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Analysis and Characterization of Algal Oil by Using Different Chromatographic Techniques for the Higher Production of Biodiesel from Scenedesmus Dimorphus Algal Species

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Abstract

Algae are the fastest-growing plants on the earth, this study demonstrates the culturing of algal strain on MBM, CHU13 media and production of algal biodiesel from Scenedesmus dimorphus, biodiesel is an alternative fuel for conventional diesel that is made from natural plant oils, animal fats, and waste cooking oils. This paper discusses the economics of producing oil from algae by soxhlet, ultrasonic wave, and expeller method grown in open ponds. Microalgae have been identified as a potential biodiesel feedstock due to their high lipid productivity and the process conditions are milder than those required for pyrolysis and prevent the formation of by-products. Algae are very important as a biomass source. Analysis of algal oil by TLC, and paper chromatography, algae will someday be competitive as a source for biofuel. Algae can be grown almost anywhere, even on sewage or salt water, and does not require fertile land or food crops, and processing requires less energy than the algae provides. Algae can be a replacement for oil based fuels, one that is more effective and has no disadvantages. About 50% of algal oil converted to biodiesel by transesterification process. This microalgal oil can be used to make biofuels for bus, and other vehicles.

Keywords: Microalgae; Biofuels; Lipid; Biomass; Glycerol; Transesterification

Abbreviation: GHC: Green House Gas; FAME: Fatty Acid Alkyl Ester; TAGs: Try Acyl Glycerol's; MFC: Microbial Fuel Cell; MAO: Microalgae Oil; TG: Triglycerides; MBM: Modified Basal Medium; TLC: Thin Layer Chromatography

Introduction

A constant rising worldwide demand of motor and power generation fuels, together with environmental concerns in terms of Green House Gases (GHG), has motivated the scientists and technologists to think about various alternate sources of energy [1]. With the increasing amount of waste originating from human activities comes the negative impact on the environment and in particular the water quality. Waste streams, which are rich in car-bon, nitrogen and other minerals, have potential for use as a substrate for microalgae cultivation [2,3]. Biodiesel is derived from the transesterification of mono-, di- and tri-acyl-glycerides (TAGs) and the esterification of free fatty acids (FFAs) that occur naturally in biological lipids, such as animal fats and plant oils. As a result, biodiesel has the potential to be a carbon neutral fuel [4-6].

Although industrial-scale facilities for biodiesel production from microalgae have not been built, there has been substantial re-search performed on the feasibility, design and requirements for such a production system. A near-complete design for a large (400 ha) production system to produce biodiesel from algae is in [7], as well as recommendations on exactly where in Australia such facilities could be situated, whilst [8] contains additional information on algal production, including economic considerations and identifies several additional pieces of equipment necessary for production not outlined in [7].

It would be valuable to be able to extract and convert triglycerides in microalgae into biodiesel in a single step, bypassing the use of large quantities of organic solvents. Such in situ or direct transesterification approaches have been used as an analytical technique to prepare FAMEs for the determination of the fatty acid composition of lipid containing tissues [9-11].

Higher biomass productivity and lower production costs will also encourage production in the tropics. Therefore, biofuels have the potential to provide opportunities for economic development and improved energy access for developing countries. However, the negative impacts of increased global demand for biofuels are of increasing concern, and include direct and indirect land use change, competition with food production, and land tenure conflicts [12-18].

Materials and Methods

Materials

The proposed study was done at School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh. All the chemicals and glasswares used in the proposed study were procured from Himedia and VK traders respectively. All the techniques and protocols used in the proposed study were standardized according to the literature available.

Algae sample collection

Collect algae sample from various (Upper lake, Colar dam, pond near by bhanpur dumping sites, Narmada hosangabad) places.

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Methods

Identification of suitable strain:

A. Media for *Scandasmus dimorphus* algae growth

There were two types of media used in desire algal culture. Elemental compositions are following (Tables 1and 2) [19].

B. Media preparation

Take above medium and make it (100 ml), Autoclave it at 121°C, 15 lbs pressure, for 15 min. Inoculate algal sample into four (100 ml) different conical flasks. Incubate it at normal RT, for 24 hour, Observe growth into different conical flasks. Select one algae containing flask which one have maximum growth and transfer it into 1000 ml media containing flask, Monitor growth of algae (day/day). After 10-15 days algal strain has to be used to further processing [19].

Algae harvesting:

A. Micro-screening

Algae with media in open pond take carefully on (250 µ, 500 µ) size sieves, filter and discard excess water, dry it in shade[19,20].

S.No.	Chemical	Quantity(mg)
1	$(NH_2)_2CO$	1800
2	KH_2PO_4	1250
3	$MgSO_4.7H_2O$	1000
4	EDTA	500
5	H_3BO_4	114.2
6	$CaCl_2.2H_2O$	111
7	$FeSO_4.7H_2O$	49.8
8	$ZnSO_4.7H_2O$	88.2
9	$MnCl_2.4H_2O$	14.2
10	$CuSO_4.5H_2O$	15.7
11	$Co(NO_3)_2.6H_2O$	4.9

Table 1: Composition of modified basal medium.

S.No.	Composition	Stock Media 20X	Working media 1X mg/L
1	KNO_3	8 gm/L	400
2	K_2HPO_4	1.6 gm/L	80
3	MgSO4 hepthydrate	4 gm/L	200
4	$CaCl_2$	2.14 gm/L	107
5	Ferric citrate	0.4 gm/L	20
6	Citric acid	2 gm/L	100
7	$CoCl_2$ dihydrate	2.14 gm/L	107
8	$H3BO_3$	114.4 gm/L	5.72
9	$MnCl_2$ tetrahydrate	73.4 gm/L	3.67
10	$ZnSO_4$ hepthydrate	8.8 gm/L	0.44
11	$CuSO_4$ pentahydrate	3.2 gm/L	0.16
12	$NaMoO_4$	1.68 gm/L	0.084
13	0.72N H_2SO_4		1drop

Table 2: Composition of modified CHU 13 medium.

S.No.	Name of methods/ sample	Distance travel by solute	Distance travel by solvent	RF value
01	Expeller	10 cm	10.7 cm	0.93 cm
02	Soxhlet	9.2 cm	10.7 cm	0. 85 cm
03	Utrasonicator	5 cm	10.7 cm	0.46 cm
04	Crude jatropha oil	9.7 cm	10.7 cm	0.90 cm
05	Jatropha biodiesel	9.2 cm	10.7 cm	0.85 cm
06	Crude karanja oil	9.7 cm	10.7 cm	0.90 cm
07	Karanja biodisel	10.2 cm	10.7 cm	0.95 cm

Table 3: RF value of different oil sample using TLC.

S.No.	Name of method	Distance travel by solute	Distance travel by solvent	RF value
01	Soxhlet	13.7 cm	18.7 cm	0.73 cm
02	Utrasonicator	13.5 cm	18.7 cm	0.72 cm
03	Expeller	13.3 cm	18.7 cm	0.71 cm
04	Jatropha crude oil	16 cm	18.7 cm	0.85 cm
05	Jatropha biodiesel	15.7 cm	18.7 cm	0.83 cm
06	Karanja crude oil	15.7 cm	18.7 cm	0.84 cm
07	Karanja biodiesel	15.6 cm	18.7 cm	0.83 cm

Table 4: RF value of different oil sample using paper chromatography.

B. Centrifugation

Medium containing algae from open pond, if algal concentration is very low, take 30 ml centrifuge tube, transfer medium containing algae in to the tube. Centrifuge it with 4000 rpm, for 5 minutes, at room temperature. Discard supernatant and keep pellets for further oil processing [19,20].

Extraction of oil from algae:

A. Expeller press method

Take 500 g of shade dried algae. Transfer it into expeller machine for extraction of algal oil. Run the expeller machine. After some time collect algal oil from machine, make it for further transesterification process for biodiesel production [21].

B. Soxhlet extraction

Take 100 g dried powder of algae, keep it into soxhlet apparatus. Add 100 ml hexane solvent, to rapture cell wall of algae. Run the soxhlet (containing algae and hexane), after 20 hour algal oil collected from round bottom flask Algal oil has use for biodiesel production [19].

C. Ultrasonic-assisted extraction

Take 50 gm of dried algae and add 100 ml of ether in 250 ml beaker, provide ultra-sonic wave for 30 minutes, (ultrasonic waves are used to create cavitation bubbles in a solvent material) Ultra-sonic wave has also work as cell wall rapture of algae. Filter it with sieves, manually press algae to extract algal oil, algal oil has use to further transesterification process [21].

Lipid analysis:

A. Thin layer chromatography

Silica paper used as the template, mark the plates with a sharp pencil, line the chamber with chromatography paper. Prepare 202 ml of solvent system (Hexane: Ether: Acetic acid 60:40:1) in a 3000 ml chromatographic chamber. Mix and pour 202 ml into the chamber. Cover and let the chamber saturate while loading the plates. With a 10 µl capillary pipette, spot 1-2 µl of phospholipids standard onto the TLC paper. Make sure the spot remains smaller than 4 mm in diameter. Move on to the other standards. After the spots have dried, repeat loading each standard until you have loaded approx. 10 µl each. Also, load 10 µl of your lipid extract on one spot, and then the remainder of the extract as a line (i.e., a series of spots). Let dry the spots. Make sure that the loading area is above the solvent. Place the plates in the chamber to develop. Immediately close the cover and let run for approximately 60 min, until the solvent front has reached the upper line. Remove the plate and leave to dry in the rack in the fume hood. Discard the solvent in the waste container provided, remove the chromatography paper and leave in the chamber. Leave the chamber in the fume hood to dry. Now place the plate in the iodine tank in the

fume hood. You will see the lipids as yellow spots after about 5 min or so. Mark the edges of the spots with a pencil [22].

B. Paper chromatography

Take the Whatman No1 chromatography paper of appropriate size, Place it on a rough paper and with the help of pencil and scale draw a line leaving 1.5 cm from the bottom. Now on the line mark seven spots leaving 1.5 cm on either side of the edges. Now measure the distance between the spots carefully draw three small circles touching the line, below the line under each circle write the name of the standard. Samples have loaded in center of the paper. With the help of capillary tube apply standard and the sample give a feather touch and see that the solute do not spread below the line. Now fold the paper in the form of a cylinder and staple at three different positions with the help of stapler. While stapling it, be careful and check that the two ends of the paper are equal and the spots are present outside the circle and there is a gap between the two edges [23].

Transesterification: 100 ml Algal oil kept in conical flask. Add 0.5 M KOH. (Algal oil will be highly viscous, one of the most common method will be using to reduce oil viscosity in the algae oil is called transesterification. It involves chemical conversion of the oil into its corresponding fatty ester) and add 70ml methanol. Heat it with 70°C on heating mental and after 2 hour I have collect biodiesel [24].

Results and Discussion

Sample collection

Algal sample ware collected from different places of Bhopal region (Figure 1).

Identification of suitable strain

Scandasmus dimorphus algal species was grown on MBM medium and CHU13 medium. Images shows below (Figure 2).

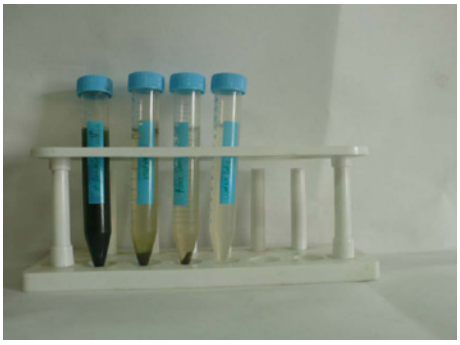

Figure 1: Different sample collection.

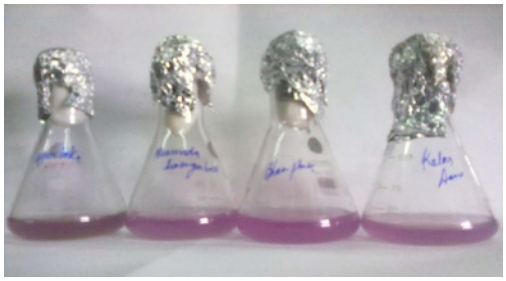

Figure 2: Culture of different algal sample.

Algal strain growth was obtained in Hosangabad sample. Select hosangabad sample and grown it 500 ml MBM solution (Figure 3).

Algae harvesting

A. Micro-screening

Algae harvesting was done after algal culturing by using sieves (250 μ, 500 μ) (Figure 4).

B. Centrifugation

In case of low growth rate of desire algal strain, used centrifuge for algae harvesting (Figure 5).

Take pallets and shaded dry, and also using lyiphilizer for drying algae and then make it in powder form.

Figure 3: Media containing algal strain.

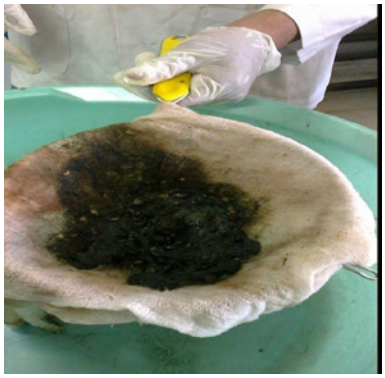

Figure 4: Algae harvesting by sieves.

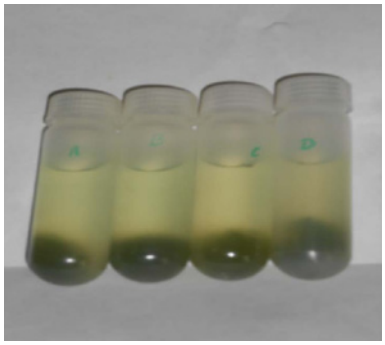

Figure 5: Algal pallets are shown in the bottom.

Algal oil extraction

A. Ultrasonic-assisted extraction

Used ultrasonicator for algal oil extraction, upper layer is algal oil shown in the figure 6.

B. Soxhlet method for oil extraction

Used soxhlet apparatus for the extraction for oil from algae shown in figure 7.

C. Expeller press method for oil extraction

Used expeller machine for the extraction for oil from algae shown in figure 8.

Oil from different technique (Figure 9)

S.No.	Quantities of algae (gm)	Quantities of oil by different method (ml)		
		Soxhlet	Ultrasonicator	Expeller press
01	500	125	108	115
02	250	60	50	55
03	100	20	28	17

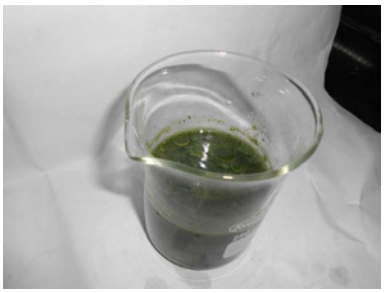

Figure 6: Ultrasonic assisted extraction.

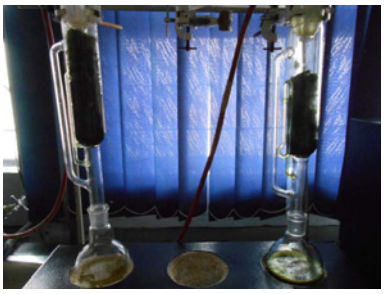

Figure 7: Soxhlet apparatus.

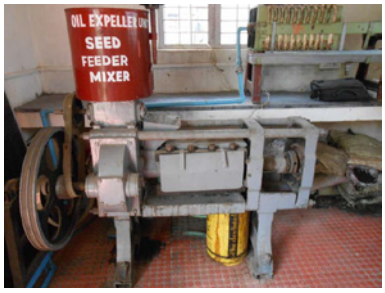

Figure 8: Expeller machine.

Chromatographic technique

A. Thin layer chromatography (Figure 10)

RF factor=Distance traveled by solute (cm)/Distance traveled by the solvent (cm)

A. RF factor of oil which was extracted by expeller method=10/10.7=0.93 cm

B. RF factor of oil which was extracted by soxhlet method=9.2/10.7=0.85 cm

C. RF factor of oil which was extracted by ultrasonicator method=5/10.7=0.46 cm

D. RF factor of crude jatropha oil=9.7/10.7=0.90 cm

E. RF factor of jatropha biodiesel=9.2/10.7=0.85 cm

F. RF factor of karanja crude oil=9.7/10.7=0.90 cm

G. RF factor of karanja biodiesel=10.2/10.7=0.95 cm

B. Paper chromatography technique (Figure 11)

RF factor=Distance traveled by solute (cm)/Distance traveled by the solvent (cm)

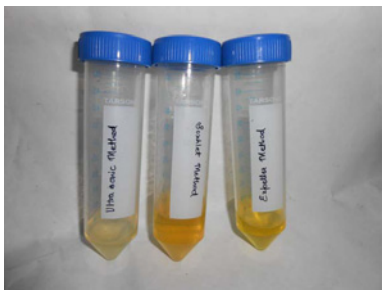

Figure 9: Extracted oil by different method.

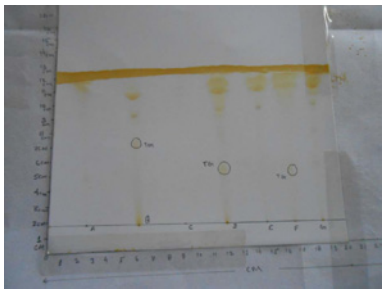

Figure 10: Thin layer chromatographic technique of different oil sample.

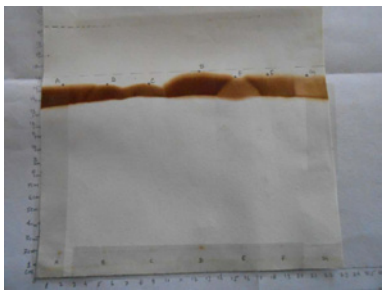

Figure 11: Paper chromatographic technique of different oil sample.

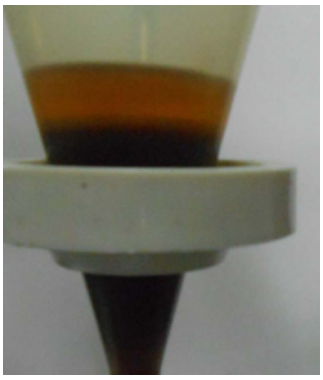

Figure 12: Upper layer shows biodiesel and lower shows glycerin.

A. RF factor of oil which was extracted by soxhlet method=13.7/18.7=0.73 cm

B. RF factor of oil which was extracted by expeller method=13.5/18.7=0.72 cm

C. RF factor of oil which was extracted by ultrasonicator method=13.3 /18.7=0.71 cm

D. RF factor of crude jatropha oil=16/18.7=0.85cm

E. RF factor of jatropha biodiesel=15.7/18.7=0.83cm

F. RF factor of karanja crude oil=15.8/18.7=0.84cm

G. RF factor of karanja biodiesel=15.6/18.7=0.83cm

Transesterification

By trasesterification algal oil converted to biodiesel shown in figure 12 upper layer shows biodiesel and lower layer shows glycerine.

Conclusion

As demonstrated hear, micro-algal biofuels is technically feasible. It is the only renewable biofuels that can potentially completely relocate liquid fuels derived from gasoline. Financial sides of producing algal biofuels need to recover significantly to be possible. Produce low-cost micro algal biofuels require primarily improvements to micro- algal biology through genetic engineering. Use of MBM medium was better than CHU-13 at the laboratory scale. Micro-screening was the best method for algae harvesting in comparison to centrifugation and other method like floculation, sieves size was 250 µ-500 µ. For the extraction of oil, best method was soxhlet method in terms of reduce labour work (125 ml oil- 500 g algae). In terms of save time best method was expeller method (115 ml-500 g). In terms of purity the best method was ultrasonicator (108 ml-500 g). Cost effective. And quantitative method was soxhlet method; it was the most reliable method for oil extraction. Extracted oil was further use for biodiesel production by transesterification process. The identification of fatty acid and triglyceride by using thin layer chromatography and paper chromatography and also calculation of RF value. Algal-biodiesel creation can be done through the use of chemical (acid, base) reaction (CH_3OH, KOH).

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