RESEARCH ARTICLE

PROSPECTIVE OF SELECTED GREEN ALGAE PERTAIN TO BIOFUEL PRODUCTION

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Received-11.05.2018, Revised-26.05.2018

Abstract: Fossil fuels are the main source of energy for transportation and industrialization. Growing population creates a huge demand of fossil fuels which become cause of exhaustion in near future. Burning of fossil fuels is also cause of climate change, global warming, pollution and various health issues in living organism, so fossil fuels become a topic of concerns for scientist and environmentalist. Petroleum based fuels can be substituted by the biofuels, which can be obtained by the trans-esterification algal oil. Algae are the most efficient producer of oil owing to its higher proficiency of CO_2 fixation, cost-effectiveness and insignificant cultivation land. This study was done to know the potential and physicochemical properties of different algal species found in local water bodies.

Keywords: Algal oil, Chemical extraction, Biodiesel, Biofuel, Transesterification, Renewable energy

INTRODUCTION

Petroleum based fuels are limited in nature and major energy crises in near futureenforced to find alternative sources of energy. Algae provide a beneficial prospective for biofuel, waste water treatment and mitigation of Greenhouse Gas (GHG). Algae are more efficient in bio-fixation of CO_2 than the terrestrial plants. The price of crude petroleum reached 145 $ US/barrel in July 2008 the highest price ever achieved in 30 years. The average price of crude oil was 51 $ US/ barrel in 2015.The demand for energy is growing worldwide especially in many of the rapidly developing countries such as in China and India. Deficit fossil fuels influence the growth of developing country so we have to look forward for the alternate source of energy. Furthermore, the continued combustion of fossil fuels has created serious environmental concerns, burning of fossils fuels are cause of various respiratory diseases like Asthma, irritation in eyes and nose and skin inflammation of human. The rapid increase in combustion of fossil fuels becomes a cause of climate change and global warmingso this is mandatory to sequester atmospheric CO2 and find out technique to reduce CO2 emission to the atmosphere. Algae are not only feedstock of biofuel but it helps in mitigation of GHG.

The ethanol and biodiesel are the two liquid of biofuels that can replace gasoline and diesel respectively (Dwivedi et. al. 2013). Now a day, biodiesel is produced from various crops such as Jatropha, Karanja, Sunflower, Palm, Coconut, Soybean, Animal fat and Rapeseed, but these crops have food demand, require more space to cultivate and a lot of money to process. Among the various biomass sources, microalgae are the most successful and suitable for biofuel production. The algae are the autotrophic thalophytes; they can fix CO2 from the environment efficiently in the form of lipid (Maltsev et. al. 2017) and other organic substances by the process of photosynthesis. Algal cultivation does not require fertile soil so with the production of biofuel soil management is also possible. The ex-President India, Late Dr. Abdul Kalam said that out of the 600,000 km² of wasteland that is available in India over 300,000 km² are suitable for Jatropha cultivation, While other 300,000 km² can be used for the algal cultivation.

In 1942 Harder and Von Witsch were the first to propose that microalgae be grown as a source of lipids for food or fuel. The use of microalgae can be a suitable alternative because of higher photosynthetic efficiency, higher biomass production highest CO2 fixation, O2 production and a faster growth rate than plants. Algae grow much faster than food crops, and can produce hundreds of times more oil per unit area than conventional crops Rapeseed, Palms, Soybeans, or Jatropha. Algae can also grow on the surface of the ocean in bags or floating screens. Algae are the most efficient producer of oil on the planet and a versatile biomass source and may soon be one of the earth's most important renewable fuel crops. (Campbell, CJ, et al. 1997). However, fossil fuels and biofuels both are the hydrocarbon and release CO2 after combustion but the released CO2 by the combustion of biofuels were already fixed through photosynthesis so ultimately CO2 do not increase in environment, while combustion of fossil fuels increase the level of carbon dioxide, nitrogen oxides, methane, sulfur dioxide and volatile organic compounds in environment. (Gavrilescu and Chisti, 2005) Studies have determined that replacing fossil fuels with renewable energy sources, such as biofuels, have the capability of reducing CO2 emissions by up to 80%. Furthermore, compared to fuels like diesel and petroleum, and even compared to other sources of biofuels, the production and

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combustion of algal biofuel does not produce any sulphur oxides or nitrous oxides, and produces a reduced amount of carbon monoxide, unburned hydrocarbons, and reduced emission of other harmful pollutants. (Hemaswarya et. al. 2012)

Local water bodies contain a diverse group of aquatic species of algae which can be used as source of oil. Pithophora is the example of macro algae which is a eukaryotic free floating filamentous alga, which can easily grow in different water bodies. They efficiently fix CO2 in form of lipid and other organic substances, and store in cells. Lipid can be extracted by various physical and chemical mean, after that extracted lipid can be converted in biodiesel through the process of transesterification. Transesterification is a process of conversion of triglycerides in to fatty acid methyl ester (FAME/biodiesel). Transesterification reaction can be catalyzed by both homogeneous (alkalis and acids) and heterogeneous catalysts. Biodiesel is mono-alkyl ester made from natural and renewable vegetable oil and animal fats based feedstock. The biodiesel is similar in fuel characteristics to conventional diesel. Biodiesel is compatible with petroleum diesel and can be blended in any proportion with diesel to create suitable biodiesel blend. In addition, Pithophora takes more time to oxidize and best suited for biodiesel production while the other oil needs antioxidant to increase their stability for biodiesel production. (Kumar and Sharma et. al. 2013)

The purposes of current study comprise collection of those algae which are copiously occurred in water bodies of Agra, extractionof lipidsvia chemical extraction method,to find out efficient alga from *Pithophora*and*Chlorococcum* and trans-esterification of lipid.

MATERIALS AND METHODS

Collection and processing of raw material

Macro and micro-algae samples have been collected from the water bodies of Agra, India. After microscopic observations two *Pithophora* and *Chlorococcum*were found significantly. *Pithophora* washed thoroughly with tap waterand stay under sunlight for three days to dry. After drying *Chlorococcum* found in the form of flakes and *Pithophora* as wool. Both were grinded separately by electric grinder to powder it. If water molecules present in processed material, it prevents the dissolution and extraction of lipid in the non-polar solvent so kept in oven at 80°C for 2hours for the complete dehydration.

Lipid extraction process (chemical method)

Oil extraction is done by Soxhlet extraction method. 100 g. of both algal powders weighed and kept in extraction tube. 500 ml of petroleum ether measures as a solvent for each sample and placed in to the flask. Condenser tube placed on the extraction tube and water supply provided properly, and heat source ran till the complete extraction of lipid.

Extracted oil in solvent separated by the evaporation of solvent. I have been developed a very primitive improvised apparatus with glassware’s of lab for the recovery of solvent. Solvent evaporation and recovery took less than 30 minutes; lipid still contained a little amount of solvent so kept in open air over night for the complete evaporation of solvent. Obtained lipid content is weighted.

Lipid purification and testing

Extracted lipid contained cell debris and other unwanted solid material which can cause errors in result so are they removed by centrifugation at 3000 rpm for 5 minutes. After centrifugation lipid and cell debris were weighted and kept in air tight container.

There are various easy and cheap methods are developed for lipid testing, some of them are Sudan regent which soluble with lipid and give red color appearance. Lipid dissolves in methanol and makes a homogenous suspension. Red color with Sudan reagent and complete dissolution of lipid with methanol confirmed the presence of lipid.

Production of biodiesel

Preparation of catalyst: Transesterification is a catalytic process. 20 ml of methanol measured and 0.20 gram of sodium hydroxide (NaoH) weighted put in conical immediately covered, stirred the mixture properly for 20 minutes.

Transesterification: For the completion of transesterification prepared catalyst poured in to 100 ml of lipid in the 250 ml of conical flask and put the conical flask on the electric shaker for 3 hours at 300 rpm. Algal oil is triglycerides molecules. In the presence of methanol and sodium hydroxide triglycerides converted in to fatty acid methyl ester (FAME)/ biodiesel and glycerol.

Washing: Transesterified lipid kept overnight to the settling of glycerol and pigments. Glycerol and pigments are separated carefully with the help of dropper. Biodiesel put in flask for washing and measured 5 ml of distilled water. Now water is poured in to the biodiesel containing flask and shaken well. Water soluble contamination dissolves in water. And water was separated by the dropper. Biodiesel kept under running fan for 12 hours. Finally biodiesel measured by the measuring cylinder and kept in air tight container and stored for analysis.

RESULTS AND DISCUSSION

The aim of this study is to find out the potentiality of algae in production of biofuel collected from local water bodies. The targeted algae were *Pithophora* and *Chlorococcum* However; lipid extraction was done in the same solvent with same apparatus and method for both algae. But they were significantly different to each other.

Table 1. Required time to complete extraction of lipid by Soxhlet apparatus

S.No.	Treatment	Solvent	Extraction Time
1. 2.	***Pithophora species*** ***Chlorococcum species***	**Petroleum ether** **Petroleum ether**	**48 hours** **56 hours**

Pithophora took less time in total extraction of lipid and *Pithophora* also contains relatively less amount of lipid while *Chlorococcum* took much time, it supposed to be lipid content is directly proportional to the extraction time, because more lipids takes more time to dissolve in solvent.

Table 2. Extracted lipid from different algae

Treatment	Dry weight	Extracted oil (ml)	Extracted oil (g)	Percentage of oil
Pithophora species	**100gm**	**7.5**	**6.38**	**6.3%**
Chlorococcum species	**100gm**	**24.7**	**20.40**	**20.4%**

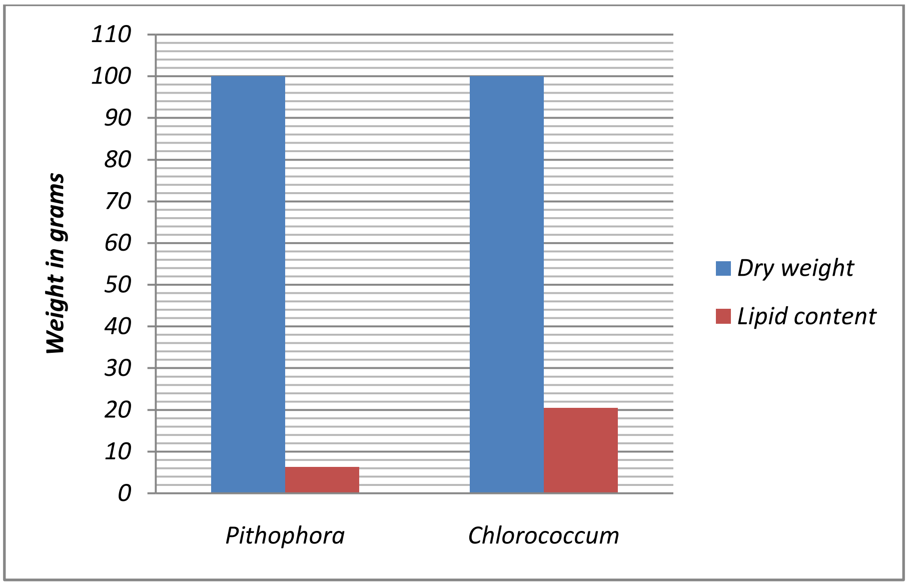

Fig.1: Lipid content in *Pithophora* and *Chlorococcum*

For the lipid extraction dry weight of *Pithophora* and *Chlorococcum* was the same but the lipid found significantly high in *Chlorococcum* than the *Pithophora*. A.B.M. Sharif Hossainet. al. (2008) reported that *Spirogyra* contained 7.3 % of lipid and *Oedogonium* contained 9.2% of lipid while *Pithophora* contained only 6.3% of lipid which is relatively low, while *Chlorococcum* produces relatively much higher lipid among them and it is a microalga.

Density of lipid

Formula $\mathbf{D} = \frac{M}{V}$ where, **D** is the density of lipid, **M** is mass of lipid, and **V** is the Volume of lipid.

Density of lipid of Pithophora $\mathbf{D} = \frac{M}{V}$, $\mathbf{D} = \frac{6.38}{7.5} = \mathbf{0.850 g/_{cm}3}$

Density of lipid of Chlorococcum $\mathbf{D} = \frac{M}{V}$, $\mathbf{D}\ \frac{4.132}{5} = \mathbf{0.826\ g/_{cm}3}$

Lipid of *Pithophora* is relatively denser than the lipid of *Chlorococcum.* Density of lipid is $0.850 g/_{cm}3$ and $0.826\ g/_{cm}3$ in *Pithophora* and *Chlorococcum* respectively. Density of all algal oils approximately matches the density ranges of a biofuels given by EN 14214 and ISO 15607 (0.86-0.90 $g/_{cm}3$)thus our results are compatible with these standards (*Pathak et. al., 2015)*

Table 3. Biodiesel extracted from different algae

S.No.	Treatment	Biodiesel (ml)
1.	***Pithophora species***	**7.6**
2.	***Chlorococcum species***	**27.5**

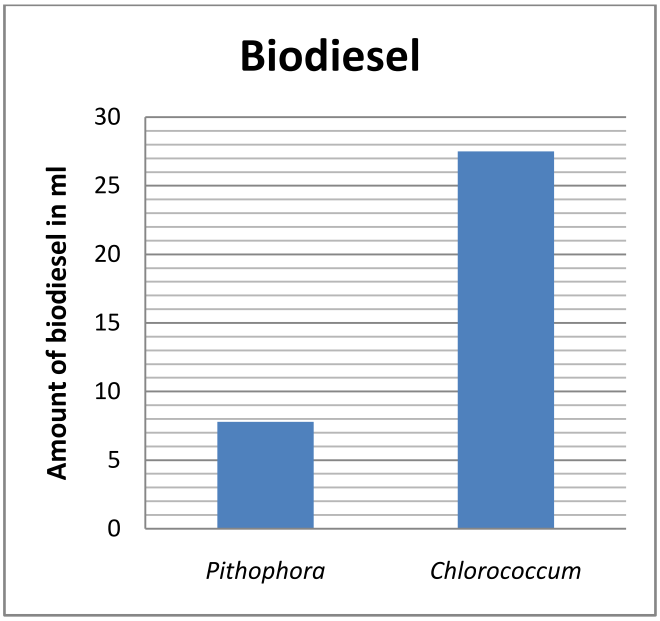

Fig.2: Biodiesel production in *Pithophora* and *Chlorococcum*

(a) (b) (c) (d)

Fig.3: (a) Extracted lipid (b) residual biomass after lipid extraction (c) cell debris after lipid purification (d) biodiesel.

CONCLUSION

Now a days demand of fuels increasing day by day as population increasing and burning of fossil fuels create serious health issues and environmental hazards, so we have to look for the sustainable use fuels and we left with ecofriendly fuels which are biofuels and Algae are the efficient producer of lipid which can be used as source of biofuel. Results proved that microalgae as well as macroalgae both can be used as ecofriendly and renewable energy. Although, *Pithophora* and *Chlorococcum* both produces lipid but there is huge difference in amount of lipid content. Results showed that *Chlorococcum* produce approximately 4 times more biodiesel than the Pithophora. So it is concluded that microalga is the efficient producer of lipid than the macroalga. And it is strongly recommend that further researches should be done in this field, becauseit will decrease the dependency on the fossil fuels. Algal biofuel will also provide economic fuel, secure future and a pollution free environment.

ACKNOWLEDGEMENT

Experiment conducted in phycology laboratory and the financial assistance provided to the completion of dissertation by the Dept. of Botany, Dayalbagh Educational Institute, Agra.

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