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Review Article

Algae as a Potential Candidate for Biofuel Production: a Review

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Abstract: With the growing rate of world population, the energy consumption and demand has also been increasing day by day. Therefore, researchers are looking for more reliable sources of energy other than fossil fuels. Biofuels are more sustainable sources of energy. There are three generations of biofuels out of which the most promising is third generation which involves the use of algae (microalgae) for fuel production. Algae are used for the production of different forms of bioenergy like bioethanol, biodiesel, biohydrogen and biogas. Microalgae are used for the production of biodiesels in excess. The quality and quantity of biodiesel produced from microalgae depend upon the type of species used for fuel production process. Microalgae are more advantageous than plants for the production of biodiesel, as they have more lipid and oil contents in their body mass, and they can easily be cultured in open water ponds or even in bioreactors where controlled conditions are provided. There are some factors which affect the growth of algae like temperature, light, gas exchange, nutrients and contaminants etc. which further affect the yield of biofuel. Now new developmental techniques like DNA recombination techniques are in practice to increase the overall yield (biodiesel) by increasing the lipid contents of algal body mass. This review article mainly focuses on the use of algae for production of biofuel as well as discussing its significance in this regard.

Keywords: Biodiesel, biofuels, bio-refinery, microalgal biomass, process development, renewable energy

1. INTRODUCTION

Continuous population increase, especially in developing countries of the world, has led to increase in energy consumption as well as energy demand (especially the energy derived from fossil fuel). In year 2010, it was found that the world's consumption of prime energy has increased by 5.6%, which is the highest record from last 40 years [1]. The usage of fossil fuels is now considered as unsustainable because of its resource depletion and also because of accumulation of greenhouse gases in the environment due to burning of these fossil fuels [2, 3]. Energy safety, inflation in prices of different oil products, resources exhaustion and alteration in climatic conditions are few of the utmost challenges, which our communities have to face in near future. It is probable that sources like biofuels can help to alleviate these challenges and can produce more stable and sustainable economies [4].

Biofuels (fuels from biological sources) are referred as renewable fuels which are used for generation of heat and electricity [2, 5, 6]. They are also replaceable of petroleum based transportation fuels and play a vital role in reducing long-term CO_2 discharge, so considered as environmentally and economically sustainable fuels [2, 6]. "Biofuel" word refers to the energy produced from metabolism of living organisms (i.e., autotrophs convert solar energy into chemical energy via photosynthesis) [5, 7].

Three kinds of biofuels (i.e., bioethanol, biodiesel and bio-hydrogen) have been comprehensively deliberated upon by the scientists. According to estimates of international agencies, by

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the year 2030 the demand of energy may increase by up to 53% [8, 9]. Biodiesel is considered as a better-suited option compared with other fossil fuels because of its high heating values, flash point, and kinematic viscidness [9, 10]. Biodiesel is a renewable and bio-degradable fuel; it is non-toxic, non-flammable, and environment friendly; thus, can help reduce net carbon-dioxide release by up to 78%. Therefore, it is considered as demanding likable fuel option nowadays [9, 11]. It has same energy contents and properties as diesel fuels of conventional use and can be used on its own or in combination with other fuels [12]. Different types of oils like canola oil, soy bean oil, palm oil, sunflower oil, and cotton-seed and waste vegetable oils are some of the extensively used edible and non-edible oils for the purpose of biodiesel production [13, 14].

So far, three generations of biofuel producing crops have been established [4] as shown in Fig. 1. First generation energy crops include rapeseed oil, sugar beet, sugarcane, soybean, and maize [4, 15]. The oils extracted from vegetables and animal fats are also included in first generation biofuels [15]. Currently, 1% of the world's transportation fuel is obtained from 1% (14 million hectares) land out of the total world's available arable land [15]. The problem with the use of these first energy crops is that they lead to competition of land with food crops and also result in loss of biodiversity because of the removal of present forests for cultivation purposes [16].

Second generation energy crops of biofuels include lignocellulosic feed stocks like miscanthus, switchgrass or poplar which have advantages over first generation energy crops as they require lower land in both quality and quantity [4]. Biofuel resources should be cheaper than those of petroleum fuel sources, they should be cultivable on less or no land at all (can grow on water), they should also be environment friendly and should use less water for irrigation purposes [15, 17].

A number of third generation feed stocks for biofuel production are explored as worthwhile substitutes to traditional energy sources but microalgae represent a promising alternative [18, 19]. Third generation energy crops include micro- and macro- algae which are more promising than previous two as they have high biomass yields on less arable land and have potential to grow on offshores. Even some can be grown on saline and brackish water, which make them more advantageous over terrestrial energy crops [4]. Algae can also be cultivated on wastewater so they can also be used for wastewater treatment purposes making them as environment friendly [20]. They have also higher productivities than land plants and possess huge quantities of triacylglycerides (TAGs), so they are considered as a main feedstock for production of biodiesel [21]. When microalgae are utilized for the production of biodiesel then different types of methods are employed in order to harvest the biomass of microalgae like flocculation, filtration, gravity sedimentation, floatation, centrifugation etc. In spite of the significance of harvesting to the economy, no universal method of harvesting is yet designed [22, 53].

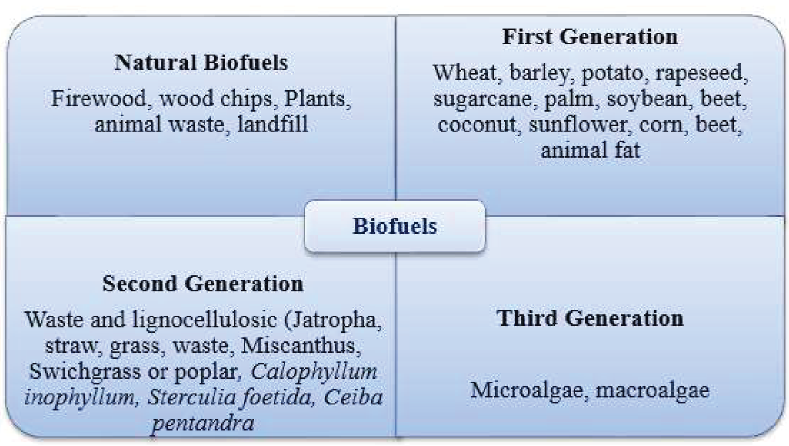

Fig. 1. Three generations of biofuel production [9].

Most of the work on algae for production of biofuels is done majorly at two grounds: one is production of ethanol by fermentation process from feedstock of algae and second is synthesis of biodiesel from oil contents of algae [4]. Algae are able to tolerate a variety of environmental extremities like heat, cold, drought, salinity, photo-oxidation, anaerobiosis, osmotic pressure and UV radiation, and are able to produce many different types of biofuels [1, 23]. Microalgae use sunlight more efficiently and their capability to produce oil is double than those of land crop plants. By using thermochemical and biochemical processes, different types of biofuels like biodiesel, bio-syngas, bioethanol, bio-hydrogen along with many vegetable oils can be produced from microalgae [10, 13, 24]. Common nutrients required for building the body biomass of microalgae are phosphate, nitrogen and CO_2, thus micro algae are also considered as suitable means for CO_2 alleviation and for reduction of the pollution and toxic chemicals from waterways [25].

From an experimental project it was found that 200 barrels of oil can be obtained from an area of 1 hectare (2.47 acres) by cultivating photosynthetic green algae on it. If the quantity of this yield produced from green algae is compared with that of the most commonly used feedstock for production of biodiesel (Soybean crop), then it is found that this yield is 100 times greater than soybean feedstock [26]. Algae are photosynthetic, so they use sunlight and carbon dioxide in order to produce biofuels, food and other bioactive compounds of higher significance [27].

Production of biofuel from microalgal cells is a sustainable source of energy. Microalgae have 2% to 40% of lipid contents (may be 50% in some) and the fuel produced by them are nontoxic sulphur free (constituents of dry mass of few algae are mentioned in Table 1, which depicts the percentage of lipids, carbohydrates and protein constituents in algal dry mass.). It is also biodegradable which makes it very useful for biofuel production [19].

Many different kinds of renewable biofuels which are produced from microalgae are bio methane (derived from anaerobic consumption of biomass of algae), biodiesel (produced from oil contents of algae), and bio-hydrogen (derived by photo biological process) [19, 27]. Electricity, charcoal, bio-gas and bio-oils can also be obtained from microalgae biomass using thermochemical procedures. Bioethanol can also be obtained using fermentation process from few macroalgal sugar contents which have little quantity of lignin in them [25].

For biofuel production, biomass of microalgae

Table 1. Constituents of algae (% of dry matter) [26].

Algae	Lipids	Proteins	Carbohydrates
Anabaena cylindrical	4-7	43-56	25-30
Aphanizomenonflos-aquae	3	62	23
Arthrospira maxima	6-7	60-71	13-16
Botryococcusbraunii	86	4	20
Chlamydomonasrheinhardii	21	48	17
Chlorella ellipsoidae	84	5	16
Chlorella pyrenoidosa	2	57	26
Chlorella vulgaris	14-22	51-58	12-17
Dunaliellasalina	6	57	32
Euglena gracilis	14-20	39-61	14-18
Prymnesiumparvum	22-38	30-45	25-33
Porphyridiumcruentum	9-14	28-39	40-57
Scenedesmusobliquus	12-14	50-56	10-17
Spirulina maxima	6-7	60-71	13-16
Spirogyra sp.	11-21	6-20	33-64
Spirulinaplatensis	4-9	46-63	8-14
Synechococcus spp.	11	63	15

or microalgal biomass (MAB) is a favorable feedstock [28, 29]. In dry biomass of microalgae the lipid contents may be more than 80% of its weight [30]. By using different manufacturing processes, not only lipid contents but also all of the constituents of microalgal biomass like carbohydrates, proteins etc. can be transformed into different kinds of biofuels [28, 29].

By direct combustion of dried algal biomass energy can be generated but it is least attractive method. Thermochemical (e.g. through gasification, pyrolysis, hydrogenation and liquefaction of the algae biomass, oil and gas based fuels are obtained) and biochemical (e.g. through the fermentation process, biomass is converted into bioethanol or biomethane) conversion processes are more attractive for energy generation purposes [20, 31].

Microalgal species include cyanobacteria (Chloroxybacteria), and eukaryotic microalgae like green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta) [33]. Use of biofuels produced from microalgae has many advantages like: (1) these are very proficient to grow round the year therefore more biodiesel is extracted from them as compared to other energy producing crops; (2) microalgae are easy to cultivate as they need very little supply of water as compared to other oil producing crops for irrigation purpose; (3) microalgae can also be grown on non-cultivable land or on brackish water, therefore they do not compete for land with food crops as compared to other oil yielding crops; (4) they have rapid growth rate; (5) they help in air quality maintenance and improvement by bio-fixation of waste CO_2 [32, 33]; (6) they can well grow even on waste water which makes them environment friendly as they help in removal of toxic organic chemicals from waste water released from different industries; (7) the growth of microalgae is also very cost effective as it does not require any extra application of herbicides or pesticides as other biofuel yielding crops do; (8) algae are also of much significance to grow as they produce many valuable products that may be used as a substitute of synthetic fertilizers and animal feed and also for production of methane or ethanol; (9) algae are also of great significance as by changing certain environmental conditions the oil contents of microalgae can be increased in order to get higher yield of biofuel [15].

2. BIOLOGY OF MICROALGAE

Alga is documented as very oldest form of life that exists on earth [34]. Algae are commonly called as thallophytes means those plants that lack proper stem, roots and leaves. They also lack proper sterile covering on outside of their reproductive cells and possess chlorophyll for photosynthetic purposes [35]. According to an estimate out of more than 50,000 species of microalgae only 30,000 species have been studied and explored [36]. Different structures of algae in the form of simple cells are used for the energy transformation purpose, and because of their simple structure these are easy to cultivate in wide range of unusual environmental conditions [34].

On the nutrition base, algae can be divided into two categories i.e. autotrophic and heterotrophic; autotrophic algae require CO_2, nutrients and sunlight from environment while heterotrophic algae feed on external organic sources [35]. Photosynthesis is the main phenomenon which is necessary for autotrophic algae to survive as they absorb sunlight energy by chlorophyll of chloroplast and convert it into adenosine triphosphate (ATP) [15]. In Fig. 2 the lipid contents of various freshwater and marine microalga species are shown, which indicate the potential of a particular species to produce biodiesel.

3. MICROALGAE LIPID CONTENTS AND PRODUCTIVITIES

Lipid contents in microalgae range from 1 to 70% but sometimes under certain specific conditions lipid contents of some microalgae species may reach up to 90% of their dry weight [37]. Table 2 indicates the oil contents of various microalgae species.

4. SELECTION OF ALGAL STRAIN

Microalgae are of great interest in terms of oil productivity as compared to plantsbecause they need little quantity of water supply for irrigation purposes as do the terrestrial plants [38]. They are also easy to cultivate even in saline water and do not require any pesticide application for normal

Table 2. Oil content in various microalgae [26].

Microalgae	Oil content (% of dry weight)
Botryococcusbraunii	25-75
Chlorella sp.	28-32
Crypthecodiniumcohnii	20
Cylindrotheca sp.	16-37
Dunaliellaprimolecta	23
Isochrysis sp.	25-33
Monallanthussalina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochlorisoleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylumtricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmissueica	15-23

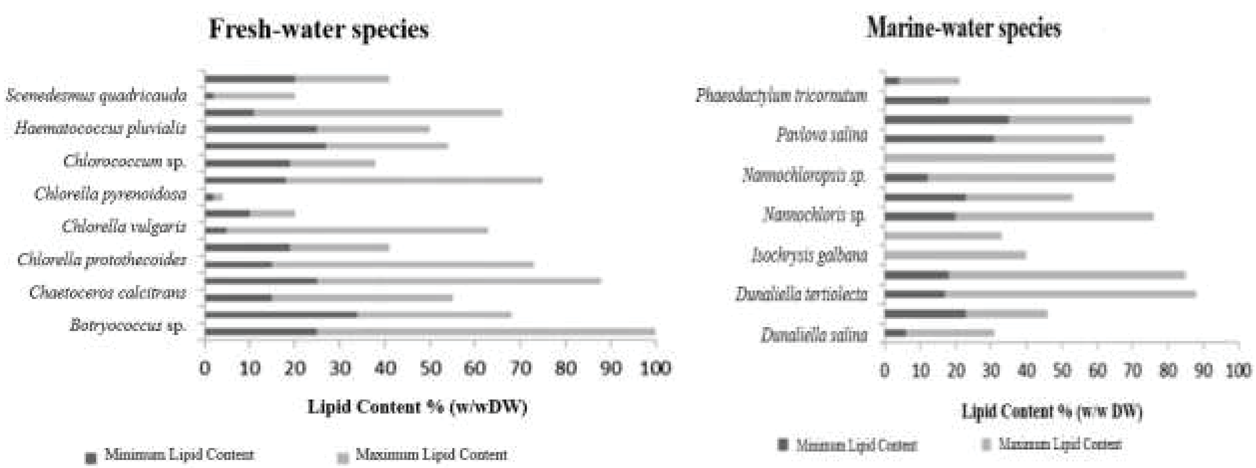

Fig. 2. Lipid contents of various marine and freshwater microalga species [23].

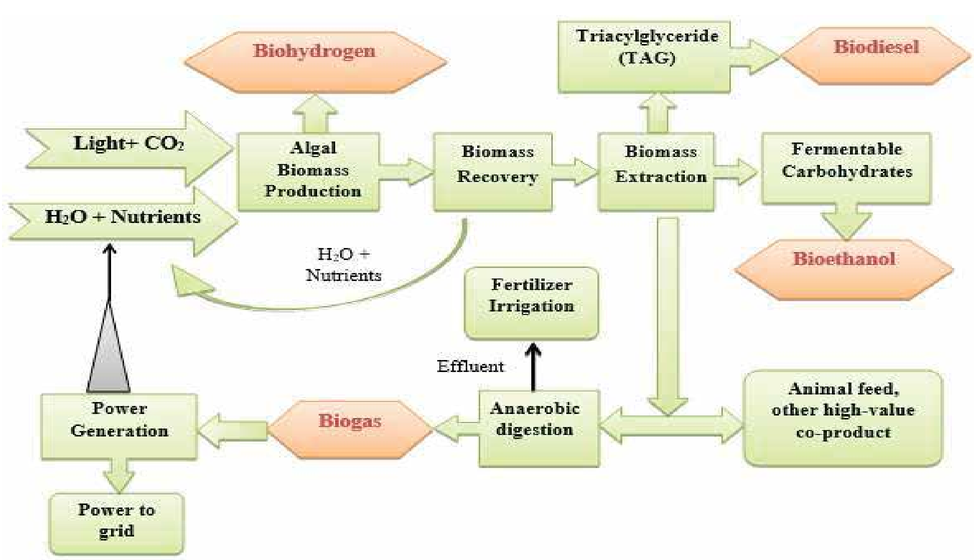

Fig. 3. A conceptual model for integrated microalgal biomass and biofuel production [5].

growth [39].

Those microalgae which are capable to live in heterotrophic conditions and depend on external source of carbon have chemical energy stored in the form of lipids [40]. Under certain conditions quantity of lipids in cells builds up, so it is very necessary to remember the aspects that result in increased deposition of lipids in algae, like nutrients and sun light, etc. [3]. The quality of biodiesel produced greatly depends upon the quality of oil produced from algae. Modern techniques like use of genetic engineering of certain basic enzymes involved in lipid biosynthesis pathway help to improve both quality and quantity of lipids equally [3]. Continuous culture technique is used for extensive production of microalgae biomass in day time. In this technique continuous supply of fresh culture is made sure, also the equal quantity of microalgae broth is drawn out constantly with every addition of new culture medium [2].

5. ALGAE BIOENERGY PRODUCTION OPTIONS

Algae are used for the production of many energy products like biodiesel, bioethanol, biogas, and biohydrogen. A conceptual model for integrated microalgal biomass and biofuel production is shown in Fig. 3.

5.1 Bioethanol

Algae are considered as more suitable candidates for bioethanol production as they have low quantity of lignin and hemicellulose as compared to plants which have high lignocellulosic content [1].

On the base of size and morphology, algae are classified as microalgae and macroalgae. Microalgae as obvious from its name are very small sized organisms as compared to macroalgae, which are multicellular organisms and look somewhat plant like e.g. kelp. As macroalgae grow in aquatic environment so they do not have same lignin cross linking in their structure as plants [41]. By having little quantity of lignin and abundant quantity of sugar contents (at least 50% of body mass), macroalgae are considered as very significant for the production of bioethanol by fermentation process [1].

After the oil extraction from the biomass of microalgae an enzyme alpha-amylase and gluco-amylase and yeast are utilized for the fermentation of sugars to CO_2 and ethanol [33]. The ethanol produced by this way accounts for 11-15% of total fermented mash produced. Ethanol can also be produced by applying dark and anaerobic conditions in which the starch is fermented inside the microalgal cells to produce bioethanol [42].

Microalgae are also investigated for the production of bioethanol. For example photosynthetic algae like spirogyra and Chlorococum sp. have high contents of polysaccharides (starch) in their cell walls. This starch is useful for production of bioethanol [41].

5.2 Biodiesel

Algae are also utilized for the production of an important biofuel i.e. biodiesel. Most of the algal species have abundant amounts of lipids that may be 50–60% of their total dry weight as storage products. By transesterification process these lipids of algae are converted into biodiesel [1]. Production of biodiesel from microalgae is advantageous because of high algal growth rate, low water requirement for cultivation, high proficiency for CO_2 alleviation and also because of being very cost effective farming. But it also has some disadvantages like low concentration of biomass [43].

5.3 Biohydrogen

In past, more focus was on the production of liquid type of biofuels like diesel and ethanol however now biohydrogen and biogas (biomethane) are also produced from algae which are further utilized for different purposes like generation of electricity and also used as gas fuel [1]. Macroalgae are the main source for the production of electricity generating biogas because of their higher growth rates and also because of their capability to develop in saltwater (marine) and also because of absence of cross linking of lignin in their structure [44]. Autotrophic microalgae and cyanobacteria have the ability to produce biohydrogen directly by anaerobic fermentation followed by oxidation of ferredoxin in presence of hydrogenase enzyme [1].

5.4 Biogas

Microalgae are also considered as the main source of biogas production through the process of fermentation [1]. Biogas production from algae also plays a very important role in bioremediation process. Currently, the biogas production from algae is not practiced on large scale because of the requirement of heat for digester and also large area for cultivation for obtaining equal quantity of energy as from biodiesel [1].

6. BIODIESEL FROM ALGAE OIL

Biodiesel is usually composed of chains of alkyl esters and produced by transesterification of oil or fat contents of algae. When biodiesel is obtained from plants or animals sources then it has 90 to 98% of triacylglycerides, and very little amount of monoglycerides, diglycerides and free fatty acids [45]. Fig. 5 depicts the complete process of biodiesel production in schematic form.

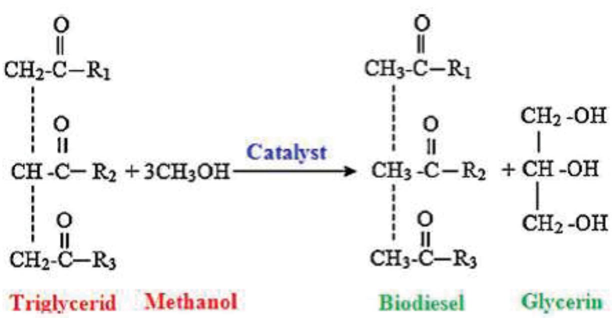

Fig. 4. The transestrification process of conversion of triglyceride into biodiesel [57].

6.1 Cultivation of Microalgae

Microalgae require an adequate amount of carbon dioxide and sunlight for photosynthesis. However all microalgae are not photosynthetic, some are heterotrophic, mixotrophic or photoheterotrophic type and they change their feeding mode on the base of environmental conditions [38]. For example Chlorella vulgaris, Haematococcus pluvialis and Arthrospira platensis (Spirulina), all of these can grow under photoautotrophic, heterotrophic and mixotrophic conditions; also the Selenastrum capricornutum and Scenedesmus acutus species can act photoautotrophically, heterotrophically or photoheterotrophically [23]. Under phototrophic cultivation, lipid contents of algae have wide ranges from 5 to 68%, mainly depends on the type of microalgae species [38].

Autotrophic microalgae are cultivated in open ponds or even in enclosed photobioreactors. Table 3 indicates the comparison of microalgae cultivation in open pond cultivation with cultivation in bioreactor. Those species of microalgae which

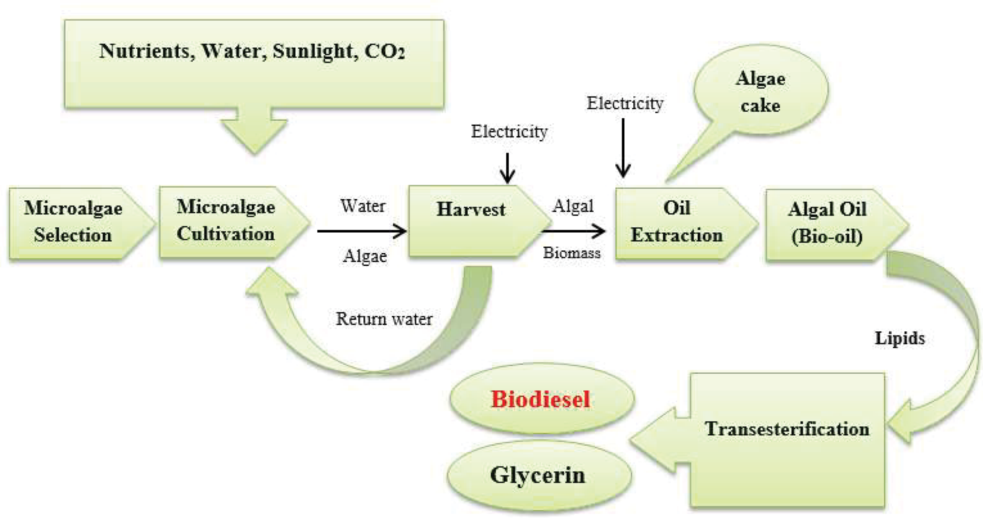

Fig. 5. Schematic representation of the various stages of manufacture of microalgal biodiesel [30]

Table 3. Open pond cultivation of microalgae versus bioreactors [60].

Factor	Open pond	Photobioreactor
Required space	High	For PBR itself low
Water loss	Very high, may also cause salt precipitation	Low
CO_2-loss	High, depending on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgassing	Build-up in closed system requires gas exchange devices (O_2 must be removed to prevent inhibition of photosynthesis and photo oxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (by spraying water on PBR or immersing tubes in cooling baths)
Shear	Usually low (gentle mixing)	Usually high (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR lifetime
Contamination risk	High (limiting the number of species that can be grown)	Low to medium
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g/l	High, generally between 0.5 and 8.0 g/l
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing, temperature only by pond depth)	Possible within certain tolerances
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Start-up	6-8 weeks	2-4 weeks
Capital costs	High ~ $100000 per ha	Very high ~ $250000 to 1000000 per ha (PBR plus supporting systems
Operating costs	Low (puddle wheel, CO_2 addition)	Higher (CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Harvesting costs	High, species dependent	Lower due to high biomass concentration and better control over species and conditions

are liable to contamination are cultured in photobioreactors, while those which are able to bear the harsh environmental conditions such as high pH (e.g., Spirulina) or salinity (e.g., Dunaliella spp.), or which grow very rapidly (e.g., Chlorella spp.) are cultivated in open ponds [46]. Heterotrophic cultivation seems more advantageous as it requires no light for growth and it also has higher mass production with lower cost of harvesting [23].

6.1.1 Environmental Conditions for Microalgae Biomass Production

Under specific environmental conditions different species of microalgae grow differently. Main factors which affect the growth of microlalgae are intensity and wavelength of light, temperature, CO_2 concentration, nutrients, salinity and mixing conditions [5].

6.1.1.1 Light: Light is the main factor that affects the growth of photosynthetic algae. Lower the intensity of light, lower will be the growth rate similarly higher the intensity of light, higher will be the growth rate of algae. With increasing intensity of light growth also increases until a point of maximum growth reaches [47] and at this point further increase in intensity will results in no more increase in growth rate rather it will cause damage to algae [5].

6.1.1.2 Temperature: In general, with increase in temperature the algal growth also increases exponentially and at a certain temperature its growth rate reaches at maximum value. Further increase in temperature will leads to decline in growth rate. For open pond culture systems, it is not easy to control temperature and it is greatly influenced by atmospheric temperature and humidity levels [5].

6.1.1.3 Gas exchange: CO_2 level in air is directly related to growth rate of algae. When CO_2 percentage is low i.e. (0.033%) then algae will limit their growth [48]. To enhance the growth of algae CO_2 is mixed with air in case of open aired pond cultures or may directly be injected into the culture medium of photobioreactors through exchange of air [5]. Oxygen concentration also affects the growth rate of algae. If oxygen level is enhanced beyond its saturation value then it leads to photo-oxidative damage to chlorophyll of cells and ultimately stops the process of photosynthesis [49].

6.1.1.4 Nutrients: Algal culture grows in presence of nutrients like macronutrients, vitamins and trace elements. However in order to prevent nutrient deficiency, different nutrients in different ratios are directly added into the culture medium [5].

6.1.1.5 Contamination: Open pond culture system has a disadvantage as it is prone to contamination by other species. When a new pond is established then to start the reproduction, inoculation of a desired strain of alga is done. But later on with passage of time some other species may also be introduced by some environmental factors which lead to reduction of yield of desired strain [3].

6.2 Harvesting of Microalgae

After algae cultivation, next step is harvesting of algal cell mass from the cultivation medium. Harvesting usually accounts approximately 20 to 30% of the total production cost [16]. Many

Table 4. Comparison of inorganic and organic flocculants [13]

Parameter	Inorganic flocculants	Organic flocculants
Nature of flocculants	Multivalent salts	Polyelectrolytes/ Polymers
Key characteristic of an effective flocculants	Increasing molecular weight and charge	High charge density
Sensitivity to pH	Highly sensitive to pH	Less sensitive to pH
Dosage of flocculants required	High dose required	Low dose required
Applicability	Applicable to some microalgal species	Applicable to wide range of microalgal species

Table 5. Comparison of microalgal harvesting methods [22].

Different Methods	Advantages	Disadvantages	Dry solids output conc. (%)
Centrifugation	Can handle most algal types with rapid efficient cell harvesting	High capital and operational costs	10-22
Filtration	Wide variety of filter and membrane types available	Highly dependent on algal species, best suited to large algal cells. Clogging or fouling an issue	2-27
Ultrafiltration	Can handle delicate cells	High capital and operational costs	1.5-4
Sedimentation	Low cost, potential for use as a first stage to reduce energy input and cost of subsequent stages	Algal species specific, best suited to dense non-motile cells. Separation can be slow. Low final concentration	0.5-3
Chemical flocculation	Wide range of flocculants available, price varies although can be low cost	Removal of flocculants, chemical contamination	3-8
Flotation	Can be very rapid than sedimentation. Possibility to combine with gaseous transfer	Algal species specific. High capital and operational cost	7

different methods are used for the harvesting of algal cell mass e.g. sedimentation, centrifugation, filtration, ultra-filtration, flocculation and flotation. Flocculation method is more promising for aggregation of algal cells (thus leads to faster sedimentation), centrifugal recovery or filtration as compared to other methods [23]. Table 5 shows the advantages and disadvantages of different harvesting techniques along with the %age of dry solid output of microalgae dry mass.

6.2.1 Flocculation

The microalgal cells carry negative charge on their surface so when these cells are neutralized then they appear in the form of clusters which are also called as “flocks” and then these agglomerated cells or flocks are then separated easily [5]. Some algal species do not require artificial method of flocculation for harvesting of cells [50] rather their cells agglomerate naturally in response to certain changes in environment like nitrogen concentration, change in pH and changes in dissolved oxygen level [5]. Flocculation can be done by different methods like, chemical flocculation (inorganic chemicals, polyelectrolytes), bio flocculation and electro flocculation [5]. Flocculation procedure is further categorized as;

6.2.1.1 Auto flocculation: In this type of flocculation the algal particles settled down spontaneously after aggregation [51]. At high level of pH the CO_2 which is to be used during photosynthesis, precipitates down in form of carbonate salts along with algal cells [52]. Hence carbon level and certain other factors induce auto flocculation [51].

6.2.1.2 Chemical flocculants: Chemical flocculation is used for isolation of a wide range of algal species [53]. On the bases of nature of chemicals, flocculants are categorized as organic and inorganic. Comparison of inorganic and organic flocculants is mentioned in Table 4. Inorganic flocculants: in case of inorganic flocculants the negative charge on the exterior of algal cells can be neutralized using iron or aluminum based chemicals or coagulants [53]. Aggregation through these inorganic chemicals is called as inorganic flocculation.

Organic flocculants: the type of organic flocculants to be used depends upon the type of algal species, their pH and concentration of algal biomass. Organic flocculants include chemicals like cationic polymers which link the algal cells together. Cationic polymers give better flocculation as compared to anionic polyelectrolytes where no flocculation obtained [54]. These flocculants are subjected to biodegradation so cause no contamination of algal biomass [53].

6.2.2 Centrifugation

Centrifugation process uses gravitational force for the collection of algal cells. Efficiency of this process greatly depends upon the form and size of the algal cells e.g. large sized filamentous cells settled down rapidly and can be collected easily as compared to small single sized cells which do not settle quickly [50].

6.2.3 Filtration

Process of filtration is also used for harvesting of cell biomass. In this process the culture medium is poured on a screen having holes of specified size. The algal cells either passed out of the filtering screen or remain on surface of it on the base of their sizes. Filtration process is also aided by vacuum or pressure in order to increase its efficiency [5].

6.2.4 Electrolytic Process

Harvesting of algal particles can also be obtained through electrolytic process. This process takes place in three steps

1. In first step electrolytic oxidation takes place to generate coagulant inside culture medium.
2. In next step the particulates are destabilized and their emulsion is broken down.
3. Lastly the destabilized particulates are restabilized or they aggregate to form flocks.

It is a very efficient procedure and almost 80–95% of algal cells can be removed or get isolated from culture medium [53].

6.2.5 Gravity Sedimentation

Through this method gravity is applied which leads to the settling of particles with clear liquid above. This is very energy efficient process [51] and commonly used for isolation of microalgal particles from water [53]. The disadvantage of this method is that it is very time consuming [55].

6.2.6 Floatation

Floatation is also a very common process of harvesting of microalgal particles. On the basis of size of air bubble, this process of harvesting algal cells is further divided into two categories i.e. [53].

1. Dissolved air flotation
2. Dispersed air flotation

Dissolved air flotation: it involves the pre-saturation of water with air at high pressure. The air bubble size is 10–100 mm [53]. In this method of harvesting air bubbles are passed through the culture medium, which stick with the algal cells while passing through the medium and make the algal particles to float at the surface of culture medium which is later collected easily [56].

Dispersed air flotation: this process involves the direct introduction of air through specific system or some other mechanical agitator system. The size of air bubble is 700–1500 mm [53]. Efficiency of this process can be improved by reducing the charge on the surface of air bubbles [51].

6.2.7 Electrophoresis Techniques

This harvesting process involves the application of electric field on the culture medium due to which hydrogen ions are generated by electrolysis of water molecules. These hydrogen ions further stick to the micro algal cells and brings them to the surface in the form of flocks [53].

6.3 Processing of Microalgal Biomass

After harvesting of algal cells from the culture medium, cells are subjected to further processing for extraction of oil and fat contents. This process should be done in a very energy efficient and cost effective way in which less use of organic liquids is made for the extraction of maximum biodiesel producing lipid contents without contamination of other by-products like DNA and chlorophyll [21, 23].

Cell disruption is the first step in processing of microalgal biomass and it is more important as many microalgal cells have stronger cell walls and also the net yield is based upon the extent of disruption of cells. Different procedures of cell disruption are used and the method to be practiced depends upon type of targeted microalga cells. Many mechanical actions (e.g. cell homogenizers, bead mills, ultrasound and autoclaving and spray drying) or non-mechanical action (e.g. freezing, organic solvent extraction, osmotic shock and acid/base or enzyme-mediated reactions) are used for cell disruption [16,23].

After cell disruption next step is extraction of lipids from cell debris. For lipid isolation specified methods for lipid extraction should be used in order to prevent the isolation of side non-lipid products along with lipids [23, 38].

A typical solid/liquid extraction method which is also very fast and efficient one can be done using organic solvents directly on the collected algal cells. Several solvents e.g. hexane, ethanol (96% v/v in water) or a combination of these can be used for extraction purposes [23].

6.4 Production of Biodiesel

The triacylglycerols obtained as a result of cell disruption are further processed to get biodiesel. These triacylglycerides are usually non-volatile, and their transesterification with short chain alkyl groups, e.g. methyl or ethyl groups, results in production of biodiesel. This is a multiple steps process i.e. formation of diglycerides from triglycerides, then monoglycerides from diglycerides and finally formation of free fatty acid and glycerin (a byproduct) from monoglycerides as shown in Fig. 4 [23,57].

This procedure is of great significance as it involves the transformation of free fatty acids into their relative alkyl esters even when they are present in algal biomass. Thus it saves time as there is no need to wait for the algal biomass to get dry after harvesting and also reduces the need of solvent extraction [58]. Glycerin is a byproduct produced in biodiesel industry (1 L of glycerol is produced as a byproduct with every 10 L of biodiesel) as a result of esterification [59].

7. DEVELOPMENTS IN ALGAL CULTIVATION FOR FUEL

Algae are cultured for over more than 50 years and now new methods are introduced in order to enhance the yield of algae. The most promising new method is genetic engineering by which transgenic algal strains (having desired characters) with enhanced protein expression, engineered photosynthesis and increased metabolism are obtained. Some of the techniques used for genetic transformation are agitating algal cells in glass bead mixture coated with DNA. It also involves propelling of micro sized DNA coated gold or tungsten particles [2].

8. CONCLUSIONS

Microalgae are promising organisms for the production of biofuels, like biodiesel, and are a sustainable resource as compared to fossil fuels. They are advantageous as they require a very little or no attention for its cultivation and growth. Freshwater and pesticides are not required for cultivation of microalgae. Also, they also hardly require arable land for cultivation; thus, microalgae do not compete for land with the food crops. In short, microalgae are a cost effective source for biodiesel production. The quantity and quality of biofuels which can be produced from these microalgae vary from species to species. Some may have up to 98% of oil content, on dry weight basis, which makes them an advantageous resource for biodiesel production.

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