PRODUCTION OF BIODIESEL FROM FRESH WATER ALGAE AND COMPARISON OF ALGAL BIODIESEL WITH STANDARD BIODIESEL AND DIESEL

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Abstract: Biodiesel is Biodegradable, Non toxic, less CO2, NOx emission. Continuous use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of Carbon dioxide in the environment. Biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Unfortunately, Biodiesel from oil crops, waste cooking oil animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Algae have emerged as one of the most promising sources for Biodiesel production. It can be inferred that Algae grown in CO2 enriched air can be converted to oily substances. Such an approach can contribute to solve major problems of air pollution resulting from CO2 evolution and future crisis due to a shortage of energy sources. In the current study, Algal species were collected from Fresh water Algae from Bam Khadi at Shanker talav, Dungri, Valsad. In the first step oil from Algae species was extracted using n-hexane and Di-ethyl ether and the mixture of both as Solvents, while in the second stage extracted oil was converted into biodiesel via Transesterification reaction. This study was undertaken to know the proper transesterification, amount of biodiesel production and physical properties of biodiesel.

IndexTerms- Biodiesel, renewable energy, Algal oil, biomass, transesterification, glycerol

I. INTRODUCTION

Increasing population and industrialization has created serious problems of energy requirement. The current scenario of consumption of fuel has led to a situation that will be no oil reserves beyond 2050. Though, oil remains may be in a surplus amount, environmental pollution inclusive of CO2, emission could be a jeopardizing effect globally, which might lead to climatic change (Yen et al., 2013). The petroleum reserves are highly concentrated in certain regions of the world, therefore those countries not having these resources are facing energy/foreign exchange crisis, mainly due to import of crude petroleum. It is predicted that 45% of the total energy requirements would be fulfilled by oil and gas which has a vital role in satisfying energy needs of the world (Khan et al., 2009). The petroleum reserves are highly concentrated in certain regions of the world, therefore those countries not having these resources are facing energy/foreign exchange crisis, mainly due to import of crude petroleum (Bisen et al., 2010).

Petroleum product consumption is increasing day-by-day because of tremendous increase in vehicle on road. Thus hydrocarbon consumption increase the environmental pollution. Fossil fuel depletion causes increase in diesel demand and uncertainly of its availability is considered as an important factor, which has triggered research towards searches for alternative energy (can be supplemented or replaced for fossil fuels). Petroleum diesel combustion is a major source of green house gas (GHG). Apart from these emissions, petroleum diesel is also major source of other air contaminants including NOx, SOx, CO, particulate matter and volatile organic compounds. As biodiesel production and use increase, new feed stocks are being developed and may soon be introduced into the market. Some examples include pennycress, camelina, cuphea, brown grease, and various strains of algae. Biodiesel is a non-toxic and biodegradable alternative fuel derived from renewable sources (Hossain et al., 2008).

Biodiesel is the mono-alkyl esters of long chain fatty acids, which is derived from transesterification of biological matter. It is an excellent renewable and safe alternative fuel with environment friendly nature (Patil et al., 2011). Biodiesel production from renewable sources can also boost farming and fuel production industries (Xue et al., 2006). The algae are now becoming the main source of biofuel production in the world. They are considered as the safer, non-competitive & rapidly growing organisms among those could be used for biodiesel production. They have the abilities to grow without much care on waste nutrients (Robert, 2013), and are considered the better source of biodiesel production as other sources can cause food problems as they are mainly including those plants which are used for food (Patil et al., 2008). Sources of commercial biodiesel include oil from waste cooking, corn, palm, animal fat, canola and jatropha. However, using plant oil for biodiesel production is not only controversial but also requires substantial quantity of land (Lee et al., 2011). Studies showed that tobacco seeds can also be used for biodiesel production (Veljkovic et al., 2006). The main advantages of biodiesel, other than being a renewable energy source, is that its burning is much cleaner than that of fossil fuel, and it can be used in the present diesel engines without modifications. Algae are one of the most exciting future solutions for our energy crisis, especially that of transportation fuel (Schenk, 2008). Algae need very low requirements to grow including carbon dioxide, sun light and water (Schenk, 2008).

Algae grow very fast and have lipid content higher. They have short generation time, i.e., they can double their mass every few hours (Schenk, 2008). Biodiesel can be produced through direct transesterification of algal biomass or by a two-step process which lipids are
extracted, collected, and transesterified (Johnson and Wen, 2009). Either process requires lipid extraction using combinations of solvents and alcohols, such as chloroform/methanol, hexane/isopropanol, or petroleum ethers and methanol (Johnson and Wen, 2009; Mulbrey et al., 2009). The direct method is advantageous, because it combines lipid extraction and transesterification into one process, making it less time-consuming than extraction transesterification processes (Johnson and Wen, 2009).

However, Johnson and Wen (2009) discovered that biodiesel yield from wet biomass processed using direct transesterification was significantly less that obtained from dry biomass, indicating that biomass drying is required for this process. In contrast, the two-step extraction transesterification process yielded similar results for both wet and dry biomass (Johnson and Wen, 2009). Energy–efficient and inexpensive lipid extraction and transesterification processes also are needed for algal biodiesel to be cost-competitive with petroleum fuels. Several studies have suggested that biogas generated from anaerobically digested algal cells following lipid extraction can be used to offset energy demands from algal biodiesel production processes (Chisti, 2007, 2008a, 2008b; Sialve et al., 2009).

However, the effects that extraction solvents will have on the digester microbial community are unknown. Toxicity issues might be avoided using environmentally friendly, solvent-free algal biodiesel processes suggested by Amin et al. (2009) and Xiong et al. (2009). These techniques use osmotic shock to rupture algal cells, enabling oil fractionation, while enzymes from Candida sp. are used for transesterification.

II. MEDIA
Reagent for oil extraction
- n-hexane
- Di-ethyl ether
Reagent for transesterification
- NaOH
- Methanol

III. RESEARCH METHODOLOGY
Collection of sample
Algae was primarily collected from Bam khadi at Shanker talav (Dungri), Valsad. In Collection of Algae was done by hands in clean plastic containers in large quantity.

Identification of Algae
Samples of Algae which were collected from Fresh water, underwent microscopic examination for the identification by using wet mount preparation techniques. Algae were identified according to their morphological characters by using as reference Bold and Wynne (1978) classification.

Drying of Algae
To make this process eco-friendly & economically cheap, collected Algal sample were divided to form small chunks & partially dewatered manually by pressing. Further samples were kept in the sun on the roof top for 3-5 days for complete drying at 25-28°C.

Grinding of Algae
Dried Algal biomass was grind to powder by using mortar and pestle. Further grinded Algal powder was pass through a sieve of 4mm diameter, from which the oversized particles were removed to obtain fine powder. Oversized particles were again grind to powder & sieved to obtained desired fine powder.

Extraction of oil from Algae
Solvents are used for extraction of oil from the obtained Algal powder. Oil from Algae was extracted by using n-hexane and Di-ethyl ether solvents. 50g Algal powder collected from fresh water mixed with 200 ml solvents. Solvents used were n-hexane, Di-ethyl ether and their mixture. Then above mixture were kept at room temperature for 24 hours.

Oil separation from Biomass
A layer of oil was formed on the surface of solvent, which was separated from the biomass using a funnel separator.

Evaporation
The extracted oil was separated from solvent by evaporation process in hot air oven at 60°C for 10-15 minutes.

Mixing of catalyst & methanol
5ml of 1N NaOH (catalyst) was mixed with 50ml of methanol. The mixture obtained was vortex for 5-10 minutes for proper mixing.

Biodiesel production
The mixture of catalyst & methanol was poured into the Algal oil in a conical flask. The above mixture was kept in waterbath at 60°C for 10-15 minutes. In this incubation the Transesterification reaction was carried out which formed two different layers. Top layer was of biodiesel where as bottom layer was formed of Glycerol. The reaction process is known as transesterification.
CH₂-OCOCR₁  |  CH₂-OH  |  R₁-COOCH₃  
|----------------------|-----------|-------------------|
CH-OCOR₂  +  3 HOCH₃  |  CH₂-CH₂OH  +  R₂-COOCH₃  
|----------------------|-----------|-------------------|
CH₂-OCOR₃  |  CH₂-OH  |  R₃-COOCH₃  

Separation of biodiesel
Flask separator was filled with mixture and was allow to kept for around 16 hours to settle the impurities and separate the Biodiesel and Glycerol layer. The top clear layer obtained was Biodiesel while as bottom layer obtained was Glycerol and impurities which was separated with help of flask separator.

IV. RESULTS AND DISCUSSION

4.1 Identification of Algal species
Microscopic identification of the obtained samples was indicated that the presence of different Algae species, which morphologically similar to *Spirogyra sp.* and *Oscillatoria sp.* from fresh water sample.

Table 1: Morphological characteristics of Algae by Microscopic Examination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Morphological characteristics</th>
<th>Name of Algae</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water Algae</td>
<td>Long, filamentous, multicellular green algae.</td>
<td><em>Spirogyra sp.</em></td>
<td><img src="image_url" alt="Spirogyra" /></td>
</tr>
<tr>
<td>Fresh water Algae</td>
<td>It forms long filaments of cells which can break into fragments.</td>
<td><em>Oscillatoria sp.</em></td>
<td><img src="image_url" alt="Oscillatoria" /></td>
</tr>
</tbody>
</table>

4.2 Drying and Grinding of Algae
Algae were subjected to sundry for complete removal of water for around 3-5 days. The samples were placed on plastic sheet and direct sun exposure was provided during day time while in evening samples were kept in shade. The dried samples were grinded with mortar and pestle, and samples were passed through sieve of 4 mm diameter.
4.3 Oil Extraction from Algal species

Oil extracted from Algae by using Solvents n-hexane and Di-ethyl ether. Algae powder was mixed with solvent and their mixture and kept at room temperature for 24 hours. After 24 hours layer of oil was formed on solvent surface which was separated through flask separator. Oil extracted from Fresh water Algae by using different solvent and their combination gave different amount of oil which were given bellow.

Table 2: Oil Extracted using different solvent combination

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Algal sample</th>
<th>Solvent used</th>
<th>Extracted oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantity</td>
<td>Place</td>
<td>n-hexane</td>
</tr>
<tr>
<td>1.</td>
<td>50 g</td>
<td>Fresh water</td>
<td>200 ml</td>
</tr>
<tr>
<td>2.</td>
<td>50 g</td>
<td>Fresh water</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>50 g</td>
<td>Fresh water</td>
<td>-</td>
</tr>
</tbody>
</table>

4.4 Biodiesel production through transesterification

The mixture of NaOH (catalyst) and Methanol were poured into Algal oil so transesterification reactions occur. The above mixture was kept in waterbath at 60°C for 10-15 minutes which formed two different layers of Biodiesel and Glycerol, which was separated through flask separator. Top layer formed of Biodiesel and bottom layer was of Glycerol. Different layers of Biodiesel and Glycerol shown bellow.
Table 3: Observing different layers of biodiesel and Glycerol

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Place</th>
<th>Solvent used</th>
<th>Observation of layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fresh water</td>
<td>n-hexane</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Fresh water</td>
<td>di-ethyl ether</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Fresh water</td>
<td>n-hexane + di-ethyl ether</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Biodiesel production from algae using different solvent

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Place</th>
<th>n-hexane</th>
<th>Di-ethyl ether</th>
<th>Biodiesel production (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fresh water</td>
<td>200 ml</td>
<td>-</td>
<td>46 ml</td>
</tr>
<tr>
<td>2.</td>
<td>Fresh water</td>
<td>-</td>
<td>200 ml</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Fresh water</td>
<td>100 ml</td>
<td>100 ml</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

Figure 2: Biodiesel production from different solvent mixture with Algae

Above graph shows the amount of Biodiesel produced by using different solvents. When using n-hexane it shows the highest amount of Biodiesel production.
Table 5: Comparative study of physical properties of biodiesel

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Properties</th>
<th>Standard Biodiesel</th>
<th>Petro-Diesel</th>
<th>Biodiesel from Fresh water Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yellow</td>
<td>White (transparent)</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td>n-hexane + Diethyl ether</td>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Color of Biodiesel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>pH of Biodiesel</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>3.</td>
<td>Flame test</td>
<td>Orange flame</td>
<td>Orange flame</td>
<td>Yellow flame</td>
</tr>
<tr>
<td>4.</td>
<td>Stability test</td>
<td>160 seconds</td>
<td>147 seconds</td>
<td>87 seconds</td>
</tr>
<tr>
<td>5.</td>
<td>Density (kg/m³)</td>
<td>797</td>
<td>802</td>
<td>679</td>
</tr>
<tr>
<td>6.</td>
<td>Kinematic viscosity (kg/m³)</td>
<td>2.38</td>
<td>2.35</td>
<td>0.91</td>
</tr>
<tr>
<td>7.</td>
<td>Specific gravity</td>
<td>-</td>
<td>-</td>
<td>0.85</td>
</tr>
<tr>
<td>8.</td>
<td>Cloud point (°C)</td>
<td>-4°C</td>
<td>-4°C</td>
<td>-4°C</td>
</tr>
</tbody>
</table>

IV. CONCLUSION

Fresh water Algae was successfully used as a raw material for production of biodiesel. It is the only renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum. Economics of producing microalgal biodiesel need to improve substantially to make it competitive with petro-diesel, but the level of improvement is necessary appears to be attainable. The process involved two steps e.g., oil extraction and transesterification. It was noted that the maximum amount of biodiesel was obtained from the algal biomass using n-hexane.

Algae are an economical choice for biodiesel production, Because of its availability and low cost. Our results prove that biodiesel can be produced from Fresh water Algae In this way alage can be used as renewable energy.

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REFERENCES
