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A Review on Microalgae Based Biofuels

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Abstract: ***In the present day scenario of soaring oil prices, depleting oil reserves and increasing global warming, biofuels present a renewable and alternative source for energy requirements. It is reported that biofuel produced from microalgae provides better yield among other sources like soybean, jatropha, corn etc. CO_2 fixation and bioremediation by removal of several inorganic compounds from waste water are additional benefits. Faster growth rate and ability to grow in harsh conditions and waste land makes microalgae more interesting for biofuel production. This paper presents a review on various microalgae species that can be used for production of biofuels, methods of cultivation, harvesting techniques, lipids extraction technologies from algae biomass and catalytic conversion of lipids into biofuel.***

Keywords: Microalgae, Biofuels, Biodiesel, Bioethanol

1. Introduction

The existing energy system is heavily dependent on fossil fuels which are geographically concentrated in few regions of the world. Dependence on imported fuels leaves many countries vulnerable to disruption in supply, which might pose physical hardships and economic burdens; the weight of fossil fuels imports on the balance of payments is unbearable for many poorer countries [1]. India, a developing country with crude oil reserves of 759.59 million tones (MT) (as on 31.03.2012) is highly dependent on import for fulfilling its crude oil requirements. More than 70% of its crude oil requirement and part of the petroleum products is met from imports. During 2011-12, India produced 38.09 MTs crude oil against an import of 171.73 MT [2]. Apart from economic issues, pollution is a major disadvantage of consuming fossil fuels. Burning of fossil fuels releases carbon dioxide, nitrogen monoxide, nitrogen dioxide, sulfur dioxide, carbon monoxide etc. which have severe consequences on the habitats and human health. Global warming is a serious consequence of greenhouse gases (GHG) emitted by vehicles and factories. Fossil fuels are non-renewable sources of energy as they are derived from pre-historic fossils and are no longer available if once used. Their source is limited and they are depleting at a faster rate. Progressive depletion of conventional fossil fuels with increasing energy consumption and GHG emissions have led to a move towards alternative, renewable, sustainable, efficient and cost-effective energy sources with lesser emissions [3]. Biofuels are therefore rapidly being developed. Biodiesel is currently produced from oil synthesized by conventional fuel crops that harvest the sun's energy and store it as chemical energy. This presents a route for renewable and carbon-neutral fuel production [4]. Biofuels can be classified as primary and secondary biofuels. Primary biofuels are those which are used in unprocessed form for heating and cooking purposes. They involve firewood, wood chips and pellets, etc. The secondary biofuels are those which are obtained by processing of biomass e.g. ethanol and biodiesel, and can be used in vehicles and industries. The secondary biofuels are further divided into first, second and third generation biofuels based on the raw material and technology used for their synthesis (Fig. 1). The first generation biofuel are produced from food crops such as soybeans, corn, potato, sugarcane etc. and thus compete with agriculture for arable land used for food production. They also need high fertilizers and pesticides for production of feedstock, thus imposing both economic and environmental limitations. The second generation biofuel use agricultural residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugarcane. Their synthesis requires pretreatment with special enzymes followed by costly technology, which makes them economically unfeasible [5]. The third generation of biofuels, i.e. microalgae based biofuels are devoid of drawbacks of first and second generation. Microalgae are able to produce 15-300 times more oil for biodiesel production than traditional crops; they have very short harvesting cycle (≈1-10 days) and they do not compete with food or feed crops [5].

1. Microalgae

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure [6]. They constitute more than 40,000 species and have been described as nature's own power cells and could provide alternatives to petroleum based fuels without competing with crops. They harvest the power of sun through photosynthesis and convert into biomass including oil (Fig. 2).

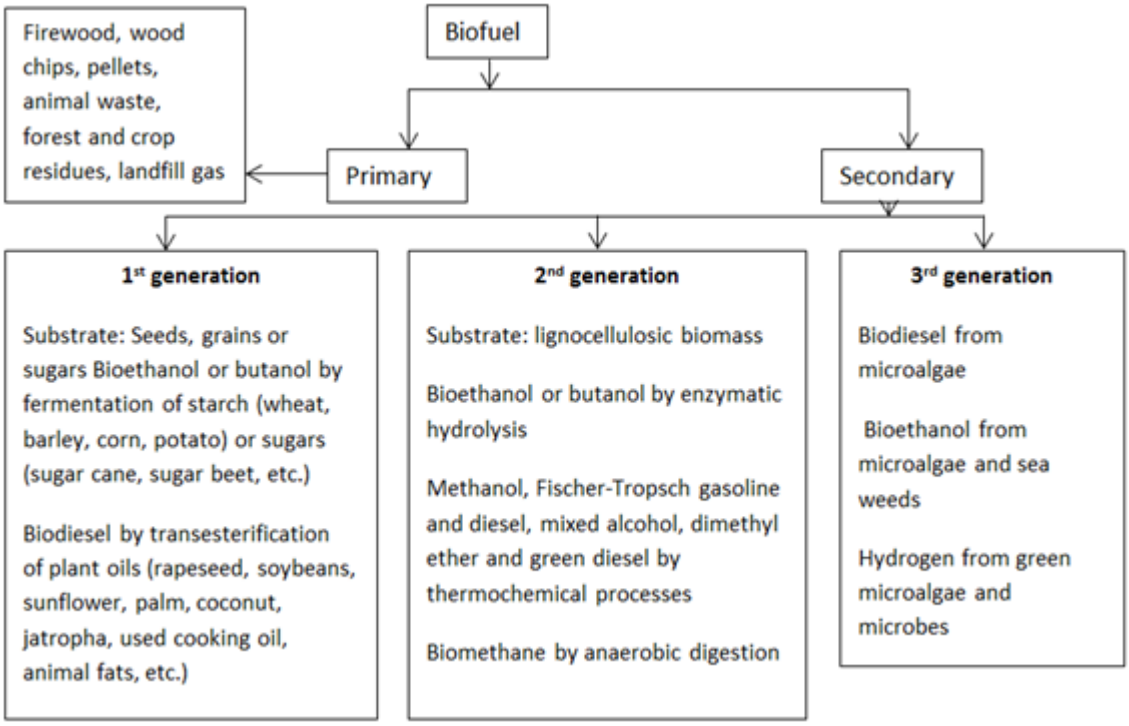

Figure 1: Classification of biofuels [5]

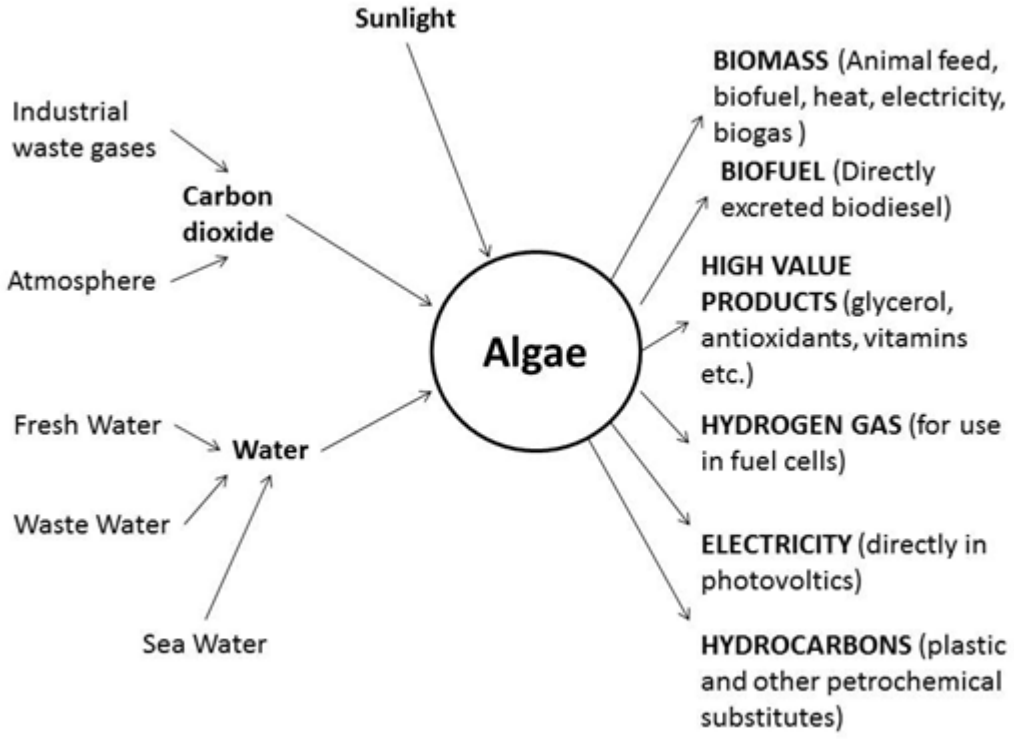

Figure 2: Conversion process for biofuel production from microalgal biomass

Microalgae have received considerable interest as a potential feedstock for biofuel production because, depending on the species and cultivation conditions, they can produce useful quantities of polysaccharides (sugars) and triacylglycerides (fats). These are the raw materials for producing bioethanol and biodiesel transport fuels. Microalgae also produce proteins that could be used as a source of animal feed, and some species can produce commercially valuable compounds such as pigments and pharmaceuticals [8]. Some microalgae have unique abilities such as producing hydrogen gas, which can be used in fuel cells [9]. These micro-organisms use CO_2 present in the atmosphere for cell growth. Industrial exhaust contains up to 15% CO_2, providing a CO_2 rich source for microalgal cultivation, thus providing an efficient route for CO_2 fixation [5,7]. Inorganic minerals such as NH_4^+, NO_3^-, PO_4^{3-} are also used by microalgae for fulfilling nutritional requirements, thus helping in bioremediation [6].

Both open system and closed system methods have been used for cultivation of microalgae. Strain selection is a key step for

successfully developing algal derived biofuels. Fast growing species which have high oil accumulation are preferred. Various microalgae strains and their oil content are shown in Table 1.

Table 1: Oil content of some microalgae [6, 10]

Microalga	*Oil content (% dry wt.)*
Ankistrodesmus sp	24.0-31.0
Botryococcus braunii	25.0-75.0
Chaetoceros muelleri	33.6
Chaetoceros	14.6-16.4
Chlorella emersonii	25.0-63.0
Chlorella protothecoides	14.6-57.8
Chlorella sorokiniana	19.0-22.0
Chlorella vulgaris	5.0-58.0
Chlorella pyrenoidosa	2.0
Chlorella	18.0-57.0
Chlorococcum sp	19.3
Crypthecodinium cohnii	20.0-51.1
Dunaliella salina	6.0-25.0
Dunaliella primolecta	23.1
Dunaliella tertiolecta	16.7-71.0
Dunaliella sp.	17.5-67.0
Ellipsoidion sp.	27.4
Euglena gracilis	14.0-20.0
Haematococcus pluvialis	25.0
Isochrysis galbana	7.0-40.0
Isochrysis sp.	7.1-33
Monodus subterraneus	16.0
Monallanthus salina	20.0-22.0
Nannochloris sp.	20.0-56.0
Nannochloropsis oculata	22.7-29.7
Nannochloropsis sp	12.0-53.0
Neochloris	29.0-65.0
Nitzschia sp	16.0-47.0
Oocystis pusilla	10.5
Pavlova salina	30.9
Pavlova lutheri	35.5
Phaeodactylum tricornutum	18.0-57.0
Porphyridium cruentum	9.0-18.8
Scenedesmus obliquus	11.0-55.0
Scenedesmus quadricauda	1.9-18.4
Scenedesmus sp.	19.6-21.1
Skeletonema sp.	13.3-31.8
Skeletonema costatum	13.3-51.3
Spirulina platensis	4.0-16.6
Spirulina maxima	4.0-9.0
Thalassiosira pseudonana	20.6
Tetraselmis suecica	8.5-23.0
Tetraselmis sp.	12.6-14.7

Dunsliella (adaptable to very high salinity) and *Chlorella* (adaptable to nutrient rich media) have been successfully grown in commercially open pond systems [8].

The interest in microalgae for oil production is due to the high lipid content of some species, and to the fact that lipid synthesis can be modulated by varying growth conditions. Factors such as temperature, irradiance and, most markedly, nutrient availability have been shown to affect both lipid composition and lipid content in many algae [11]. It has been seen that high irradiances stimulate triacylglyceride accumulation, while under low irradiances, mainly polar lipids (phospholipids and glycolipids), structurally and functionally associated with cell membranes are synthesized [12].

Nutrient deficiency has been regarded as the most efficient approach to increase lipid content in algae. Up to 70% increase in lipid content has been reported with several species in response to limiting nitrogen supply in batch cultures [13]. It is believed that increases in lipid content during N-starvation are mainly obtained at the expense of other components, particularly proteins [12].

Combining the cells of different strains of algae to obtain a genetically modified algae strain that can give high yield of lipids is also a subject of research [14]. The technique has been used on plants and yeasts and researchers are now investigating if stable algal hybrids can be created using this technique.

2. Advantages of Microalgae as Source of Biofuel

Various advantages of using microalgae as feedstock for biofuel production are:

i. Algal productivity can offer high biomass yields per acre of cultivation. Comparison of oil yield per acre per year from various biomass feedstocks is shown in Table 2.

Table 2: Comparison of oil yields from biomass feed stocks [15]

Crop	*Oil Yield (Gallons/ Year/Acre)*
Soybean	48
Camelina	62
Sunflower	102
Jatropha	202
Oil Palm	635
Algae	1,000-6,500

ii. Faster growth rate of microalgae compared to other feed stocks for biofuels.
iii. Removal of CO_2 from industrial flue gases by algae bio-fixation, reducing the GHG emissions process while producing biodiesel.
iv. Algae can grow in very nutrient rich environments that are toxic to other plants so they could be used for treating 'waste waters', from a range of industrial sources. The contaminants present in the waste water such as NH_4^+, NO_3^-, PO_4^{3-} are used up in cell growth and thus removed from the waste water.
v. The biomass remaining after oil extraction from algae can be used for production of ethanol, methane, livestock feed or as organic fertilizer (because of high N: P ratio). It can also be burned for heat [7, 16].
vi. Microalgae can be cultivated in waste land or non-arable land; in harsh conditions using waste water for supply of nutrients thus neither competing with food crops non needing fresh water and additional fertilizers supply.

Depending on the algal species being cultivated other commercially important compounds can also be obtained, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants etc. [7].

3. Synthesis of Biofuel

Biofuel production from microalgae includes a production unit where cells are grown, followed by the separation of cells from the growth medium and subsequent lipid extraction for biodiesel production through transesterification.

Following oil extraction, amylotic enzymes are used to promote hydrolysis and formation of fermentable sugars [17]. These sugars are fermented and distilled into bioethanol [18].

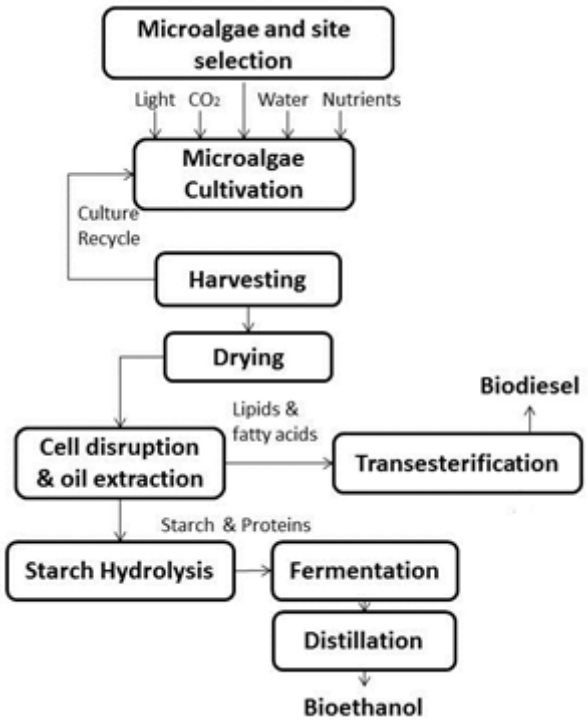

Figure 3: Integrated process for biodiesel and bioethanol production from microalgae

4.1. Microalgae cultivation

Microalgal culture is one of the modern biotechnologies. Open systems and enclosed bioreactors (photobioreactors) are two principal methods of cultivating microalgae. The major difference between the two is the trade-off of cost versus control [8].

4.1.1. Open Systems

Open system comprise of lakes and natural ponds, circular ponds, raceway ponds and inclined systems. They have larger capacity than most closed systems. The nutrients can be provided by runoff water from industries or sewage treatment plants, thus helping in waste water treatment [19]. Raceway ponds equipped with paddle wheels are the most commonly used systems for mass cultivation of microalgae. Problem encountered in open ponds is contamination by protozoa, ciliates and bacteria etc. Most microalgae that have their optimum growth at neutral or lower pH cannot be operated for long time in open ponds, but species which grow under extreme conditions of pH (e.g., Spirulina) or salinity (e.g., Dunaliella) are able to withstand contamination even in low density cultures. However contamination can occur in those cultures due to pH gradients and this could be attributed to inefficient mixing system [20]. For large scale cultivation, microalgae are usually produced in open ponds due to lower investment and production costs.

4.1.2. Closed Systems

Closed PBRs are characterized by the regulation and control of nearly all the biotechnologically important parameters as well as by the following fundamental benefits: a reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature, and flexible technical design. High cell density is also obtained in algae cultivated in closed systems [21]. Tubular PBR, bubble-column and flat plate PBR are commonly used closed systems. Tubular PBR has been well used for outdoor microalgae cultures, the main reason being large illuminated surface area.

Tubular bioreactors that circulate the culture by using an airlift device are especially attractive for several reasons: circulation is achieved without moving parts and this provides a robust culture system with a reduced potential for contamination; the cell damage associated with mechanical pumping is avoided; and the airlift device combines the function of a pump and a gas exchanger that removes the oxygen produced by photosynthesis. Continuous removal of oxygen is essential, as excessive dissolved oxygen in the broth inhibits photosynthesis [22]. In spite of many advantages, the scale up difficulties has restricted the use of tubular PBRs on commercial level [20].

Both methods consume an identical amount of carbon dioxide, if the losses to atmosphere are disregarded [10]. Various advantages and disadvantages of both the systems are mentioned in Table 3.

Table 3: Advantages and Disadvantages of open and closed algal cultivation plants [23]

Parameter	*Open ponds (Raceway Ponds)*	*Closed systems (PBR systems)*
Contamination risk	High	Low
Water losses	High	Low
CO_2 losses	High	Almost none
Process control	Complicated	Less complicated
Standardization	Difficult	Possible
Weather dependence	High	Less because protected
Maintenance	Easy	Difficult
Construction costs	Low	High
Biomass concentration at harvesting	Low	High
Overheating problem	Low	High

4.2. Harvesting and Biomass Concentration

Harvesting of microalgae is a two-step process involving: (1) Bulk Harvesting: aimed for separating biomass from bulk suspension using methods like flocculation, floatation or gravity sedimentation; and (2) Thickening: the aim is to concentrate the slurry using methods such as centrifugation, filtration and ultrasonic aggregation [24].

The cost of biomass recovery from broth can make up to 20-30% of the total cost of producing the biomass [25].

4.3. Processing and Components Extraction

Numerous methods for extraction of lipids from microalgae have been applied; most common methods are oil press/expellers, solvent extraction, supercritical fluid extraction (SFE) and ultrasound techniques.

Oil presses or expellers can be used to extract oil from microalgae by use of high pressure to break cells and compress oil out. This method extracts almost 75% of the oil, but is reported less effective because of long extraction time and workability on dry algae only [26].

In solvent extraction method organic solvents, such as benzene, cyclo-hexane, hexane, acetone, and chloroform are added to algae paste. Solvent destroy algal cell wall, and extract oil from aqueous medium because of their higher solubility in organic solvents than water. Solvent extract can then be subjected to distillation process to separate oil from solvent. Latter can be reclaimed for further use. Hexane is reported to be the most efficient solvent in extraction based on its highest extraction capability and low cost [27].

Supercritical extraction makes use of high pressures and temperatures to rupture the cells. This particular method of extraction has proved to be extremely time efficient and is commonly employed [27].

Another promising apparatuses to be used in extraction of microalgae is via ultrasound. This method exposes algae to a high intensity ultrasonic wave, which creates tiny cavitation bubbles around cells. Collapse of bubbles emits shockwaves, shattering cell wall thus disrupting latter and releasing desired compounds into solution. This method is under research stage for application on commercial scale [28].

The advantages and limitations of different extraction methods are given in Table 4.

Table 4: Advantages and limitations of different extraction methods [28]

Extraction methods	*Advantages*	*Limitations*
Oil press	Easy to use, no solvent involved	Large amount of sample required, slow process
Solvent extraction	Solvent used are relatively inexpensive; reproducible	Most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy intensive; large volume of solvent needed
Supercritical fluid extraction	Non-toxicity (absence of organic solvent in residue or extracts), 'green solvent' used; non-flammable, and simple in operation	Often fails in quantitative extraction of polar analytes from solid matrices, insufficient interaction between supercritical CO_2 and the samples
Ultrasound	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improves the release of cell contents into the bulk medium	High power consumption; difficult to scale-up

4.4. Biofuel Production

The ideal diesel fuel molecules are saturated non-branched hydrocarbon molecules with carbon number ranging from 12 to 18 whereas algal oil molecules are triglycerides generally with no branched chains of different lengths and different degrees of saturation [29]. Triglycerides are highly viscous fluids and their direct use as fuel in IC engines leads to problems like injector coking, more engine deposits, ring sticking, and thickening of engine lubricant [30]. To avoid these problems, various upgrading techniques mentioned in the literature are as follows:

(1) Blending
(2) Microemulsions
(3) Thermal cracking
(4) Transesterification

4.4.1. Blending:

Triglycerides can be directly mixed with diesel fuel for running an engine. A blend of 20% oil and 80% diesel was found to be successful [28].

4.4.2. Microemulsions:

Viscosity can be reduced by forming microemulsions of triglycerides with solvents such as methanol, ethanol and butanol. A microemulsion is defined as the colloidal equilibrium dispersion of optically isotopic fluid microstructures with dimensions generally in range of 1-150 nm formed spontaneously from two normally immiscible liquids and one or more non-ionic amphiphiles. These can improve spray characteristics by explosive vaporization of low boiling point constituents of the micelles [32].

4.4.3. Thermal Cracking:

Pyrolysis or thermal cracking is the process of converting one substance into another by means of heat or with the aid of catalyst. It is performed by heating in the absence of air or oxygen. Pyrolysis of fats and vegetable oils has been investigated for more than 100 years, to produce low molecular weight hydrocarbons [31].

4.4.4. Transesterification:

Transesterification or alcoholysis is the reaction of fat or oil with an alcohol [32]. Methanol is commonly used because of low price. However other alcohols such as ethanol and butanol can also be used. The yield of transesterification reaction depends on ratio of alcohol-oil, catalyst types and concentration, reaction time, temperature and agitation rate.
In this reaction, the triglycerides present in the lipids are

converted into esters, giving glycerol as byproduct [33]. Thus the molecular weight and viscosity of lipids is reduced and volatile biodiesel is obtained. Excess methanol is added to shift the reaction towards right.

Usually NaOH is used an alkaline catalyst, other alkanine catalysts include KOH, CH_3ONa and CH_3OK [24, 33]. The commonly used acid catalysts are H_2SO_4, HCl and sulphonic acids ($R\text{-}SO_3H$) [33].

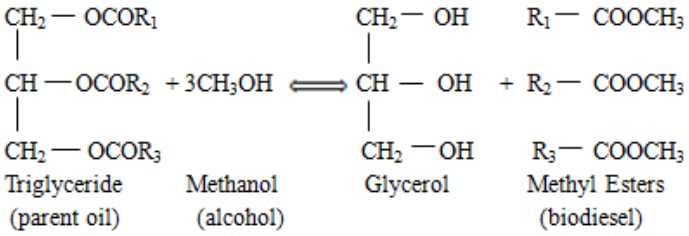

Figure 4: Transesterification of triglycerides [33]

Excess methanol is used to achieve high degree of conversion. When acid catalysts are used a higher alcohol to oil ratio is needed [34]. The heating value of biodiesel obtained from algal oil (41 MJ/Kg) is comparable to heating value of petroleum based diesel (42.7 MJ/Kg), and it is superior to the heating value of biodiesel obtained from seed oils (39.5 MJ/Kg) [35].

Bioethanol Production:

Besides biodiesel, algae can be cultivated and can be used as a feedstock for the production of bioethanol. After extraction process the residual biomass can be used as feedstock for the fermentation process for bioethanol production. In the fermentation reaction cellular starch is converted into ethanol, acetate, hydrogen and carbon dioxide [36].

The main fermentation reaction can be expressed as:

$$C_6H_{12}O_6 \rightarrow 1.5\ C_2H_5OH + 0.5CH_3COOH + 1.1H_2 + 1.9CO_2$$ [36]

As the importance of microalgae in biodiesel production is growing, an equal or more attention is needed for the efficient use of these easily cultivable microorganisms to generate the green fuel bioethanol.

Table 5 summarizes the starch or fermentable biomass content of some microalgae.

Table 5: Algal sources for bioethanol production [37]

Algal source	**% starch or biomass after oil extraction (g/dry weight)**
Saccharina latissima	~50.0 (reserve food material)
Green alga	>50.0 (starch)
Laminaria hyperborea	55.0 (reserve food material)
Spirogyra sp.	43.3 (biomass after oil extraction)
Oedigonium sp.	33.6 (biomass after oil extraction)
Chlamydomonas reinhardtii	53.0 (starch)
C. reinhardtii	17.0 (starch)
Chlorella vulgaris	12.0–17.0 (starch)
Chlorella sp.	21.5 (starch)
Synechococcus sp.	15.0 (starch)
Chlorococcum sp.	26.0 (starch)
Scenedesmus sp.	20.4 (starch)
S. acuminatus	7.3 (starch)
S. acutiformis	16.4 (starch)
S. acutus	18.6 (starch)
S. arcuatus	12.9 (starch)
S. armatus	15.4 (starch)
S. obliquus	23.7 (starch)
Nostoc sp.	30.7 (starch)
N. maculiforme	30.1 (starch)
N. muscorum	33.5 (starch)
N. paludosum	32.1 (starch)
N. piscinale	17.4 (starch)
Oscillatoria	19.3 (starch)
O. jasorvensis	9.7 (starch)
O. obscura	12.6 (starch)
O. okeni	8.1 (starch)
Phormidium angustissimum	28.5 (starch)
Spirulina fusiformis	37.3–56.1 (starch)

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