Algal Biodiesel Production: Comparison, Characterization and Optimization of Various Extraction Processes

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Abstract- The paper presents the production of Algal biodiesel from three processes namely Flask- magnetic Stirrer set up, Soxhlet apparatus and Ultrasonication Technique. The algal sample is identified to be Rhizoclonium heiroglyphicum (C.A.Ag.). The extraction time in case of extraction using ultrasonication technique is minimum which is 50 min followed by 6 hrs in case of flask-magnetic stirrer set up and 12 hrs in case of extraction through soxhlet apparatus. Also the percentage recovery in case extraction through ultrasonication is maximum which is 56.2% followed by 51.43% (Flask magnetic stirrer set up) and 49.52% (soxhlet apparatus). Ultrasonication at 70% amplitude and at 0.7 cycle per sec for extraction time of 50 min proves to be superior among three processes in terms of reduction in time and percentage recovery. Also, FESEM of algae before and after extraction of algal oil has been analyzed.

Keywords Algae, Energy, Renewable Energy, Ultrasonication, Biodiesel, Bio-oil.

1. Introduction

There is a limited availability of non renewable energy sources in the world and also their exploration, processing and usage have an adverse impact on the environment.

Combustion of fossil fuels leads to carbon and GHG emission. The use of fossil fuels increases the pollution by emitting wide range of gaseous pollutants like NOX, SOX, CO, particulate matters and volatile organic compounds [1,2]. Reduction in the usage of fossil fuels as energy sources can considerably lessen the amounts of pollutants produced. This could be achieved either through using less energy or through replacing fossil fuels by something more renewable. Therefore, more emphasis is being given to use renewable instead of fossil fuels. Technology is being redefined to make this possible. In last few decades, lots of efforts have been made in the alternate energy sources production and Algae have come out to be of great interest. Recently, bio-fuel has been identified as an alternate renewable source of energy as they are biodegradable, renewable, non-toxic and significantly produces less emissions than petroleum based diesel upon combustion [3,4] Biodiesel can also be produced from different feedstock such as corn, canola, jatropha seeds, lignocellulosic biomass, soybean, palm, sunflower seeds and algae etc. [5] However, Algae is considered as one of the most promising non-food feedstocks for bio-fuel production because of their higher rate of productivity and high lipid content in some species [6].

Technologies based on algae as fuel could be used as a key tool to reduce GHG emissions from power plants based on coal and other carbon intensive industrial processes. Algae can also be used to produce methane, ethanol as well as syngas etc. Some algae can also produce hydrogen by photo synthesis [7]. Microalgae are fast growing with an appetite for carbon dioxide and have the capacity to produce more oil per acre as compared to any other biodiesel feedstock. Also, they can be grown on land which is not very suitable for food crops. Microalgae are unicellular

photosynthetic microorganisms, living in saline or fresh water environments that convert sunlight, water and carbon to algal biomass. They produce storage lipids in the form of TAGs. Rapid growth and high productivity are exhibited by many species whereas substantial quantities of lipids, often greater than 60% of their dry biomass are accumulated in many other species. Algae contain anywhere between 2% and 40% of lipids/oils by weight.

2. Method

2.1. Algae collection, pre-treatment and Identification

Algae were collected from a pond in Bhagwanpur, Roorkee, India in the month of August, 2015 using sieves in polythene bags wearing nitrile gloves. The algae were washed with water to remove insects, sand, pebbles, and other impurities. The algae were then preserved in 4% formaldehyde solution to prevent decaying. 4% formaldehyde solution was prepared using 10 ml of 37-41% of formaldehyde solution and 90ml distilled water. Also, authentication of algal sample was done to identify the presence of algal species in the biomass.

2.2. Lipid Content Estimation

The lipid content of algae was estimated using Bligh and Dyer estimation method [8]. For this, algae were dried by pressing and keeping it in the hot air oven. Then grinding of dried algae was done and one gram of dried algae was taken for the process. Also a mixture of 5 milliliter chloroform, 10 milliliter methanol and 5 milliliter distilled water was taken in a conical flask of 500 ml and blended. To this blended mixture, dried algae sample of 1 gram was added. The mixture was kept for 17 hrs continuously on a magnetic stirrer. After 17 hrs, the stirrer was shut off and the mixture was filtered by using whatman filter paper of pore size 11 micrometer and the filtrate was collected in a beaker and was left undisturbed for almost 24 hrs . After 24 hrs, there were two layers formed. The upper layer was of methanol and water which was discarded and the bottom layer was of lipid and chloroform. Chloroform was evaporated or distilled out by heating at 50 deg C [9]. The left over was the algal oil which is lipid It is then weighed and lipid estimation was done using Bligh and Dyer method by Eq. (1)

Lipid Content = (Mass of algal oil formed/ mass of dry powder algae sample) (1)

2.3. Oil Extraction using Flask Condenser setup

For extraction of algal oil from algae, hexane solvent extraction method was first used. 100 gram of this algal slurry was taken in a double neck conical flask. To this slurry, 200 milliliter of hexane was added and the flask was attached to the condenser. The condenser was attached with water inlet and outlet using rubber tubes. The setup was kept on a magnetic stirrer and was kept at 80 deg C as the boiling point of hexane is 68 Deg 0C and constant stirring was done using magnetic stirrer. The set up was kept on for around 6 hrs. After 6 hrs, the heater was shut off and the mixture was allowed to cool to room temperature. Then, the mixture was filled in centrifuge tubes and was centrifuged at around 9100 rpm and for 13 minutes.

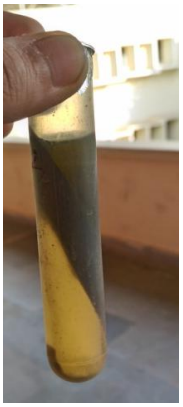

Fig. 1. Centrifuge tube showing three layers

Three layers were formed, the upper layer being of lipid and hexane, the middle layer was of algal biomass and the bottom layer was of water (as shown in the Fig. 1). The middle and bottom layer were discarded and the upper layer was collected and distillation was carried out to separate lipid from hexane.

2.4. Oil Extraction using Ultrasonication technique

For the extraction of algal oil in ultrasonic processor equipment, 100 grams of slurry was taken and to this slurry, 200 milliliter of hexane was added as a solvent. The beaker was then put in a ultrasonic processor equipment and the equipment was set for 70% amplitude and 0.7 cycle/sec and was kept for 50 minutes [10]. Mixture was allowed to cool to room temperature. Mixture was then centrifuged at around 9000 rpm for about 12 minutes. Lipid was finally separated as explained above [11]

2.5. Oil Extraction using Soxhlet Apparatus

Apparatus consisting of condenser , Soxhlet extractor of volume 100 milliliter and solvent flask of volume 250 milliliter, were cleaned with distilled water and dried at room temperature. Hexane was used as solvent. For every batch, 50 gram of the grinded algae biomass slurry was poured on the whatman filter paper of pore size 11 micrometer, which was at the bottom in soxhlet extractor. 100 milliliter of hexane was taken in flask and all three parts were compiled. Filter paper was used so as to prevent biomass slurry from flowing down into flask through siphon. Heater was started, heating round bottom flask and temperature of 100 deg C was maintained. Hexane after attaining its boiling point temperature evaporated to soxhlet extractor through pipe on the side of extractor part which got cooled in condenser and collected in extractor drop wise on algal slurry and lipid part is extracted out. When hexane in extractor part got filled up to certain height, hexane along with lipids siphoned out into flask at bottom through siphon. This cycle continuous and lipid got extracted. Extraction through this process was carried out in batches of 12 hours each and total 4 batches were carried out. In every batch, after 12 hours heater was

stopped and apparatus was allowed to cool at room temperature. Afterwards the double layered solution in flask was collected in conical flask and allowed to settle for 4 hours. Lipid was separated then by same procedure as explained above.

2.6. Transesterification of algal oil to biodiesel

Algal oil samples formed by above mentioned three processes were made to undergo transesterification process to obtain algal biodiesel. For this purpose basic catalytic transesterification process using methanol and sodium hydroxide (NaOH) was done because methanol is cheap and reason behind choosing sodium hydroxide was time factor. Basic catalytic conditions are favorable for reaction and time to achieve equilibrium is less as compared to acidic catalyst conditions. 100 milliliter of methanol was taken and 1 gram of sodium hydroxide pellets were added to this flask and stirred for an hour on magnetic stirrer keeping neck of flask sealed so that protonated catalyst and sodium methyl oxides are formed. Then 10 milliliter of algal oil is taken and 100 milliliter solution of methanol and sodium hydroxide prepared earlier is added to this flask. Condenser was fixed over flask and magnetic stirrer was started, maintaining temperature of 60 0C and stirrer was set ON. Stirring continued for 2 hours after which heater was closed and conical flask was removed from setup and left sealed and undisturbed for 20 hours for separation process. After 20 hours two separate layers were obtained. Upper layer observed is of methyl ester that is the required biodiesel and lower layer consists of glycerol and traces of sodium hydroxide and unused methyl oxides. Biodiesel obtained has impurities like methanol traces and soaps which were removed further. Methanol was vaporized and soap particles were removed by water washing. Water washing of biodiesel was carried out carefully while preventing formation of emulsion. Water washing was done multiple times till clear water was obtained after water washing. This process was carried out for all algal oil samples and hence biodiesel from three different processes was formed.

3. Results and Discussion

3.1. Algal species Identification and Lipid Content Estimation

The algal sample is identified to be Rhizoclonium heiroglyphicum (C.A.Ag.). It is a alga (forming dark green scum) belonging to class Chlorophyceae. Also, the lipid content was around 17.5%.

3.2. FESEM before and after extraction of algal oil

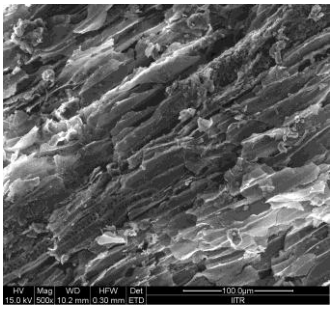

Fig. 2. FESEM of fresh algae

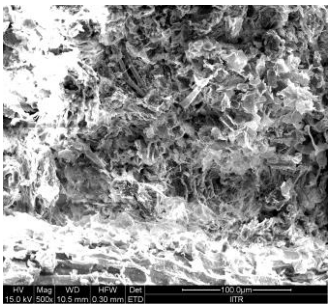

Fig. 3. FESEM of used algae- Flask Magnetic Stirrer setup

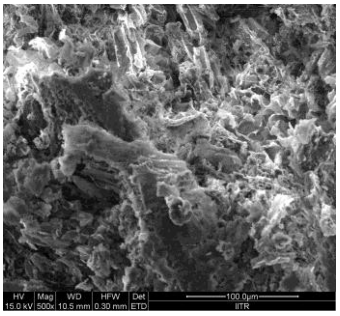

Fig. 4. FESEM of used algae- Ultrasonication

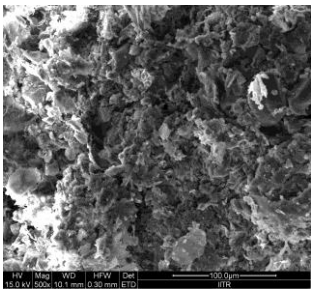

Fig. 5. FESEM of used algae- Soxhlet Apparatus

The FESEM results shows morphological changes in the algae before and after extraction of algal oil.

3.3. Comparison of extraction time and percentage recovery for three processes

Table 1. Comparison of extraction time

Oil Extraction Method	Algae slurry (Grams)	Water content 40% by weight (grams)	Algae content (grams)	Hexane taken (ml)	Solvent to biomass ratio	Extraction time
Soxhlet Apparatus	50	20	30	100	3	12 hrs
Flask-Magnetic stirrer set up	100	40	60	200	3	6 hrs
Ultrasonication	100	40	60	200	3	50 min

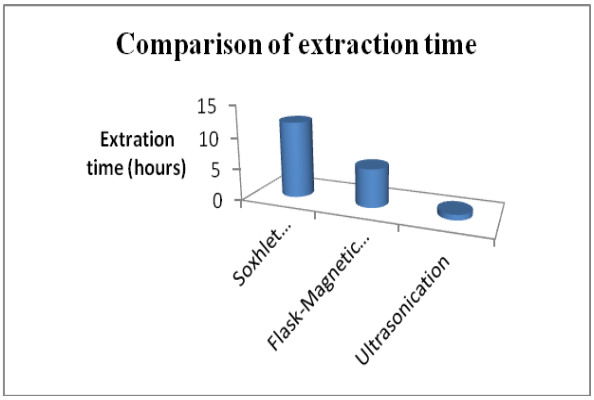

Fig. 6. Comparison of extraction time

The extraction time for the extraction of algal oil from algae is minimum in case of ultrasonication as compared to the other two methods as the high pressure cycles of the ultrasonic waves support the diffusion of solvents, such as hexane in our case into the cell structure. And it improves the mixing and increases the chemical reactivity of the reactant and hence reduces the time upto 93 percent as compared to soxhlet apparatus. [12]

Table 2. Comparison of recovery percentage

Oil Extraction Method	Algae content (grams)	Estimated Lipid (%)	Estimated Lipid Content	Bio-diesel formed (grams)	% recovery
Soxhlet Apparatus	30	17.5	5.25	2.60	49.52
Flask-Magnetic stirrer set up	60	17.5	10.5	5.4	51.43
Ultrasonication	60	17.5	10.5	5.9	56.2

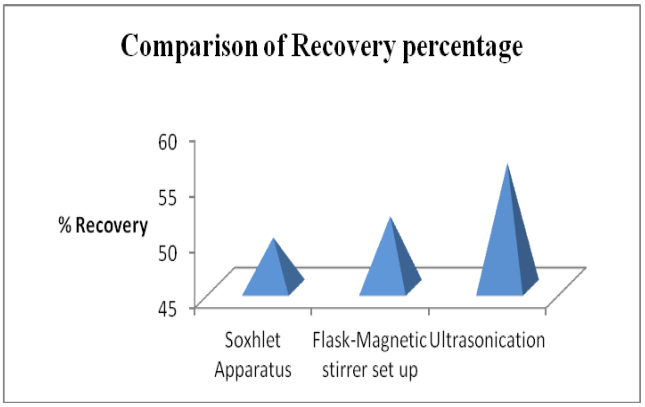

Fig. 7. Comparison of recovery percentage

Ultrasonication technique used for extraction of oil specifically at 70% amplitude and set at 0.7 cycle per sec proves to be the better than soxhlet and simple extraction using flask and magnetic stirrer set up as the technique of ultrasonication improves the reactant mixing and also helps to increase the chemical reactivity. Also, the high pressure cycles of the ultrasonic waves support the diffusion of solvents, such as hexane in our case into the cell structure. Since ultrasound ruptures the cell wall mechanically by the cavitation shear forces, it therefore facilitates the transfer of lipids from the cell into the solvent. [13]

4. Conclusion

The estimated lipid content in the algae, calculated using Bligh and Dyer Protocol is 17.5%

The extraction time in the extraction of algal oil using ultrasonication process is the least among the three processes that is 50 min (0.833 hrs) whereas for Soxhlet apparatus and Flask –magnetic Stirrer set up, it was around 12 hrs and 6 hrs respectively.

The percentage recovery is maximum in case of the extraction of algal oil using ultrasonication technique which is around 56.2 % followed by 51.43% for extraction by Flask –Magnetic stirrer setup and 49.52% for extraction by Soxhlet apparatus keeping the solvent to biomass ratio same.

Ultrasonication technique has highest extraction efficiency when the amplitude is set at 70% and extraction time is around 50 minutes.

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