Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Biomass utilization of waste algal consortium for extraction of algal oil

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Abstract

Biofuels are considered to be the best way to reduce green house gas emission and alternative to pollutant fossil fuels. At present research is being conducted by culturing algae to produce different fuels like biodiesel, bioethanol etc. Using algae to produce biodiesel would be the only viable method to replace the need of gasoline used for automotive today. Experiments were planned to produce sustainable alternative fuel energy (algal oil) from naturally available waste algal consortium. Thus using algal blooms served two purposes firstly bioremediation by reducing eutrophication caused by algal blooms and secondly an attempt to produce a cheap alternative algal oil. The use of algal blooms for algal oil extraction is not reported till now. Combination of mechanical and chemical method gave varied results. Steam distillation using Clevenger apparatus gave better results in comparison to the other method used. It proved to be a cheaper method of oil extraction and not reported till now. Lipid tests conducted for the presence of lipid contents in the sample were positive. That confirms the presence of oil in the extracted sample. Therefore, an attempt was done for the use of waste natural resource to the purposeful way.

Keywords: Algal consortium, Bio fuel, *Spirulina*, lipids.

Introduction

Bio fuel demand is unquestionable in order to reduce gaseous emissions (fossil CO_2, nitrogen and sulphur oxides) and their purported green house, climate change and global warming effects, to face the frequent oil supply crisis, as a way to help non –fossil fuel producer countries to reduce energy dependence. Finding sufficient supply of clean energy for the future is one of society's most daunting challenges and is intimately linked with global stability, economic prosperity and quality of life.

However, the use of microalgae can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and versatile biomass source and may soon be one of the Earth's most important renewable fuel crops, due to the higher photosynthetic efficiency, higher biomass productivities ,a faster growth rate than higher plants, highest CO_2 fixation and O_2 production, growing in liquid medium which can be handled easily ,can be grown in variable climates and non- arable land including marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands), in non-potable water or even as a waste treatment purpose, use far less water than traditional crops and do not displace food crop cultures; their production is not seasonal and can be harvested daily.

Bio fuels are referred to solid, liquid or gaseous fuels derived from organic matter. They are generally divided into primary and secondary bio fuels. While primary bio fuels such as fuel wood are used in an unprocessed form primarily for heating, cooking or electricity production, secondary bio fuels such as bio ethanol and biodiesel are produced by processing biomass and are able to be used in vehicles and various industrial processes. The secondary
bio fuels can be categorized into three generations: first, second and third generation bio fuels on the basis of different parameters, such as the type of processing technology, type of feedstock or their level of development.

First generation bio fuel includes Bio ethanol or butanol by fermentation of starch (from wheat, barley, corn, potato) or sugars (from sugarcane, sugar beet, etc.) Biodiesel by transesterification of oil crops (rapeseed, soybeans, sunflower, palm, coconut, and used cooking oil, animal. Second generation bio fuel includes Bio ethanol and biodiesel produced from conventional technologies but based on novel starch, oil and sugar crops such as *Jatropha*, cassava or *Miscanthus*; Bioethanol, biobutanol, produced from lingo cellulosic materials (e.g. straw, wood, and grass) . Third generation Biodiesel from microalgae
Bio ethanol from microalgae and seaweeds, Hydrogen from green microalgae and microbes.

As a matter of fact, average biodiesel production yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and vegetables. Some microalgae have high oil content and can be induced to produce higher concentration of lipids .The ability of algae to fix CO_2 can also be an interesting method of removing gases from power plants and thus can be used to reduce green house gases with a higher production microalgal biomass and consequently higher biodiesel yield.

Microalgae are the fastest growing photosynthesizing unicellular organisms and can complete an entire growing cycle every few days. Some algae species have high oil content (up to 60% oil by weight and can produce up to 15,000 gallons of oil per acre per year under optimum conditions. Microalgae contain lipids and fatty acids as membrane components, storage products metabolites and sources of energy. The lipids and fatty acid contents of microalgae vary in accordance with culture conditions.

In some cases, lipid content can be enhanced by the imposition of nitrogen starvation or other stress factors. Microalgae are known to accumulate more lipids in nutrient deficient conditions. Biochemical studies have suggested that acetyl-CoA carboxylase (ACCase), a biotin-containing enzyme that catalyzes an early step in fatty acid biosynthesis, may be involved in the control of this lipid accumulation process. Therefore, it may be possible to enhance lipid production rates by increasing the activity of this enzyme via genetic engineering.

For oil extraction from algae harvest the algae from its growth medium (using an appropriate separation process) and extract the oil out of it. Extracting can be done by using Mechanical methods (Expression or Ultrasonic assisted extraction) or by Chemical methods (Hexane solvent method, soxhlet extraction or super critical fluid extraction). Many manufactures of algae oil use a combination of mechanical pressing and chemical solvents in extracting oil.

Chemical conversion of the oil into its corresponding fatty ester is known as transesterification. Transesterification of algal oil is normally done with methanol and sodium hydroxide serving as the catalyst. Sodium methanoate can be produced by reacting methanol with sodium. Thus methanol is reacted with the algal oil (the triglyceride) to produce bio-diesel & glycerol. The end product of this reaction is hence biodiesel, sodium methanolate and glycerol.

In the present study we used the algal consortium of pond scum of D.E.I. Faculty of science. The basic idea behind this selection is to make a humble effort to produce sustainable alternative fuel energy (algal oil) from waste algal consortium. *Spirulina platensis* was also taken for oil extraction under laboratory conditions.

METHODOLOGY

1) Mass Cultivation

Algae are easy to grow under varieties of conditions and environments. Open ponds, shallow race ways, horizontal photo bioreactors, vertical photo bioreactors, plastic bags containers, open and closer circulations systems are some of the containers and devices used to grow algae.

Pond scum is an example of open cultivation system. In our present study, we used algal consortium from open pond of Dayalbagh Educational Institute, Faculty of science. In this pond larvae of insects, leaves of plants along with dust and mud were also present as contamination. So algal blooms grown under natural condition without adding any specific chemical medium were used for oil extraction.

Mass production of *Spirulina* sps. was done in cemented tanks of 200-300 litre capacity and PVC transparent tubs of 40 litre capacity in mist house. The medium used was CFTRI medium which is as follows.

Table 1: Composition of CFTRI medium

Nutrients	Quantity (g/L)
Sodium Bicarbonate ($NaHCO_3$)	40
Di potassium Hydrogen Phosphate	0.5
Sodium Nitrate ($NaNO_3$)	1.5
Potassium Sulphate (K_2SO_4)	1.0
Sodium Chloride (NaCl)	1.0
Magnesium Sulphate ($MgSO_4$)	0.2
Calcium Chloride ($CaCl_2$)	0.04
Ferrous Sulphate ($FeSO_4$)	0.01

2. Identification of Algae

An attempt was made to identify the algal consortium obtained from the pond scum with the help of microscope and identification was done with the help of book the structure and reproduction of algae **(Fritch, F.E.).** The slides were prepared & captured with the help of imaging microscope.

3. Harvesting and drying of Algae

The algae were harvested from growth medium and pond.

I. The algal blooms of pond were harvested manually by sieves and then allowed to dry in shade.

II. *Spirulina* was harvested weekly by gravity filtration method and allowed to dry in shade for oil extraction.

4. Extraction of oil

Extraction was achieved by a combination of two methods

i. Mechanical Method

ii. Chemical Method

In our present study algal sample was dried so that it retains its oil content. The algal blooms powder and *Spirulina* powder was pressed in manual Hydraulic Press and Ball Mill. These helped in cell disruption by applying pressure on the wall of algae. After the cell disruption using hydraulic press and ball mill, oil extraction with different solvents like n- hexane, benzene, petroleum ether was attempted. The algal sample was dissolved in respective solvents and was allowed to stir for 5- 6 hours on magnetic stirrer. The residual pulp was removed from the solvent through filtration. Then it was allowed to separate in separating funnel. Continuous shaking of sample resulted in the separation of two layers on the top of separating funnel. After that the solvent was allowed to evaporate.

Besides the use of solvents a known amount of dried algal consortium powder was kept for steam distillation in Clevenger apparatus. The temperature was maintained at 50^0 C and the whole system was run for 8-10 hours with continuous running water. The ring of oil gathered on the top of condensed material in the apparatus was eluted out. The algal cake left after extraction was kept for further use as a bio fertilizer for soil reclamation of barren land.

The extracted oil after the evaporation of respective solvents was dissolved in DMSO (Di Methyl Sulphoxide) and kept for storage for further studies.

5. Tests for the presence of Lipids

There are some qualitative tests for the presence of lipids in a sample. Some of these tests were performed for the confirmation of lipids in the extracted sample.

- ***Translucency test***

This test involves a piece of filter paper and a hot plate and ether. Take the piece of filter paper and place a drop of the test solution on it. Then place the filter paper on the hot plate and heat to 60 degrees Celsius or 140 degrees Fahrenheit for 5 minutes. Remove the filter paper and immerse in ether. After the paper is air dried, look at the spot. If the spot is translucent, there are lipids in the solution.

Sudan Red Test:

Sudan red is a lipid soluble dye. When Sudan red is added to a mixture of lipids and water, the dye will move into the lipid layer colouring it red. Add 2 ml of water in 2 ml of the test sample. Add 5-6 drops of Sudan reagent. Red colour of the sample confirms the presence of lipid in the sample.

Results

1. Mass production

Spirulina platensis was cultivated *in vitro* in large scale. Its growth was very good during Jan- Apr. Due to high temperature and contamination of mosquito larvae and amoeba its growth was depleted. Therefore, the growth performance of *spirulina* sps. was excellent in winter and gradually decreased as the temperature rises.

In case of algal consortium the growth of algal blooms was excellent in winter but did not survive last week of May. Therefore, whole of algal blooms were collected during Feb-May.

2. Identification of algal blooms

The algal blooms were identified by slide preparation in imaging microscopes. Some of algal sps. were identified with the help of book "The structure and reproduction of algae" (Fritch, F.E.). The identified sps. were:*Vaucheria aquatica*, Anabaena *sps. , Calothrix sps., Ulothrix sps*. Most of these algae were filamentous and some of them were globular green algae which might be the members of Chlorophyceae. Cyanobacterial species were identified on the basis of the structure and position of heterocysts.

The unidentified expected globular algae may be *Pandorina, Eudorina, Coleochaete, Pediastrum, Gymnodinium.*

3) Harvesting and drying of algae

Harvesting of algal blooms was done manually by sieves, irrespective of the fact whether it contained larvae and other contamination. After draining these were allowed to shade dry in mist house. So the flakes of algal blooms were left.

Similarly *Spirulina* was also harvested by muslin clothes and then allowed to dry in shade. After that it was grind with the help of mixer grinder.

Total dry weight of algal blooms after drying was **950 gm.**

Total dry weight of Spirulina obtained was **1.150 Kg**.

1. Extraction of Oil

The simplest method of extraction is mechanical crushing. In our experiment, we used ball mill for cell disruption and further the sample was allowed to crush in hydraulic press. It was observed that dried matter did not show any significant results in mechanical extraction. Therefore, we applied moist sample to the hydraulic press.

This mechanically crushed algal sample was then kept for chemical extraction using solvents like n-hexane, Benzene, petroleum ether A clear difference was observed among the colour of the algal oil extracted by the three solvents. n-hexane extracted oil appeared light green, Benzene extracted oil appeared blackish where as Petroleum ether gave dark green algal oil. Most significant results were observed in case of Petroleum ether after evaporation a slime greasy layer was left in the bottom and wall of the flask.

Similarly in case of Clevenger apparatus, quantity of oil extracted is greater than the solvent extraction.

Total extracted quantity of oil from Clevenger apparatus is **1.5ml/ 250 gm** of sample.

Table 2: A comparative results of Extracted oil with different solvents

Algal sample	n- hexane	Benzene	Petroleum ether
Spirulina platensis	0.4 ml/ 250 gm	0.5 ml / 250 gm	0.7 ml/ 250 gm
Algal Blooms	0.5 ml/250 gm	0.4 ml/ 250 gm	0.8 ml/250 gm

1. Preliminary test for lipid confirmation

Two tests were performed for the confirmation of lipid in the extracted sample i.e. Translucency test and Sudan red tests. Both the tests were positive. This proves that the sample contained lipid contents.

Discussion

1. Mass cultivation

Different algal species were selected by different researchers, because of known lipid contents *Spirulina* is taken as the experimental algal species. *Spirulina platensis* was also used for oil extraction by Andrich *et al,* 2006.

Use of algal blooms for oil extraction is not reported till now. Most of the oil extraction reports are on individual algae such as *Chlorella protothecoides* (Miao *et al* , 2006) and (Wei Xiong *et al*, 2008).

2. Identification

Most commonly reported algal species used for oil extraction are *Chlorella vulgaris* (Gouveia & oliveria, 2009), *Chlorella protothecoides* (Wei Xiong *et al* 2008 & Miao *et al* , 2006), *Spirulina maxima* and *Spirulina platensis* (Andrich *et al* 2006). *Nannochloropsis sps. Dunoliella tetriolecta* (Ferrentino *et al* , 1990), *Oedogonium* and *Spirogyra* (Hossain *et al* 2008). Most of identified species (*Vaucheria, Anabaena, Calothrix, Ulothrix* etc.) of waste algal consortium have not been reported for the use of oil extraction

3. Harvesting and drying

Many methods are adopted for harvesting like filtration, centrifugation, flotation, flocculation etc. we used gravity filtration method for algal bloom extraction and filtration method for *Spirulina* extraction.

4. **Extraction of oil**

There are many methods of oil extraction adopted by different research scientists. Thermochemical liquefaction and the extraction using supercritical carbondi oxide(Aresta *et al,* 2005), Solvent extraction combined with magnetic stirred agitation (Govindarajan et al, 2009). Dimethyl ether (DME) was also reported as solvent for oil extraction (Kandra et al, 2010)

We tried to extract oil by using a combination of mechanical and chemical methods with three solvents viz. n- hexane, Benzene, Petroleum ether respectively. Petroleum ether proved to be an effective solvent out of these three.

The high oil yield was reported in different studies. Goveia & Oliveira, 2009 reported 29.0 and 28.7% of oil

extracted from *Neochloris oleoabundans and Nannochloropsis sps*. Hossain *et al,* 2008 reported 1.8 gm and 3.0 gm of extracted oil from *Spirogyra* and *Oedogonium* sps. Kandra et al 2010 reported 40.1% of green crude oil.

In present investigation quantity of oil extracted from *Spirulina* as well as algal consortium is reported to be 0.7 ml and 0.8 ml with petroleum ether which is fairly less as compared to other reports.

Steam distillation using Clevenger apparatus proved to be most effective for algal oil extraction & petroleum ether was found to be the best solvent in our investigation. It seems that algal oil extraction from algal blooms may not be an economically viable solution for producing green sustainable energy.

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