



## MICROALGAE AS POTENTIAL SOURCES OF BIOFUELS

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Energy security and conservation of environment are two key factors, indispensable for the growth, progress and sustainability of the human race. The fossil fuels reserves are depleting at an alarming rate; however, their major role in green house gases emissions is becoming even more dangerous for the environmental and ecological balance. Driven by these facts, world over, the researchers are determined to find alternative renewable and potentially carbon neutral biofuels as alternative energy sources. The alternate energy sources like the first generation biofuels derived from food crops such as maize, sugarcane, rapeseed and sugar beet caused a great worry to world food markets and exaggerated the water shortages. Second generation biofuels obtained from lignocellulosic residues of agriculture and nonfood crops address some of the issues; but, there is concern over land use changes. As a consequence, third generation biofuels specifically derived from microalgae are considered to be commercially viable alternative energy sources, free from major disadvantages associated with first and second generation biofuels. Like plants microalgae are photosynthetic in nature, known to produce carbohydrates, proteins and lipids in large quantities in short durations of time. This review focuses on various technologies used for the production of biofuels from algae. The outcome of the study reveals that the microalgae-derived biofuels could gradually replace the petroleum diesel to meet the growing energy needs.

**Keywords:** Biofuels, CO<sub>2</sub> sequestration; Conversion; Microalgae, Production.

### **Introduction**

The deposits of fossil fuels and natural gas reserves are decreasing day by day and keeping this into consideration efforts have been initiated from past few decades to find out alternate fuels and energy sources. However, the substantial question which has come up recently is that the fossil fuels and natural gas reserves may last for next few decades, but their unregulated use has resulted in catastrophic environmental

changes which have led to global warming due to increase in the greenhouse gases (GHG) emissions especially CO<sub>2</sub>. The overall insinuation is that renewable energy resources have to be developed for energy security; but strategies for simultaneous mitigation of unregulated CO<sub>2</sub> emissions associated with the existing fossil fuels and prospective fuels/ energy sources have to be developed. These challenges can be met out by (i) increasing the energy efficiency (i.e.

decreasing energy use per unit) of product, process or service (ii) increasing use of clean fossil energy (i.e. separation of CO<sub>2</sub> from flue gases and burring it under land or water bodies for gradual release) and (iii) increasing the use of renewable energy which do not produce CO<sub>2</sub>.

Since last few decades, great success has been achieved in developing liquid renewable energy sources. Presently, the first generation biofuels have been developed to meet the commercial demands at affordable prices. However, these are mainly produced from food and oil crops including sugarcane, sugar beet and maize for bio-ethanol and rapeseed oil, vegetable oils and animal fats for biodiesel production<sup>1,2</sup>. It is therefore presumed that the use of first generation biofuels will remain restricted due to competition with food production sectors, where the same products (crops) are required as feed stocks. Besides, the lack of well managed agricultural practices, high water and fertilizer requirements will also limit their application as alternate energy sources<sup>3</sup>.

Realizing the limitations associated with the commercial viability of the first generation biofuels, the second generation biofuels were developed from the whole plant matter of non-edible crops, agricultural residues, forest harvesting residues and wood processing wastes<sup>4</sup>, leaving the food crops for human consumption. However, the technology for conversion of these wastes, specifically the lingo-cellulosic wastes into usable substrates for bioenergy (biofuel) production has not reached the commercial scales, which has so far hindered their large scale production and applications<sup>1</sup>.

So, in order to make a renewable energy source technically and economically viable, certain conditions need to be fulfilled viz. (i) it should be cost effective i.e. should

be competitive or cheaper than petroleum fuels (ii) should demand less land use or should not share any significant land use of the current crops (iii) The technology should have provisions for restricting CO<sub>2</sub> emission (e.g. CO<sub>2</sub> sequestration), and (iv) should demand minimal water use. These can be achieved through well planned exploitation of microalgae, which have the great potential to fulfill the energy demand along with the ability to enable the development of environmentally safe biofuel production technology<sup>5</sup>.

#### **Microalgae Used for Biofuels Production**

In the present review, the term microalgae refers to both prokaryotic (Cyanobacteria) and eukaryotic (green algae, red algae and diatoms) microalgae. The most prominent classes of microalgae for the production of biofuels are: green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta)<sup>6,7</sup>. Algae have both autotrophic, mixotrophic and heterotrophic genera. Autotrophic algae just like plants require only simple nutrients such as salts, CO<sub>2</sub> and alight energy source for growth. Mixotrophic algae can live either as autotrophic algae or as heterotrophic algae depending on the prevailing environmental conditions. Heterotrophic algae on the other side are non-photosynthetic and therefore require reduced organic compounds as source of energy and carbon as well as other synthetic nutrients. Some photosynthetic algae are mixotrophic, i.e. they can do photosynthesis as well as use exogenous organic nutrients as energy sources<sup>6</sup>.

#### **Microalgae as Better Resources for Biofuels**

There are many advantages of using microalgae as sources of biofuels: (1) microalgae grows throughout the year, so, oil productivity of microalgal cultures is higher than the best oilseed crops<sup>8</sup> (2)

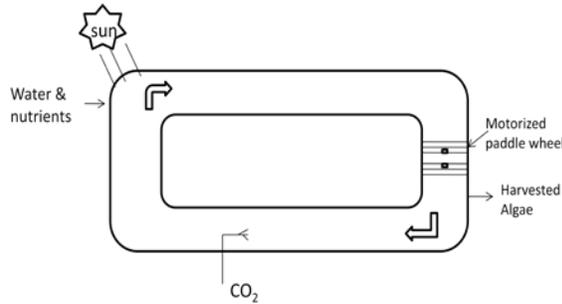


Fig. 1: Open pond system for microalgae cultivation

Although, their cultivation require water (based media), but the volume of water required is quite less than that required for the terrestrial crops, therefore, reducing the load on freshwater sources<sup>9</sup> (3) microalgae can be cultivated in saline waters not fit for drinking, and therefore may put minimal burden on potable water, minimizing associated environmental impacts<sup>9</sup> (4) microalgae have fast growth rates compared to plants, (biomass doubles in periods as short as 3.5 h)<sup>11-13</sup> and many species have oil content in the range of 20–50% of their dry biomass weight (5) they can maintain and improve the air quality by biofixation of waste CO<sub>2</sub> (1 kg of dry algal biomass consumes about 1.8 kg of CO<sub>2</sub>)<sup>11</sup> (6) microalgae can obtain nutrients (especially nitrogen and phosphorus) from waste water, therefore, organic effluent from the agri-food industry can be remediated using microalgae cultivation<sup>14</sup> (7) there is no input of cost in terms of herbicides or pesticides application for algae cultivation, besides it also eliminates the instances of land and water pollution through herbicides or pesticides leaching due to rain water runoff<sup>15</sup> (8) microalgae can be used to produce valuable co-products such as proteins, carbohydrates and residual biomass after oil extraction, which may be used as feed or bio-fertilizer<sup>13</sup>, or fermented to produce ethanol or methane<sup>16</sup> and (9) microalgae are capable of producing biohydrogen<sup>17</sup>. These

are some of the potentials of microalgae which make them most suitable candidates for the production of biofuels.

### Current Methods for Microalgal Biomass Production

Cultivation of microalgae under natural conditions has certain advantages such as the free availability of energy source in the form of sunlight and soluble nutrients naturally present in the water body<sup>18</sup>. However, the productivity under such conditions may have limitations due to non-availability of sunlight during night and seasonal variations in the availability of solar radiations. Such production plants can be successful in only those areas which have high availability of solar radiations<sup>19</sup>. Therefore, the confines of sunlight have been solved by employing artificial means of light (fluorescent lights) for the production of photoautotrophic algae at pilot and production scale stages<sup>20</sup>. Selection of an artificial source of light, is dependent on the absorption spectra of major accessory pigments present in different algal groups. For example, the common photosynthetic pigments in green algae are chlorophylls a, b and zeaxanthin; whereas, in diatoms chlorophylls a, c and fucoxanthin are the main pigments.

Microalgae can fix CO<sub>2</sub> from various sources such as atmosphere, discharge gases from heavy industry and soluble carbonates<sup>6</sup>. Generally, CO<sub>2</sub> is fed into the algae growth media either externally from the powerplants<sup>21,22-25</sup> or directly into the media in the form of soluble carbonates such as NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub><sup>26,27</sup>.

Other nutrients necessary for cultivation of algae are nitrogen and phosphorus<sup>28</sup>. Some algal species are capable of fixing nitrogen gas present in the air<sup>29,30</sup>, but majority of microalgae need it in the soluble form, such as solubilised urea<sup>31</sup>.

Phosphorus is assimilated in very small amounts during algal growth cycle<sup>32</sup>; however it should be supplied in excess of basic requirements, because phosphate ions combine with metals ions, due to which their bioavailability is reduced<sup>10</sup>.

#### **Phototrophic Production of microalgae**

At present, phototrophic production is the only method which is both technically and commercially viable for pilot or industrial scale production of algae biomass<sup>33</sup>. There are two main methods that have been developed viz. the open cultivation system (natural or modified ponds/water bodies) and closed the closed cultivation system (photobioreactor)<sup>34</sup>. The technical viability i.e. process development and scale-up of each system is dependent on the inherent properties of the selected microalgae strain under used, as well as environmental conditions and the economy of land and water used<sup>35</sup>.

#### **Open Production Systems**

Production of algae in open production systems i.e. open ponds has been practiced from last several decades<sup>34</sup>. These production systems can be classified into natural water bodies (ponds, lakes and lagoons) and artificial ponds. A typical artificial ponds is made up of a closed loop, oval shaped recirculation channels (Fig. 1), with provision for mixing and circulation to facilitate sustained algal growth and productivity. In a continuous production cycle, the liquid nutrient medium is introduced in front of the moving paddlewheel and circulated through the loop to the harvest point. The continuously moving paddlewheel prevents sedimentation. The CO<sub>2</sub> required for microalgal growth is generally meet out from the surface air; however, CO<sub>2</sub> pumps may be used to incorporate surplus CO<sub>2</sub><sup>36</sup>. Open pond system is a cost effective

method for large-scale algal biomass cultivation as compared to photobioreactors. Open pond production can be implemented in areas with marginal crop production potentials, thereby preventing the use of agriculturalland<sup>37</sup>. They also have lower energy input requirements<sup>15</sup>, and cleaning and regular maintenance are easier<sup>38</sup> and may have the propensity to provide with substantial net energy yield<sup>15</sup>. However, open pond systems are less efficient than the closed photobioreactors<sup>11</sup>. This can be due to several factors such as temperature fluctuation, evaporative losses of the media, CO<sub>2</sub> shortage, improper mixing, and inadequate light. Temperature fluctuations during day and night and during different seasons are hard to control in open ponds<sup>11</sup>. Considerable losses of CO<sub>2</sub> into the atmosphere due to diffusion may cause reduction in biomass yield due to suboptimal assimilation of CO<sub>2</sub>. Further, poor mixing by less efficient mixing instruments may turn into low biomass productivity due to poor mass (CO<sub>2</sub>) transfer rates<sup>38</sup>. Poor light penetration owing to thick top algal biomass layer may result in reduced biomass productivity. However, this can be resolved by reducing the thickness of biomass layer using thin layer inclined type culture systems, and by improving the mixing<sup>11,38-40</sup>.

#### **Closed Production Systems**

The limitations of open pond system gave the idea for developing closed photosystems i.e. the photobioreactors for the production of microalgae. In fact there are certain problems of open pond system such as pollution and contamination risks, which prohibit their application in pharmaceutical and cosmetics industries for the production of high-value products<sup>38</sup>. Further, monocultures which are based on single species of microalgae are possible with photobioreactors only, but not with the open

pond systems<sup>11</sup>. Examples of closed systems include the flat plate, tubular and column photobioreactors. These systems are more suitable for the production of high value products using single species of microalgae, as these prevent the microbial contamination. Due to the higher biomass yields attained downstream processing costs can be reduced substantially. However, the costs of closed systems are significantly higher than that of the open pond systems<sup>41</sup>.

A typical photobioreactor consists of an array of straight glass or plastic tubes<sup>38,42</sup>. The tubes are generally 0.1 m or less in diameter<sup>11</sup>. Re-circulation of algal cultures is more commonly carried out with mechanical pumps; however airlift systems are better, as they allow exchange of CO<sub>2</sub> and O<sub>2</sub> between the liquid medium and aeration gas besides the mixing of culture and media components<sup>43</sup>. Examples of photobioreactors includes Flat-plate photobioreactors and Column photobioreactors.

In recent years, closed photobioreactors have become main focus of research in algal production technologies. The increasing use of closed photobioreactors compared to the open pond systems in industrial production strategies is due to the precise process control and substantially higher biomass yields and corresponding higher production of biofuels.

### **Factors Affecting Microalgal Biofuels Productivity**

The major factors which are crucial for economical production of biofuels from microalgae, are described below.

*Photosynthetic Efficiency:* Photosynthetic efficiency (PE) is expressed as the fraction of light energy that is fixed as chemical energy during photoautotrophic growth<sup>44</sup>. Only the wavelengths between 400 and 700 nm,

representing around 42% of the total solar radiations is captured by algae for photosynthesis. The captured energy is used for fixing the CO<sub>2</sub> into carbohydrates via the Calvin cycle as per the reaction scheme is summarized below.



Most terrestrial plants attain average PE levels between 1% and 2%<sup>45</sup>. Whereas, algae achieve substantially higher PE values compared to terrestrial plants. For example, PE levels for *Chlorella* sp. has been recorded in the range between 6 to 9 %<sup>46-48</sup>. Even higher PE values of 15% and 21 % have been recorded for some algal sp., indicating significantly higher PEs for algae compared to terrestrial plants. These reports clearly support the fact that the microalgae can act as the most competent resource for biofuels production<sup>49</sup>.

*Microalgal Strain selection:* Selection of suitable algal strains is crucial to the overall success of biofuel production from microalgae<sup>50-52</sup>. The ideal algae strain for biofuel production should: (i) have high lipid yield or productivity (ii) be able to outgrow contaminating strains in open pond production systems (iii) be robust enough to survive the shear stresses common in photobioreactors (iv) have high capacity for CO<sub>2</sub> assimilation (v) have limited nutrient requirements (vi) be tolerant to a wide temperature range due to diurnal cycle and seasonal variations (vii) have a short life cycle (for high productivity) (viii) have a high photosynthetic efficiency and (ix) possess self-flocculation ability. At present, there is no such strain reported which meets all these requirements simultaneously.

Another important factor for commercial microalgal production is the adaptability of algae to the (production) site<sup>51</sup>. The adaptability in the prevailing

environmental conditions, gives the native algal strain a distinct advantage over the imported ones<sup>51</sup>. Two algae viz. *Chlorella kessleri* and *Scenedesmus obliquus*, isolated from effluent treatment ponds near a power plant have been reported to carry out substantial biofixation of CO<sub>2</sub> in the open system<sup>53</sup>. In another study on algal biofuel production, variation in the utilization of CO<sub>2</sub> has been reported, wherein the algae *Botryococcus braunii* was found to be most suitable for biodiesel production, the other algae *Scenedesmus* sp. was shown to be more suitable for CO<sub>2</sub> mitigation<sup>54</sup>.

The wild type strains of algae isolated from nature may have limitations for the production of lipids, therefore, in order to improve the production capabilities of algal strains genetic manipulation may be used<sup>50</sup>. Interest in creating the transgenic microalgae as green bio-factories is growing day by day because of their potential to produce biofuels and other value added products such as proteins and metabolites<sup>54</sup>.

### **Lipid productivity**

Many microalgal strains constitutively produce high levels of lipid (~20–50% of dry cell weight). The lipid concentration can further be increased by optimising the physic-chemical factors<sup>56</sup> such as regulating the light intensity<sup>57,58</sup>, temperature<sup>57</sup>, nitrogen level<sup>58-61</sup>, salinity<sup>57,61</sup>, and CO<sub>2</sub> concentration<sup>59,62,63</sup>. The most suitable method of increasing lipid accumulation in microalgae is nitrogen limitation, which not only helps in lipid accumulation, but also promotes a gradual change in lipid composition from free fatty acids to triacylglycerols (TAGs)<sup>59</sup>. TAGs are more suitable for production of biodiesel<sup>63</sup>. Lipid accumulation in microalgae occurs under growth limiting conditions e.g. due to nutrient limitation (typically nitrogen). Under such conditions cell

propagation stops but carbon assimilation continues and converted to TAG lipids that are stored within the cells, thereby gradually increasing the lipid concentration<sup>63</sup>. The effects of nitrogen concentration, salinity and light intensity on lipid productivity has been studied, showing a recorded increase in production of lipids up to 76% for specific growth conditions<sup>61</sup>. Contrarily higher lipid productivity has been reported for some microalgae under nitrogen sufficient conditions and high light intensity compared to nitrogen deficient conditions<sup>68</sup>. In case of CO<sub>2</sub> a concentration of 2% (v/v) was optimal for *Nannochloropsis oculata* for obtaining maximum biomass and lipid productivity<sup>62</sup>.

### **Recovery of Microalgal Biomass**

The method used for recovery of microalgal biomass depends on the physical properties of microalgae, e.g. size, concentration and buoyancy and on the cost of the target products<sup>64</sup>. Generally, microalgae harvesting involves:

*Bulk harvesting* – this is performed for separation of biomass from the bulk suspension. The concentration factors for this operation are generally 100–800 times to reach 2–7% total solid matter. This will depend on the initial biomass concentration and technologies employed, including flocculation, flotation or gravity sedimentation.

*Concentration*—the aim is to concentrate the slurry through techniques such as centrifugation, filtration, hence, is generally a more energy intensive step than bulk harvesting

*Flocculation*: Flocculation is generally the preparatory step in the harvesting of microalgal cells at the industrial scale. It causes aggregation of the microalgal cells to create flocs which are big enough in size for easy and effective removal of the

biomass in the subsequent steps of filtration, flotation or gravity sedimentation<sup>65</sup>. Flocculants neutralizes or reduces the negative charges present on the surface of microalgae cells thereby facilitating their aggregation. Multivalent metal salts like ferric chloride, aluminium sulphate and ferric sulphate are generally used as flocculants.

*Flotation:* In this method micro-air bubbles are dispersed into the media, which naturally cause floatation of the microalgal cells. This method does not require any addition of chemicals as in the case of flocculation<sup>6</sup>. Some microalgae naturally float at the surface of the water due to high lipid contents<sup>52</sup>.

*Sedimentation by Gravitation and Centrifugation:* Sedimentation of biomass by gravitation is the most common method of harvesting of algal cells produced using waste water obtained from waste treatment plants<sup>66</sup>. However, this technique is only suitable for large microalgae with size greater than 70  $\mu\text{m}$ <sup>67</sup>. On the other side, centrifugal recovery is the preferred method for biomass harvesting associated with high value metabolites and products<sup>68</sup>. The process is fast but consumes high electrical energy; besides biomass recovery depends on the factors viz. density of the cells and residence time of slurry in the centrifuge<sup>65</sup>. For instance, harvesting efficiency of centrifugation of a 15% (w/v) biomass slurry is greater than 95%<sup>68</sup>, and a concentration factor 150 times is technically possible<sup>69</sup>.

*Filtration:* The conventional filtration process is a quite suitable method for harvesting of relatively large (>70  $\mu\text{m}$ ) microalgae such as *Spirulina* and *Coelastrum*. It cannot be used to harvest small sized algae cells like that of *Chlorella* and *Dunaliella* (size less than 30  $\mu\text{m}$ )<sup>69</sup>. The conventional filtration is

carried out under applied pressure or suction, filtration aids such as cellulose or diatomaceous earth may be added to improve the efficiency<sup>65</sup>.

Recovery of algal cells with size less than 30  $\mu\text{m}$  can be performed through microfiltration and ultra-filtration techniques<sup>70</sup>. This method takes care of small size and also of the fragile nature of some microalgae that need the low pressure conditions<sup>33</sup>. Membrane filtration is more cost effective than centrifugation for processing of low broth volumes (less than 2000 liters per day). On the other side centrifugation is economical than micro & ultra-filtration for harvesting of biomass from larger scale productions (greater than 20,000 liters per day)<sup>71</sup>.

### **Methods for Conversion of Algal Biomass to Biofuels**

The technologies for conversion of microalgal biomass to biofuels can be separated into two basic categories of thermochemical and biochemical conversion. Factors that influence choice of conversion method include: the nature, water content and amount of biomass feedstock, the preferred form of the bio-energy and cost effectiveness of the method<sup>72</sup>.

*Thermochemical Conversions:*

Thermochemical conversion refers to thermal treatment of biomass to produce fuel to extract out biofuels and other co-products, and is performed using different methods such as direct combustion of the biomass, thermochemical liquefaction, gasification and pyrolysis<sup>73</sup>. The methods are described below.

*Gasification:* Gasification refers to treatment of the algal biomass at very high temperatures (800–1000 °C) to yield a mixture of combustible gases<sup>74</sup>. During gasification, the biomass is oxidised and

hydrolysed (with steam) to produce syngas i.e. a mixture of CO<sub>2</sub>, CO, H<sub>2</sub>, N and CH<sub>4</sub><sup>75</sup>. Syngas is a convenient to use fuel that can directly be used as a fuel for gas engines or gas turbines<sup>76</sup>. Gasification studies have been carried out for many microalgal biomass. Gasification studies on *Spirulina* biomass were performed between 850 to 1000 °C and the results showed that gasification at 1000 °C produced the highest possible theoretical yield of methanol i.e. 64 % of the biomass weight<sup>77</sup>. In another investigation<sup>78</sup> gasification of the microalgae *Chlorella vulgaris* was performed using a novel process, wherein a CH<sub>4</sub> rich syngas was obtained, besides the total nitrogen content of the microalgae was converted into fertilizer grade NH<sub>4</sub>.

**Thermal Liquefaction:** Thermal liquefaction refers to a process that converts wet algal biomass into liquid fuel<sup>79</sup>. Thermal liquefaction is catalysed in the presence of hydrogen at temperatures ranging from 300–350 °C and pressure ranging from 725- 2900 psi<sup>80</sup>. The process decompose biomass materials down to shorter and smaller molecular materials i.e. the bio-oil with a higher energy density<sup>81</sup>. A study on thermal liquefaction of *Botryococcus braunii* biomass at 300 °C reported a maximum yield of 64% (dry wt. basis) of bio-oil with Higher Heating Value (HHV) of 45.9 MJ kg<sup>-1</sup> and a positive energy balance of 6.67:1 (output/input ratio) for the process. In another study, a bio-oil yield of 42% (of the dry wt.) was achieved from *Dunaliella tertiolecta* with a HHV of 34.9 MJ kg<sup>-1</sup> and positive energy balance of 2.94:1<sup>49</sup>. These results show that thermal liquefaction is a commercially feasible process for the production of liquid fuel from the algal biomass.

**Pyrolysis:** The pyrolysis process used to convert biomass to bio - oil, syngas and

charcoal is carried out anerobically at temperatures ranging from 350–700 °C<sup>80</sup>. This process has been considered to be competent enough for commercial production of biofuels with potential to replace petroleum based liquid fuels<sup>80</sup>. However, there are technical issues, as oils obtained through this process are acidic, viscous, unstable and contain chemically dissolved water<sup>82</sup>. Therefore, the process needs refinements to lower the content of water and other residues<sup>75</sup>. However, in comparison to other conversion technologies, extensive research has been performed on pyrolysis of algal biomass which suggests that this process can be successfully applied at commercial scale.

#### Biochemical Conversions

The biological process of energy conversion of biomass into other fuels includes anaerobic digestion, alcoholic fermentation and photobiological hydrogen production:

**Anaerobic digestion:** Anaerobic digestion (AD) refers to the anaerobic bio-conversion of organic wastes into biogas, which is mainly comprised of CH<sub>4</sub> and CO<sub>2</sub><sup>83</sup>. The energy content of the gas produced through this process is about 20–40% of the feedstock. It is an appropriate process for organic wastes with high moisture content (80–90%)<sup>84</sup>, so it is suitable for the conversion of wet algal biomass to biofuels. The process has three sequential stages viz. hydrolysis of biomass, fermentation of released sugars followed by methanogenesis. In hydrolysis the complex organic matter is degraded to soluble sugars. Then, fermentative bacteria convert these into alcohols, acetic acid, and gases like H<sub>2</sub> and CO<sub>2</sub>, which are then metabolized by methanogens into CH<sub>4</sub> (60–70%) and CO<sub>2</sub> (30–40%)<sup>14</sup>. Conversion of algal biomass into methane is an efficient energy extraction process, besides the waste product

obtained is rich in nutrients that can be reused for algal biomass production<sup>85,86</sup>.

**Ethanolic fermentation:** Ethanolic fermentation is the anerobic conversion of biomass materials like sugars (mono- and disaccharides), starch and cellulose into ethanol in the presence of the yeast<sup>84</sup>. The dilute alcoholic product (10–15% ethanol) is distilled to remove the water and other impurities. The concentrated ethanol (95% v/v) is condensed into liquid form, which can be used as a supplement or substitute for petrol incars<sup>75</sup>. The solid waste from the process can be used for animal feed preparation or for conversion to syngas through gasification<sup>84</sup>. This helps to compensate for the feed stock costs which typically make up 50–80% of the final ethanol selling price. *Chlorella vulgaris* a good source of ethanol due to its high starch content (upto 37% of the dry cell wt.) and for which upto 65% conversion efficiency (from starch to ethanol) has been reported<sup>16</sup>. From the above account it can be determined that ethanol production from microalgae is technically feasible

#### Photobiological production of Hydrogen Gas

Hydrogen (H<sub>2</sub>) gas is a clean fuel with high energy efficiency<sup>74</sup>. Microalgae have the potential to produce H<sub>2</sub> gas in the presence of light<sup>17</sup>. Eukaryotic microalgae produce H<sub>2</sub> under anaerobic conditions, either as an electron donor in the CO<sub>2</sub> fixation process or it is evolved in both light and dark conditions<sup>87</sup>. The microalgae convert water molecules into hydrogen ions (H<sup>+</sup>) and O<sub>2</sub> during photosynthesis; the hydrogen ions produced are then anaerobically converted to H<sub>2</sub> by hydrogenase enzyme<sup>14</sup>. The enzyme hydrogenase is sensitive to O<sub>2</sub>; therefore the O<sub>2</sub> produced during photosynthesis causes rapid inhibition of the

enzyme, and the photosynthetic hydrogen production

is hampered<sup>14,44,88-90</sup>. Therefore, for the purpose of H<sub>2</sub> production, microalgae must be cultured under anaerobic conditions. There are mainly two approaches for photosynthetic H<sub>2</sub> production from water. The first H<sub>2</sub> production process is a two stage process where photosynthetic oxygen production and H<sub>2</sub> gas generation are temporally separated<sup>17</sup>. In the primary stage, algae are grown photosynthetically under normal conditions. In the secondary stage, the algae are grown under anaerobic conditions, which stimulate the consistent production of H<sub>2</sub><sup>91</sup>.

The second approach involves the photosynthetic production O<sub>2</sub> and H<sub>2</sub> gas simultaneously. Here, the electrons released during photosynthetic splitting of water are fed directly into the H<sub>2</sub> evolution process<sup>17</sup>. Although, the H<sub>2</sub> productivity of this process is theoretically higher than the two-stage photosynthetic process; but this method of simultaneous production of O<sub>2</sub> and H<sub>2</sub> suffers from severe hydrogenase inhibition after a very short period of initiation during photosynthesis<sup>17</sup>.

#### Biodiesel from Algal Biomass

Biodiesel is mixture of fatty acids methyl esters (FAMES) generally produced via transesterification reaction between triglycerides (vegetable oil/ animal fat/algal oil) and alcohol (methanol/ethanol) in presence of alkali such as potassium hydroxide or sodium hydroxide as catalyst<sup>74,92</sup>. Biodiesel can be used as a pure fuel (100%) in any engine running with petroleum diesel or blended with petroleum diesel in different ratios such as B20, B50 and B80 representing respectively 1:5, 1:2 and 4:5 biodiesel/petroleum diesel on volume/volume (v/v) basis. The Production process consists of two steps: 1<sup>st</sup>- extraction

methanol (or ethanol) into biodiesel using a suitable catalyst like alkali or enzyme (lipase).

Algal biodiesel has similar physical and chemical properties to petroleum diesel. However, it has many benefits compared to petroleum diesel, viz. it is derived from biomass and therefore is renewable, biodegradable, and semi-carbon neutral, it is non-toxic and contains reduced levels of carbon monoxide, soot, particulates, hydrocarbons and sulphur oxides. It is important to note that compared to the 1<sup>st</sup> generation biodiesel, algal biodiesel is a better fuel for the aviation industry where low freezing points and high energy densities are crucial requirements<sup>93</sup>. The second major benefit of algal biodiesel is that the CO<sub>2</sub> emissions can be reduced up to 78% of that of the petroleum diesel<sup>94</sup>.

#### Mitigation of CO<sub>2</sub> Emissions

Microalgae can efficiently capture CO<sub>2</sub> released from different sources such as atmospheric CO<sub>2</sub>, CO<sub>2</sub> emissions from transportation, power plants and industries and CO<sub>2</sub> released from soluble carbonates. The economic viability of the bio-mitigation process is dependent on the selection of appropriate microalgal strains. The important prerequisites for high CO<sub>2</sub> fixation are high rates of CO<sub>2</sub> assimilation, high tolerance of trace constituents of flue gases such as sulphur oxides and nitrogen oxides. Besides, the microalgae should have the propensity for resource generation for the production of biofuels and co-products; should be easy to recover or harvest; should be tolerant to high temperature, as the exhaust gases which acts as source of CO<sub>2</sub> carry high temperature; should be suitable for generation of value-products from wastewater treatment processes. Sufficient data are available which signifies the carbon

sequestration potential of under various conditions. In this respect *C. vulgaris* grown on wastewater discharge from a steel plant successfully sequestered 6.2 % (w/v) of CO<sub>2</sub> per day<sup>95</sup>. In another paper, the use of *Chlorella* sp. has been reported for reduction of CO<sub>2</sub> concentration in flue gases by 10–50%<sup>24</sup>. These results were in line with the finding of other researchers. For example, de Moraes and Costa<sup>95</sup>, used flue gas as source of CO<sub>2</sub> for the production of *Spirulina* sp. and reported a maximum CO<sub>2</sub> biofixations of around 53 % and 45 % for 6% (v/v) CO<sub>2</sub> and 12% (v/v) CO<sub>2</sub>, respectively.

Despite the potential of microalgae as resources for the production of biofuels, the high cost associated with the production technology, makes it costlier than the petroleum based fuels and therefore, hinders its commercialization<sup>37</sup>. However, integration of bio-mitigation of CO<sub>2</sub> emissions as a part of the production technology may plausible solution to reduce the cost of algal biofuels.

#### Conclusions

This review highlights the viability of the current technological innovations in biofuels' production technology from microalgae as a renewable energy resource and for bio-mitigation of CO<sub>2</sub> emissions from petroleum based fuels. High contents of cellular lipids combined with some useful co-products when properly utilized, would improve the commercial feasibility of microalgae as a resource for the production of biofuels. Phototrophic production is the most effective in terms of net energy balance. Combination of the two processes viz. Microalgal-mitigation of CO<sub>2</sub> emissions and its biofuels' production process may result in cost reduction for the production of biofuels. It is also suggested that thermal liquefaction and

pyrolysis are the most technically viable

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biofuels, after the extraction of oils from them.

The research outcomes presented in this review suggests that the continued improvement in production technologies to optimize the microalgae biomass production, increasing the oil content and extraction of the oil and biomass processing would open the avenues for utilization of microalgal biofuels over the petroleum fuels for meeting out various energy demands.

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