been carried out.

1. Introduction

Diesel is one of the most principal fuel used in developing countries. These fossil fuel-based reserves are gradually nearing completion in the midst of increasing consumption and global climate changes[1,2]. In addition to their limited supplies, chemically synthesized fuels are not feasible for long term applications as they are generated through an expensive process, resulting in greenhouse gas (GHG) emissions threatening environmental pollution, generate radioactive wastes[3]. Therefore, there is a steady increase in research for finding new sources of biological fuels that are cost-effective, carbon-neutral (without any toxic effluents), more compatible with modern transportation engines, easily available and can be used in combinations with other fuels [4]. In addition to this, fuels like biodiesel have higher combustion efficiency owing to their higher cetane number [5,6], reduce the overdependence on petroleum and encourage the application of natural

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products. Bio-diesel can be produced from animal fats/ plant lipids, vegetative oils, tallow, lard and waste oils as raw materials [7]. The lipids extracted possess a very high viscosity restricting their flow characteristics. Hence Transesterification facilitates reduction in the viscosity of the parent oils comparable to that of commercial high-speed diesel oil [8]. The catalysts used for Transesterification can be alkaline or acidic, with alkaline catalyzed methods offering economic viability[9]. Biodiesel belongs to the class of fatty acid methyl or ethyl esters obtained by transesterification reaction of complex lipids (triglycerides) and is the principal energy fuel for automotive purposes (aircraft, cars, military vehicles) [10], cleaning of oil spills[11], heating oils and generators [1]. Based on their source, biodiesel is categorized into- i) first generation (edible plant foods such as sugarcane, soybean), ii) second generation (waste feedstocks such as wheat, corn, switchgrass as well as animal fats), iii) third generation (lignocellulosic microalgae, weeds) and iv) fourth-generation (genetically engineered microorganisms for improved fuel efficiency) [12]. There have been extensive reports on biodiesel produced from different sources as seen in- soya [13], rapeseed [3], Jatropha [14], waste catfish oil [7,15], microalgae [5,13,16], flax and genetically engineered microorganisms [17]. Production of biodiesel involves cultivation, harvesting, and pretreatment of raw material followed by extraction of lipids (triglycerides), conversion of lipids to fatty acid methyl esters (FAME) and purification of fuel [18]. There are different strategies used to derive oil which include-1) mechanical extraction using screw press, 2) organic solvent, 3) ionic, 4) osmotic solutions, 5) supercritical carbon dioxide, 6) ultrasound and 7) microwave radiation [19]. Srinivas *et al.,* [20] have used waste vegetable oil as the raw material for production of biodiesel with ethyl hexyl nitrate is added as an additive. Jaspreet Singh *et al.,* [21] have opined that usage of 0.5 % KOH during the transesterification reaction, the biodiesel yield could be maximized. Studies on the water kelp *Laminaria digitate* identified the fatty acids as acetic, propionic and butyric acids which are the intermediate of the acidogenic and acetogenic stages [22].

In addition to the well-known method of transesterification, biodiesel is also produced by various techniques such as- a) dilution (direct mixing of vegetable oils with commercial diesel), b) microemulsion- colloidal mix of two immiscible liquids, c) Pyrolysis (anaerobic decomposition of organic matter in the presence of a catalyst [23], d) ultrasound and non-catalytic processes involving supercritical methanol at high temperatures, pressure, and e) BIOX co-solvent methods [8,24].

Transesterification reaction in biodiesel production involves the conversion of triglycerides in the presence of alcohol and catalyzing agent (acid, alkali, enzyme, ionic liquids and bi-functional catalysts) into glycerol and esters of fatty acid [5]. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel and crude glycerol [3]. In most production methanol or ethanol is the alcohol used to respectively produce methyl or ethyl esters. Transesterification can be performed by three processes- a) chemical method, b) enzymatic and c) microbial [5]. The efficiency of this process is dependent on several parameters such as reaction temperature, concentrations of alcohol and oil, presence of catalyst, time and presence of moisture and free fatty acids[14]. Acidic catalyzed reactions involving sulfuric, hydrochloric or phosphoric acid are helpful in the conversion of lipids with high free fatty acids (FFA) content. They result in direct conversion of free fatty acids without saponification. Alkali mediated transesterification using sodium or potassium hydroxide is the most commonly used method for the production of biodiesel. This method results in yield of esters with good purity [25]. Lipases catalyze specific conversion of triglycerides to give high quantity biodiesel at moderate temperatures of 30 to 50 °C. In this process, it is easier to separate the glycerol from the final product. Ionic liquids are reusable organic chemicals that speed up the process of lipid conversion as seen in case of Fe_3O_4/ Poly (styrene-methacrylic acid) [26]. Bi-functional catalysts are amphoteric compounds that convert free fatty acids to biodiesel [27]. The final biodiesel usually contains good proportion of glycerol which needs to be purified by either of several well-established

methods- i) washing with warm/normal water and acids, ii) dry washing in solid adsorbent (resin) and iii) membrane reactors.

There have been several studies on the application of biodiesel in compression ignition engines either as a standalone fuel or a blending option with high-speed diesel [2,6,20].

Salvinia molesta commonly known as "Kariba Weed" or "Giant Salvinia" after it infested a large portion of the reservoir of the same name, is an aquatic fern, native to south-eastern Brazil. It is a free-floating plant that does not attach to the soil (Figure 1) but instead remains buoyant on the surface of a body of water[28]. The fronds are 0.5–4 cm long and broad, with a bristly surface caused by the hair-like strands that join at the end to form eggbeater shapes. It prefers nutrient-rich waters such as those found in eutrophic water or those polluted by wastewater. It copes well with dewatering, and while it prefers to grow in moderate temperatures, it will tolerate low or very high temperatures and hence a tropical aquaphyte[29]. Freshwater is better than saline water for nurturing these hydrophytes as the elevated presence of sodium and chloride concentrations in salty water will negatively influence growth and lipid metabolism in the host plant [5]. These metal ions inhibit the growth of shoots and leaves due to external osmotic pressure and toxic elemental accumulation. High salinity also results in increased nitrogen which inhibits triacylcerol (TAG) production [7].

Fig. 1. *Salvinia molesta* hydrophyte

In the current work, *Salvinia molesta* was used as the algal feedstock for the extraction of lipids owing to the faster growth potential and oleaginous. The hydrophyte was cultivated in-house under different conditions to study the growth, harvested and then used for the lipid extraction. The extracted lipid was subjected to the transesterification reaction to produce the bio-diesel. Physico-chemical analysis and performance analysis of the biodiesel was carried out and compared with that of high-speed diesel oil.

2. Methodology

2.1 Cultivation

Salvina molesta (an aquatic weed) was grown in spacious circular vessels filled with freshwater respectively under sunlight for a duration of two months as seen in (Figure 2). The aquatic weed selected in this study is phototrophic as it effectively utilized sunlight and available carbon dioxide present in the surrounding environment as a nutrient source for growth and photosynthesis. Their growth was monitored periodically. The fern was found to fully mature after 2 weeks and the vegetative cover area was doubled after 4 weeks indicating the fast-growing nature of the aquatic fern. The presence of nutrients (carbon and nitrogen) and environmental factors such as aeration, sunlight (for photosynthesis) and water salinity (pH) play a major role in aquatic plant growth and

subsequent FFA production [30]. This form of autotrophic nutrition in the presence of sunlight and good aeration is reported to have significantly increase FFA concentration as seen in case of *Jatropha* [14].

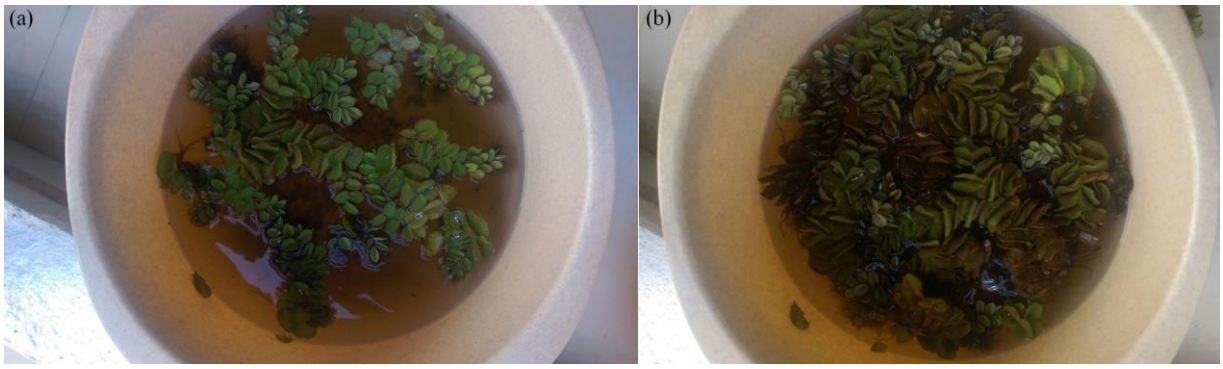

Fig. 2. Cultivation of *Salvinia molesta* (a) After 2 weeks (b) After 4 weeks

2.2 Harvesting and Pretreatment

Fully matured pods were harvested (Initial weight 1200 g) and splashed with saline water for the removal of contaminants. From each pod, the stem, petioles and leaves were selectively separated. The separated substrates were then placed in a drying oven at 45°C for 3 hours to remove moisture. The dried substrates were then powdered and sieved to yield fine particle size of about 1 mm and final weight of 180 g (Figure 3(a)).

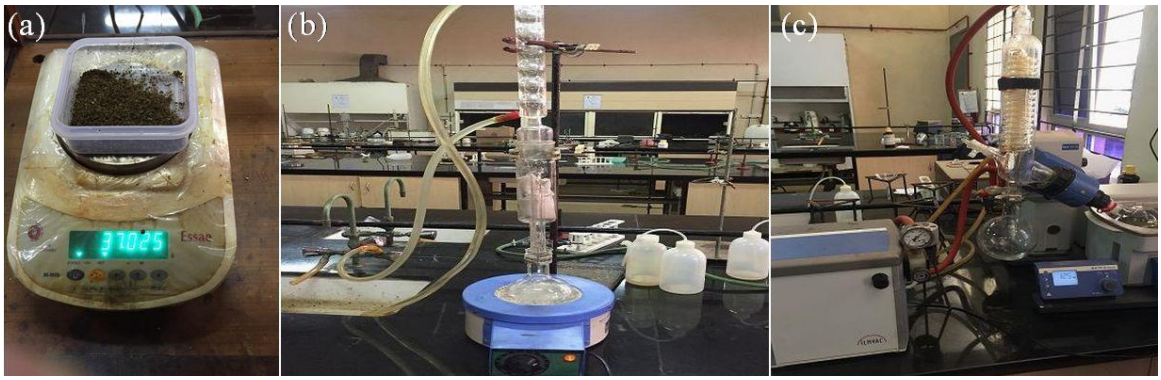

Fig. 3. (a) Biomass particles (b) Lipid Extraction in Soxhlet Apparatus (c) Separation of lipid from Hexane

2.3 Lipid Extraction

Lipids are extracted from the weed by hexane treatment similar to (*Konga et al.,* 2016). For this, the leaves were crushed into fine particles and taken in an airtight container for Soxhlet's method. The concentrated mass (180 g) was transferred to a thimble of the Soxhlet extractor (Figure 3(b)). 300 mL of hexane (solvent) was taken in a round-bottomed distillation flask placed on heating mantle and connected to a reflux condenser at the top. The solvent was heated for 4 hours and the generated vapors were allowed to condense in the reflux and the extracted lipids dissolved into the solvent. The solution containing hexane and lipids is separated by distillation using a rotary evaporator (Figure 3(c)). Hexane with a lower boiling separates out from the lipid-containing extract under low pressure and is collected in a separate flask. The FFA (free fatty acid) content of extracted lipid (~20 g with specific gravity 0.73) was analyzed.

2.4 FFA Estimation using Titration

The lipid extracted from rotary distillation was subjected to acid-base titration using phenolphthalein to determine its concentration in percentage. 1 g of lipid was taken with 50 mL ethanol in a round-bottom flask and 3 drops of phenolphthalein were added. 0.1 N KOH was slowly added through titration until the coloration of the solution turned light pink (Figure 4). The procedure was repeated to obtain the average acid value. The acid value (mass of KOH required to completely neutralize 1 g of substance) of FFA was calculated following the EN-14104 standard given by Eq.(1) based on the normality of the alkali 'N_{KOH}' , Volume of the alkali 'V_{KOH}' and the mass of the lipid 'W_{lipid}'

Fig. 4. Assessment of FFA of the extracted lipid

$$AN = \frac{(56.1 \times N_{KOH} \times V_{KOH})}{W_{lipid}} \quad (1)$$

$$FFA = \frac{AN}{2} \quad (2)$$

Eq. (2) gives the value of the Free fatty acid content (%). Since the lipid content obtained from the hydrophytes was of less quantity than that required for the different tests, blending with waste cooking oil was carried out by varying the lipid content between 5%-10% by volume. Waste cooking oil is an ideal blending choice, Table 1 shows the comparison between the physicochemical properties of high-speed diesel and waste cooking oil. The blend with 5 vol. % was termed L5 and that with 10 vol. % was termed L10 for distinction. The acid values were re-determined for each of these blends.

2.5 Transesterification and Biodiesel Recovery

Each blend was transferred to a three-necked round-bottomed flask. 0.9 % KOH and 34.475 mL of methanol were added to it. The contents were initially preheated at 100°C to remove any moisture present in the flask. The catalytic reaction was allowed to take place for 1 h at 60 °C (Figure 5 (a)). At the end of the reaction (Figure 5(c)), there were two separate layers with the upper one containing biodiesel and lower one made up of glycerol, methanol respectively. The solution was transferred to a decantation flask and allowed to settle down for 1-2 hrs. The glycerol and methanol content settled

down at the bottom and was collected in a separate flask (Figure 5 (c)). The biodiesel for each blend was obtained and subjected to physiochemical analysis.

Table 1
Comparison of the physical and chemical properties of high-speed diesel and waste cooking oil [21]

Properties	ASTM D6751-02	High-speed Diesel	Waste Cooking Oil
Density(kg/m^3)	~800	850	875.8
Kinematic viscosity (centistokes)	1.9-6.0	2.049	4.336
Flash point (ºC)	>130	78	154
Fire point (ºC)	>53	83	160
Calorific value(kJ/kg)	>33000	42000	38896.2
Carbon residue(%)	<0.05	0.0214	0.0179
Ash Content (%)	0.02% max	0.02	0.02

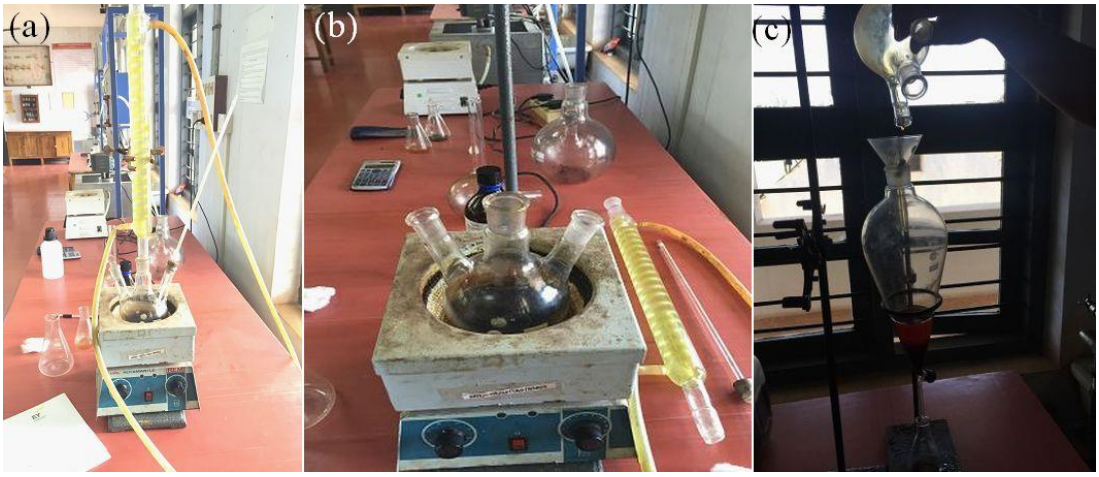

Fig. 5. (a) Transesterifcation reaction (b) Completed reaction (c) Separation of biodiesel from glycerol

2.6 Physiochemical Analysis

The viscosities, flash, fire points and calorific values of commercial high-speed diesel and produced biodiesel from L5 and L10 blends were found out using Redwood viscometer, Cleveland flash, and fire point apparatus and Bomb calorimeter respectively (Figure 6).

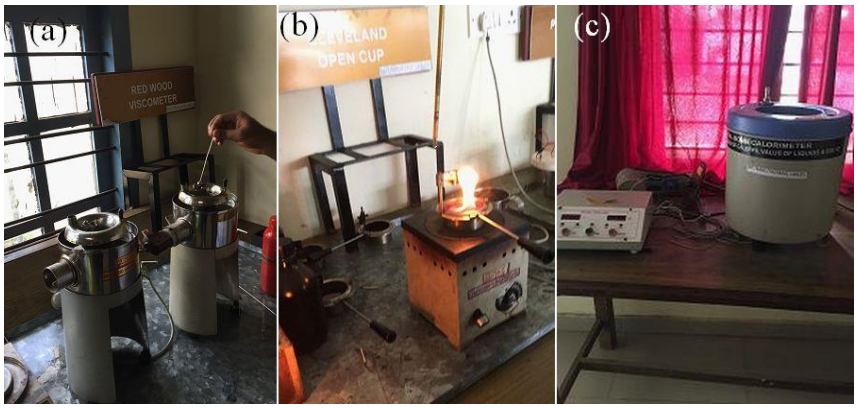

Fig. 6. (a) Red Wood Viscometer (b) Flash/Fire Point testing (c) Bomb Calorimeter

3. Results

3.1 FFA Analysis

Table 2 shows the FFA analysis of the lipid and the L5, L10 blends. The lipid can be subjected to transesterification provided the FFA is closed to 1 %. In this case, the FFA values of the L5 and L10 blends satisfied the condition and were therefore subjected to the transesterification reaction.

Table 2
FFA Analysis of the raw extracted lipid and the blends

Parameters	Lipid	L5	L10
Acid Value (g)	8.415	2.042	1.874
FFA (%)	4.207	1.021	0.937

3.2 Physico-Chemical Analysis

Figure 7 shows the products of the transesterification reaction. The glycerol was separated from the biodiesel (ester) and the biodiesel was decanted into a conical flask for the physicochemical experiments.

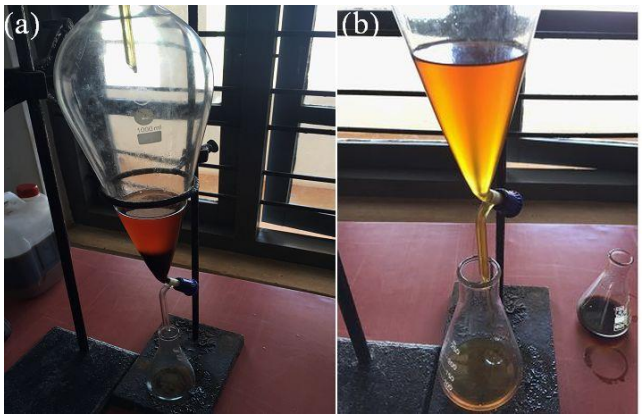

Fig. 7. (a) Mixture of ester and glycerol (b) Separated biodiesel

Table 3 shows the physical and chemical properties of biodiesel blends in comparison with that of high-speed diesel. The density of the L10 blend was higher than that of diesel by 6% while L5 blend was 4% denser. The kinematic viscosity, as well as dynamic viscosity of the blends, was ~55-70% higher than that of diesel for both the blends. The Flash Point and Fire Point of the biodiesel blends were almost 2 times that of the high-speed diesel which shows the significant drop in the volatility which offered an advantage from the safety standpoint of storage and transportation [31]. As per the Bio-diesel standard EN 14214 [31,32], the flash point and the viscosity values of the L5 and L10 blends are in good agreement with the specifications. The drop in the calorific value was 17% and 11% for L5 and L10 blends respectively. The diminish in the properties could be attributed to the purity of the methyl ester phases and the type of glycerides corresponding to the fatty ester types in the waste cooking oil and lipid blends[33–35].

Table 3
Experimental values of the physical and chemical properties of high-speed diesel and biodiesel blends

Properties	High-speed Diesel	Biodiesel (L5)	Biodiesel (L10)
Density (g/cc)	0.84	0.87	0.89
Kinematic Viscosity @ 36ºC (centistokes)	2.146	3.22	3.62
Absolute Viscosity @ 36ºC (kg/ms)	0.18	0.28	0.32
Flash Point (ºC)	87	157	174
Fire Point (ºC)	91	162	181
Calorific Value (kJ/kg)	43140	35780	37290

4. Conclusions

The oleaginous hydrophyte *Salvinia molesta* was successfully cultivated and harvested for lipid extraction. The FFA content of 4.21 necessitated blending of the extracted lipids with waste cooking oil. The calorific value of the L10 blend was found to match that of the high-speed diesel, but higher viscosity and volatility of the L10 blend could be advantageous for blending with high-speed diesel from the safety standpoint of storage, transportation and handling. The study showed that aqueous ferns and hydrophytes which are otherwise an ecological menace could be harvested and utilized in biodiesel production.

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