

## A note on algae as potential source for alternate fuels – Biodiesel

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**Abstract:** In the view of faster rates of depletion of fossil fuels and increased energy demand the world is looking for clean alternate fuels. Many researchers are exploring new ways of exploring the potent fuels like biofuels from various sources. The most used sources for the production of biofuels are the plants. Due to the inconsistency and cost effectiveness of the production of seeds for biodiesel production, the attention is now shifted to many other natural resources. Our rich source of algal and planktonic wealth has been providing niche for satisfying the future demands. A meagre amount of work has been carried out in this potential area, in terms of exploring the new resources of algal & planktonic wealth. Out of many algal species like *Monolithus Salina*, *Botrycoccus braunii*, *Dunliella primolecta*, *Chlorella vulgaris* *Botrycoccus braunii* which yields a percentage of about 75 which is a suitable in production of biodiesel<sup>1</sup>. This article provides the information in detail about the potential algal sources & the procedures for separation of lipids and other hydrocarbons from different algal species and their importance.

**Keywords:** Alternate fuels, Biofuel/biodiesel, algae, planktonic wealth

### Introduction:

The need of energy is increasing continuously, because of increases in industrialization and population<sup>1,2</sup>. With the exponential growth in population, there is immense pressure on energy production and over the past century this has led to over exploitation of non-renewable resources. The basic sources of this energy are petroleum, natural gas, and coal, hydro and nuclear. The major disadvantage of using petroleum based fuels is atmospheric pollution created by the use of petroleum diesel. Petroleum diesel combustion is a major source of greenhouse gas (GHG). Apart from these emissions, petroleum diesel is also major source of other air contaminants including NO<sub>x</sub>, SO<sub>x</sub>, CO, particulate matter and volatile organic compounds<sup>3</sup>. Biomass is one of the better sources of energy. Hence much of modern research focuses on means of realizing the potential of non-conventional and renewable sources of energy. Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, globally, socially and economically.

The world's population has been increased in the few decades globally, the increase is predicted to be from 7 billion in 2013 to 9.2 billion by 2050, due to which the usage of energy fuels from different sources is being utilized at a maximum rate. Due to this the requirement for the fuels is increasing day by day. The fossil fuels are being deprived and the usage of it has caused the increase in the need of fuels globally<sup>22</sup>. As this process continues in future the necessity of fuels will be increased very highly. Replacing fossil fuels with any other source is finding a hunting task for the researchers<sup>1</sup>. There were many sources available in the past for the production, but these are depleting day by day. There has arisen a crisis between food and fuel as many of the crops are being utilized for the production of fuels<sup>10</sup>. The fuels were being produced by variety of crops such as Sunflower, *Jatropha* seeds, Palmolein seeds etc. This has made a crisis for in acquiring a large land for the

production of these plants. In this direction, the production of biodiesel with the help of the photosynthetic organisms was found to be more efficient. Biofuels are considered as the efficient source for the usage because of the environmental benefits which it possess as it is processed from the renewable energy sources<sup>5</sup>. Biofuels can be classified based on their production technologies like: First generation biofuels, second generation biofuels, third generation biofuels and fourth generation biofuels. The examples of first generation biofuels are Bio alcohols, vegetable oil, biodiesel, bio gas. For second generation: Bioalcohols, bio-oil, and bio hydrogen. For Third generation: Vegetable oil, biodiesel (source algae). Fourth generation: Biogasoline<sup>1</sup>. Biofuels such as Bioethanol had a replacement of the fossil fuels but with the invention of Biodiesel from the microalgae finds to be a very potent source for the transportation<sup>7</sup>. The photosynthetic organisms are the microalgae species which utilize much amount of sunlight, carbon dioxide from the atmosphere for their growth. The idea of using biodiesel from microalgae is a new idea as it involves less emission of carbon dioxide into the environment thus giving a great impact on greenhouse effect. The microalgae are the unsurpassed ideal contender for the production of biodiesel<sup>6</sup>. After a thorough research scientists have found that Biodiesel from microalgae is the best suited one and still much research is going on it. The idea of biofuels is not new but due to the escalating prices of petroleum has made them to make an extensive research over it<sup>8</sup>.

### Microalgae:

Algae are the aquatic plants which lack perfect roots, stems, shoots etc. and they vary in size from microscopic to many metres in length. The algae are considered as a group of plants which are adapted to aquatic environments, and their cellular components may vary from unicellular to large multicellular forms<sup>9</sup>. They can survive by synthesising their own foods from the process of photosynthesis available from sunlight, which are capable of storing lipids in the form of Tri Acyl Glycerides (TAG's) for production of substantial amount of biodiesel<sup>5</sup>. The green microalgae prefer in rich N/P ratio ponds because the rate of biomass generation is higher in these ponds when compared with the low N/P ratio. In the low N/P ratio the presence of cyanobacteria prevails at a higher rate, as these have a better adaptability towards the light environments. The microalgae have many nutritional values in terms of the lipid content, protein content, amino acids etc.

The following table shows, microalgae as a source for valuable products:

Product	Content (% of dry mass)
Colouring substances	
B Carotene	10
Xanthophylls	1
Astaxanthin	1-6
Canthaxanthin	>1
Phycocyanin	Up to 5
Phycoeythrin	Up to 5
Biomass	
Health food	100
Feed additives	100
Soil Inoculum	100
Poly unsaturated fatty acids	
Arachidonic acid	2-30
Eicosapantenoic acid	2-30
Docosahexaenoic acid	2-30
Anti Oxidant	Up to 3
Tocopherol	>2
SOD	>1

### Preference of Microalgae:

- ✓ The microalgae have a high efficiency for photosynthesis with an adaptability to wide range of light and temperature sources.
- ✓ The microalgae can grow in water with different levels of nutrients and can adjust with the change in the growth characteristics and nutrient uptake ability.
- ✓ The microalgae have a surface to volume ratio, thus enhancing them to grow very efficiently.
- ✓ Fixation of CO<sub>2</sub> at different water levels is achieved very easily.

**Advantages of Microalgae as a Biofuel<sup>6,8</sup>:**

- ✓ Microalgae can be harvested for more than once a year. Biomass is doubled for every 24 hrs.
- ✓ Growth rate is much faster than trees.
- ✓ It can be grown in wide range of temperatures (-2 °C to 50 °C)
- ✓ It contains the high amount of lipid content from which biodiesel is produced.
- ✓ Nutrients from waste water and atmospheric carbon dioxide produce useful lipids in algae
- ✓ There are many 1000's of different kinds of algal strains available which are capable of producing biodiesel.
- ✓ Utilizes Carbon Dioxide from the atmosphere, a process also for cleaning the environment.
- ✓ It takes the Carbon Dioxide from the atmosphere and gives us a CO<sub>2</sub>free Biodiesel.
- ✓ It grows with the help of natural resources which are abundantly available around us.
- ✓ Microalgae farming could be more cost effective than conventional farming.
- ✓ Potential of growing in many places like forests, farmlands etc. thus reducing the damages to ecosystem and food chain supply.
- ✓ It gives high yield and hence the product is of lower cost.
- ✓ It can run well efficiently on cold temperatures.

**Limitations of using Microalgae as a Biofuel<sup>33</sup>**

When amount of particulate matter in the biodiesel is decreased then there is a corresponding increase in the nitrogen oxides which is the reason of heavy smog formation.

- ✓ It is relatively newer technology.
- ✓ Produces biodiesel with many polyunsaturates

**Other Applications of Microalgae:**

Apart from the production of biodiesel, microalgae have a potent application in various other fields of biotechnology. With the gene modification in any of the potent organism we can achieve new products. Microalgae can be used in the production of various products related with proteins, antigens, antibodies etc.

**Reduction of CO<sub>2</sub> by biomass and its conversion into energy<sup>32</sup>****Diversity of microalgae:**

Microalgae are the prokaryotic or eukaryotic organisms which can grow in any type of harsh conditions like extreme cold, extreme hot or moderate temperatures<sup>9</sup>. There are many different species of microorganisms available in fresh water or marine habitats. A few examples of prokaryotic organisms are Cyanobacteria whereas the examples of some of the eukaryotic organisms are the green algae (Chlorophyceae, Chlorophyta etc.). The availability is in the range of 25,000 different species of algae available on the surface of the earth. A number of algae species are capable of producing terpenoid oils one of which is *Botryococcus braunii* which is reported to accumulate upto 86% of dry weight as oil<sup>12</sup>.

The following table gives an overview of lipid content of different microalgae species<sup>27</sup>.

Species	Maximum lipid content (% ; w/w)
<i>Monalanthus salina</i>	72
<i>Botrycoccus braunii</i>	53-75
<i>Dunliella primolecta</i>	54
<i>Dunliella bardawil (salina)</i>	47
<i>Navicula pelliculsa</i>	45
<i>Radipsphaera negevensis</i>	43
<i>Biddulphia aurtia</i>	40
<i>Chlorella vulgaris</i>	40-58
<i>Nitzschia palea</i>	40
<i>Ochromonas dannica</i>	39-71
<i>Chlorella pyrenoidosa</i>	36
<i>Peridinium cinctum</i>	36
<i>Neochloris oleabundans</i>	35-54
<i>Oocystis polymorpha</i>	35
<i>Chrysochromulina sp.</i>	33-48
<i>Phaeodactylum tricornutum</i>	31
<i>Stichococcus bacillaris</i>	32

#### Algal Fuel Crop Candidates:

Researchers are failing in identifying the most promising candidates for the biofuel production. They have constructed lipid profiles of ideal species but none of it till date has found to be satisfactory. These strains should be genetically modified for efficient lipid production and many of the industrialists are aiming for this type<sup>11</sup>. Algae biodiesel is still not yet economically competitive with petroleum diesel. The cost of algae biodiesel is proportional to the species specific efficiency of algae to sequester carbon dioxide as lipids<sup>1</sup>. Microalgae have found to be the potential source for the fuels, chemicals and industrial waste water treatment. The elements required for the growth of microalgae include like Carbon (Glucose), Nitrates, Phosphates and some of the trace elements like Zn, Fe, Ni, Co, Mg etc.<sup>14</sup>. Recently a strain from chlorella species named *C.protothecoides* has gained a much attention, for possibility of production of biodiesel in large scale<sup>16</sup>.

#### Diversity and different types of Algae forms:

Algae are known to be the oldest form of species living on the earth. It dates back many years back for its existence. They are mainly divided into two major forms.

- A) Macro algae
- B) Micro algae.

#### Macro algae:

These are extensively found in the marine environments, these are used in the preparation of many of the products which are useful for the mankind. Few species (*Porphyra*, *Cladophora*, *Ulva* etc.) are found to have significant quantities of lipids for consideration of them in the biofuel market<sup>17</sup>.

#### Microalgae:

These microalgae are found to be available both in fresh water and marine environment. These are single celled organisms which can grow profusely in the fresh water and marine sources by anchoring the ecosystems. They also provide extensive food system for the organisms which are thriving in these kinds of environments. These microalgae fall into the different groups<sup>12</sup>. They are

- a) Diatoms
- b) Green Algae
- c) Blue green algae
- d) Golden Algae.

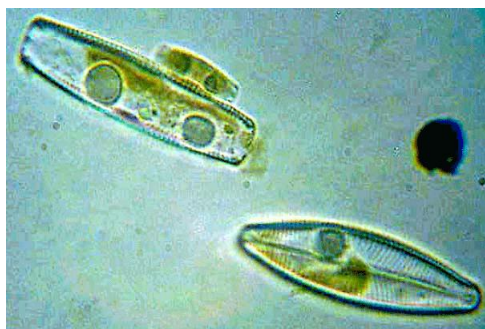
The green algae include many different species for about 8000 species having their habitats in both marine and fresh water environments<sup>8,12</sup>.

#### Different Algae Species available:

There are many different kinds of algae species available on this earth. These species help in maintaining the ecosystems in the marine environment, by providing food for the small organisms or animals available in the marine environment.

#### Diatoms:

The diatoms are the major group of algae which are among the most common type of phyto plankton. These kinds exist in the form of colonies along with together<sup>18</sup>.



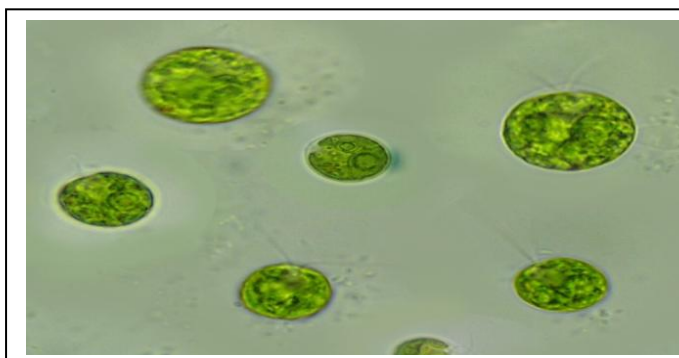
#### *Navicula lanceolata*

They have wide variety range of pH and salinity where they will grow. They are extensively used as a bio-indicator in environmental valuation and monitoring. Diatoms were shown to produce an average of 60% of cellular mass as their triacyl glycerols (TAG'S) under certain growth conditions. These TAG'S can be easily converted into biodiesel with the help of the process of transesterification. Diatoms can yield a total of about 100-200 of oil when compared with soya bean oil, 7 to 31 of oil from the palm oil. Thus sustainable energy could be made with the help of diatoms<sup>13</sup>.

These diatoms are found to have many applications in the pharma, genetic engineering, biofuel etc. These have found to have a substantial amount of lipid in them for the production of biodiesel. These can accumulate a considerable amount of lipid when compared with other algal sources<sup>15</sup>.

#### Green Algae:

The green algae are the large group of algae from which algae have emerged. There are about 8000 different species of algae available on this planet<sup>12</sup>. Many of the species live in either colonized forms, single celled, long branched chains<sup>2</sup>. The green alga has the colour variation of colour from dark green to light green in colour. The reason for this one is for the presence of the chlorophyll a and b. The coloration of these is due to Xanthophylls and  $\beta$ -Carotenes. These are available in the areas where light is available abundantly. These will grow in shallow ponds where the light can sufficiently pass through and very less in no. in the depth of the ocean.

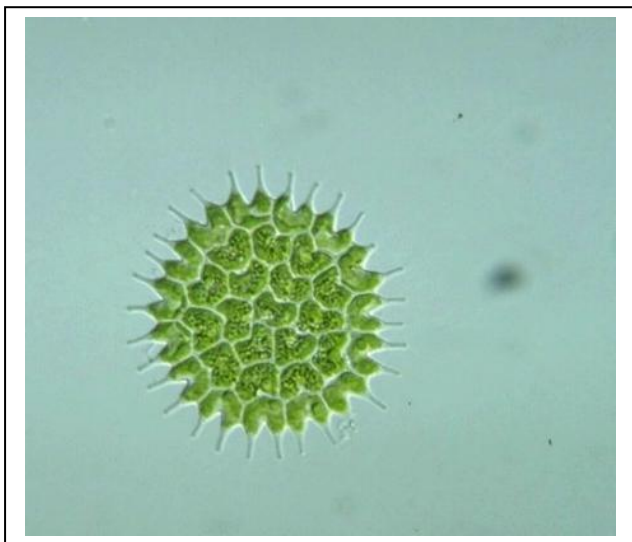


#### *Chlamydomonas reinhardtii*<sup>34</sup>

There are many species of green algae available in many of the fresh water and marine environment. Some of the species include like *Pediastrum boryanum*, *Chlamydomonas* sp. and *Cladophora* etc. Among the different kinds of species available the *Chlamydomonas reinhardtii* is found to be the potent one for the storage of lipids within itself. It has encountered a total of about 76% on basis of its dry weight. The *Chlamydomonas* species is considered as a model species for other organisms.

### *Pediastrum boryanum*<sup>35</sup>

This species exist in colonies with circular, star like in shapes. These are found on the surface of the ponds, lakes, rivers etc. These appear green in colour and appear sharp to the nakedeye.



*Pediastrum boryanum* is the scientific name for the green algae species. These when are present in groups release tiny things called the Zoospores. Each of the zoospores are released at different time intervals and they are going to form a new algal colony/ies.

### **Chlorophyta:**

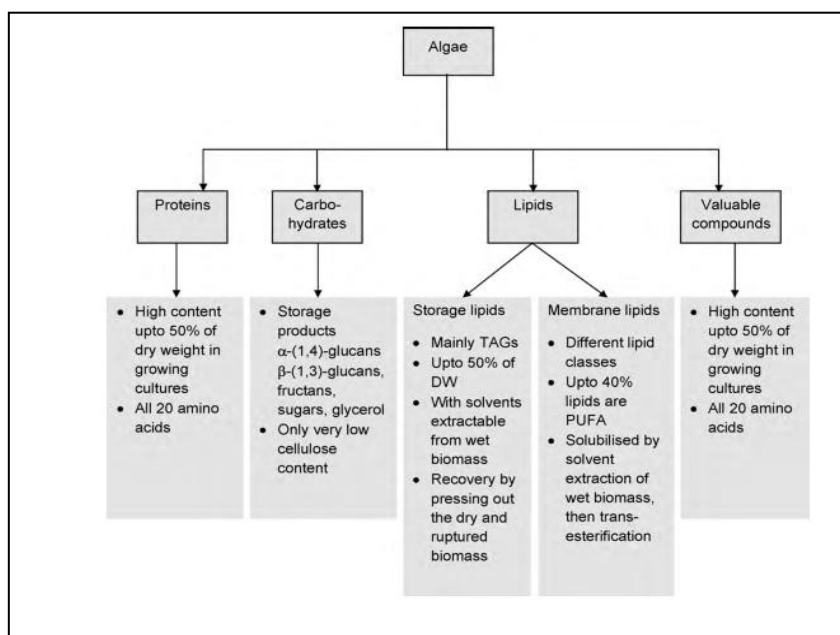
Chlorophyta is a division of green algae. These Chlorophyta species are inhabited to many of the marine, terrestrial and fresh water places. The division includes both unicellular and multi cellular organisms. Some of these species have adapted to the extreme environments such as deserts, hyper saline environments, under vents etc. Two species of the alga can cause disease in humans as well as animals, the disease is very dangerous and is known as protothecosis<sup>12</sup>.

### **Cultivation of Microalgae:**

The farming of microalgae takes place in various methods like open pond systems, in Photo bioreactors. The open pond systems are the most common forms or types of cultivation. These are man-made ponds which are exposed to atmosphere for enough amount of sunlight which makes these microalgae to grow. These open pond systems can take many different forms of lagoons, raceway pond system etc. The primary requirements of open pond systems are Light, Nutrients and water. This kind of cultivation offers a lot of constrains/limitations<sup>36</sup>. They are

- Risk of Bacterial contamination is high.
- It cannot be considered for a scale up process.

## Components of Microalgae<sup>19</sup>



## Biofuel:

Biofuel is any type of fuel derived from organic matter. Now a days there is a rapid decrease in the fossil fuels and many of them are looking onward for economic fuels. Due to the decrease in these fossil fuels there is a rapid increase in the fuel price. Still there is a lot of confusion among the people for the terms of biodiesel and biofuel. Biofuel has been known to humans since many years, but this term biodiesel is a new term which is component of biofuel. These biofuels are made from the components other than petroleum products<sup>37</sup>. These energy resources have found an advantage over the environmental conditions and thus found the adaptable organisms for the efficient producers of biomass and biodiesel<sup>16</sup>. Biodiesel is a specific type of fuel which can be used in the diesel engines. Production has increased from 25 million gallons in 2000 to 1.5 billion gallons in 2012<sup>38</sup>.

## Global Biodiesel Production

Country or Region	Biodiesel Production (Million Litres)	Share Total (%)
Germany	1,921	54.5
France	511	14.5
Italy, Austria, Denmark, United Kingdom, Czech Republic, Poland, Spain, Sweden	9-227	0.1-6.4
Europe (Total)	3,121	88.6
United States	290	8.2
Other	114	3.2
<b>Total</b>	<b>3,524</b>	<b>100.0</b>

Global biofuels production has been increasing almost exponentially over the last 5 years to reduce the dependency on oil and combat increasing and uncertain oil prices [20]. By displacing petroleum based gasoline with renewable alternatives. Biofuels production uses a wide range of industrial processes with multi-feedstock sources to manufacture Bioethanol, Biodiesel, Biobutanol and other fuels. Feedstock sources are not limited to, but include agricultural crop waste biomass, various vegetable oils, algae biomass and algae oils.

World energy makes two things clear: Most of the humanity needs to consume a great deal more energy in order to experience reasonably healthy lives and to enjoy at least a modicum of prosperity<sup>21</sup>. In contrast, affluent nations in general and U.S. and Canada in particular, should reduce their excessive energy. The U.S. and Canada are the only two major economies where average annual per capita energy usage

surpasses 300 Giga Joules (Approximately 80 tonnes of crude oil). This is twice as average in the richest E.U economies (Bordeaux and Berlin).

### Biofuel Scenario in India:

India is one of the fastest growing economies in the world. The Development Objectives focus on economic growth, equity and human well-being. Energy is a critical input for socio-economic development. The energy strategy of a country aims at efficiency and security and to provide access which being environment friendly and achievement of an optimum mix of primary resources for energy generation. Fossil fuels will continue to play a dominant role in the energy scenario in our country in the next few decades. However, conventional or fossil fuel resources are limited, non-renewable, polluting and, therefore, need to be used prudently. On the other hand, renewable energy resources are indigenous, non-polluting and virtually inexhaustible. India is endowed with abundant renewable energy resources. Therefore, their use should be encouraged in every possible way<sup>23</sup>.

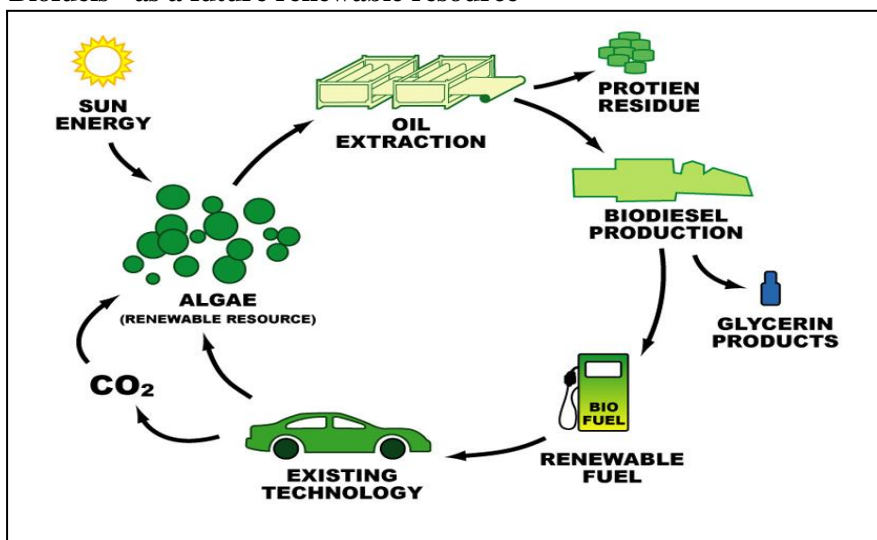
Biofuels can increasingly satisfy these energy needs in an environmentally benign and cost effective manner while reducing dependence on import of fossil fuels and thereby providing a higher degree of National Energy Security.

The growth of biofuels around the globe is spurred largely by energy security and environmental concerns and a wide range of market mechanisms, incentives and subsidies have been put in place to facilitate their growth. Developing countries, apart from these considerations, also view biofuels as a potential means to stimulate rural development and create employment opportunities. The Indian approach to biofuels, in particular, is somewhat different to the current international approaches which could lead to conflict with food security. It is based solely on non-food based feed stocks to be raised on degraded or wastelands that are not suited to agriculture, thus avoiding a possible conflict of fuel vs. food security.

### Biodiesel:

The biodiesel is defined as the mixture of fatty acids alkyl esters obtained by Transesterification of vegetable oils or from animal fats. These contain a major amount of triglycerides (90-98%), mono and diglycerides for about (1-5%), and some small traces of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds and traces of water<sup>22</sup>. The products obtained on the reaction with any of the strong acid or base such as sodium or potassium hydroxide, produces new chemicals called as esters, this product is said to be known as biodiesel. Biodiesel (monoalkyl esters) is one of such alternative fuel, which is obtained by the Transesterification of triglyceride oil with monohydric alcohols. It has been well-reported that biodiesel obtained from canola and soybean, palm, sunflower oil, algal oil as a diesel fuel substitute<sup>24</sup>. Biodiesel is a nontoxic and biodegradable alternative fuel that is obtained from renewable sources. Biodiesel fuel can be prepared from waste cooking oil, such as palm, soybean, canola, rice bran, sunflower, coconut, corn oil, fish oil, chicken fat and algae which would partly decrease the dependency on petroleum-based fuel. The burning of an enormous amount of fossil fuel has increased the CO<sub>2</sub> level in the atmosphere, causing global warming<sup>25</sup>.

### Biofuels - as a future renewable resource<sup>39</sup>





The above picture depicts on how the algae grows and how it is used in the process of biofuel production.

The interest in using this biodiesel as a future fuel is to compensate the usage of the fuel with the fuel from microalgae. This biodiesel shares similar properties with the petro diesel for its usage in the engines without any modification. The best used mixture is B20 which contains 20% of biodiesel and the remaining 80% of petrodiesel. This B20 is the most popular blend because of its good balance cost, lower emissions than petro-diesel, and weather cold performance<sup>26</sup>.

#### Characteristics of Algal Biofuel that differs from petro diesel<sup>40</sup>

- It has no sulphur content.
- It has more aggressive solvent properties than petro diesel will dissolve the leftover varnish residue.
- Biodiesel has superior lubricating power which can increase the lifetime of a fuel injecting equipment.
- Biodiesel flashpoint is significantly higher when compared with the petro diesel.
- Biodiesel reduces particulate matter upto 47% when compared with petroleum diesel.

#### Biodiesel physical and chemical properties<sup>27</sup>

Name	Biodiesel
Chemical Name	Fatty acid methyl esters
Chemical formula range	C <sub>14</sub> - C <sub>24</sub> methyl esters
Kinematic viscosity range	3,3-5,5
Density Range	860-894
Boiling point Range (K)	>475
Flash point Range (K)	430-455
Distillation Range (K)	470-600
Vapour pressure (mm Hg at 295K)	<5
Solubility in water	Insoluble
Physical Appearance	Light to dark yellow.
Odour	Light Soapy and oily odour
Biodegradability	More than conventional diesel
Reactivity	Stable, avoid strong oxidising agents

The oils usually contain sterols, free fatty acids, sphingolipids, phospholipids, water molecules and other impurities which cannot be used as a fuel directly hence the oil need to be transesterified for its application in the fuel engines<sup>28</sup>.

There are many kinds of works which have been carried out till now like characterisation of microalgae which were obtained from various sources (Marine and Freshwater Sources). They have isolated and characterized many of such species from the various sources and have included in the database of algae. The species they selected was from *Cyanobacteria*, two different species of *Chlorella vulgaris*, another strain from *Rhodophyta*, and from *Nannochloropsis oculata* which was an unknown strain<sup>29</sup>. E.A Ehmin and his team in the year 2010 had extracted the biofuel from microalgae using the non-edible microalgal lipids using the acid catalysed insitu Transesterification process<sup>30</sup>. They have found that increasing reaction volume and temperature led to improvement in the FAME conversions and hence was easier for them to predict the outcomes<sup>31</sup>. Much of the work is still being enhanced for the production of biodiesel from microalgae with the help of photo bioreactors. The use of photo bioreactor has gained much attention these days because the growth of the microalgae is enhanced in the bioreactor and it assures for contamination free culture. Due to this property many of the researchers are concentrating much on these closed bioreactor systems so that the lipid yield in the microalgae will increase, hence the oil content is increased in it<sup>32</sup>.

#### Conclusion, Future scope and Significance:

The biodiesel from microalgae has gained a prominent significance in today's world as it is very cheap in obtaining, cost effective, harmless to the health and many more. These various kinds of advantages have made the biodiesel from microalgae very efficient. Many of the researchers and many big companies are practising these on a large scale to make it practically available globally. The process used in obtaining the biodiesel from microalgae is very cheap process which makes it available for many users. The property of

biodiesel makes it to be used in any kind of diesel engines with or no modification of engines, this is another added advantage.

The species like *Botryococcus braunii*, *Chlorella vulgaris*, *Chlorella pyrenoidosa* etc. and many more to come and to be invented need to be studied more on how to increase the lipid content in it by the genetic modification of genes and other molecular biology techniques which are available, so that they can produce a rich amount of lipid content in themselves and which are further involved for higher production of oil which results in the production of biodiesel<sup>4,32</sup>.

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### References:

1. VeerAmuthu Ashok Kumar, Ramasamy Rengasamy 2011; Mass culture of *Botryococcus braunii* Kutz under open raceway pond for bio fuel production; *Bioresource Technology*; January 2012, Vol 104; 394-399.
2. Magnesh G.Kulkarni, Ajay K. Dalai; Waste cooking oil; American Chemical Society Publications; *Catalysis and Chemical Reaction*; March 24 2006.
3. Donald.L.Klass; *Biomass for Renewable Energy and fuels*; Encyclopedia of Energy; 2004.
4. Carla S Jones and Stephen P Mayfield; Algae biofuels: Versatility for the future of Bio energy; *Current Opinion in Biotechnology* 2012; 346-351.
5. M.Prathima Devi, G.Venkata Subash, S.Venkat Mohan; Heterotropic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: Effect of nutrient supplementation; *Renewable Energy* 43(2012) 276-283.
6. W. S. Grayburn, R. A. Tatara, K. A. Rosentrater, G. P. Holbrook; Harvesting, oil extraction, and conversion of local filamentous algae growing in wastewater into biodiesel; *International Journal of Energy and Environment*; Volume 4, Issue 2, 2013 pp.185-190.
7. Yanqun Li, Mark Horsman, Nan Wu, Christopher Q. Lan, Nathalie Dubois-Calero; Articles: Biocatalysts and Bioreactor Design; *Biotechnol. Prog.* 2008, 24, 815-820.
8. Sadeq Emeish; Production of Biodiesel From Microalgae; *Journal of Energy Technologies and Policy*; Vol.3, No.10, 2013.
9. Mike Packer; Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy; *Energy Policy*; 37 (2009) 3428–3437.
10. Teresa M. Mata, Antonio A. Martins, Nidia. S. Caetano; *Microalgae for biodiesel production and other applications: A review*; *Renewable and Sustainable Energy Reviews* 14 (2010) 217–232.
11. Jeffrey D. Palmer, Douglas E. Soltis, Mark W. Chase; The Plant Tree of Life: An Overview and Some Points of View; *American Journal of Botany* 91(10): 1437–1445. 2004.
12. Michael D. Guiry; How Many Species of Algae are There; *Journal of Phycology*; 1057–1063 (2012).
13. Mark Hildebrand, Aubrey K Davis, Sarah R Smith, Jesse C Traller & Raffaella Abbriano; The place of diatoms in the biofuels industry; *Biofuels* (2012) 3(2), 221–240.
14. Aurelien Tartar, Drion G. Boucias, Byron J. Adams and James J. Becnel; Phylogenetic analysis identifies the invertebrate pathogen *Helicosporidium* sp. as a green alga (Chlorophyta); *International Journal of Systematic and Evolutionary Microbiology* (2002), 52, 273±279.
15. Evan Stephens, Ian.L.Ros, Jan H.Musssung, Liam D.Wagner, Micheal A. Borowitzka, Clemens Posten, Olaf Kruse and Ben Hankamer; Future Prospects of microalgal biofuel production systems; *Trends in Plant Science*; Vol 15, no 10; 554-564.
16. Yusuf Chisti; Biodiesel from microalgae; *Biotechnology Advances* 25 (2007) 294–306.
17. Juan M. Lopez-Bautista, Fabio Rindi and Michael D. Guiry; Molecular systematics of the subaerial green algal order Trentepohliales: an assessment based on morphological and molecular data; *International Journal of Systematic and Evolutionary Microbiology* (2006); 56; 1709–1715.

18. Sanjay K. R., Nagendra Prasad M. N., Anupama S. , Yashaswi B. R and Deepak B; Isolation of diatom *Navicula cryptocephala* and characterization of oil extracted for biodiesel production; *African Journal of Environmental Science and Technology*; Vol. 7(1), pp. 41-48, January 2013.
19. E.W.Becker, *Micro Algae: Biotechnology and Microbiology*, Cambridge University, 1994,293pp.
20. Ayan Dermibas; Relationships derived from physical properties of vegetable oil and biodiesel fuels; *Fuel* 87 (2008) 1743–1748.
21. Carl Safi, Alina Violeta Ursu , Céline Laroche ,BacharZebib, Othmane Merah, Pierre-Yves Pontalier , Carlos Vaca-Garcia; Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods; *Algal Research*; 3 (2014); 61-65.
22. E.A. Ehimen, Z.F. Sun, C.G. Carrington; Variables affecting the in situ transesterification of microalgae lipids; *Fuel* 89 (2010) 677–684.
23. *National Policy on Biofuels*; Government of India Ministry of New & Renewable Energy. (2006).
24. Shakeel A. Khan, Rashmi, Mir Z. Hussain, S. Prasad, U.C. Banerjee; Prospects of biodiesel production from microalgae in India; *Renewable and Sustainable Energy Reviews* 13 (2009) 2361–2372.
25. Jasvinder Singh, Sai Gu; Commercialization potential of microalgae for biofuels production; *Renewable and Sustainable Energy Reviews*; 14 (2010); 2956-2610.
26. Jon Van Gerpen; Biodiesel processing and production; *Fuel Processing Technology* 86 (2005) 1097–1107.
27. Kalpesh K. Sharma, Holger Schuhmann and Peer M. Schenk High Lipid Induction in Microalgae for Biodiesel Production *Energies* 2012, 5, 1532-1553.
28. Stephens, E.; Ross, I.L.; Mussgnug, J.H.; Wagner, L.D.; Borowitzka, M.A.; Posten, C.; Kruse, O.; Hankamer, B. Future prospects of microalgal biofuel production systems. *Trends Plant Sci.* 2010, 15, 554–564.
29. Reitan, K.I.; Rainuzzo, J.R.; Olsen, Y. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *J. Phycol.* 1994, 30, 972–979.
30. Ehimen EA, Sun ZF, Carrington CG. Variables affecting the in situ transesterification of microalgae lipids. *Fuel* 2010;89:677e84.
31. Amish P. Vyas, Jaswant L. Verma, N. Subrahmanyam: A review on FAME production processes; *Fuel*. 89 (2010) 1–9.
32. Richmond A. *Handbook of microalgal culture: biotechnology and applied phycology*(Ed.). Blackwell Science; 2004.588p.

#### Website sources:

- 33.<http://www.oilgae.com/algae/oil/biod/char/char.html>.
- 34.<http://cdn.bionews-tx.com/wpcontent/uploads/2013/10/Chlamydomonas.jpg>
- 35.[http://www.fcps.edu/islandcreekes/ecology/green\\_algae.htm](http://www.fcps.edu/islandcreekes/ecology/green_algae.htm).
- 36.<http://www.co2algaefix.es/?q=node/63&language=en>.
- 37.<http://www.wisegeek.com/what-is-the-difference-between-biodiesel-and-biofuel.htm>.
- 38.<http://www.biodiesel.org/what-is-biodiesel/biodiesel-basics>.
- 39.<http://refuelingthefuture.yolasite.com/third-generation-biofuels.php>.
- 40.<http://www.oilgae.com/algae/oil/biod/char/char.html>.

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