Advances in Biotechnology

Chapter 3

Microalgae: A Potential Candidate for Biodiesel

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Abstract

Modern way of life intimately depends on fuels that are derived from fossil resources. With the depletion of resources and to meet the demand of the diesel fuel industry, alternative oil sources are being explored and developed in recent days. Biofuels derived from renewable biomass, organic matter could minimize the use and reduce the dependency on fossil fuel. It is eco-friendly, non-toxic, bio-degradable, stable, reduces the level of potential or probable carcinogens and has a favourable emission profile. Oleaginous microorganisms such as fungi and microalgae with 20% or more lipids in their cell have emerged as a potential feedstock for biodiesel production. Microalgal biodiesel production is considered to reduce the overall production costs of biodiesel in the global market, which is the major reason for researchers focusing their attention on oleaginous microalgae. Of late, combinatorial approaches such as genetic engineering and molecular engineering have been implemented in order to develop efficient microalgal platforms for the production of biodiesel. The present chapter describes the rapid progress made in this area in the past ten years.

1. Introduction

High energy prices, global warming, burgeoning population and uncontrolled urbanization are drawing considerable attention to find a renewable biofuels. The basic sources of energy are fossil fuels- petroleum, diesel, natural gas, coal and nuclear energy. Over 1.5 trillion barrels

of oil have been produced since Edwin Drake drilled the world's first oil well in 1859 [1]. It is estimated that, the same amount is required to meet the global demand in the next 25 years alone. In 2008, the annual world primary energy consumption was estimated as 11,295 million tonnes of oil equivalent. Fossil fuels accounted for 88% of the primary energy consumption, with oil (35%), coal (29%) and natural gas (24%) as the major fuels, while nuclear energy and hydroelectricity account for 5% and 6% of the total primary energy consumption respectively. It is estimated that the global demand for petroleum will be increased to 40% by 2025 [2].

Extensive use of fossil fuels for transport, electricity and thermal energy generation has led to the emission of greenhouse gases (GHGs) to the atmosphere, thus contributing to global warming. They account for 98% of total carbon emission [3]. Combustion of fossil fuels emits more than 6 billion tonnes of carbon-di-oxide annually in the atmosphere (Fig.1) In 2006, associated GHGs emissions were 29G tonnes [4]. It is estimated that natural processes confiscate only about 12G tonnes. Petroleum diesel combustion also contributes for green house emissions. Furthermore, it is also a major source of other air contaminants including nitric oxide, sulphur oxide, carbon monoxide, particulate matter, carcinogens and volatile organic compounds. Therefore, it is important to develop suitable strategies and stringent policies to minimise the impact of excess GHGs [5]. Another disadvantage with petroleum based fuels is their uneven distribution in the world (Fig.2), followed by decline in its reservoirs (at a rate of 2-3% predicted per year starting in 2010 [6].

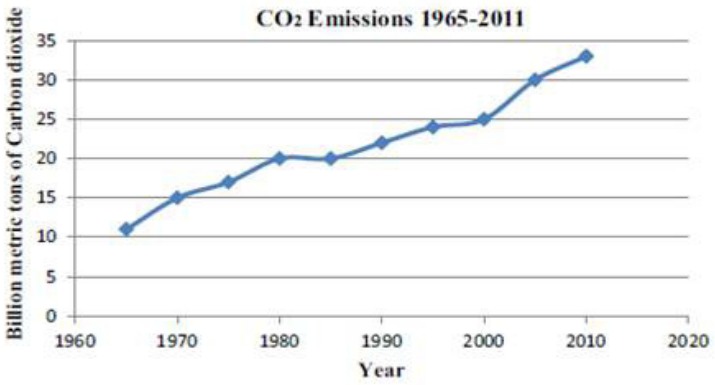

Figure 1: Graph showing global increase in carbon-di-oxide emission

Source: [7]

Of late, with the rapid increase in the price of crude oil and projected decrease in fossil fuel and petroleum reserves, followed by the growing concern of the environmental hazards of the non-renewable fuels has stimulated researchers to quest for alternative, sustainable and renewable energy sources.

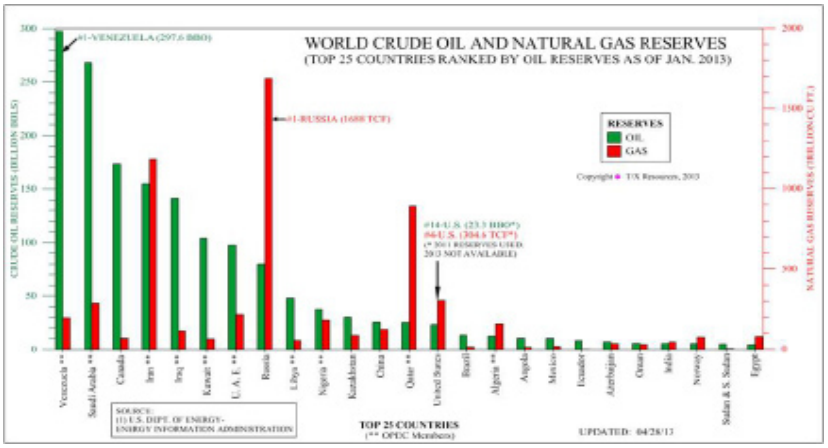

Figure 2: Global distribution of crude oil and natural reserves

Source: [6]

1.1. Development of biofuel resources

Finding suitable auxiliary clean energy for the future is one of the society's most daunting challenges and is associated with global stability, economic prosperity and quality of life. Of late, production of biofuels from renewable resources such as plants or organic waste, oleaginous micro-organisms has received considerable attention. It is eco-friendly, biodegradable and sustainable renewable resources.

1.2. Classification of biofuels

Biofuels are classified as primary and secondary biofuels. Primary biofuels are used in crude form, primarily for heating, cooking or electricity production such as fuel wood and wood chips etc. Whereas, secondary biofuels are produced by biomass processing (e.g. bioethanol, biodiesel etc). It can be blended with petrol to drive the vehicles and in various industrial practices. Secondary biofuels are further divided as first, second and third-generation biofuels on the basis of raw material and the technology used for their production (Fig.3). Biofuels can be solid, such as fuel wood, charcoal, and wood pellets, in liquid form such as ethanol, butanol and biodiesel and gaseous such as biogas (methane) [8].

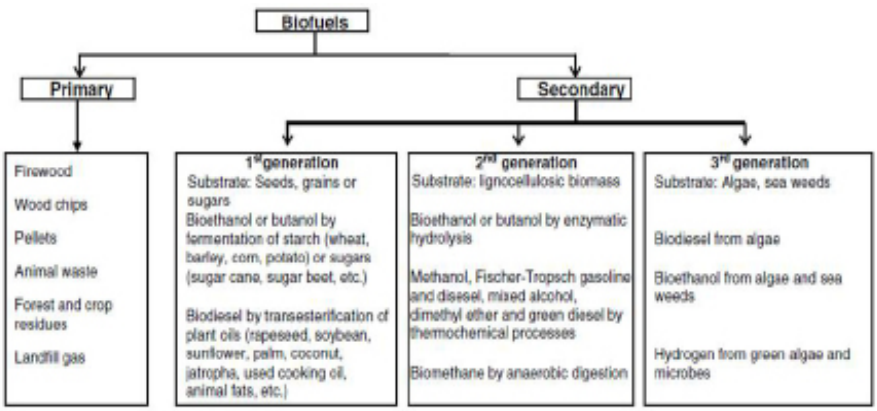

Figure 3: Classification of biofuels

Source: [8]

1.2.1 First Generation Biofuels

First generation biofuels such as bioethanol is the most promising alternative renewable energy source and has attained commercial level production in several countries like Brazil and United States Of America [9].Together, these countries account for 89% of the current global bioethanol production [10]. It is a liquid fuel produced by fermenting sugar extracted from lignocellulose [10], corn starch [11], sugarcane bagasse [12], sugar beets [13] and molasses [14], grains or seeds [15, 16]. It improves fuel combustion in vehicles, thereby, reducing GHGs. In Brazil, bioethanol accounts for 40% fuel needs [17].

Biodiesel, a monoalkyl esters of long chain fatty acids with short chain alcohols, primarily methanol and ethanol, resulting in fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs) [18]. It is obtained from dedicated oleaginous plants such as pongamia, jatropa etc by transesterification processes. It is eco-friendly, biodegradable, stable, reduces GHGs emission, low flammability and good lubrication properties [19]. Pure biodiesel or biodiesel blended in any ratio with petroleum-based diesel can be used in conventional diesel engines with no or only marginal modifications.

However, the first generation biofuels seemed to create scepticism to scientists. As vegetable oil is used for human consumption, harnessing it for biodiesel production could lead to an increase in price of food-grade oils. The extensive plantation of oil yielding plants could lead to land competition and biodiversity loss [20]. The cost of biodiesel production mainly depends on the price of the feedstocks that accounts for 60-75% of the total cost of biodiesel production [21]. To become a potential alternative fuel, biodiesel must compete economically with diesel.

1.2.2 Second Generation Biofuels

Transition to second generation biofuels has attracted great attention. It is produced from two methods i.e. biochemical or thermochemical processing from agricultural ligno-cellulosic biomass (non-edible crop residues or whole plant biomass) and industrial or municipal organic waste. It is eco-friendly, inexpensive, renewable, reduces land requirement and limits the direct food versus fuel competition [22]. Biomass conversion by thermochemical method is achieved at extreme temperatures and pressures. The fuel thus obtained can be used directly in engines. Whereas, biochemical conversions, also called as saccharification involves application of array of enzymes such as cellulase, amylase, β-glucosidases, xylanase [23] obtained from fungi [24] and bacteria [23] on residual substrates such as ligno-cellulosic biomass [25], rice straw [26], sugarcane bagasse [27], molasses [28], sugar beet pulp [29] and starch [30]. However, it is cost effective, requires sophisticated equipment and larger-scale facilities which limits its economic feasibility and commercial production [31].

1.2.3 Third Generation Biofuels

Growing lines of evidence suggest that, micro-organisms such as yeast, fungi and microalgae can accumulate large amount of lipid. This has attracted great attention and can be used as potential candidate for third generation biofuel production. Bacteria, in general, do not produce triacylglycerols but instead, accumulate poly-β-hydroxy-butyrates and alkanaoates as storage polymers [32]. Several benefits can be envisioned from yeast, algae and fungi due to their advantages over higher plants such as similarity in fatty acid profiles with plant seed oils, easy to grow, simple cultural conditions and nutrients for growth, no requirement of agricultural land and consistency of the product yield has been shown to be an ideal alternative owing to its amicability for the separation, purification and industrialization [33]. Furthermore, it is devoid of the major drawbacks associated with first and second generation biofuels. Screening the potential oleaginous microbial cell factories or engineered strains for biodiesel production could be a promising way for renewable energy. The manipulation and regulation of microbial lipid biosynthesis opens a new avenue for academic researchers and harness its potential in its commercial application for biodiesel production.

2. Microalgae for Biodiesel Production

Microalgae comprises several groups of unicellular and multicellular, colonial or filamentous, photosynthetic or heterotrophic micro-organisms containing chlorophyll and other pigments. It can grow autotrophically or heterotrophically with a wide range of tolerance to different temperature, salinity, pH and nutrient [34]. More than 40,000 microalgal species have been classified as prokaryotes (cyanobacteria) and eukaryotes such as green algae, diatoms, yellow–green algae, golden algae, red algae, brown algae, dinoflagellates [35,36]

2.1. Classification

Algae is classified into four types

1. Prokaryotic Algae: **Cyanophyta-** Cyanobacteria are the only prokaryotic algae. It consist of chlorophyll and phycobiliproteins.

2. Eukaryotic Algae: It consist of chloroplasts which is surrounded by two membranes of the chloroplast envelope.

a. Phylum Glaucophyta: It includes algae that represent transitional position in the evolution of chloroplasts; photosynthesis is supported by modified endosymbiotic cyanobacteria. Example- *Glaucocystis*

b. Phylum Rhodophyta: It comprises Chloro phyll *a,* phycobiliproteins, flagellated cells are absent, storage product is floridean starch. Example - Red algae

c. Phylum Chlorophyta: It comprises chlorophylls *a* and *b*, storage product is starch. It is found inside the chloroplast. Example: Green algae

3. Eukaryotic algae: It consist of chloroplast which is surrounded by one membrane of chloroplast endoplasmic reticulum.

a. Euglenophyta : It comprises chlorophyll *a* and *b*, one flagellum with a spiral row of fibrillar hairs and proteinaceous pellicle in strips are present under the plasma membrane; storage product is paramylon; character istic type of cell division. Example: Euglenoids

b. Dinophyta (dinoflagellates)): it comprises mesokaryotic nucleus, chlorophyll *a* and *c*. Cell is commonly divided into an epicone and a hypocone by a girdle and helical transverse flagellum.

a. Apicompexa : they are heterotrophic flagellates with colorless plastids.

4. Eukaryotic algae with chloroplasts are surrounded by two membranes of chloroplast endoplasmic reticulum.

a. Cryptophyta: Nucleomorph present between inner and outer membrane of chloroplast endoplasmic reticulum. Starch is stored in the form of grains between inner membrane of chloroplast endoplasmic reticulum and chloroplast envelope. It consist of chlorophyll *a* and *c*, phycobiliproteins are present. Periplast are seen inside the plasma membrane. Example : Cryptophytes

b. Heterokontophyta : It usually consist of anterior tinsel and posterior whiplash flagellum. It consist of chlorophyll *a* and *c* along with fucoxanthin. Storage product is in the form of chrysolaminarin, present in the heterokonts.

Example : *Paraphysomonas sigillifera*.

Heterokontophyta consist of the following classes Chrysophyceaeec, Synurophyceae, Eustigmatophyceae, Pinguiophyceae, Dictyochophyceae, Pelagophyceae, Bolidophyceae, Bacillariophyceae, Raphidophyceae, Xanthophyceae, Phaeothamniophyceae, Phaeophyceae, Prymnesiophyta.

2.2 Selection and screening of oleaginous microalgae for biodiesel production

Due to variation and diversity of microalgal lipids in nature, selection of oleaginous microalgal strains suitable for biodiesel production requires screening of large number of

microalgal strains. In 1978, the first large-scale collection and screening of oleaginous algae was started, when the Aquatic Species Program, launched by U.S. National Renewable Energy Laboratory. Over 3000 strains were collected and eventually around 300 species were identified as oleaginous algae [37]. Screening of oleaginous microalgae and optimizing culture conditions to enhance lipid accumulation and evaluation of its potential for biodiesel production is well studied [38, 39, 40, 41]. Screening of microalgae encompass the following steps [Figure 4].

1. Sampling from the field i.e. isolation or collection from algal collection library

2. Identification and maintenance of the culture

3. Biomass harvesting

4. Determination of lipid content oil extraction [42, 43, 44, 45]

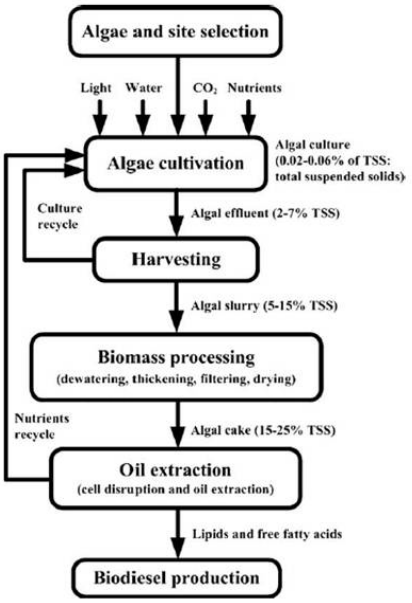

Figure 4: Process of biodiesel production in microalgae

Source: [43]

2.3. Harnessing microalgae for biodiesel production

The advantages of microalgae as an alternate source for biodiesel production over high plants are as follows

1. Rapid growth, accumulates high content of lipid

2. Non-requirement of arable land for its growth

3. Phototropic microalgae marks it to be economical than oleaginous heterotrophic microorganisms that utilize glucose and other organic carbon sources [46]

4. It utilizes large amounts of carbon-di-oxide emitted by power plants and other industrial

sources, thereby contributing to GHG mitigation [36]

5. It also produces other types of biofuels such as alkanes, ethanol, butanol and hydrogen [47]

6. Production of biodiesel from microalgae results in minimal release of sulphur dioxide, nitrous oxide and other contaminants when compared to petroleum-derived diesel [48,42].

2.4. Biochemistry of lipid accumulation in microalgae

The process of lipid accumulation in microbial cells is well documented [49]. Microorganisms in general, are able to synthesize lipids for essential functioning of their membrane structures. However, a few microbes in the microbial kingdom have the ability to accumulate more than 20% lipids in their cells. These are called as oleaginous organisms and they store lipid in oil vacuoles as triacylglycerol. The process of lipid accumulation is known as lipogenesis. The pattern of lipid accumulation and fatty acid profile in microalgal species varies significantly **(Table 1)**. It is influenced by factors such as light intensity [50], nitrogen concentration [51,52], carbon-di-oxide concentration [53], salinity [54], temperature [35], pH [39] etc. Overview of the metabolites and representative pathways in microalgal lipid biosynthesis is depicted in Figure.5.

Table 1: Lipid content in selected microalgae

Marine and freshwater microalgae species	lipid content (% dry weight biomass)	Lipid productivity (mg/L./clay)	Volumetrk productivity of biomass (g/L/day)	Areal productivity of biomass (g/&/clay)
Ankistrodesmus sp.	24.0-31.0	-	-	113-17.4
Botryococcus braunii	25.0-75.0	-	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	-
Chaetoceros cakitrans	14.6-16.4/39.8	17.6	0.04	-
Morella emersonii	25.0-63.0	10.3-50.0	0.036-0.041	0.91-0.97
Chlorella protothecoides	14.6-57.8	1214	2.00-7.70	-
Chlorella sorokiniano	19.0-22.0	44.7	0.23-1.47	-
Chlorella vulgaris	5.0-58.0	11.2-40.0	0.02-020	0.57-0.95
Chlorella sp.	10.0-48.0	42.1	0.02-2.5	1.61-16.47/25
Chlorelia pyrenoidosa	2.0	-	2.90-3.64	72.5/130
Moreno	18.0-57.0	18.7	-	3.50-13.90
Chlorococcum sp.	19.3	53.7	0.28	-
Crypthecodinium cohnii	20.0-51.1	-	10	-
Dunaliella sauna	6.0-25.0	116.0	0.22-0.34	1.6-3.5/20-38
Dunaliella primotecta	23.1	-	0.09	14
Dunaliella tertioleao	16.7-71.0	-	0.12	-
Dunaliella sp.	17.5-67.0	33.5	-	-

Ellipsoidion sp.	27.4	47.3	0.17	
Euglena gracilis	14.0-20.0	-	7.70	
Haematococcus pluviatis	25.0	-	0.05-0.06	10.2-36.4
Isochrysis galbana	7.0-40.0	-	0.32-1.60	
hoc/trysts sp.	7.1-33	37.8	0.08-0.17	-
Monodus subterraneus	16.0	30.4	0.19	-

Source: [48, 45, 60]

Lipids are classified into phospholipids, spingolipids and neutral lipids. Triacylglycerols, main constituents of biodiesel are packed in neutral lipids. Biosynthesis of triglycerides in microalgae may consist of the following three steps:

(a) Formation of acetyl coenzyme A (acetyl-coA) in the cytoplasm

(b) Elongation and desaturation of hydrocarbon chain

(c) Synthesis of triglycerides

(a) Formation of acetyl coenzyme A (acetyl-coA) in the cytoplasm

Microalgae, in the presence of photon energy fix the carbon-di-oxide into sugars. Acetyl-coA is formed during the light reaction and Calvin cycle. It is synthesized in the chloroplast [55]. Further, the 3-PGA is exported to cytoplasm for consumption. Subsequently, carbon is directed for glucose synthesis via glycolysis and is further converted into starch, which acts as a storage product in cells [56].

After Calvin cycle, 3-phosphoglycerate (3-PGA) is synthesized in the chloroplasts followed by the glycolytic pathway to form pyruvate (Fig.5). Pyruvate releases CO_2, generates acetyl-CoA (acetyl coenzyme) in the presence of pyruvate dehydrogenase (PDH). Acetyl-CoA serves as the precursor for fatty acid synthesis in the chloroplast [55].

(b) Elongation and desaturation of carbon chain of fatty acids

In most of the organisms, the elongation of carbon chain of fatty acids is achieved by two enzyme machineries namely acetyl-coA carboxylase enzyme [ACCase] and fatty acid synthase [49]. During fatty acid synthesis, acetyl-coA acts as a primer and malonyl-coA serves as a substrate. Fatty acid synthesis is initiated by ACCase enzyme, it synthesizes malonyl-CoA from acetyl-CoA and bicarbonate. Malonyl-CoA group is transferred to malonyl-ACP (acetyl carrier protein) catalyzed by an acyl carrier protein malonyltransferase. The C16 and C18 fatty acid thio-ester is formed after a series of elongation reactions [57]. Growing body of evidence suggest that, synthesis of short-chain fatty acids in microalgae is similar to other living organism such as plants, animals, fungi and bacteria [49,57]. Desaturation of carbon

chain of fatty acid occurs from C18 and further elongation of carbon chain occurs thereby leading to the synthesis of long-chain fatty acids which are unusual in normal plant oils (Fig.5). Thus, selection of a potential strain is a crucial step for algal biodiesel production.

Triacylglycerol is synthesized by the sequential acylation of glycerol-3-phosphate (G3P) backbone with three acyl-CoAs catalyzed by the enzyme acyltransferases. Acylation of G3P using glycerol-3-phosphate acyltransferase results in the synthesis of lyso-phosphatidic acid. This is further acylated to phosphatidic acid by (lysophosphatidic acid acyltransferase). Furthermore, phosphatidic acid phosphatase removes the phosphate group from phosphatidic acid to generate DAG (diacylglycerol). The oil synthesis is catalyzed by DGAT (diacylglycerol acyltransferase) from DAG to triacylglycerol [55, 57].

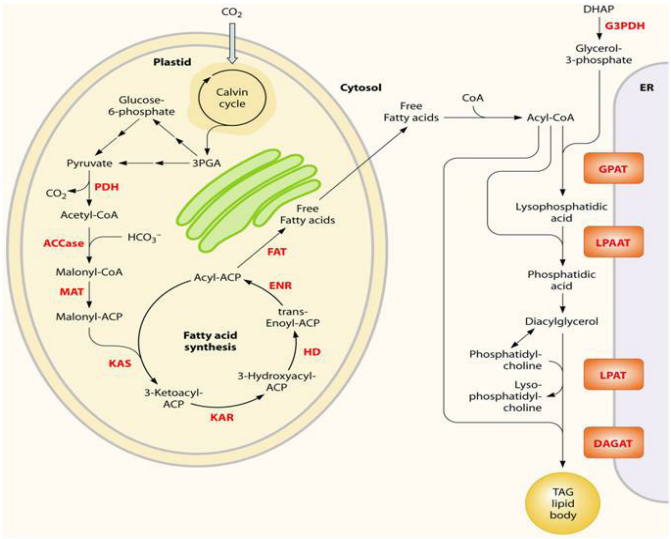

Figure 5: Overview of the metabolites and representative pathways in microalgal lipid biosynthesis shown in black and enzymes in red.

(Free fatty acids are synthesized in the chloroplast, while TAGs may be assembled at the ER. ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; ENR, enoyl-ACP reductase; FAT, fatty acyl-ACP thioesterase; G3PDH, glycerol-3-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; HD, 3-hydroxyacyl- ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; LPAAT, lyso-phosphatidic acid acyltransferase; LPAT, lyso-phosphatidylcholine acyltransferase; MAT, malonyl-CoA:ACP transacylase; PDH, pyruvate dehydrogenase complex; TAG, triacylglycerols).
Source: [43]

2.5. Microalgae lipid accumulation and oil production

Microalgal species can be induced to accumulate substantial quantities of lipids [58, 59] thus contributing to high oil yield. Average lipid content ranges between 1%-70%. However, under certain physiological conditions few species can reach up to 90% of dry weight (Table 1). Although microalgae oil yield is strain-dependent it is generally much higher than other vegetable oil crops **(Table 2, 3** and **4)**.

Table 2: Oil content in selected microalgae

Sl. No	Microalgae	Oil content (wt% of dry basis)
1	*Botryococcus braunii*	25–75
2	*Chlorella sp.*	28-32
3	*Crypthecodinium cohni*	20
4	*Cylindrotheca sp.*	16-37
5	*Dunaliella primolecta*	23
6	*Isochrysis sp.*	25-33
7	*Monallanthus salina*	>20
8	*Nannochloris sp.*	20-35
9	*Nannochloropsis sp.*	31-68
10	*Neochloris oleoabundans*	35-54
11	*Nitzschia sp.*	45-47
12	*Phaeodactylum tricornutum*	20-30
13	*Schizochytrium sp.*	50-77
14	*Tetraselmis sueica*	15-23

Source: [61]

Table 3: Comparison of microalgae with other biodiesel feedstocks

Sl. No	Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year	Land use (m2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ ha year)
1	Corn/Maize (Zea mays L.)	44	172	66	152
2	Hemp (*Cannabis sativa L.*)	33	363	31	321
3	Soybean (*Glycine max L.*)	18	636	18	562
4	Jatropha (*Jatropha curcas L.*)	28	741	15	656
5	Camelina (*Camelina sativa L.*)	42	915	12	809
6	Canola/Rapeseed (*Brassica napus L.)*	41	974	12	862
7	Sunflower (*Helianthus annuus L.)*	40	1070	11	946
8	Castor (*Ricinus communis*)	48	1307	9	1156
9	Palm oil (*Elaeis guineensis*)	36	5366	2	4747
10	Microalgae (low oil content)	30	58,700	0.2	51,927
11	Microalgae (medium oil content)	50	97,800	0.1	86,515
12	Microalgae (high oil content)	70	1,36,900	0.1	1,21,104

Source: [43]

Table 4: Yield of various plant oils

Sl. No	Crop	Oil in litres per hectare
1	Algae	1,00,000
2	Castor	1413
3	Coconut	2689
4	Palm	5950
5	Safflower	779
6	Soy	446
7	Sunflower	952

Source: [61]

3. Properties of biodiesel

Physicochemical properties of microalgal biodiesel are nearly similar to diesel fuel. Important properties of biodiesel are cetane number, heat of combustion, viscosity, oxidative stability, cold flow properties and lubricity [62]. The main properties of microalgal biodiesel compared with diesel and first generation biodiesel is shown in Table 5.

3.1. Cetane number

It determines the quality of ignition of a fuel which increases with the number of carbon and decreases with the number of unsaturated carbon bounds [63]. A higher unsaturated biodiesel like microalgae biodiesel would have a lower cetane number.

3.2. Heat of combustion

It indicates if a biodiesel is suitable to burn in a diesel engine. The heat of combustion increases with the length of the carbon chain [64]. In 2004, Miao and Wu reported that, lipids extracted from heterotrophic microalgae in the presence of sulphuric acid in methanol, obtained a biodiesel with a heat of combustion of 35.4 MJ/L which is in the range of diesel fuel (36-38 MJ/L) [65].

3.3. Viscosity

It increases with the number of carbon and decreases with the degree of unsaturation. A higher kinematic viscosity would create engine problems like engine deposits [64]. Transesterification decreases the viscosity of the oil at values usually between 4 to 6 mm/s (40°C) [66].

3.4. Oxidative stability

When fatty acid methyl esters (FAME) reacts with oxygen, hydrogen peroxides, aldehydes, acids and other oxygenates are formed, which could deposit in the engine [64]. It

entirely depends on the degree of unsaturation [63]. Oxidation stability of microalgal lipids is therefore a real problem [67]. It can be overcome by adding antioxidants if the biodiesel blend is stored more than a few months [66].

3.5. Cold flow properties

It is an important parameter for biodiesel production in European countries such as Canada. Decrease in temperature could lead to the formation of visible crystals in the biodiesel at a limit called as cloud point [64]. Cloud point temperature decreases with the mole fraction of unsaturated compounds and slightly increases with the length of the carbon chain [68].

3.6. Lubricity

Lubricity for a fuel is "the ability to reduce friction between solid surfaces in relative motion" [69]. The lubricant of diesel fuel is influenced by the viscosity, acidity, water content and the sulphur compounds [70]. For microalgae biodiesel, no lubricant study is yet reported from the literature.

Table 5: Comparison of properties of microalgal oil, conventional diesel fuel, and ASTM biodiesel standard

Sl. No	Properties	Biodiesel from microalgal oil	Diesel fuel	ASTM biodiesel standard
1	Density (Kg/l)	0.864	0.838	0.84-0.90
2	Viscosity (mm^2/s, cSt at 40°C	5.2	1.9-4.1	3.5-5.0
3	Flash Point (°C)	115	75	Min 100
4	Solidifying Point (°C)	-12	-50 to 10	-
5	Cold filter plugging point (°C)	-11	-3.0 (max -6.7)	Summer max 0; winter max <-15
6	Acid value (mg KOH/g)	0.374	Max 0.5	Max 0.5
7	Heating Value (MJ/Kg)	41	40-45	-
8	H/C ratio	1.81	1.81	-

Source : [71]

4. Mass Cultivation of Microalgae

Large-scale production of microalgal biomass generally uses continuous culture system during daylight. In this method, fresh algal culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously. However, feeding ceases during the night, but the mixing of the culture medium should continue to avoid flustering of the biomass [72]. As much as 25% of the biomass produced during daylight, may be lost during the night because of respiration. The extent of this loss depends on intensity of sunlight under which the biomass was grown, temperature during day and night time. In general, for large-scale production of microalgae, raceway ponds [3, 73] and tubular photobioreactors [3, 74] are widely used.

4.1 Open Pond System

It is also known as "Raceway Pond System". At present, about 98% of commercial algae are cultivated using this system [75]. It is made up of a closed loop recirculation channel which is 0.3m deep (Fig.6). Mixing and circulation is mechanically achieved by paddlewheels, which are limited to 20cm- 30cm in depth (Fig.6). Flow is directed around bends by baffles placed in the flow channel. They are constructed from concrete, however, compact earth-line ponds lined with plastic have also been used [3]. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins (Fig.6). On completion of the circulation loop, broth is harvested behind the paddlewheel, which is operated continuously to prevent sedimentation.

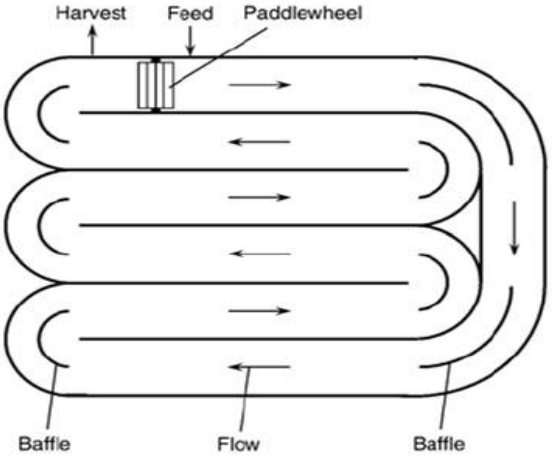

Figure 6: Aerial view of raceway pond

Source [45]

Raceway ponds is most suitable for mass cultivation of microalgal species which can tolerate high salinity and pH such as *Dunaliella salina*, *Spirulina*, *Chlorella species* etc [76]. Microbial contamination, seasonal variation and temperature fluctuations directly impede the biomass production [77, 45]. Due to low productivities, large areas of land may be required to meet the desired output of cultivation [76]. Maintenance and cleaning of open ponds are easier and less energy intensive than photobioreactors [3]. Although raceways are economical, they have a low biomass productivity compared with photobioreactor [77, 3].

4.2. Photobioreactor

Photobioreactors (PBRs) have received much attention because of its versatility, high biomass productivity and ease to control culture conditions [78,79]. Various types of photobioreactors used in microalgal mass cultivation are horizontal tubular PBRs, stirred PBRs, airlift and bubble column photobioreactor [79,80,81]. They are more versatile than open ponds as they can use sunlight, artificial light and various combinations and intensities of light sources. Advantages and disadvantages of the respective PBRs are summarized in Table. 6

Tubular PBRs is commonly used for mass cultivation of microalgae [81]. The productivities of PBRs are influenced by the light supply, carbon-di-oxide and fluctuations in temperature, pH, and dissolved oxygen levels [82]. It consist of a series of straight, transparent solar tubes which allows the light to pass through the dense culture **(Fig.7)**. It is made up of plastic or glass with 0.1m in diameter. The orientation of the solar collector may be horizontal, vertical, inclined or as a helical coil around a supporting frame [3,79]. Microalgal broth is circulated from a reservoir (i.e. the degassing column in **Fig.7**) to the solar collector and back to the reservoir. The solar tubes are placed parallel to each other and flat above the ground (Figure.7). Horizontal, parallel straight tubes are sometimes arranged like a fence (Figure. 7). The tubes are always oriented North–South direction **(Fig.7)**.

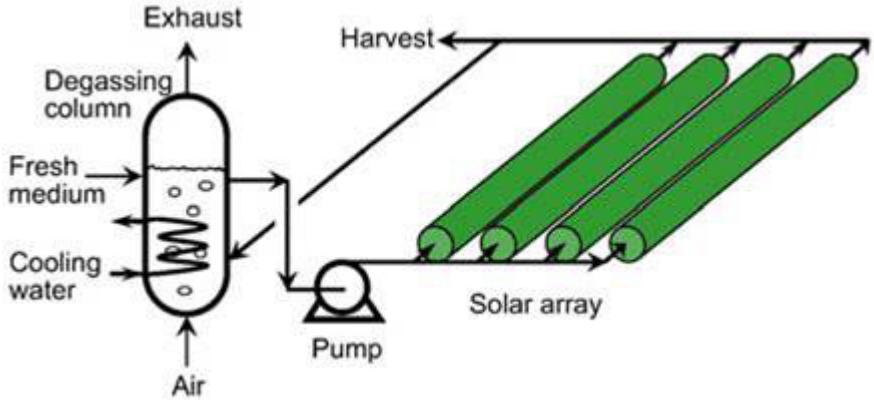

Figure 7: A tubular photobioreactor

Source: [45]

Photosynthesis generates oxygen. Therefore, cultures are generally reticulated by pump passing through a degasser at regular intervals in order to remove excess oxygen (Fig .7). Higher levels of oxygen lead to lower productivities due to photo-oxidative stress. As the broth moves along a photobioreactor tube, pH increases because of consumption of carbon dioxide [83]. Additional carbon dioxide injection points is required to prevent carbon limitation and an excessive rise in pH [72]. As much as 25% of the biomass produced during day light could be consumed during the night to sustain the cells until sunrise. However, this problem can be overcome by lowering the temperature at night.

Table 6: Advantages and disadvantages of different Photobioreactors

Sl. No	Type of photobioreactor	Advantages	Disadvantages
1	Horizontal tubular PBR	High light conversion efficiency	a. Causes photo bleaching due to high concentration of dissolved oxygen and thus reduces photosynthesis efficiency
2	Strirred PBR	a. Expedient b. Carbon-di-oxide can be supplied efficiently	a. Lack of internal light b. Mechanical agitation limits its use c. Low surface area
3	Airlift PBR	a. High biomass production b. uniform mixing can be achieved c. low hydrodynamic stress d. Best suitable for immobilization of algae	Cost- effective
4	Bubble column PBR	a. Economical b. Efficient release of oxygen	a. Lack of internal light b. Lack of mixing

4.3. Advantages and limitations of raceway ponds and photo bioreactors

In contrast to open ponds, photobioreactors have the advantages of low contamination, high productivity, minimal evaporation, reduced CO_2 losses and better control over culture conditions (Table.7). The major drawbacks of photobioreactors are the high costs of construction, fluctuations in temperature [85], pH [84,85], oxygen [86], light [87] and carbon-di-oxide [85]. Although these can be partially compensated by higher productivity, they still limit the cost-effective production of microalgal biomass on a scale required for biodiesel production. Hybrid algae production system comprising photobioreactors and open ponds may be a promising way. Sufficient contaminant-free inoculum can be produced in photobioreactors, followed by transfer to open ponds or raceways to attain the biomass needed for biodiesel production [88].

Table 7: Comparison between open ponds and photobioreactor

Sl. No	Culture systems for microalgae	Open Ponds	Photobioreactors
1	Contamination control	Difficult	Easy
2	Contamination	High	Low
3	Energy consumption	Low	High
4	Process control	Difficult	Easy
5	Species control	Difficult	Easy
6	Mixing	Very poor	Uniform
7	Operation regime	Batch / semi-continuous	Batch / semi-continuous
8	Space required	More	Less
9	Population (algal cell) Density	Low	High
10	Investment	Low	High
11	Operation costs	Low	High
12	Light utilization Efficiency	Poor	High
13	Temperature control	Difficult	Easy
14	Productivity	Low	3–5 times more productive
15	Hydrodynamic stress on algae	Very low	Low–high
16	Evaporation of growth Medium	High	Low
17	Gas transfer control	Low	High
18	CO_2 losses	PBRs _ Ponds	Depends on pH, alkalinity, etc.
19	Cultivation of algae	Limited to few strains	Versatile
20	Biomass productivity	Low	High

Source: [3,43,79, 81]

4.4. Hybrid production systems

This technique combines distinct growth stages in photobioreactors and as well as in open ponds. The first stage is in a photobioreactor where controllable conditions minimize microbial contamination and favour monocell culture system [89, 90]. Further, the production stage is carried out in raceway pond. In this stage, microalgal cells are exposed to various nutrient stress, which enhances synthesis of the desired lipid product [3].

5. Methods of Recovery of Microalgal Biomass

The fiscal recovery of microalgal biomass still remains as a major challenge. It is documented that, harvesting accounts to 20–30% of the total cost due to small size of microalgal cells (2-20 μm in diameter) and high water content of the broth [43]. Various methods such

as flocculation, sedimentation, flotation, filtration, centrifugation and drying have been under practice for harvesting the biomass.

5.1. Flocculation

It is the most cost-effective and reliable method used for harvesting different species of microalgae. It is achieved by addition of chemicals (organic and inorganic), micro-organisms and rarely by auto-flocculation to form larger clumps, which ease the process of separation (Table.8). An ideal flocculent should be non-toxic, inert and economical. For the recovery of most of the unicellular microalgae cultured in open or raceway pond system, flocculation is used as a pre-treatment step to increase the particle size [74, 91].

5.2. Sedimentation

It is widely used separation technique in wastewater treatment processes. Lamella separators and sedimentation tanks are used for gravity sedimentation. Gravity sedimentation results in high microalgal harvesting efficiency only when preceded by flocculation. Factors influencing particle settling velocity of untreated microalgae are gravity force, particle diameter, density of the medium, density of particle and medium viscosity. It is the most appropriate method due to low capital costs even in large scale operations [74]. However, it is suitable for microalgal species with high sedimentation rates. The advantage of this technique is it is inexpensive, process control is easy with only a requirement of a settling tank and is amenable for large scale biomass harvesting [81].

Table 8: Different types of flocculants used for harvesting microalgae

Sl. No	Method	Advantage	Disadvantage
1	Gravity sedimentation	1. Inexpensive 2. Low energy consumption	1. Not suitable for all types of microalgal species 2. Low reliability 3. Low efficiency
2	Flocculation	1. High recovery 2. Reliable 3. Low energy consumption	1. Flocculants may be expensive 2. Not suitable for all types of microalgal species 3. Time consuming
3	Floatation	1. Does not require addition of chemicals 2. Relatively fast	1. Particle size should be less than 500μm
4	Centrifugation	1. High recovery 2. Corrosion resistance 3. Rapid	1. High energy consumption 2. Expensive 3. Cannot be used for species <30 μm
5	Filtration	**1.** Reliable **2.** Able to harvest species of low density	1. Filters may have to be replaced periodically 2. Membrane fouling & clogging 3. Time consuming 4. Expensive
6	Electrolytic method	1. Inexpensive 2. Low risk of contamination 3. High efficiency 4. No addition of chemicals 5. Reduces operation time	1. Cathode fouling 2. Unsuitable for large scale operations
7	Immobilization	1. More stable 2. High efficiency	1. Expensive 2. Unsuitable for large scale operations
8	Drying	1. No addition of chemicals	1. Requirement for large drying surfaces 2. Risk of material loss

5.3. Floatation

It is a process in which the algal cells are attached to the micro-air bubble surface and are carried on to the surface [104, 109]. Unlike flocculation, floatation does not require addition of chemicals [110]. Hanotu et al in 2012 reported that small bubbles take longer time to rise making them more susceptible to aggregate with the microalgae particles compared to large bubbles [111]. To achieve higher efficiency, the particle size should likely be less than 500μm [112]. Chen et al noted that flotation was more beneficial in microalgal removal than sedimentation and furthermore, it is relatively fast compared to sedimentation [32].

5.4. Centrifugation

The use of centrifugation for biomass recovery and dewatering is considered to be rapid, easy, non-disruptive and high efficiency technique [81, 113]. Cell separation is achieved by increasing the gravitation field subjected to the microalgal suspension thereby concentrating the biomass into a cake with >95% cell harvest efficiency at 13000/g [88]. However, this technique requires high energy consumption and therefore it is not suitable for large scale and commercial scale operations [92, 45].

5.5. Filtration

Filtration is influenced by the size of microalgal cells and the nature of the filter used. Various types of filters are used for harvesting microalgae. Conventional filtration methods such as rotary drum pre-coat filters and press filters are unsuitable for harvesting all microalgal species, as the size range of microalgae range between 2-30μm [92]. Therefore, micro-filtration (pore size ranges from 0.1-10 μm) is appropriate for biomass recovery process. Macro-filtration (pore size is >10 μm) is suitable for flocculated and larger microalgal cell biomass recovery [104]. However, these methods are unsuitable for large-scale operations [114].

5.6. Electrolytic Method

It is another potential approach to separate microalgal cells without the addition of chemicals. In this method, an electric field drives algae to move out of the solution. Water on electrolysis generates hydrogen, binds to the microalgal cells, forms complexes and carries to the surface. Advantages of electrochemical method are highly efficient, versatility and safe. Limitations are high energy consumption and unsuitable for large scale purpose [74, 115].

5.7. Immobilization

Several microorganisms have a natural tendency to attach to surfaces and grow on them [116]. This property is used for immobilizing microbial cells on immobilizing agents such as sodium alginate. Immobilization of the microalgal cultures provides a ready-to-retrieve ancillary platform for biomass recovery [117]. Immobilized biomass can be used for biofuel conversion by thermal or fermentative means. For example, immobilization of hydrocarbon rich microalgae, *Botryococcus braunii, Botryococcus protuberance* on alginate beads yielded a significant increase in chlorophyll, carotenoids, dry biomass weight and lipids during the stationary and resting growth phases compared to free living cells. In addition, the immobilized cells are more stable than free cells.

5.8. Drying

Harvested biomass must be processed immediately after harvest. Dehydration or drying is commonly used to extend the viability depending on the final product required. Various methods of dehydration are sun drying [118], low-pressure shelf drying [118], spray drying [119], freeze drying [120].

Sun drying is the most economical drying method. However, the main disadvantages is time consuming , requirement for large drying surfaces and the risk of material loss [118]. Spray drying is commonly used for extraction of high value products, but it is relatively expensive and can causes significant deterioration of certain algal pigments [119]. Freeze drying is equally expensive, especially for large scale operations, but it is unsuitable for the extraction of oils. Intracellular elements such as oils are difficult to extract from wet biomass with solvents without cell disruption, but are extracted more easily from freeze dried biomass [92, 120].

Table 9: Advantages and disadvantages of different microalgal harvesting methods

Sl. No	Method	Advantage	Disadvantage
1	Gravity sedimentation	1. Inexpensive 2. Low energy consumption	1. Not suitable for all types of microalgal species 2. Low reliability 3. Low efficiency
2	Flocculation	1. High recovery 2. Reliable 3. Low energy consumption	1. Flocculants may be expensive 2. Not suitable for all types of microalgal species 3. Time consuming
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6. Extraction Techniques

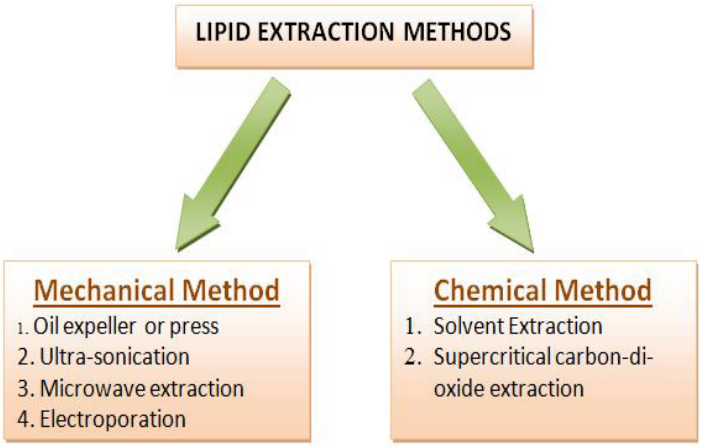

Figure 8: Types of lipid extraction methods

6.1. Press/ Oil expeller method

It is one of the simple, mechanical crushing method commonly used for extracting oil from plant seeds. Oflate, this method is also employed to extract lipid from algal biomass [61]. In this method, high mechanical pressure is applied for crushing and breaking the cells. This results in release of oil contents from the algal biomass. However, high mechanical pressure results in decreased lipid recovery, increased heat generation and choking problems .Oil recovery ranges between 70–75% [121]. To increase the extraction efficiency, occasionally solvents are used. The major drawback is unlike plant seed oil, extraction of oil from microalgal cells is hindered by the rigid cell wall. Furthermore, along with the oil, algal pigments also get extracted. Before conversion to oil, the pigments have to be separated, thus making the entire process cumbersome and expensive.

6.2. Solvent Extraction

Solvent extraction is simple, rapid and inexpensive method compared. The choice of solvent for lipid extraction depends on the type of the microalgae grown. Solvents used should be inexpensive, volatile, non-toxic and non-polar and poor extractors of other cellular components. The most commonly used solvents for microalgal lipid extraction are n-hexane, benzene, diethyl ether and chloroform [122, 123, 124]. Some of the common methods used for the extraction of lipids are Bligh and Dyer method, Soxhlet extraction and Folch et al method [125, 126, 127] .

6.3. Ultrasonication

It is simple, rapid, imparting higher purity to the final product, economical, less energy consumption and can be operated under lower temperature [128]. Ultrasonic waves are produced that propagate in the liquid medium resulting in alternating high pressure and low pressure cycles. During high pressure cycle, the vacuum air bubble produced during the low

pressure cycle ruptures and emits shock waves. This process is known as cavitation [129]. The shockwaves thus produced damage the microalgal cell wall and thereby favours the leakage of intracellular components. In addition, ultrasonic waves aid in the penetration of solvents such as hexane and facilitate the high efficiency transfer of lipids from the cell into the solvents. The disadvantage of this method is cost effective for large scale application [130].

6.4. Supercritical Carbon Dioxide Extraction

It is a promising technology for lipid extraction and could potentially replace the use of traditional organic solvents [131]. In this technique, carbon-di-oxide is compressed beyond its supercritical point (31°C, 74 bar). Now, the supercritical carbon-di-oxide is brought in contact with algal biomass in an extraction vessel. Due to its high penetrating power, it efficiently extracts oil from algae with less solvent residues compared to other extraction methods [132]. Advantages of SCCE extraction are high penetrating power, high efficiency, low toxicity of the supercritical fluid and minimum solvent residues. Carbon-di-oxide generated during the process can be used for the cultivation of microalgae. This gives further value to the process [61]. Disadvantages are requirement of elevated pressure, high capital and operating costs for a high-pressure SCCE [133].

6.5. Microwave Assisted Extraction

Application of microwave assisted lipid extraction in seeds was first established in the mid-1980s. Microwaves are electromagnetic radiation of frequency ranging from 0.3 to 300 GHz. The contact between a dielectric or polar material such as water (present in the microalgal cells) and a rapidly oscillating electric field, produced by microwaves generates heat, thus producing water vapour within the cell. Eventually, it results in cell disruption. It further leads to electroporation effect which promotes cell membrane damage, thus releasing the cellular constituents [134]. This method is relatively safe, rapid and high efficient in extracting microalgal oils under small scale production [103].

Table 9: Advantages and disadvantages of different extraction methods

Sl .No	Method	Advantages	Disadvantages
1	Oil expeller	1. Easy to use	1. Large amount of biomass is required 2. Time consuming 3. Less efficiency
2	Ultra sonication	1. Reduced extraction time 2. Economical 3. Reduced solvent usage 4. Higher penetration power	High energy consumption
3	Supercritical carbon-di-oxide	1. Easy to use 2. Rapid method	3. Cost effective 4.
4	Microwave	5. Economical 6. Safe and rapid method 7. Reduced solvent usage 8. Improved extraction yield	Filtration/centrifugation is required to remove the solid residue
5	Solvent	High efficiency	1. Cost effective 2. Solvent recovery is energy intensive 3. Not rapid 4. Toxic and highly flammable

7. Conversion of Lipid to Biodiesel

7.1 Hydrothermal Liquefaction

It is employed using subcritical water close to its critical point. Under this condition, hydrogen bonding within the water phase is reduced, transforming it from a polar, hydrogen-bonded solvent to a non-polar solvent, capable of extracting and dissolving organic components from the biomass [135]. However, as shown in the phase diagram of water (Fig.9), HTL also requires high reaction pressures to maintain water in the liquid phase and minimise steam formation, in order to prevent the latent heat losses associated with vaporisation [136].

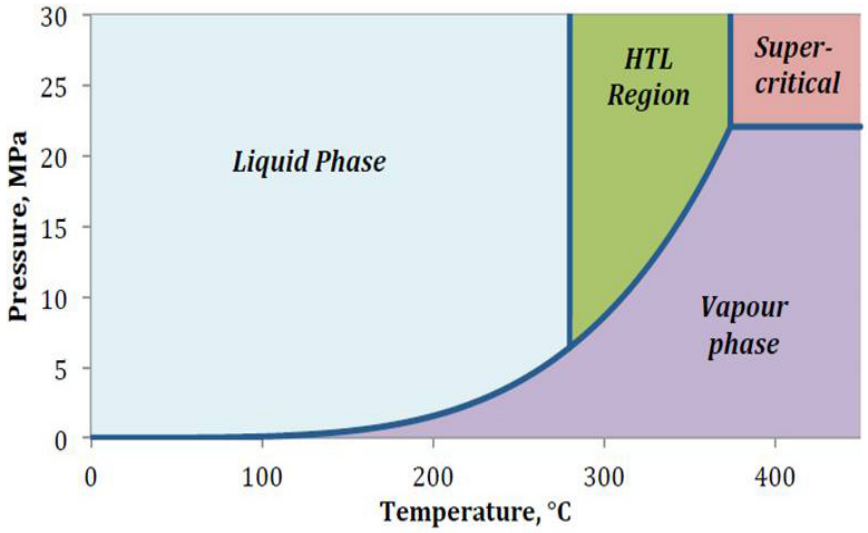

Figure 9: Hydrothermal Liquefaction

Source: [136]

Hydro-thermal liquefaction encompasses four different product phases: solid ash, bio-crude oil, water-soluble compounds and reaction gases. These reactions can be divided into three different stages namely:

First stage: Hydrolysis of the biomass macromolecules (lipids, proteins and carbohydrates) into smaller, water-soluble fragments

Second stage: Rearrangement of the fragments through decarboxylation, deamination and dehydration reactions

Third stage: Dehydration, condensation, cyclisation and polymerization reactions to form the desired bio-oil [135, 137].

The overall process is influenced by temperature, reaction time, biomass concentration and lipid content. The main advantage of this technology is it does not require pre-drying of the biomass and ensures a relatively high product yield [138].

Thermochemical liquefaction of microalgae species such as *Botryococcus braunii*, *Dunaniella tertiolecta* and *Spirullina platensis* yielded 30-80% dry weight basis of oil. This shows that the thermal conversion of biomass to biofuel is an attractive method for liquid fuel production. However, the major disadvantages are reactors for thermochemical liquefaction and fuel-feed systems are complex and expensive [139].

7.2. Pyrolysis

Pyrolysis involves chemically reducing triglyceride to fatty acid alkyl esters (FAAEs) by the application of heat and in the absence of oxygen [19]. In 1986, pyrolysis of microalgal biomass to produce biofuel was first demonstrated in Germany [140].

There are two types of pyrolysis namely slow pyrolysis and fast pyrolysis. In slow pyrolysis, the biomass is associated with liquid fuels, at low temperature (675-775K) and in the presence of air [141]. However, in fast pyrolysis, biofuel is produced in the absence of air at atmospheric pressure, with a relat ively low temperature (450–550°C). In slow pyrolysis, the yield is 15–20% and the main products are char and char-oils whereas, the products of fast pyrolysis are oils and gases with a yield of approximately 70% respectively [142]. Fast pyrolysis has proved to be a promising way to produce bio-oils compared to slow pyrolysis for the following reasons:

(1) Low yield

(2) The viscous bio-oils obtained from slow pyrolysis is not suitable for liquid fuels

(3) Fast pyrolysis process is rapid and less energy intensive

However, the major disadvantage of this process is high equipment cost for separation of various fractions. Also the product obtained was found to be similar to gasoline containing sulphur which makes it less eco-friendly [65].

7.3. Transesterification

It is a multi-step process, wherein, triacylglycerides present in the lipid reacts with methanol in the presence of a catalyst to produce diglycerides, monoglycerides and finally yielding corresponding fatty acid methyl ester (FAME) and glycerol as a by-product (fig.9). Short chain alcohols such as ethanol, propanol, butanol, and amyl alcohol are also used for transesterification. However, ethanol is most frequently used solvent because it is inexpensive and physical and chemical advantages. The production of biodiesel through transesterification can also be achieved by using an alkali catalyst such as sodium hydroxide, potassium hydroxide, sodium ethoxide and an acid catalyst such as sulfuric, sulfonic acid, hydrochloric acid and enzyme catalyst such as lipases (Table.10). Transesterification process is influenced by lipid content, temperature, moisture content, amount of free fatty acids, alcohol etc [115].

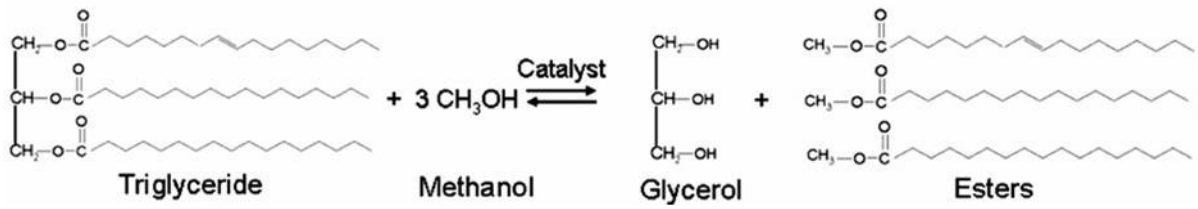

Figure 10: Transesterification reaction of triacylglycerides extracted from microalgal oils for fatty acid methyl ester (biodiesel) production

Table 10: Types of transesterification methods

Sl. No	Types of transesterification	Advantages	Disadvantages	Reference
1	Chemical catalysis	a. Reaction condition can be well controlled b. Large-scale production c. Methanol produced can be reused	a. High temperature b. Energy intensive	[143, 144]
2	Enzymatic catalysis	a. Moderate reaction condition b. High yield c. Eco-friendly d. Small amount of chemicals is required for the process	a. Conversion process is high b. Chemicals hinders the enzymatic activity	[145, 146, 147, 44]
3	Supercritical fluid technique	a. Reaction condition can be well controlled b. Eco-friendly c. Rapid	a. Energy intensive b. Expensive	[148, 149, 44]
4	In situ transesterification	a. High yield b. Rapid c. Eco-friendly d. Economical	a. Energy intensive	[150, 151, 152]

8. Genetic Engineering of Microalgae

Enhanced lipid synthesis and accumulation is pivotal to achieve economic viability of biodiesel production from microalgae. However, such a robust strain remains elusive for researchers even after decades of screening natural strains [153]. Most of the strains known to-date possesses either one or few of the required characteristics. The first pioneer work on genetic manipulation of microalgae was isolation and overexpression of Acetyl CoA Carboxylase (ACCase) from *Cyclotella cryptica*. This enzyme catalyzes a key metabolic step in the synthesis of fatty acid in algae. Although the full-length ACCase gene was overexpressed in yeast and *C cryptica*, no increased lipid production was observed [37]. Many attempts to up-regulate the ACCase encoding gene and other genes in the pathway of fatty acid synthesis failed to achieve anticipated results, showing that direct manipulation of the fatty acid synthesis pathway is not a hopeful strategy. However, up-regulation of TAG assembly genes, such as glycerol-3-phosphate acyltransferase or diacylglycerol acyltransferase had enhanced oil content in many plant seeds suggesting that enzymes in TAG assembly pathway are interesting candidates for genetic manipulation to enhance lipid biosynthesis in microalgae [47].

Table 11: Various studies on genetic engineering of microalgae for lipid synthesis

Sl. No	Target protein	Host	Type of medication	Gene source	Primary phenotype change	Reference
1	Acetyl-CoA carboxylase	*Cyclotella cryptica*	Nuclear over expression	Endogenous *Navicula saprophila*	No increase in total lipid accumulation	[154]
2	Malic enzyme (ME)	Chlorella pyrenoidosa	Overexpression of the gene PtME	Phaeodactylum tricornutum, a diatom	Lipid content increased by 3.2 fold	[155]
3	Malic enzyme (ME)	*Phaeodactylum tricornutum*	Putative malic enzyme gene	Endogenous	Lipid content increased by 2.5-folds	[156]
4	Pyruvate dehydroganase kinase	*Phaeodactyllum tricornutum*	Antisense Cdna	Endogenous	82% increase in neutral lipids	[157]
5	Malic enzyme	*Phaeodactyllum tricornutum*	Nuclear overexpression	Endogenous	2.5-fold increase in total lipids	[156]
6	Lipogenesis transcription factor	*Chlorella ellipsoidea*	Nuclear overexpression	Soybean	52% increase in total lipids	[158]
7	Overexpression of DGAT enzyme	*Chlamydomonas reinhardtii*	RNAi	Endogenous	34% rise in TAG production	[159]

9. Commercialization of Microalgae

Oflate, many attempts have been done to commercialize microalgal biofuels. In 2010, the U.S. Department of Energy (DOE) announced an investment of up to $24 million for three research groups aimed at commercializing biofuels derived from algae. The Sustainable Algal Biofuels Consortium of Mesa, Arizona, led by Arizona State University was funded with $6 million to investigate biochemical conversion of algae to biofuels and other value-added products. Another team led by the University of California, San Diego, is received $9 million to develop algae as a robust biofuel machinery. Several companies are also attempting to commercialize microalgal biodiesel. For example, in July 2009, Exxon Mobil Corporation announced an alliance with Synthetic Genomics Inc. to develop next generation biofuels from photosynthetic algae. In U.K., Carbon Trust Company has invested millions of dollars in the commercialization and utilization of algae-based biofuel through Algae Biofuels Challenge project. The U.K. government announced it would contribute to the further funding of this project. Although the investments in biofuel production from algae are being increased worldwide, several challenges must be tackled before commercial-scale production of biofuels from algae can be achieved [79, 161, 162].

10. Conclusion

Microalgae are considered as the most promising microbial cell factories for biodiesel production. It is the only renewable biodiesel that can potentially replace liquid fuels derived from petroleum Adequate oleaginous microalgal strains with increase tolerance to varying environmental stress can be grown in photobioreactors or open ponds on large scale for biodiesel production. However, new technologies have to be developed and improved, involving the harvesting of microalgal biomass, dewatering, extraction of microalgal oil, transesterification and downstream processing. The main hurdle of microalgal biodiesel production is lowering the cost to make it competitive with petroleum derived fuels. Producing low-cost microalgal biodiesel requires primarily improvements to algal biology through genetic and metabolic engineering. However, these technologies are still in the infancy stages and most have not been applied on a commercial scale. Therefore, further research in the development of novel upstream and downstream technologies will benefit the commercial production of biodiesel from microalgae.

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