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# A Review Paper: Potential of Microalgae as Source of Biofuel in India

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***Abstract:*** **- Day by day, our existing fossil fuels are depleting. There is an urgent need to focus and find out the alternative and renewable sources for sustainable environment, which can substitute the petroleum derived fuels. In the past few years, biodiesel has come up as a potential alternative source of energy. Biodiesel feedstocks derived from microalgae have emerged as one of the most promising alternative sources of lipid for use in biodiesel production in India. Second generation microalgal systems have the advantage that they can produce different algal species with different potential for the production of biodiesel, bioethanol, biomethane and biohydrogen. The present paper gives an overview of the potential of microalgae biodiesel in India. The paper discussed about fundamental aspects of various algal species that have been found to have high oil (or lipid) content. In this regard, we have examined the chemical composition and suitability of microalgal oil for synthesis of biodiesel by transesterification reaction. Finally the review research paper covers the approach for making algal biodiesel more economically and competitive with petrodiesel.**

***Keywords*****: Biodiesel, Petroleum derived fuels, Microalgae, India, Feedstock, Lipid, and Transesterification**

## I. INTRODUCTION

Day by day increasing demand of fuels for different purposes as transport, agriculture etc has been created to think for other alternative sources for sustainable development [20]. According to an estimate, 70% of the total petroleum is only consumed by automobiles. This is an alarming stage that the country definitely is in problem because of increasing demand of transportation (Grow diesel consortium news circular, 2008). Petroleum products consumed during the period of 2011-12 in India was 147.995 million metric tonnes (MMT). According to annual report (2011-12) of Ministry of petroleum & Natural Gas, 171.73 MMT of crude oil was imported by India. This creates a heavy burden foreign exchange [15].

Table-1 and Graph-1 shows that the pattern of production of crude oil during the period 2004-05 to 2011-12. During this period, the production of crude oil has remained in the range 33 to 38 MMT with year to year variations. It has been analyzed that the production of 38.09 MMT crude oil during the year 2011-12 is about 1.08% higher than the actual crude oil production of 37.684 MMT during 2010-11. There were also various reasons like environmental legislations and legal issues in Assam, power disturbances in Gujarat, created problem in production of oil in Andhra Pradesh.

| Year | Crude oil Production (MMT) | % Growth |
|---|---|---|
| 2004-05 | 33.981 | 1.82 |
| 2005-06 | 32.19 | -5.27 |
| 2006-07 | 33.988 | 5.59 |
| 2007-08 | 34.118 | 0.38 |
| 2008-09 | 33.508 | -1.79 |
| 2009-10 | 33.69 | 0.54 |
| 2010-11 | 37.684 | 11.86 |
| 2011-12 | 38.09 | 1.08 |

Table- 1. Crude oil Production

(Source: MoPNG, 2011-12, India)

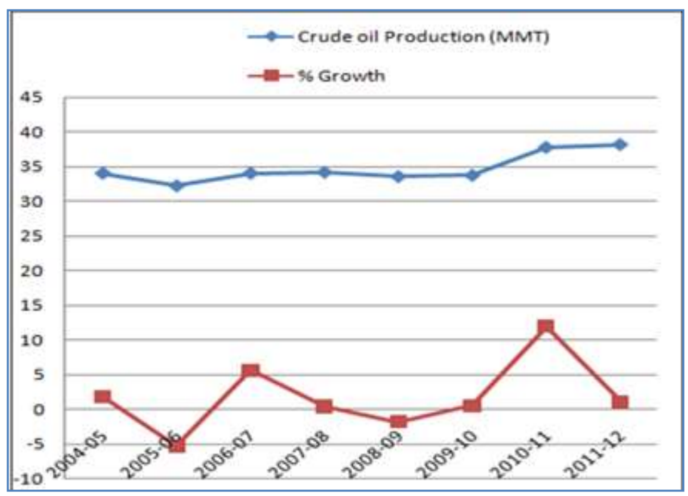


Figure- 1. Crude Oil Production

There was an increase by 3.37% in production of total petroleum products, including fractioners, during 2011-12 compared to the year 2010-11. The indigenous consumption of petroleum products increased by 4.93% during 2011-12 at 147.995 MMT compared to the previous year (2010-11) at 141.04 MMT. Year-wise production and consumption of petroleum products during 2004-05 to 2011-12 are shown in Table-2 and Graph-2 below.

| Year | Production of Petro Products (MMT) | Growth (%) | Consumption of Petro Products (MMT) | Growth (%) |
|---|---|---|---|---|
| 2004-05 | 120.819 | 4.35 | 111.633 | 3.60 |
| 2005-06 | 121.935 | 0.92 | 113.214 | 1.42 |
| 2006-07 | 137.353 | 12.64 | 120.748 | 6.65 |
| 2007-08 | 146.990 | 7.02 | 128.948 | 6.79 |
| 2008-09 | 152.678 | 3.87 | 133.599 | 3.61 |
| 2009-10 | 182.011 | 19.21 | 137.808 | 3.15 |
| 2010-11 | 192.433 | 5.73 | 141.040 | 2.35 |
| 2011-12 | 198.920 | 3.37 | 147.995 | 4.93 |

Table- 2. Production and Consumption of Peteroleum Products

(Source: MoPNG, 2011-12, India)

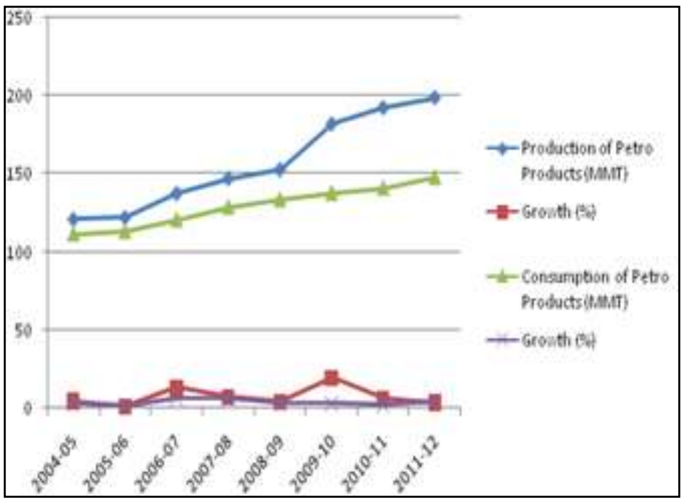


Figure- 2. Production and Consumption of Petroleum Products

Day by day consumption of petroleum has increased enormously. Thus, in India, search for alternatives to petrodiesel is of special importance and the use of biodiesel is comparatively much more important for us than for rest of the countries. Due to higher demand for fuel it is expected that crude oil production will start declining from the beginning of 2012. Different types of microalgae are able to grow in a variety of environmental conditions, even on the limited areas of land [19]. Therefore, alternative biodiesel is the only option to fulfill the requirements in future.

## II. MICROALGAE AS ALTERNATIVE FUEL

According to the recent research report, it has been analyzed that the microalgae are the one of the earliest life creatures on the earth [8]. These are both prokaryotic or eukaryotic photo driven cells that covert $CO_2$ and water to rich biofuel and other important products [4, 5]. Microalgae are the large group of photosynthetic unicellular species, which exist individually or in chains, or groups. There are at least 40,000 known species of algae in India. These organisms are remarkably adaptable and occupy virtually every environment on the planet. They can be found in waters of widely varying temperature, pH, and salinity (from freshwater to hyper-saline). Algae essentially harness energy via photosynthesis. They capture $CO_2$ and transform it into organic biomass which can be converted to energy. The global energy demand, especially petroleum derived fuels (PDF) has been increasing day by day because of several reasons reason is the increased levels of green house gases (GHG) emissions [11]. Day by day burning of fossil fuel is responsible for release of large amount of carbon di oxide $CO_2$ and contributing major role towards global warming. Biofuel from Microalgae are an alternative solution in India. It is estimated that about 120 million tones per year of total petroleum product consumed by India. If we compare microalgae with other peterocrops as a substitute of petroleum products, it has been found that the potential and efficiency of microalgae are much higher than other sources [1]. Like other biomass resources it is theoretically a carbon neutral source of energy. Microalgae are being widely researched as a fuel due to their high photosynthetic efficiency and their ability to produce lipids, a biodiesel feedstock. Microalgae biomass, the subject of this review, is thought to be a promising next generation biofuel feedstock [3]. The process of biodiesel production from microalgae is clearly shown in below mentioned line diagram (Figure 3).

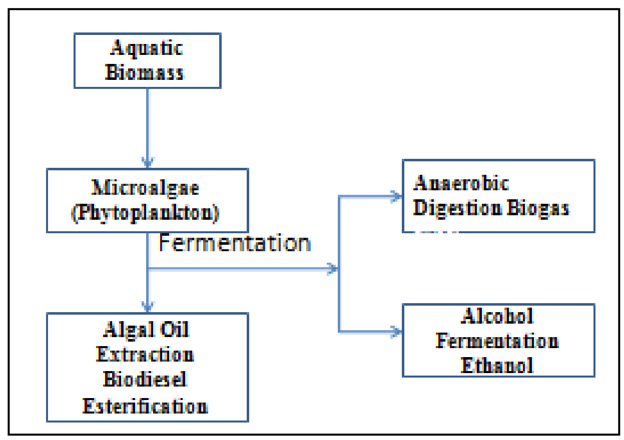


Figure- 3. Biodiesel Production from Microalgae

For some time it has been known that many species of microalgae have the ability to accumulate lipids in the form of triacylglycerols (TAGs) [5]. This oil can reach 20%–60% of the dry cell weight and is typically synthesized in response to conditions of stress, such as lack of nitrogen. This response allows cells to store carbon and energy during times when cell division is limited.

It is clearly shown in the Table 4 that the microalgae are efficient alternative as a raw material as compared to the other raw material for large scale biodiesel production (Table 4) [5].

| Crop | Oil in Litres per hectare |
|---|---|
| Corn | 172 |
| Castor | 1413 |
| Sunflower | 952 |

| | |
|---|---|
| Jatropha | 1892 |
| Oil Palm | 5950 |
| Soybean | 446 |
| Coconut | 2689 |
| Microalgae | 136900 |

Table- 4. Availability of oil present in different feedstock in liter per hectare

Two basic techniques are applied in the production biofuel from microalgae which depends on the type of specific organisms and the hydro-carbon which they produce. During the primary stage we simply convert the biomass (nutrients) into lipids or hydrocarbons [2, 10, 21].

In secondary procedure, algal biomass is converted through the thermo-chemical process of liquefaction into lipid or hydrocarbons. Lipids and hydrocarbons can normally be found throughout the micro algal biomass [17]. The microalgal rich in high lipid content are of great interest of research for sustainable raw material for production of biodiesel in India [31].

## III. ANALYSIS OF MICROALGAE

### *3.1 General properties of microalgae*

Some basic properties of methyl esters produced from microalgae are described below (Table 5)

| | |
|---|---|
| Density (kg/L @ 15.5 C) | 0.862 |
| Viscosity (cSt @ 40 C) | 4.2 |
| Cetane No. | 56 |
| Heating Value (MJ/kg) | 41.4 |

Table- 5. Basic properties of methyl esters produced from microalgae

### *3.2 Chemical aspects of Microalgae*

Different algal species have different chemical compositions (Table 6) [1, 21, 10].

| **Strain** | **Protein (%)** | **Carbohydrates (%)** | **Lipids (%)** |
|---|---|---|---|
| Scenedesmus obliquus | 51-55 | 11-18 | 13-14 |
| *Scenedesmus quadricauda* | 47 | - | 1.9 |
| *Scenedesmus dimorphus* | 8-18 | 21-52 | 16-40 |
| *Chlamydomonas rheinhardii* | 48 | 17 | 21 |
| Chlorella vulgaris | 51-58 | 12-17 | 14-22 |
| *Chlorella pyrenoidosa* | 57 | 26 | 2 |
| *Spirogyra sp.* | 5-20 | 31-64 | 12-21 |
| *Dunaliella bioculata* | 49 | 4 | 8 |
| *Dunaliella salina* | 57 | 32 | 6 |
| *Euglena gracilis* | 39-61 | 14-18 | 14-20 |
| *Prymnesium parvum* | 28-45 | 25-33 | 22-38 |
| *Tetraselmis maculata* | 52 | 15 | 3 |
| Porphyridium cruentum | 28-39 | 40-57 | 9-14 |
| Spirulina platensis | 46-63 | 8-14 | 4—9 |
| *Spirulina maxima* | 60-71 | 13-16 | 6-7 |
| *Synechoccus sp.* | 63 | 15 | 11 |
| *Anabaena cylindrica* | 43-56 | 25-30 | 4-7 |

Table- 6. Chemical Composition of Different Species of Microalgae

(Source:Baker, 1994)

Most common algae like *Cyl-indrotheca*, *Dunaliella*, *Chlorella*, *Crypthecodinium*, *Isochrysis*, *Nannochloris*, *Nan-nochloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum*, *Por-phyridium*, *Schizochytrium*, *Tetraselmis*, *Botryococcus braunii* and *Scenedesmus* have rage of 20 to 50% of oil levels. (Table 7).[14, 32].

| **Species** | **Oil (%)** |
|---|---|
| Botryococcus braunii | 50 |
| Chlorella sp. | 30 |
| Crypthecodinium cohnii | 20 |
| Cylindrotheca sp. | 27 |
| Dunaliella primolecta | 23 |
| Isochrysis sp. | 29 |
| Monallanthus salina | 20 |
| Nannochloris sp. | 28 |
| Nannochloropsis sp. | 50 |
| Neochloris oleoabundans | 45 |
| Nitzschia sp. | 46 |
| Phaeodactylum tricornutum | 25 |
| Schizochytrium sp. | 64 |
| Tetraselmis sueica | 20 |

Table- 7. Oil content (% dry weight) in different microalgae

The graphical representation of different species of microalgae shows that Schizochytrium sp. are rich in oil percentage as compared to other species (Figure 3).

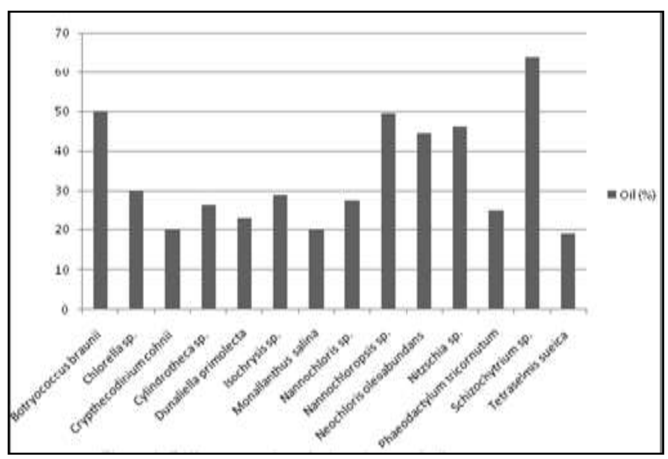


Figure- 4. Different Species of Microalgae and Oil Production

The lipid content in some of micro-algae may be increased under adverse growth conditions such as nitrogen limitation, low temperature, high light intensity, high salt concentration and high iron concentration [12, 18].

### *3.3 Biological aspects of algae*

Microalgae are a large group of microscopic photosynthetic organisms, many of which are present in a unicellular manner and found in diverse environments. Microalgae could be further subdivided into prokaryotic cyanobacteria which clearly lack the nuclear structures and eukaryotic algae. Eukaryotic algae can be classified into at least 12 major divisions, in which microalgae find their locations. Among those divisions, some frequently mentioned classes include diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), red algae (*Rhodophyceae*), yellow-green algae (*Xanthophyceae*), golden algae (*Chrysophyceae*), brown algae (*Phaeophyceae*), and Euglenoids. Except *cyanobacteria* whose lipid contents are believed to be as low as less than 10% of dry algal weights, species have efficiency to produce high levels of lipids could be identified from each of these eukaryotic algal classes [13].

## IV. PRODUCTION OF MICROALGAL BIOMASS

Growth of microalgae is feasible both in open-culture systems such as ponds, lakes and raceways, or in highly controlled closed-culture systems like photo bioreactors. Different environmental as well as other parameters are important for growth of microalgae such as light, carbon dioxide, water and inorganic salts. Microalgae require temperature generally within 20- 30°C for their growth. Biodiesel production is much more feasible and cheaper during the sunny days [20]. Raceway ponds [22] and tubular photobioreactors [30] are very efficient for of large-scale production of microalgae.

*4.1 Raceway Pond:* Raceway type open pond systems are primarily used for industrial scale cultivation of microalgae (Figure 4). They are usually built in concrete and operated at water depths of 15–20 cm. The open pond cultivation systems are relatively cheaper to construct and can be constructed in non agricultural or waste lands. The cost of construction could be significantly reduced by building plastic lined compact earthen raceway ponds and such low cost ponds may be suitable for production of microalgal biomass for biofuel applications [3].

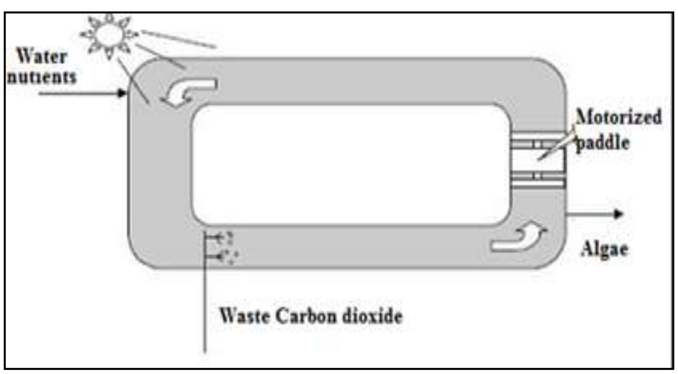


Figure- 5. Raceway Type Open Pond System

*4.2 Photobioreactor:* Closed photobioreactor systems are being used for cultivation of microalgal species for the production of high value products. Closed photobioreactors have been discussed for theoretical advantages in terms of protection from contamination, attaining higher cell culture densities and facilitating better control over physico-chemical conditions e.g., light and temperature [9]. Various designs of photobioreactors have been discussed [3, 7] and tubular, flat plate, airlift tubular and bubble column reactors are the commonly used closed photobioreactor designs (Figure 5). As per the reports available in literature, the largest closed tubular photobioreactor is the 25 $m^3$ plant of Mera Pharmaceuticals, Hawaii [24] and 700 m3 plant in Klotze, Germany [25]. The cost of closed photobioreactos is significantly higher than open ponds and is difficult to scale up. Reports on biomass productivity of other microalgae with photobioreactor systems are largely based on laboratory studies as currently the relatively high construction and operating costs, and complexity of operation of closed PBRs limit their large-scale commercialization [9]. There is no research available on large scale commercial production of microalgae in India using closed photo bioreactors.

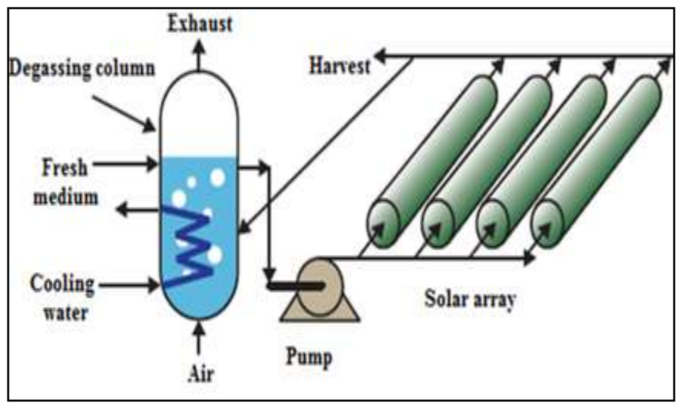


Figure- 6. Photobioreactor

## V. PROCESS OF BIOFUEL PRODUCTION FROM MICROALGAE

Figure 6 shows that the triglycerides in which three fatty acid molecules are esterified with a molecule of glycerol. In making biodiesel, triglycerides and methanol are reacted to form glycerol amd methyl esters as biodiesel. The process is known as are reacted with methanol in a reaction known as transesterification or alcoholysis [26].

The reaction occurs stepwise:

Triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol.

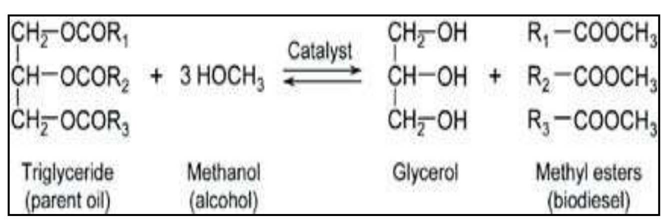


Figure- 6.

The main parameters affecting the base-catalyzed transesterification process are:

- Alcohol formulation
- Alcohol/oil molar ratio
- Catalyst formulation and concentration
- Reaction temperature
- Reaction time
- Agitation
- Presence of moisture and FFA

## VI. BIODIESEL QUALITY AND STANDARDS AT NATIONAL & INTERNATIONAL LEVEL

Biodiesel quality depends on both physical and chemical properties. Table 8 presents a list of the most important biodiesel quality standards in the world and the required test methods are presented.

| Country/Area | Specifications | Title |
|---|---|---|
| E U | EN 14213 | Heating fuels - Fatty acid methyl esters (FAME) - Requirements and test methods |
| E U | EN 14214 | EN 14214 Automotive fuels - Fatty acid methyl esters For diesel engines - Requirements and test methods |
| US | ASTM D 6751 | ASTM D6751 - 11a Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels |
| Brazil | ANP 42 | Brazilian Biodiesel Standard (Agência Nacional do Petróleo) |
| India | IS 15607 | Bio-diesel (B 100) blend stock for diesel fuel - Specification |
| Japan | JASO M360 | Automotive fuel - Fatty acid methyl ester as blend Stock |
| South Africa | SANS 1935 | Automotive biodiesel fuel |

Table- 8. Biodiesel Standards

The diversity in these technical specifications is primarily related to the origin of the feedstock and the characteristics of the local markets [23].

## VII. BIODIESEL IS ENVIRONMENT FRIENDLY

In view of environmental considerations, biodiesel is considered as ‘carbon neutral’ because all the carbon dioxide ($CO_2$) released during consumption had been sequestered from the atmosphere for the growth of vegetable oil crops [28]. Proper source of carbon is very important parameter for growth of microalgae as compare to other nutrients. The tolerance of $CO_2$ of various Species are described in Table 9.

| Species | Known maximum $CO_2$ concentration | References |
|---|---|---|
| *Cyanidium caldanum* | 100% | [16] |
| *Scenedesmus sp.* | 80% | [18] |
| *Chlorococcum littorale* | 60% | [24] |
| *Synechococcus elongates* | 60% | [27] |
| *Euglena gracilis* | 45% | [20] |
| *Chlorella sp.* | 40% | [18] |
| *Eudorina spp.* | 20% | [18] |
| *Dunaliella tertiolecta* | 15% | [13] |
| *Nannochloris sp.* | 15% | [20] |
| *Chlamydomonas sp.* | 15% | [13] |
| *Tetraselmis sp.* | 14% | [23] |

Table- 9. $CO_2$ Tolerance of Various Species. *Source: Mark E. Huntley (University of Hawaii) and Donald G.Redalje (University of Southern Mississippi)*

Commercial experience with biodiesel has been very promising. There is no requirement to change the engine type for blending of biodiesel. It is the biggest advantage of biodiesel as compare to other alternative sources. Biodiesel performs as well as petroleum diesel, while reducing emissions of particulate matter, carbon monoxide (CO), hydrocarbons and oxides of sulphur (SOx) [28]. Emissions of oxides of nitrogen (NOx) are, however, higher for biodiesel in many engines. Biodiesel virtually eliminates the notorious black soot emissions associated with diesel engines and the total particulate matter emissions are also much lower. Other environmental benefits of biodiesel include the fact that it is highly biodegradable and appear to reduce emissions of air toxics and carcinogens (relative to petroleum diesel). Usage of biodiesel will allow a balance to be sought between agriculture, economic development and the environment.

## VIII. OPPORTUNITY OF BIODIESEL FROM MICROALGAE IN INDIA

Now a day’s India is one of the fast growing country both in terms of its population as well as in its economy. In terms of economy, energy plays a very important role. As discussed above in research paper, microalgae are an opportunity to produce sustainable green energy especially in India. Economic growth in India is directly correlated with both the demand and supply of energy in different sectors. Use of fossil fuel and environmental protection are the burning topic of discussion for sustainable development of a country. As the consequence of use of fossil fuel, there is severe increase in air and water pollution, deforestation, water shortages, carbon emissions, global warming as well as other environmental issues. This is the high time to move towards the renewable sources of energy and switch off the use of fossil fuel [17]. In this context, microalgae are good and potential source for biodiesel production. In India different species of microalgae are available with different chemical composition. This is an opportunity to find out the efficient species of microalgae and carried out the further research.

## IX. CONCLUSION

Algae, as a source of biofuel, offer many advantages over traditional biofuel crops including the potential to be grown on the marginal land, the use of water sources not suitable for agriculture (e.g. high salt content could be tolerated), as well as high growth rates, and relatively high lipid content [6]. In the application of algae to remove carbon dioxide ($CO_2$ biofixation) and energy production, tolerance to the $CO_2$, is of great importance [29]. Literature reports on the $CO_2$ effect are greatly contradictory. Some of them showed that the concentration of $CO_2$ aeration above 5% could be harmful to microalgae cells and inhibit the microalgae growth [1]. Tang et al. (2011) reported that *Scenedesmus obliquus* and *Chlorella pyrenoidosa* could grow well under $CO_2$ concentrations ranging from 5% to 30%. They even could grow at the $CO_2$ level of 50%. Without an effective policy and control, large scale algal biofuel production is likely to impact food security and therefore needs to be cultivated in areas that do not conflict with food security. lot more research and technology develop is required in areas of algal cultivation, harvest and processing before large-scale substitution of petroleum derived fuel by algal biofuels can be realized. Waste management efforts can be efficiently adapted to agriculture and algal biofuel production to fufill the dual objectives of environmentally benign wastewater processing and C neutral biofuel production. This requires that multiple goals of biofuel production, sustainability and waste management besynchronized.

## REFERENCES

[1]. Banerjee A., Sharma R., Chisti Y., Banerjee U.C., Botryococcus braunii : a renewable source of hydrocarbons and other chemicals, *Crit. Rev. Biotechnol*, **44**, 245-79 **(2002)**

[2]. Becker E.W., In Microalgae: biotechnology and microbiology, Ed. Baddiley, *J.et al. Cambridge Univ. Press, Cambridge NewYork*, 198 **(1994)**

[3]. Borowitzka M.A., Commercial Production of microalgae: pond, tanks, tubes and fomenters, *Journal of Biotechnolgy* **70**, 313-21 **(1999)**

[4]. Chisti Y., Microalgae as sustainable cell factories, *Environ. Eng. Manag.*, **5**, 261-274 **(2006)**

[5]. Chisti Y., Research Review paper: Biodiesel from microalgae, *Biotechnology Advances*, **25**, 294-306 **(2007)**

[6]. Chiu S.K., Kao C.Y., Tsai M.T., Ong S.C., Chen C.H., Lin C.S., Lipid accumulation and $CO_2$ utilization of Nannochloropsis oculata in response to $CO_2$ aeration, *Bioresource technology*, **100**, 833- 38 **(2009)**

[7]. Eriksen N.T., The technology of microalgal culturing, *Biotechnol. Lett.* **30**, 1525-36 **(2008)**

[8]. Fukuda H., Kondo A., Noda H., Biodiesel fuel production by transesterification of oils, *J Biosci Bioeng*, **92**, 405-16 **(2001)**

[9]. Greenwell H.C., Laurens M.L. Shields R.J., Lovitt R.W., Flynn K.J., Placing micralgae on the biofuels priority list: A review of the technological challenges, *J. Roy. Soc. Interfa,.* doi:10.1098/rsif. 0322 **(2009)**

[10]. Guschina I.A., Harwood JL. Lipids and lipid metabolism in eukaryotic algae. Prog. Lipid. Res. 2007; 45: 160-86.

[11]. Hill J., Nelson E., Tilman D., Polasky S., Tiffany D., Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels, *Proc Natl Acad Sci USA,* **103**, 11206 -210 **(2006)**

[12]. Hsieh C.H., Wu W.T., Cultivation of microalgae for oil production with a cultivation strategy of urea limitation, *Biores. Technol*, 100, 3921-926 **(2009)**

[13]. Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Seibert M., *et. al.* Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, *Plant J*, **54(4)**, 621-39 **(2008).**

[14]. Illman A.M., Scragg A.H., Shales S.W., Increase in Chlorella strains calorific values when grown in low ni-trogen medium, *Enz. Microb. Technol.*, **27**, 631-35 **(2000)**

[15]. Indian petroleum & natural gas statistics, 2011-2012. *Ministry of Petroleum and Natural Gas (MoPNG)*, Govt. of India, **(2012)**

[16]. Kleinschmidt M.G., McMohan V. A., Effect of growth temperature on lipid composition of *Cyanidium caldanum, Plant Physiology*, **46,** 290-293 **(1970)**

[17]. Laura L., *et. al.* Engineering algae for biohydrogen and biofuels production, *Current opinion in biotechnology*, 264-71 **(2009)**

[18]. Li Y., Horseman M., Wang B., Wu N., Lan C.Q., Effects of nitrogen sources on cell growth and lipid accumu-lation of green alga Neochloris oleoabundans, *Appl. Micro-biol. Biotechnol*, **81**, 629-36 **(2008)**

[19]. Mata T.M., Martins A.A., Caetano S.N., Micro-algae for biodiesel production and other applications: A re-view, *Renew. Sustain. Energy. Rev.*, **14**, 217-32 **(2010)**

[20]. Meher L.C., Vidya Sagar D., Naik S.N., Technical aspects of biodiesel production by transesterification -a review, *Renew Sustain Energy Rev.*, **10**, 248-68 **(2006)**

[21]. Metzger P, Largeau C., Botryococcus braunii: a rich source for hydrocarbons and related ether lipids, *Appl. Microbiol.Biotechnol*, **66**, 486-96 **(2005)**

[22]. Molina Grima E., Acién Fernández F.G., García Camacho F., Chisti Y., Photobioreactors: light regime, mass transfer, and scaleup, *J. Biotechnol*, **70**, 231-47 **(1999)**

[23]. NREL. Biodiesel Handling and Use Guide – Fourth Edition, *National Renewable Energy Laboratory*, REL/TP-540-43672, Available from: http://www.osti.gov/bridge **(2010)**

[24]. Olaizola M., Commercial development of microalgal biotechnology: From the test tube to the marketplace, *Biomolecular Engineering*, **20**, 459-66 **(2003)**

[25]. Pulz O., Photobioreactors: Production systems for phototrophic microorganisms, *Appl. Microbiol. Biotechnol*, **57**, 287-93 **(2001)**

[26]. Sharma R., Chisti Y., Banerjee U.C., Production, purification, characterization, and applications of lipases, *Biotechnol Adv*, **19,** 627–62 **(2001)**

[27]. Suzuki E., Ohkawa H., Moriya K., Matsubara T., Nagaike Y., Iwasaki I., et al. (2010). Carbohydrate metabolism in mutants of the cyanobacterium *Synechococcus elongatus* PCC 7942 defective in glycogen synthesis. Appl. Environ. Microbiol. 76, 3153–315910.1128/AEM.00397-08

[28]. Takagi M., Watanabe K., Yamaberi K., Yoshida T., Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of Nannochloris sp. UTEX LB1999, *Appl. Microbiol. Biotechnol*, **54**, 112-17 **(2000)**

[29]. Tang D., Han W., Li P., Miao X., Zhong J., $CO_2$ biofixaton and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different $CO_2$ levels, *Bioresource Technology*, **102**, 3071-76 **(2011)**

[30]. Tredici M.R., Bioreactors, photo. In: Flickinger M.C., Drew S.W., editors. Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation. *Wiley*, 395-419 **(1999)**

[31]. Wawrik B**.,** Harriman B., Rapid, Colorimetric Quantification of Lipid from Algal Cultures, *The Journal of Microbiological Methods*, **80**, 262-66 **(2010)**

[32]. Xiong W., Li X., Xiang J., Wu Q., High-density fermentation of microalga Chlorella protothecoides in bio-reactor for microbiodiesel production, *Appl. Microbiol. Bio-technol*, **78**, 29-36 **(2008)**