International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **2(6),** 46-50, June **(2013)** *Int. Res. J. Biological Sci.*

A New Dimension to Algae Fuel: Far from Light and closer to Human Needs

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Available online at: www.isca.in
Received 18th April 2013, revised 23rd April 2013, accepted 23rd May 2013

Abstract

The source and sink balance of fuel on earth, has been time and again challenged by the growing consumerism of fuel by people. To meet these demands, alternative fuel technology is currently a growing industry of research, especially the use of organisms like algae to harvest fuel, has been one of the recent developments in plant biotechnology. Algae being photosynthetic in nature, utilizes the sugar they produce, to harvest fuel in a scientifically driven manner. However there is a limitation to the variety of oil produced from the same algae decreasing the yield efficiency. But thanks to science, we have come up with new skills to harvest different types of oil products from the same algae using different sugar inputs in a dark acclimatized metabolic pathway. A few algae like Chlorella protothecoids and Botryococcus braunii are fed with rich carbohydrate sources (grass and woodchips) in dark, where it gets converted into oil using underlying principles of fermentation. The heterotrophic growth mode of algae favors an oil yield 3.4 times higher than autotrophic one, with a cetane rate of the fuel obtained as 74, which is ideal for any fuel. The Oil can further be upgraded using nano-sized catalysts like commercially available calcium oxide nano-crystals which can increase the reaction rate up to 99%.

Keywords: Pyrolysis, oil yield, Heterotrophic, Lipid, glucose, nano-catalysts.

Introduction

The term "algae" encompasses a variety of organisms found throughout the world in or near bodies of water. Though most algae are photosynthetic or autotrophic, some are heterotrophic, deriving energy from the uptake of organic carbon such as cellulosic material. Once considered to be a part of PLANT Kingdom is currently labeled under the kingdom “PROTISTA". The feedstock involved in the bio fuel production is primarily a plant derived product.

Descending back to the time, when the first life reported to have evolved on earth was algae (much like chara); their metabolic pathways have been a source of fascination for detailed studies. However, when the world thinks of newer energy sources, these algae have proved to add a new dimension. Biomass is the most common form of renewable energy, having the potential to address problems related to greenhouse effect, global warming and the depletion of non-renewable and conventional sources of energy. When produced by sustainable means, biomasses emit roughly the same amount of carbon during conversion that is taken up by plants for their growth and development. The use of biomass, therefore doesn’t contribute to a buildup of CO_2 in the atmosphere[1]. Fuels from the pyrolysis of biomass have lower sulfur and nitrogen content than in fossil fuels. They are therefore, ecofriendly[2].

An ideal biofuel should have high yield with low investment in its production. Autotrophic algae biofuel production has witnessed an up-gradation to the heterotrophic form, to achieve a better control. However, most of the research is concentrated on the lignocellulosic materials such as pinewood, cotton straw and stalk[2-4]. Till date energy production from microalgae via fast pyrolysis is a rarely worked on field. It is known that of all the algae, a very few like *Chollerela protothecoids, Botryococcus braunii, Spirullina,* heterotrophically produce biofuel. Recently, few companies have started targeting the heterotrophic route that uses fermentation. Further to decide upon the ideal algae species, the parameters of consideration are to be the cellular organization and the growth pattern of these algae[5]. An algae with a higher lipid concentration and a lower crude protein content favors the fermentation pathways. Possible reasons being higher crude lipid content enable greater combination of fatty acid modification and a lower crude protein content would ensure limited nitrogen containing product.

Basically, a high quality biomass achievement is the priority because a specific carbon chain reassembling can lead to several oil products, with a varied saturation and functional group addition. To answer the generally asked question on commodity cost, it is essential to realize that commodity cost is not the only factor of production viability because a high value specialty product can also be equally viable. Alternatives to the microalgae technique, though exists inform of yeast and higher order plants, a successful manipulation of metabolic pathway of algae to make it heterotrophic from the usual autotrophic mode; is what has been the star criteria. The research has showed that bio yield from microalgae like *C. protothecoids* etc. has roughly been in a range of 2.5-3.5 times higher than that of autotrophic cells. Not only a higher yield but also a high quality bio-oil product has been achieved in-comparison to the fast pyrolysis of lignocellulosic materials. The bio oil obtained had lower oxygen content with high heating value (41 $MJKg^{-1}$), a low density

($0.092Kg^{-1}$), a low viscosity (0.02PaS) compared to those of wood. Still the practical utility of the biofuel is limited. The reason being that the existing infrastructure and engineering requires seem to be more conventional to the fossil fuels.

Material and Methods

Sample preparation: The very first step is obtaining a desired quality of microalgae strain and successful maintenance of the culture. The methods of culture apparatus and preparation of a basal medium have been stated before[6].For example, working with *C. protothecoids*, the algae was cultured by inoculating the green cells into the Erlenmeyer flasks containing basic culture medium under 25°C (±1) for 120 hours(hrs.). Air was bubbled into; at regular pressure ensuring aeration. It was found to fix CO_2 through photosynthesis. This algal suspension (green) was diverted to act as heterotrophic cells by controlling the media nutrients. To achieve this $10g^{-1}$glucose was added to the basal medium and the glycine was restricted to $0.1g^{-1}$. Gradually, the green Chollerela cells in the organic carbon medium took its heterotrophic growth by consuming glucose. This led to a yellow algal solution. Finally, dry powders of green phototrophic and yellow heterotrophic algal cells were obtained by centrifugation, water cleansing and desiccation(by a freeze dryer).

Fast Pyrolysis: These algal cells were passed through a screen of 0.5mm aperture to obtain smaller particles capable of ensuring rapid heat transfer in the reactor. 200gm of sample and 4g min^{-1} was the proportion of the reaction mixture. The experiment was carried out in 2 groups. The first was carried out with autotrophic and heterotrophic Chollerela cells at 500°C with a heating rate of $600CS^{-1}$, a sweep gas (N_2) flow rate of 0.4 m^3h^{-1} and a vapor residence time of 2-3 s. The other was performed using only heterotrophic cells with temperature variation from 400 -600°C, with a similar reaction condition of sweep gas rate and heating rate.

Selection of Algal culture and Species: The characterizations of main chemical constituents of both autotrophic and heterotrophic cells were done. The crude lipid content determination[7] and crude protein content determination was performed.

Analysis of Bio-Oil: The classification of the bio oil obtained was done by thin layer chromatography in which oil was fractioned using column liquid chromatography and on the basis of solubility in n-hexane (30ml). The n-hexane soluble fraction samples were added by adsorption onto an inert silica gel support. It was then eluted successively with n-hexane, dichloromethane/n-hexane (2:1), ethanol to produce saturated hydrocarbons, aromatics and polar fractions respectively. The elemental composition was determined by elemental analyzer by which heating value, density and viscosity were determined.

Results and Discussion

Chemical component in heterotrophic cells: The manipulation of growth mode of microalgae from autotrophic to heterotrophic was obtained by addition of glucose (organic carbon source) to the medium and decreasing the inorganic nitrogen source in the medium. When viewed under confocal scanning laser microscope most of the autoflurosence disappeared in the heterotrophic cells as compared to autotrophic ones. Crude lipid content was determined to be 81% and crude protein content was 10.57%. The heterotrophic cells (HC cells) were full of lipid vesicles which were easily observed under differential interference microscopy. High heating values is the result of higher lipid concentration as they have high chemical energy (figure 1).

Yield efficiency by Fast Pyrolysis: Fast pyrolysis, in high temperature heating of biomass in anaerobic conditions ensured a typical temperature of 500°C in the first set of experiments. The bio oil obtained was (57.2%) in HC cells, which was 3.4 times higher than in AC cells (16.6%). This oil also had lower gas yield rate (11.2%),a low density and a viscosity, making it an ideal fuel in comparison to the contemporaries.

Need for control of Temperature in Oil yield process: At a high temperature, there might be a change in the phase of specific oil subtypes, affecting the yield percent. Further algal cell exposure at 0.5mm aperture ensures efficiency of cells to persist the high temperature conditions. Strong evidence in this regards was established[6]. It suggests that there was an increase in the yield of oil when the temperature was raised from 400°C to 450°C. The oil yield increased from 41% to 57.9 %. Beyond this temperature the yield gradually declined to 54.6% at 600°C. The optimal yield was obtained at 450°C, thus making the yield efficiency to be subjective to the reaction conditions like temperature, pressure, vapor residence times etc. There was an increase in oil yield in HC cells and a collaborative decrease in char yield. This significant cross inverse relation of char yield and oil yield seem to be a result of differential cellular concentrations of varied components. However going to the initial thought about lignocellulosic materials as feed stock, the maximum oil yield was obtained as 42% -47% unlike 57% of algae at a temperature range of 500-550°C[2,3,4].The reason can be traced to the fact that, what components makes each of these type of plants. Chemically, higher plants have cellulose, lignin, hemicellulose whereas algae are mainly composed of protein, lipids and carbohydrates (water soluble) which are conducive to the pyrolysis method[8,9,10]. However from, the result suggests that the range of temperature for HC cells is more economical and easily supplied using lignocellulosic materials, thus making it more viable (figure 2).

Advantages: In simple words, the yield efficiency (3.4 times) is the biggest advantage of this oil from algae. However, the control offered by growing algae in the dark and the flexibility in the feedstock is an addendum. Anaerobic heterotrophic growth of algae results in higher secondary metabolite production and thus higher product variability. To minimize the cost of the product, the feasibility of using several wastes as a feedstock is of significance. The method of heterotrophic algae growth and thereby derived bio-oil by fast pyrolysis consumes less space and thus meets infrastructural challenges. A wide range of products have been reported to be produced. These are from soap, personal care products to surfactants, nutraceuticals, pharmaceuticals etc. These oils are also used as dielectric fluids in the transformer market. The bio fuel can also be a string competitor to the fossil fuels as it has a high cetane rate (74) and it doesn't build up CO_2 concentration, thus regulating global warming.

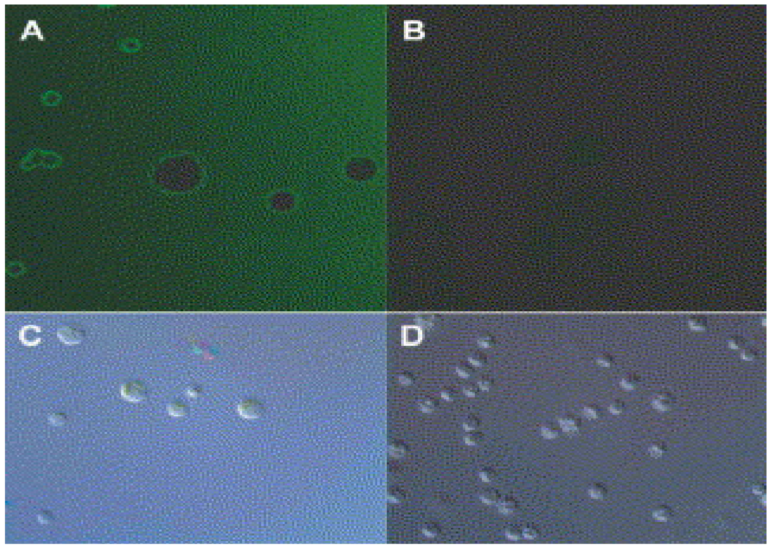

Figure-1
Confocal Scanning Microscopy (A, B); Differential Interference Microscopy (C, D) (Miao *et.al* 2004)

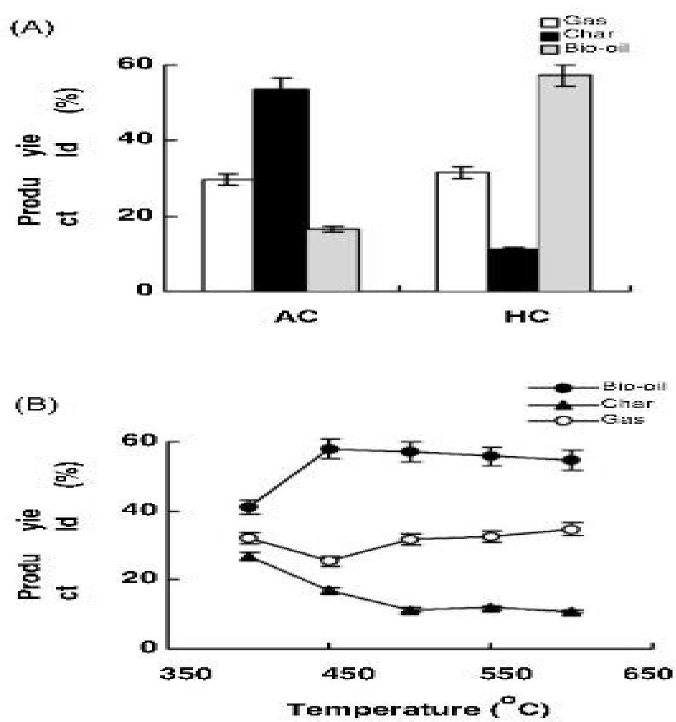

Figure-2
Product yields (on the basis of dry weight of samples) of fast pyrolysis of samples at different temperatures.(A)Product yield of AC and HC cells of *C.protothecoids* at 500°C.(B) Product yield of HC cells of *C.protothecoids* at temperature ranging from 400 to 600°C. (Miao.*et.al* 2004)

There is a kind of algal biofuel called FAME (fatty acid methyl ester based) fuel which has shown better cold properties than any other fossil fuel. So the product obtained by fast pyrolysis can be titled as "Co-product Inclusive Model" (CIM).The model outline is:
Quantity of Sugar + Quantity of algae = Main product + Multiple by-products

Disadvantages: The infrastructure conventionality is the prime limiting criteria of this method, as photosynthetic algal bio-oil generation is more conventional. Further, food chain might be disturbed if other alternatives like plant products are used. It requires the land/sea to grow sugar to feed the algae for desired product, whereas it could be more efficient to grow algae using sunlight for energy. The yield practically decreases, due to sticking of algal cells to the wall of the reactor during centrifugation. However, these reported disadvantages are much irrelevant when looked at the signifance of the product formed.

Route-Dependence Theory: As mentioned the process of oil production from algae involves a series of co related steps and there is a sequence to it. The efficiency of this process involves successful amalgamation of these steps. Thus analyzing the route taken up during the reaction suggests a route dependence theory. If plants could process Sunlight-Sugar more efficiently than Sunlight-Oil and algae could process Sugar very efficiently to biodiesel than Sugar-Oil, this process makes a sense.

Sn2OA > Sn2Sgp + Sg2OA
Sn2OP > Sn2Sgp + Sg2OA

Where,
Sn2OA- cost of sunlight to oil using algae.
Sn2Sgp- cost of sunlight to sugars by plants.
Sn2OP- cost of sunlight to Oil by plants.
Sg2OA- cost of Sugar to Oil by Algae.

Upgradation: The use of catalysts like MCM.41 based catalyst with uniform mesopore may also improve the overall oil yield and increase the oxygenated compounds. Acid trans-esterification, of crude oils derived from *C.protothecoids*, using sulphuric acid with molar ratio of methanol to oil (30-56:1) exhibited a desired reduction in the specific gravity value. Especially, when the process involves trans-esterification of nanometer sized oxide particles, there can be use of inorganic oxides as heterogeneous nano-catalyst. While considering these oxides surface area as well as catalytic activity must be parameters for consideration. For example, it has been reported that PbO_2 (Lead Oxide) has been most efficient in conversion of crude oil to methyl ester (89% efficiency) while PbO_2 is shown to have comparatively lowest surface area. Commercially available calcium oxide is known to improve the trans-esterification process by 2% whereas nano sized CaO (20nm) increases the same process by 99%. Tranesterification of crude oil obtained from *C.protothecoids*, using acid catalysts (sulphuric acid) with molar ratio of methanol to oil (30 to 56:1) showed a required lessening of specific gravity.

Conclusion

The study establishes the precise production of liquid fuel from microalgae involving a process like fast pyrolysis. The manipulation of metabolic pathway of microalgae as in *C.protothecoids* (transformed to heterotrophic) has been possible due to addition of feedstock like glucose (organic carbon source) in the medium. There was a higher lipid concentration in the cells due to heterotrophic growth mode. In addition, heterotrophic growth mode is more conventional to present microbial reactors, improving the yield and making the oil more cost effective[1,12].

The product and the by-product together make a term called "Co-Product", which results in production of several ranges of secondary metabolites, making algae a net producer. The oil yield was 3.4 times higher than autotrophic mode with a significant cetane rate of 74. This makes it potential for an ideal fuel. Meanwhile the temperature, for maximum oil production from microalgae was decreased because heterotrophic growth results in high content of materials which facilitate pyrolysis. The highest bio oil yield 57.4% was obtained at 450°C, a lower temperature than that for wood. The development of bioengineering can be added to the fast pyrolysis technique to introduce an entire metabolic pathway not present in an organism and thus would lead to development of a system for energy production, with slowing of global warming. A new thought of using the sugar from the algae itself, can be a combinational mode of operation, taking into account both autotrophic and heterotrophic mode.

Acknowledgement

I would like to acknowledge the information shared by the SOLAZYME Company on their website. I would also like to thank Mr. Sambeet Satapathy, for helping in the designing of this entire content into a systematic paper.

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