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A comprehensive insight from microalgae production process to characterization of biofuel for the sustainable energy

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ABSTRACT

The present article reviews about the algae isolation, microalgae growth, types of cultivation, oil extraction, biodiesel characterization and the advantage of microalgae biomass and its other applications. A detailed review was undertaken on various microalgae harvesting methods, types of oil extraction and biodiesel production. The merits and demerits of open pond systems and photo-bioreactors cultivation are discussed briefly. The various microalgae species and its lipids used for biodiesel production were presented and compared with conventional feedstocks. This article also discusses the key process parameter for in-situ transesterification for biodiesel production such as molar ratio, stirring rate, moisture, reaction time, catalyst type and temperature. The fundamental characterization of the biodiesel and physiochemical properties such as flash point, cetane number, density, kinematic viscosity, pour and cloud point and calorific value were studied and compared with the results of conventional diesel. This study reports that oil from microalgae can be a suitable alternative than edible oils due to ease of growth, separation, and high lipid content. The properties of microalgae biodiesel meet ASTM standards. Overall, algae are not only a potential source for biofuel but can also be used in wastewater treatment, food additives, carbon sequestration, heath care, cancer treatment and aquaculture all of which are discussed in this review.

1. Introduction

Conventional energy sources are being depleted rapidly due to increasing global population, industrialization, transportation and overuse of conventional fuels. As per literature, global consumption of fossil fuel was 88% as a whole of which the share of natural gas, coal and oil are 24%, 29% and 35% respectively. It is stated that by 2030 the global energy demand would spiral to 53%. However, conventional fossil fuels also negatively impact on global warming [1]. Biofuels derived from rapeseed, karanja, soybean, oat, laurel, ground nut, coconut, almond, barley, okra, camelina, sorghum, copra, fish oil are called first-generation sources. Present biofuels focus on second-generation oil crops namely animal tallow and waste cooking oil affects the global food market and agricultural lands. Such contradictory issues provoked researchers in finding innovative and novel liquid fuels. First and the second-generation biofuels are unable to meet the global energy demand. Recently, third generation biofuel from microalgae gained attention as an alternate feedstock for renewable energy [2]. Microalgae can be an alternative for biofuel production due to its low environmental impact, carbon sequestration, high lipid content and rapid growth. Apart from this, microalgae can survive in harsh climates, brackish water, fresh water and occupies limited land area compared to other conventional oil seeds. Microalgae grows 10 times faster than other terrestrial plants and has additional advantages like high

Abbreviations: AC, Actinium; BaO, Barium oxide; CaO, Calcium Oxide; CF_3SO_3, Trifluoro methane sulfonate; DCW, Dry cell weight; FACS, Fluorescence activated cell sorting; FTIR, Fourier transform infrared spectrometry; GCMS, Gas chromatography and mass spectrometry; HRAP, High rate algal ponds; HTL, Hydrothermal liquefaction; K_3PO_4, Tripotassium phosphate; LED, Light emitting diode; $MeSO_4$, Methyl sulfate; Mg-Al_2O_3, Magnesium-Aluminum Oxide; MgO, Magnesium Oxide; NMR, Nuclear magnetic resonance; PBR, Photobioreactors; PMMA, Polymethyl methacrylate; SrO, Strontium Oxide; TiO_2, Titanium Oxide; TMS, Tetra methyl silane; WO_3, Tungsten Oxide; ZrO_2, Zirconium Oxide.

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production rate, growth in brackish water and barren areas, storing more lipids and easy harvesting. The harvested microalgae are used to generate power and transport fuels. Microalgae also grows in wastewater through photosynthesis by absorbing necessary nutrients. It reduces CO_2 emission by absorbing carbon in wastewater and the carbon-dioxide is converted to raw material for bioproducts, fertilizers and biofuels [3–5]. For both carbon sequestration and biomass production, microalgae genetically change and exploit lipids. This biotechnology development can satisfy 30% of global energy needs without affecting the environment. Apart from this, it has benefits such as fertilizer, animal food, agar production and dyes. Microalgae are used in wastewater treatment and biofuel production by selecting appropriate strains and illuminating them through acceptable light limits. Algal lipids have fuel value, as proved by analyzing different species via thermo-conversion techniques [6]. Lipids are extracted from microalgae cells and converted to the methyl esters through transesterification [7,8]. Direct conversion of microalgae to ethanol, bio-oil, syngas was also studied [9].

The aim of the present review is to provide the reader with an overall view from microalgae isolation to spent microalgae application with specific emphasis on biodiesel. This review is broadly organized into microalgae isolation, cultivation and harvesting, oil extraction, biodiesel production and applications of microalgae biomass. The first includes various stages in microalgae isolation, two types of cultivation including open pond and photobioreactor and factors affecting microalgal growth. The second stage investigates recent developments in oil extraction, biodiesel production and applications in wastewater treatment. This review also provides an insight into microalgal bioproducts such as biorefinery products, fertilizers, pharmaceuticals and its uses in biotechnology and nanotechnology.

2. History of microalgae

The life of trees originated in three domains namely Cyanobacteria, Eco bacteria and Eukaryotes. Blue green algae are cyanobacteria and the first photoautotrophic plants which initiated oxygen evolution on earth. Then glycobacteria, prochlorophytes, glaucophytes and other algae species were formed. Unlike phototrophic plants, microalgae can be cultivated in limited light conditions called mixotrophic. It removes carbon, nitrogen and phosphorous possessing high cell densities [6,10]. Broadly algae are classified into Phaephyceae, Rhodophyceae, Chlorophyceae, Bacillariophyceae, Chrysophyceae and Cyanophyceae [11,12]. Applied phycology was first initiated by Beijerinck in 1890 with a chlorella culture. Researchers have identified 3,00,000 species and determined its lipid content and suitable environment to grow, so that one can easily get the species on one's choice. They also understood the climatic conditions for mass production of biomass. Many universities have varied culture collections with some like the University of Texas having 2300 species of fresh water microalgae, red and sea water algae, Coimbra University in Portugal has 4000 strains, and Japan 2150 strains. These species are used for fuel production through gasification, pyrolysis, hydrothermal liquefaction etc. Specifically, strains with high triacylglycerol content can be converted in to biodiesel [13]. Microalgae is a unicellular or multicellular photosynthetic organism which can produce biomass with sunlight, CO_2 and inorganic salt [11,14]. It has the ability to accumulate 77% of lipids in dry cell weight. Adenosine triphosphate was extracted from chloroplast developed from solar energy and carbon-dioxide. The photosynthetic reaction can be determined in Eq. (1).

$$6CO_2 + 12H_2O + \text{Photons} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \quad (1)$$

To ensure ideal growth conditions, microalgae cultivation temperature should be maintained between 20 and 30 °C. If it increases slightly, biomass growth increases, any further increase reduces biomass growth. Hence, to locate the optimum temperature, optimization with different growth parameters was studied with individual species. Another cultivation parameter is pH which is hard to fix in a cultivation medium. Appropriate pH value was reported as between 6 and 8 for optimum biomass yield [15]. Microalgae yields 20–50% lipids in dry cell weight under stressed conditions rather than the optimal growth conditions of 5–20%. The molecular formula for microalgae is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, so to find optimum lipid production, the strains are stressed under desired environmental conditions [16]. Fig. 1 shows a flow chart from the cultivation methodology to the characterization of oil.

2.1. Merits of microalgae over other oil plants as a source for biodiesel

- Microalgae can produce more lipids (20–50% dcw) than other plants. It also grows 1–3 times faster. The percentage of oil conversion is also higher than other crop seeds.
- Microalgae can be cultivated in deserts, arid and semiarid conditions and also in saline water, sea water, industrial and municipal waste water [17]. It utilizes nitrogen and phosphorous from waste water and provides additional benefits as bioremediation.
- The stoichiometric growth of 1 kg of algal biomass is 1.8 kg of CO_2. CO_2 emitted from coal powered power plants is fed for microalgae sequestration. It can be grown round the year in a photobioreactor.
- The applications of fresh and used microalgae are as fertilizer, proteins, polysaccharides, pigments and animal food.
- Carbohydrate fractions from algae can be used for bioethanol by direct fermentation and thermal degradation of algae biomass could be achieved to the solid, liquid and gaseous fuels. Syngas can be produced using direct combustion method process.
- Algae lipid molecule serve as a substitute for energy because it contains biochemical components such as proteins, carbohydrates, and nucleic acids and especially free fatty acids, triglycerides, phospholipids, and glycolipids.

2.2. Isolation of strains

Isolation of strains includes a collection of aquatic species from different environments like freshwater, brackish and hypersaline water, marine ponds. The isolated strains are acclimatized to both time and environment. [18]. Isolation is done through various techniques; traditionally algae are isolated from micropipettes through a microscope by cell division methods and cultivated in agar plates. This technique is time consuming as the media and equipment have to sterilize frequently. Another method is to enrich the mother culture with suitable nutrients namely nitrogen and phosphate as these cells grow faster and can be immediately sub-cultured for mass production. A flow cytometer sorts the cell automatically with the help of the chlorophyll autofluorescence and green autofluorescence [19]. Unwanted microorganisms like fungus and bacteria are removed from isolated samples through a series of dilutions, agar plating and sub-culturing. Three plating methods namely spread plate, pour plate and streak plate are used for isolation. Serial dilution method, FACS, and paper-based device are other simple methods to isolate the cells [20]. Routine microscopic analysis was undertaken to ensure purification of the culture using nutrient agar plate [21]. Water samples were collected and transferred to 30 test tube samples maintained with Bristol medium and incubated at 30 °C under 2000 Lux and photoperiods of 12 h dark/light. The samples were checked every 48 h with an optical microscope for algal growth. Tests revealed that optimal growth is maintained for sub culture [22]. Microalgae was isolated from a nearby Korean thermal power plant and named the KR-1 strain. The isolated culture was maintained under a controlled environment including a light intensity of 110 μmole/m^2-sec, pH level of 5.5 and temperature 30 °C. Algae growth was found to be 660 nm by a spectrophotometer. The species growing in natural systems have less metabolic changes resulting in a higher biomass growth [23]. For example, species isolated in contaminated water are good for wastewater treatment while the species collected from oil spot regions suits for biodiesel production [2]. Marine microalgae were isolated from the Moroccan Atlantic and Mediterranean coasts using agar plates and

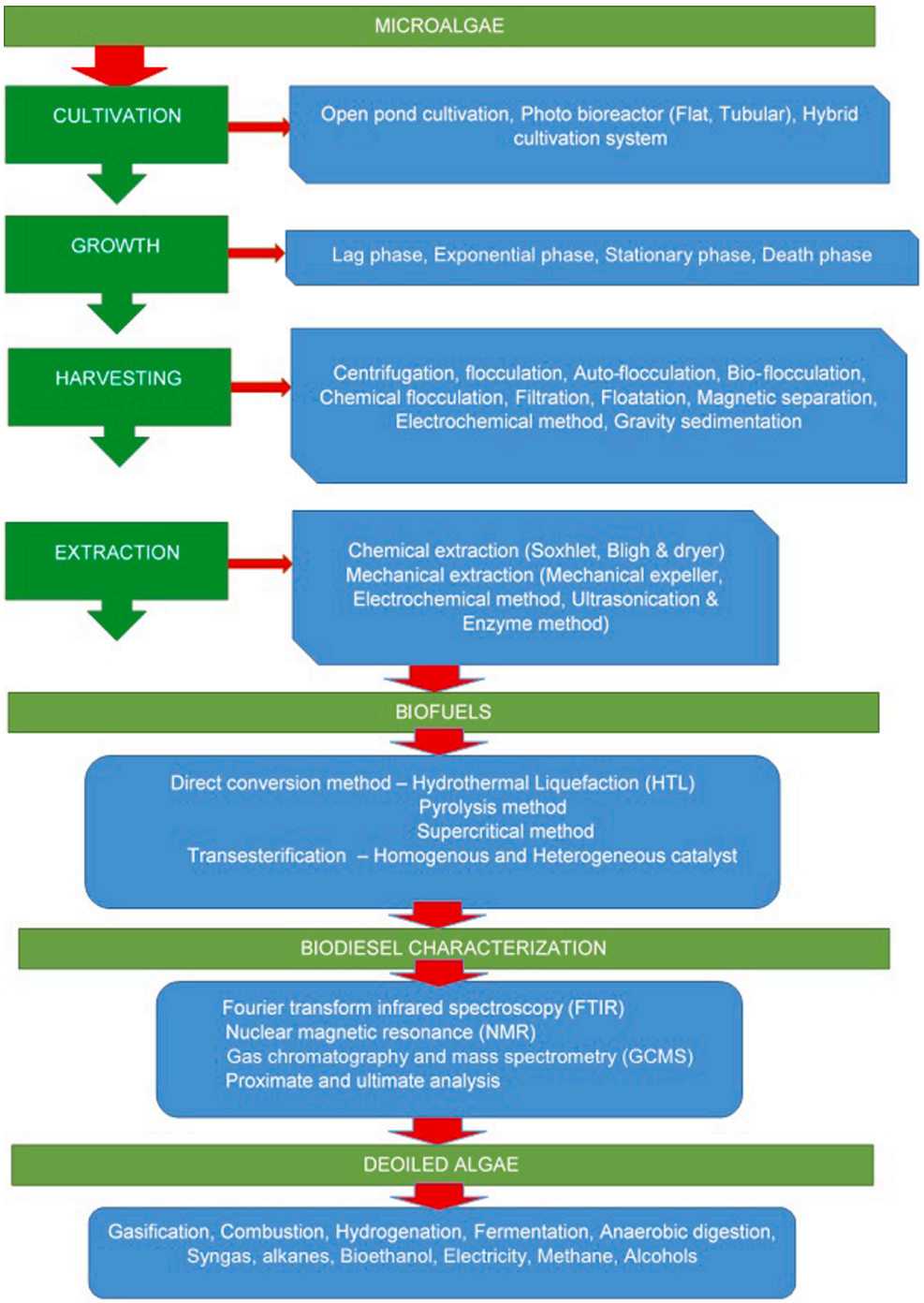

Fig. 1. Process value chain for algae.

cultivated in F/2 medium which determined that *Nannochloropsis sp, Dunaliella tertiolecta, Isochrysis sp, Tetraselmis sp T. elliptica and N. gaditana* are promising biodiesel feedstock [2,24,22].

2.3. Lipid content in microalgae

Lipids, the primary source for oil production contain triglycerides used for the transesterification of biodiesel aided by a catalyst. Lipids are categorized as neutral, crude and total lipids. The constituents of neutral lipids are triglycerides, free fatty acids, alcohols, esters and hydrocarbons. Crude and total lipids contain pigments, phospholipids and glycolipids [25,26]. Lipids production increases with the help of nutrients. Lipids develop in the absence of nitrogen and silicon [2]. The range of lipid content in microalgae was between 20 and 50% of biomass with the maximum yield being 70% under bold basal medium. So far, *Dunaliella tertiolecta* ATCC 30,929 has the highest lipid content in a range of 60.6–67.8% of dry weight [27]. Fig. 2 shows various seeds and their oil percentages and Table 1 shows the lipid content of various microalgae species [26]. Lipid yield can be maximized with nutrients like phosphate, iron and sulfate which play a major role in their generation. In nutrient deprived conditions, microalgae showed high level of carbon C16:0 and C18:0 [2] light intensity at 82 $\mu E/m^2/s$ influence development of polar and neutral lipids. Light intensity can alter lipids metabolism and fatty acid composition.

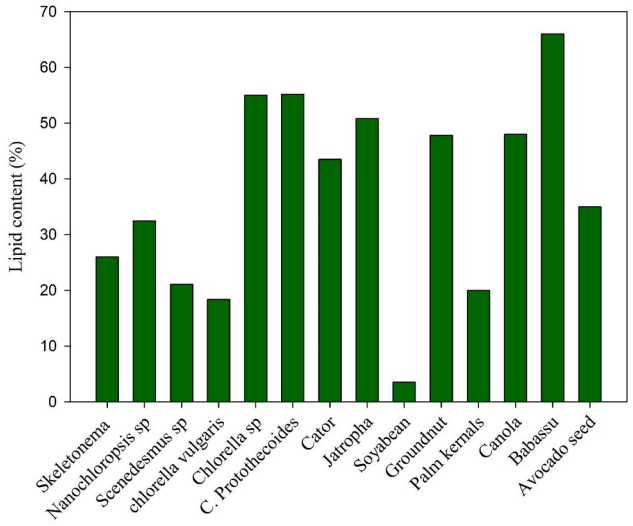

Fig. 2. Various seeds and its oil content.

3. Microalgae cultivation

Microalgae cultivation is divided into open pond and PBR. Open pond cultivation systems are used for biomass production coupled waste water treatment using raceway and HRAP. Open pond is easy to construct, operate and has low maintenance cost. The major drawbacks in this cultivation are contaminations, water vaporization, less photosynthetic effect due to light penetrating shallow depth, invasion of species, pond collapse and less control on growth parameters [29,30,17,26]. Photobioreactors are closed vessels that contain mother culture, medium, artificial light, and rotator with the advantage of contaminant free and zero exchange of gases. PBR has benefits over open pond cultivation by controlling environment conditions like pH, light, temperature and CO_2. Reduction in water evaporation can be achieved. The operational and maintenance cost of PBR systems is higher than open pond systems, but the growth of biomass concentration is between 20 and 100 g which is higher than open pond cultivation at 10–25 g of biomass/day/m^2. On comparing the pH with other parameters such as biomass concentration, biomass productivity, TN and TP consumed, pH showed the maximum significant with the probability value of ($P = 0.005$) using sodium hydroxide and ammonia nitrate. The alkaline chemicals maintain the medium in the pH range between 6 and 7.5 for *C. Sorokiniana* growth showed the maximum growth.

3.1. Open pond cultivation method

Open pond systems are classified into unstirred, circular and raceway ponds [36]. The design of open ponds is focused on mixing efficiency, gas/liquid mass transfer, light penetration and residence time. The open ponds are mixed using manual as well as paddle wheel driven motor. Huge energy loss was obtained in the sharp edge of the race way ponds, on increasing the curvature of the radius energy loss can be eliminated. In order to reduce the dead zone, wing baffles was developed and installed in the hydrodynamics manner. Though unstirred ponds are simple to construct with less than half meter depth covered by plastic films, it has a poor growth rate due to environment factors [37]. Raceway ponds have various shapes with rectangular shaped channels constructed in parallel where water flow is through a paddle wheel drive attached to a motor. Among open ponds, raceway ponds are used for mass cultivation. Raceways ponds are exposed to sunlight at depths ranging from 0.2 to 0.5 m. CO_2 in the atmosphere is captured by microalgae as a carbon source. Raceway ponds are 10 to 100 m in length, 1 to 10 m in width and 0.25–0.30 m in depth. Optimization of energy consumed by the paddle is in progress [38], whereas smaller size raceways are constructed at depths of 15–20 cm with a growth of 1 g dcw density per liter and average of 60 to 100 mg/l/d. [37]. Open raceway ponds are cheap and pertinent to the cultivation of microalgae due to its removal of nutrient from waste water [18]. To prevent debris, rainfall, water evaporation and pollution, current open ponds are constructed with greenhouse gases [15].

Big ponds are constructed with 30 × 10 m dimension and covered with plastic bags with a wall slope of 80 cm depth for better biomass production [37]. Open pond systems are shown below Fig. 3.

Table 1
Different algae species and their lipid content.

Species	Lipid in % of dry wt.	Species	Lipid in % of dry wt.
C. Vulgaris [33]	26	*Cylindrotheca* sp. [33]	16–37
C. Pitschmannii [33]	51	Spirogyra sp. [33]	11–21
Neochloris oleoabundans [33]	17	*Nitzschia sp* [33]	45–47
Cylindrotheca closterium [33]	17	*Schizochytrium* sp. [33]	50–77
Chaetoceros muelleri [1]	33.6	*E. gracilis* [33]	27.20
Chlorococcum sp. [1]	19.3	Ellipsoidion sp. [1]	27.4
Chaetoceros calcitrans [1]	39.8	*Phormidium corium* [33]	5.60
Chl. emersoni [1]	25.0–63.0	*Chaetoceros muelleri* [33]	33.6
Chl. sorokiniana [1]	19.0–22.0	*Scenedesmus* sp. [33]	21.1
Chl. sp. [1]	10.0–48.0	*Ellipsoidion* sp. [33]	27.4
Chl. pyrenoidosa [1]	2.0	*Nannochloropsis* sp. [33]	29.2
Isochrysis sp. [1]	7.1–33	*Isochrysis* sp. [33]	27.4
Dunaliella salina [1]	6.0–25.0	*Pavlova lutheri* [33]	35.5
Dunaliella tertiolecta [1]	16.7–71.0	*Ankistrodesmus falcatus* [34]	59.9
Dunaliella sp. [1]	17.5–67.0	*Chaetoceros calcitrans* [34]	40
Pavlova lutheri [1]	35.5	*Chaetoceros muelleri* [34]	43.4
Haematococcus pluvialis [34]	4	*Chlamydomonas reinhardtii* [34]	24
Koliella Antarctica [34]	22	*Chlorella minutissima* [34]	31
Nannochloris sp. [1]	20.0–56.0	*Chlorella zofingiesis* [34]	55
		Chlorococcum oleofaciens [34]	17
Nannochloropsis sp. [1]	12.0–53.0	*Ellipsoidion parvum* [34]	17
Porphyridium purpureum [34]	11	*Monodus subterraneus* [1]	16.0
cenedesmus quadricauda [32]	18	*Isochrysis galbana* [34]	20
Phaeodactylum tricornutum [1]	18.0–57.0	*Nannochloropsis oculata.* [1]	22.7–29.7
Botryococcus braunii [35]	25–75	*Monodus subterraneus* [34]	16.1
Thalassiosira pseudonana [32]	17.4	*Nannochloropsis oceanica* [34]	54.3
Spirulina platensis [1]	4.0–16.6	*Pavlova salina* [1]	30.9
Scenedesmus quadricauda [1]	1.9–18.4	*Phaeodactylum tricornutum* [34]	18.7
Tetraselmis suecica [1]	8.5–23.0	*Scenedesmus* sp. [1]	19.6–21.1
Tetraselmis sp. [1]	12.6–14.7	*Porphyridium cruentum* [32]	9.5
Hantzschia [1]	66	*Scenedesmus obliquus* [32]	19
Spirulina maxima [1]	4.0–9.0	*Stichococcus* [1]	32–40
Monallanthus salina [29]	20	*Skeletonema costatum* [32]	21.1
Euglena gracilis [31]	14–20	*Synechococcus* sp. [32]	29.0
Ankistrodesmus sp. [35]	24.0–31.0	*Tetraselmis suecica* [32]	12.9

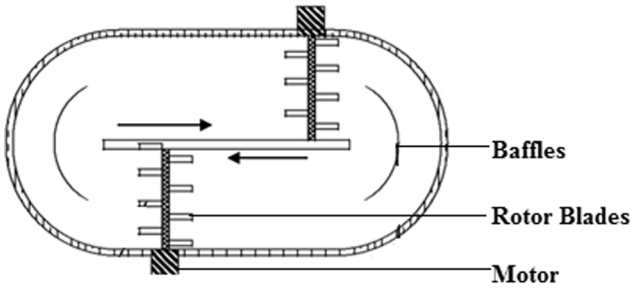

Fig. 3. Open pond cultivation System for microalgae.

3.2. Photobioreactors (PBR)

Cultivation in closed systems means isolated algae is grown in a closed reactor where all parameters are controlled externally in PBR. PBR systems requires additional cost for light, CO_2, medium and circulation systems, but controlling cultivation parameters are easy [39]. PBR can be constructed using bags, towers and tanks as they ensure culture mixing, light illumination etc. [40]. Closed bioreactor produces fivefold biomass and the choice of the reactor is the main factor for biomass production. The different types of reactors are column, flat plate, tubular and membrane PBRs. Semi-hollow-spheres are also used for biofuel production [41]. Nevertheless, lipid production of microalgae was estimated between 19,000 to 57,000 oils per acre per year using less arable land [15].

3.3. Flat plate photobioreactor

Flat plate photobioreactors are made from transparent glass, plexiglass and polycarbonate. Its advantages include high surface to volume ratio and high cell growth with a density > 80 g/l [33,25,28,32]. To increase light, flat plate photobioreactor attached to waveguides are used to increase the illuminated area volume by 21.4 – 410% higher than the conventional PBR. This results in a 220% increase in biomass production [42]. Flat plate photobioreactors which radiate red light from both sides increase biomass density by 0.326 kg/m^3 compared to conventional PBRs which produce 0.264 kg/m^3 [43]. Photosynthesis efficiency increased by 12.52% due to the PMMA tubes embedded in flat plate photobioreactors. Microalgae mixing was promoted using hollow PMMA tubes [44]. LED (Light emitting diode) attached to a flat plate PBR increases biomass productivity by 113% than a constant irradiation PBR [45]. Flat plate PBR dilution rate studied for two years reported a maximum biomass yield of 0.19 g/l day. Addition of nitrate 10.0 mM decreased biomass production and decreasing nitrate to 2 mM N/g day increased productivity of saturated acids in lipids [46]. Flat plate PBRs attached to mixers increase the medium's mixing degree and increases algal growth rate [47]. Baffles attached to flat plate PBRs increases biomass productivity by 1.88 times compared to a reactor without baffles. Optimized light path length was reported as 80 mm with higher biomass production [48]. When light irradiates the system with high waves, the system gets overheated and collapses. Splitting the infra-red in the reactor reduces heat which is bypassed to generate electricity to mix the medium [37]. Sterilization of Flat plate PBRs is difficult due to the large surface area to volume ratio [15].

3.4. Tubular bioreactor

The tubular reactor is divided into vertical and horizontal bioreactors. The vertical tubular PBR is used in cold and hot regions. A constantly controlled environment like light and temperature to the culture is difficult [49]. Vertical column PBR is made up of a transparent tube through which light is passed for photosynthesis. Vertical column PBR is further split into bubble column and airlift PBRs. Bag PBR is also used outdoor with a 100 L capacity of 10% (v/v) culture. The bag is aerated with compressed air at 34.5 kPa and maintained at ambient temperature [37]. A schematic picture of a tubular photobioreactor is shown in Fig. 4.

The photosynthetic rate of favoured reactors was eight quanta for the one molecule of oxygen. Lumostatic operation in a bubble column photobioreactor is used to maintain uptake light [50].

3.5. Hybrid cultivation system

Open pond and PBR cultivation systems have disadvantages like contamination and high cost respectively. Hence, combining PBR and open pond is recommended to avoid contamination and improve cell production. Specific algal species can be cultivated in a hybrid reactor and subsequently be transferred to the open pond where inoculum should be high to dominate contaminants [3]. Aqua search is a leading producer of astaxanthin from *Haematococcus pluvialis* by the hybrid method [15].

3.6. Growth phase of microalgae

Algae grows in photoautotrophic, heterotrophic and mixotrophic in nature. Photoautotrophic requires sunlight for algae to grow while the heterotrophic method needs organic substances to enhance cell growth metabolism. Some strains grow in both photoautotrophic and heterotrophic nature to assimilate organic substances in the mixotrophic process [51]. Eq. (2) shows a method to determine the growth rate of microalgae.

$$\mu = \frac{1}{t} ln \left[\frac{X_m}{X_o}\right] \tag{2}$$

Brennan and Philip emphasized selection of medium as very important to grow microalgae. Selecting right medium for a specific species is difficult due to continuous climate change and environmental issues [14]. Light is a prominent source for algal growth with an illumination rate of 6 h a day at 3240 lx. This produces the maximum cell density of 500 mg/L [12]. The most common commercial approach to grow algae is the suspended method. The mother culture of microalgae is freely suspended in medium in a container where artificial mixing is provided to cells till uniform distribution is achieved. This method shows less growth and high harvesting cost due to 99% water content in the suspended cultivation. This can be overcome by using non-suspended cultivation like cultivation on surfaces using biofilm. The non-suspended method has a lot of advantages namely, requires less water for growth, easy harvest, active growth in a small area with high density. The growth of *Scenedesmus obliqus* is high in suspended cultivation of 70.9 **g/m^2 per day** than that of 14 **g/m^2per day in suspended cultivation** [9].

Algae growth is measured at 450 nm using optical density and chlorophyll. Chlorophyll content measured using a fluorometer, determines units in the secondary standard of PN 800–950. According to Krohn et al. [52] biomass productivity is determined in the Eq. (3).

$$P = (A_2 - A_1)(D)^{-1} \tag{3}$$

Where A_1 and A_2 are area of the biomass growth in initial and final periods and D is the number of days of growth. The span of microalgae growth is divided into four phases: lag, exponential or logarithmic, stationary and death phases. Once the algae mother culture is fed to the Photobioreactor or open pond, growth begins in a short time and when it does not adopt to the new environment it results in the lag phase. Later microalgae sustain itself and grows exponentially by taking nutrients from the medium attaining a stationary phase by optimum fortification from the medium after which it stops growing. In the final phase, microalgae growth deteriorates leading to the death phase.

In another study, 5 different growth phases described as lag phase, exponential growth phase, linear growth phase, stationary growth phase

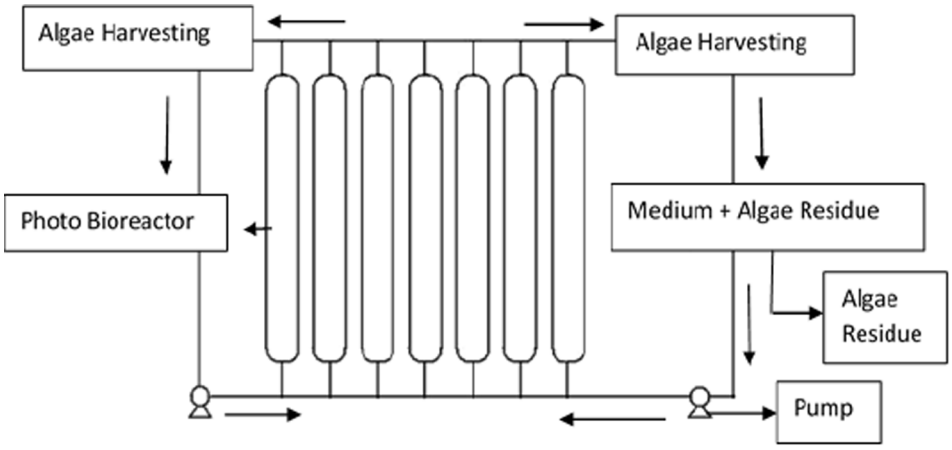

Fig. 4. Tubular photobioreactor for the growth of microalgae.

and decline or death phase were reported. Microalgae growth doubles in 3–4 h at the exponential phase with more protein [1] while in the stationary phase nutrients are depleted and carbohydrates and glycogen production increases [53].

4. Harvesting of microalgae

Microalgae harvesting is challenging specifically from a biofuel production point of view as small sized cells (1–10 μm) need to be diluted 50–200 times for better separation [2]. Biomass concentration in the medium becomes 7% and above revealing that it has mature for harvesting. Biomass harvesting cost includes 30% of its production [14] cost. Gravity force and drag force inhibit biomass harvesting and research is underway to offset these issues. There are different harvesting methods used including centrifugation, flocculation, filtration, floatation, magnetic separation and electrochemical harvesting. In recent years, polymers-based polysaccharides are used for flocculants. Three types of polysaccharides - marine, microbial and plant have been used for flocculants [54,55,56].

4.1. Centrifugation

The principle of the centrifugation is the application of centrifugal force to the heterogeneous medium through dilute suspension. A centrifugal force higher than gravity forces ensures biomass in 2–5 min with 80–90% efficiency [2]. As cells are separated non-disruptively it helps production of high value products [30]. The disadvantage of this harvesting method include slurry at settling time, depth of settling systems, high power consumption and being unsuitable for large scale systems. Though operating cost is high, pre-concentration through flocculation reduces biomass concentration for centrifugation [5]. By using pre-concentration in flocculation, operational cost is decreased to 13.8 to 1.83 MJ/kg. A combination of filtration and centrifugation resulted in total harvest energy decreased from 8 to 0.84 KWh/m^3 [57]. Methods used for the centrifugation include disc stack solid-ejecting centrifuge and solid-bowl-decanter. In multistage centrifugation, separation is done in different chambers based on particle size. Hydro cyclone type centrifuge showed less efficiency with 0.4% solids and concentration factor of 4. Mainly, it is used for pre-concentration of algae prior to the main harvesting factor. Compared to the other techniques, it can be applied to all microalgal species, high recovery rate and chemical free biomass with the penalty of high energy consumption, treatment time, maintenance, and capital cost.

4.2. Flocculation

Flocculation is used alone or with pre-concentration before harvesting. The principle of flocculation ensures that cell surface with negatively charged particles are neutralized by the addition of positive flocculants to the medium [57]. As per the manufacturer's recommendation, flocculants with starch as a cationic or polyacrylamide as an anionic was prepared through dissolution and kept for a maximum 2 weeks at 4 °C. Then the algal culture's pH was increased to 7.0 by adding 1 normality of hydrochloric acid. 100 ml of algae was suspended in an Erlenmeyer flask and stirred at 150 rpm, after which flocculants were poured into the flask. This speed was again reduced to 50 rpm for 2 min to set the flocculant completely, after which the flask was kept free for 60 min to settle the algae culture. This is called the settling phase [57]. Flocculant efficiency estimates the quantity of algal cells harvested in the total mass. Recovery efficiency is defined as the ratio of the number of recovered cells to the total number of cells as in Eq. (4).

$$RE = 1 - \left(\frac{C_t}{C_o}\right) \tag{4}$$

Cell Concentration factor is a method to find flocculation efficiency as shown in Eq. (5). The ratio of the final algal biomass to the initial algal biomass is the cell concentration factor [55].

$$CF = \left(\frac{V_0}{V_f}\right)(RE) \tag{5}$$

Flocculation is further divided into auto-flocculation, bio-flocculation and acidified-flocculation. Also inorganic chemical flocculants are multivalent cations such as aluminum sulfate, ferric chloride and ferric sulphate that form poly-hydroxy complexes at optimal pH leads to neutralization and reduction of negative surface charges on micro algal cells. Salts with lower solubility shows more effective and similarly more electronegative ion has faster coagulation process.

4.2.1. Auto-flocculation

Auto-flocculation is a chemical aid to harvest cells with the help of magnesium and calcium with maintaining a high pH. Calcium ensures a 50-fold increase in cell concentration. Literature reports that removing iron from calcium enhances cell productivity [58]. Another method of auto-flocculation is to increase/decrease pH value. When pH value is changed continuously, the charge becomes neutral at a stage. Cells are collected due to gravitational force at a specific point [59]. Auto flocculation is achieved with the help of bacteria and algae grown in the same medium. Once stationary phase is achieved, the bacteria start colliding with algae to form flocculants. This method has disadvantages like need for extra chemicals to grow bacteria and contamination in the medium. The medium should use analytical grade chemicals as otherwise the process ends hazardously. Chemical flocculants are divided into organic and inorganic flocculants. Chemical flocculants like Zn^{2+}, Al^{3+} and Fe^{3+} are used to treat waste water in industry which is inappropriate

for mass scale microalgae production as the flocculant once used cannot be reused due to excess cationic action [60]. Chi-tosan is a water purifying flocculants added to ferric sulfate and alum [61]. Heat aided flocculation is a novel derivative to harvest algae using waste heat generated from the industry to harvest algae without addition of chemicals [62]. *Chlorococcum nivale, Chlorococcum ellipsoideum, Scenedesmus* sp. were harvested with > 90% efficiency at pH 4 in 15 min. Auto-flocculation is slow and unreliable and also it could be non-toxic, also the environmental modification may be uneconomical for commercial use.

4.2.2. Bio-flocculation

Bio flocculation is a process where microorganisms are used to harvest cells. Bio-flocculation is used more compared to other flocculation methods due to its sustainability and environment-friendly nature. However, bio-flocculants are also commonly used for wastewater treatment [3]. The 3 types of bio-flocculants are plant based, microbial and microalgal fungal association. Table 2 shows microbial and bio-flocculants. Recent results reported that fungi assisted spores achieved better floc than pellets of 1.1×10^4cells/mL (spore) size [63]. Bivalent cations such as magnesium and calcium aid alkaline induced the bio-flocculation. On using the bio-flocculation cations, 96% biomass recovery was obtained with the concentration factor of 9 at calcium concentration of 5.7 mM. Sometimes decrease in biomass recovery was obtained due to the formation of calcium precipitation that increases the sludge volume. Overall, most of the research was validated at a lab scale, and very limited research was carried on large-scale micro-algal harvesting system.

4.2.3. Acidified and alkaline flocculants

Acidified flocculant is a new harvesting method for cells using coagulants like sulphuric acid and ferrous sulphate. The method is cheap, reusable and contamination free. pH reduction is done in the medium which releases the precipitates with the remaining solution being reused up to 3 times with 98% efficiency [3]. Flocculation is also possible with alkali based chemicals. Calcium phosphate precipitation suits cell harvesting and waste water treatment. Adding alkali increases harvesting up to 364 mg/L compared to conventional harvesting of 129 mg/L [64]. A high 90% efficiency was achieved for fresh water (*Chlorella vulgaris, Chlorococcum sp, Scenedesmus* sp.) and marine algae (*Phaeodactylum tricornutum, Nannochloropsis oculata*) [62]. In electro flocculation, an aluminum rod is used to ensure a positive charge for a range of pH resulting in the negative charge in the medium getting cells together [60,17].

Table 2
Microbial and plant flocculants used for microalgae.

Microorganisms	Microalgae	Flocculation Efficiency %
Kl. pneumoniae	*Synecosystis*	95
Pa. spirulina	*Scenedesmus* sp.	95
C –PGA	*Nannochloropsis oculata LICME002*	96
	Phaeodactylum tricornutum	97
	C. vulgaris LICME001	90
	Botryococcus braunii LICME 003	92
Sol. silvestris	*Nannochloropsis oceanic*	90
E-coli	*Chlorella zofingiensis*	83
CGMCC 2876 (c –PGA)	*Desmodesmus* sp. *F51*	92
Plant product		
Moringa oleifera	*Chlorella* sp.	90
Guar gum	*Chlamydomonas* sp.	84
	Chlorella sp.	92
Strychnos potatorum	*Chlorella vulgaris*	99.7
Inulin	*Botryococcus* sp.	88.6

4.3. Filtration

Screening is the first method of filtration undertaken through a given aperture. Screening efficiency is based on algal size and pore size in the screen [63]. Tangential flow filtration which recovers 70–89% algae is another filtration type. In this method, properties of recovered algae like structure and lipids do not change during filtration [65]. Recent research reveals that acrlylonitrile butadiene styrene was used for microalgae separation [66]. Microfiltration using polymethylmethacrylate can be used for cross-flow filtration [67]. Filtration has several advantages such as high recovery efficiency, low energy consumption and low shear stress. Also it has disadvantages such as high operation cost, limited by the membrane size, time consuming process and replacement of membrane pumping is difficult.

4.4. Floatation

Floatation is a gravity separation method where air/gas bubble medium is pressurized to carry suspended algae to the liquid's surface. Techniques like dispersed air flotation, ozonation dispersed flotation and electrophoresis flotation were tried to harvest suspended matter [68]. Microalgal biomass is collected on top of the suspension through the upward gas bubbles principle. Effective floatation size is in an order of 10–30 μm to 500 μm. Floatation is applied to different processes like ozoflotation, flocculants free electrolytic flotation. The principle of inverted sedimentation is used in flotation as it has the advantage of high overflow rates, small footprint, produce thicker concentration and sedimentation [62].

Ozoflotation is an important technique in microalgal cell harvest as it reduces cost by harvesting 79.6% biomass with a dosage of 0.23 mg/mg [69]. This process doubles lipid extraction quantity and FAME acquired from biomass. Ozone was generated with the help of oxygen enriched air supplied by the air separator. Ozone was pressurized (conditions studied are: gas concentration 25, 35 and 45 mgO_3/L and time in 5, 10 and 15 min) using a fixed glass diffuser. During the reaction, foam formed on the top was collected through the sides. Another flotation method is flocculants-free electrolytic floatation where flocculants are replaced with stainless steel and carbon to generate bubbles which force the cells upward to enable their collection on the surface. The achieved biomass yield was 23.72 g/h and power to collect it was 2.73 kWh/kg [70]. Air bubbles form when air is pressurized inside the medium and they mix with particulate mass. Due to increased buoyancy, algae have a tendency to float on the surface of the medium. In dissolved air flotation, air size and flow are predominant parameters defining harvest efficiency. Eq. (6) depicts the mathematical model of a hydraulic flow pattern.

$$\frac{A}{S} = \frac{RC_s(f(p+1)-1)}{QS_i} \tag{6}$$

Where $\frac{A}{S}$ is ratio of kg of air/kg of solids, $\frac{R}{Q}$ the recycle ratio, $C_s$the saturation concentration of air in water at 30 °C in liter/m^3, $p$the operating pressure, $S_i$the concentration of influent suspended solids and $f$the proportionality factor [75].

4.5. Magnetic separation

In recent years, magnetic particles received more attention in algal cells separation. They were first used to clean reservoirs. They were also used in the separation of microalgae. Naked magnetic particles and surface functionalized magnetic particles are types of algal magnetic recovery methods [58]. The magnet has an electrostatic interaction between source and cells. Particles like Fe_3O_4, magnetite and ferrous sulphate are used as precursors in algal cell harvests. The choice of magnetic particles is an important parameter, as sometimes lipids are

extracted when harvesting cells [59]. Magnetic particles factionalized with cationic groups for efficient harvest are labelled surface functionalized magnetic separation method. Methods of being attached or being immobilized was undertaken in polyelectrolyte for cell harvest [71]. Diallyldimethylammonium chloride, aminoclay, polyethylenimine, (3-aminopropyl) triethoxysilane, diethylaminoethyl, silica coated magnetic particles and modified fly ash are functionalized particles to enhance harvesting efficiency. Organic or polyelectrolyte flocculant use Cationic polymers to link algal cells together. Efficiency depends on biomass concentration, algal cells and their pH. Inorganic flocculants are negatively charged and applied on cell's surface to neutralize them. Cells are collected at the corner. Iron and aluminum are also used to coagulate cells for harvest.

Magnetic separation includes an algal solution drum, peristaltic pump, magnetic separator and collecting tank. A known amount of Fe_3O_4 nanoparticles are added to the algal solution and mixed till it collides with the substrate. Nanoparticles aggregated with algae are absorbed by the magnetic drum, where a scrapper separates the algae, with the remaining free solution being saved in a collecting tank [72].

4.6. Electrochemical harvesting

In this method, Boron doped diamond and aluminum as electrode are used to harvest microalgal cells. Complete harvest of microalgal cells was possible with direct electro floatation at 15 mA cm^{-2} [73]. Titanium based reactive electrochemical membrane filtration system was used to develop a sustainable harvesting membrane with simultaneous lipid extraction and harvesting being undertaken resulting in increasing harvesting efficiency by 15.2 ± 0.6 g-lipid g-$algae^{-1}$. With the help of an electrolyte (sodium chloride) recovery efficiency of *C. Sorokiniana* increased from 65.99% to 94.52% [74,61].

Electrolytic harvesting is a continuous process which works on the polarity exchange principle. Microbial electrochemical system is another method to harvest microalgae, generate bio hydrogen, treat wastewater etc. [75–77]. This involves two phases: Destabilization and Floatation. Aluminum ions are used to liberate the anode in the oxidation method. The ions produce monomeric products through hydrolysis. The aluminum hydrolysis ion reacts with microalgae to form flocs particles due to which microalgae move to the surface of the medium. This method consumes less power. Table 3 reveals the power consumption of different harvesting methods [78,79].

4.7. Gravity sedimentation method

This is an easy way to harvest algae by exploiting the density difference between water and the species. Eq. (7) representing the settling velocity of gravity sedimentation is shown below.

$$Settling\ Velocity = \frac{2}{9} g \frac{r^2}{\eta} (\rho_x - \rho_l) \tag{7}$$

Here r is the cell radius,η represents fluid dynamic viscosity,ρ_x and $\rho_l$density of the solid and liquid substrates. The density of microalgae is 1025 kg/m^3 which is close to salt water. This difference creates an easy way for the species to settle in the pond. The settlement rate also depends on algae size and shape as some species spheroid in shape settle easily while filamentous types find it hard to settle and float. Sometimes settlement depends on the content of different species like carbohydrates, proteins and lipids. Algae with high lipid settle less due to low density [80]. Sedimentation kinetics were determined with several samples taken in cuvette and diluted. The samples were mixed and stored at 27 °C in a spectrophotometer. During the settling period, pH value was recorded and suspension turbidity measured at 750 nm to determine the density of microalgae in the suspension. Microalgae recovery was determined using Eq. (8).

$$Recovery(\%) = \frac{(OD_{750}(t_0) - OD_{750}(t))}{OD_{750}(t_0)} \tag{8}$$

Here, $OD_{750}(t_0)$ is the sample taken at time zero and $OD_{750}(t)$ the sample taken at time 't' [60].

Table 3
Electrical energy consumption for various harvesting methods.

Harvest method	Power consumption (Wh/g)
centrifugation	1.67
Polarity Exchange	1.08
Polymer flocculation	36.81
Pressure filters	0.18
Vacuum filters	1.23
Tangential flow filtration	3.58

4.8. Analysis of harvesting for better yield

An optimal harvesting method is needed to extract sufficient lipid with low input energy cost and chemicals. Centrifugation and chemical precipitation are feasible methods for high yield than filtration, floatation, precipitation, ion exchange, electro dialysis and ultrasonic vibration [94]. Better yields are possible by screening algae based on current climatic conditions and changing or improving metabolic activities with transcriptomics, proteomics and metabolomics/metabonomics approaches. This change metabolic flux through regulated enzyme upstream and downstream in the metabolic pathway. Genes are agitated in the cell [38]. Recent results revealed that *Chlorella minutissima* grown under carbon sources like 5.96 g/L glucose, 0.73 g/L nitrate and 4.12 g/L acetate increased lipid production 10-fold [95,96]. *Chlorella sp* cultured and irradiated with f/2 sea water and ^{137}Se-γ enhanced lipid content to 54.9% [97]. pH effect in a culture medium is an important parameter for enhancement of lipid and biomass production. Sreekumar at al. reported that 30% higher lipid production was achieved at a pH of 8.5 and higher bio mass production at a pH at 6.5 [98].

Harvested algae are separated into two batch. One batch is stored long term at 80 °C and another batch is further divided into several samples and stored at temperatures of 80 °C, 20 °C, 37 °C. The samples were frozen and dried at 80 °C to determine lipid content [99]. Prasad et al. reported that increase in algal cells and Chlorophyll *A* content was observed under algae immobilization on cotton cloth for 18 months in BG 11 medium [100]. Algae can be stable long term in water-in-oil emulsion containing silica nanoparticles and Aerosil R974 [101]. Among several harvesting methods derived from in-depth literature study, the recovery of algae via flocculation proved to be a promising method with high recovering efficiency with low cost. Though, this process use chemical as a recovery agent, but the harvesting efficiency improved a lot. Chitosan is a natural polymer showed prominent flocculant can be used to eliminate the pollution.

5. Lipid extraction method

5.1. Soxhlet extraction method

In the conventional soxhlet extraction method, harvested algae are dried and chopped to fine powder which is then filled in a cellulose thimble and placed inside the reflex chamber. This is placed above a three-neck flask containing n-hexane. The solvent is heated to boiling point making the solvent vaporize and escape from the flask. The condenser above the extraction chamber condenses hexane and drops it into the thimble and washes the powder. The oil and solvent flow back to the three-neck flask. Oil is separated from hexane through a Clevenger apparatus [81]. The choice of solvent is an important parameter for optimum lipid extraction. There are several polar solvents for extraction namely chloroform, hexane, benzene, diethyl ether, acetone, methanol, ethyl acetate, ethanol and alcohol [82]. These are high energy consumers for distillation but N-dimethylcyclohexylamine is used for freeze

dried microalgal cells. Chemical methods use various solvents likely chloroform/methanol, hexane/isopropanol, dichloroethane/methanol, dichloroethane/ethanol and acetone/dichloromethane in different volume/volume (v/v) ratio to extract lipids. Every species has its own cell thickness and permeability to crack the cell wall. Hence, choosing a solvent is important to increase lipid extraction efficiency [83]. *Botryococcus braunii* is a microalgae that secretes long chain lipids directly into the cultivation medium. Obviously, the extracted lipids are long chain hydrocarbons. Hence, the advantage of this method is economy and easy conversion of long chain hydrocarbons into biodiesel. However, this species has a very low specific growth rate which make it unsuitable for biodiesel conversion [84]. Wet algae paste can be converted directly to jet fuel through the selective extraction method using hexane and ethanol and Pt/Meso-ZSM-5 as catalyst [85].

5.2. Microwave irradiation extraction

Microwave irradiation particularly heats localized hotspots called polar molecules in the algae releasing lipids through rupturing the cell wall. The principle of heating used is convention and conduction, similar to a conventional surface reactor. This method needs a large amount of energy which is insufficient to produce lipids but extraction efficiency of this method is higher than the Bligh and dryer method [86]. Microwave assisted oil extraction with azeotropic solvents increased supercritical carbon di-oxide affinity between neutral and polar lipids [87]. The effect of microwave irradiation timing with respect to the biomass concentration and irradiation power were studied to find an optimal parametric conditions on oil extraction. The higher pretreatment time attributes to the localized heating in the suspension and facilitates more cell disruption. On varying the microwave power from 635 to 1021 W, the pretreatment of biomass suspension increased. Also, higher amount of water in the biomass showed the high molecular collision and disintegrates the micro-algal cell wall.

5.3. Bligh and dryer method

Mixture of chloroform and methanol in a 3:1 v/v ratio is used to extract lipids. 2 g of dried algae is mixed with sterile sand and crushed with a pestle and mortar. Simultaneously 15 ml of chloroform and 5 ml of methanol are added to the mixture to form a suspension. To avoid binding of acid and lipids, another 6 ml NaCl solution is added to the suspension which is then shaken vigorously and poured into a separating funnel whose lower part filters lipid, methanol and chloroform using the Clevenger distillation method [88].

5.4. Lipid extraction using ionic liquids and methanol

Ionic liquids are CF_3SO_3 (trifluoro methane sulfonate) and $MeSO_4$ (methyl sulfate). Ionic lipids are used to extract lipids. Methanol is added to the solution to decrease viscosity of ionic liquids and to act as reactants for transesterification. Finally, hexane in added to extract the correct amount of lipids. The protocol for ionic liquid extraction is 500 gm of microalgae is mixed with a solution of 2.5 ml ionic liquids and 2.5 ml methanol maintained at 65 °C under a magnetic stirrer for 18 h. The solution is kept in the separation funnel for phase change [89]. Ionic liquid treatment doesn't required reactor due to its low vapor pressure and, short reaction time. Because of the hydrogen bonds, the cell walls were affected by the ionic liquids, the efficiency of lipids can be increased by blending two or more ionic liquids.

5.5. Electrochemical method of lipid extraction

Electrochemical process combines electrical and chemical energy to generate current intensity to extract lipids. For efficient lipid and protein extraction, electrochemical methods are used with different anode materials. The electrochemical cell consists of titanium and lead oxide as anode and stainless steel as cathode. Anode and cathode are submerged parallel to each other in an electrolyte solution. A 0.6 A intensity current is applied to the solution with a recycling flow rate of 394.51 ml/min and 100 min of electrolysis which are the optimum parameters for a reactor of 5 cm width, 15 cm length and 17 cm depth. Performance yield is calculated based on solution concentration, particle size, lipids and turbidity [90]. Algae depend on lipid content of different species, nature of growth and cell structure [11].

In recent research reveals many methods for oil extraction from algae. Ali et al., reported that flash hydrolysis showed enhanced biocrude yield of 90% [91]. Microwave irradiation coupled with 1-butyl-3-methylimidazolium hydrogen sulfate and imidazolium based iconic ion used to extract the lipid resulted in a 15% higher extraction rate for *Chlorella sorokiniana* [92]. Microwave assisted pyrolysis of algae revealed 66.6% of carboxylic acid [93].

6. Lipid to biodiesel conversion techniques

6.1. Direct conversion method

Wet microalgae are directly converted to crude biodiesel with supercritical ethanol reactions. Four grams of wet algae samples contain 60% water. Experiments were conducted in a PARR reactor under supercritical ethanol conditions. Here three variables: algae to ethanol (1:6–1:15), reaction time (2–30 min) and reaction temperature (245–270 °C) were taken by varying one variable and keeping the other two constant. The experiments were maintained with pressure in 1200–1350 psi range. After the process, the product mixture was cooled to room temperature and ethanol was separated using rotary evaporator. Around 5 ml hexane was added to the mixture and placed inside centrifugal tubes and rotated for 10 min at 3200 rpm. Organic compounds and waste biomass were separated in different layers. The organic component was separated for further GC analysis [102].

6.2. Transesterification method

The production of biodiesel is through transesterification, a process which converts triglycerides into Fatty acid methyl esters by mixing Short chain alcohol as acyl acceptor with triglycerides and a catalyst. Glycerol is a byproduct used in detergent manufacture applications.

The catalyst used is divided into Homogeneous and heterogeneous catalysts. Homogeneous catalysts are further divided into acid and alkaline catalysts, while heterogeneous catalysts are divided into acid and alkaline catalysts. The list of homogenous and heterogeneous catalyst used are presented in (Table 4) [103].

Catalyst is a substance used in a chemical reaction to increase the

Table 4
Types of catalyst.

Catalyst	Catalyst Name	Advantages	Disadvantages
Homogeneous alkaline Catalyst	NaOH, KOH	Mostly used and Low cost	Formation of saponification
Heterogeneous alkaline Catalyst	CaO, MgO, SrO, BaO, Mg–Al_2O_3, K_3PO_4	Higher reaction rate and Possibility to reuse the catalyst used.	Leaching in reaction
Homogeneous acid Catalyst	H_2SO_4, HCl,	Yield is not affected by water content	Reaction rate is low
Heterogeneous acid Catalyst	ZrO_2, TiO_2, WO_3	Can be used for both esterification and transesterification	It requires high temperature, more reaction time and high molar ratio
Enzymatic Catalyst	Lipases	It doesn't require excess energy.	High cost and need 75% of catalyst for reaction

rate of reaction by spending less activation energy. Catalyst are of three types: homogeneous, heterogeneous and biocatalyst. Classification of catalysts is shown in Fig. 5 [9].

6.3. Pyrolysis process

Pyrolysis process is anaerobic heating of algal biomass at a temperature ranging between 200 and 700 °C without oxidation. Pyrolysis is divided into two types: fast and slow pyrolysis. As per a literature survey, pyrolysis is a proven technology for biodiesel production from microalgal biomass with 87% rate at 300–600 °C. The stability of this microalgal biodiesel is better than other crop oils, traditional bio oil and fossil products. This process can be achieved even with a low temperature of 300 °C which ensures 40% yield [11]. Heating the oil before transesterification ensures more yield and increases reaction rate due to shortened reaction time. Due to pretreatment of oil, alcohol and catalyst come together in an immediate reaction to prevent alcohol loss. At 65 °C, alcohol is added in excess to ensure complete conversion of oil to esters. The general transesterification equation is stated in Eq. (9) [104].

$$\underset{\text{Ester}}{R\text{ - }COOR^1} + \underset{\text{Alcohol}}{R^2\text{ - }OH} \overset{\text{Catalyst}}{\leftrightarrows} \underset{\text{Ester}}{R\text{ - }COOR^2} + \underset{\text{Alcohol}}{R^1\text{ - }OH} \quad (9)$$

Eq. (9) explains various steps of the chemical reaction where the triglyceride reacts with methanol in addition to catalyst to form biodiesel and glycerol as a byproduct.

Methanol is mixed with triglycerides in acid or base to form Diglycerides which are again converted to mono-glycerides as shown in Eq. (11) [37].

Depending on residence time and reaction temperature, pyrolysis is divided into three types - fast, intermediate and slow pyrolysis. Fast pyrolysis involves less residence time and a high 500 °C temperature which ensures more yield than slow pyrolysis which has more residence time and temperature lesser than fast pyrolysis. For optimum yield, a combined effect like moderate temperature, high heating rate and shorter residence time are desired [79,105]. Some of the oil contains phospholipids which inhibit biodiesel production by forming gum where oil should be kept in a vacuum at high temperature to remove high free fatty acid of less than 0.2 mg KOH/g. Titration estimates FFA value, and if FFA is above 2.5% pretreatment is done to oil. Table 5 shows the different types of pyrolysis and their optimum conditions.

6.4. Supercritical transesterification

Supercritical transesterification is a biodiesel conversion process where triglycerides react with supercritical alcohols mainly ethanol and methanol. Manufacturing cost is high due to high consumption of alcohols in a molar ratio of 42:1 rather than 3:1 as in normal techniques. The advantage of this process is that it takes less than 10 min to convert triglycerides to methyl esters. Due to the secondary reaction of glycerol with unsaturated esters at high temperature, fuel quality improves [119]. Thus, total fatty acid methyl esters are determined using Eq. (10) [61,120].

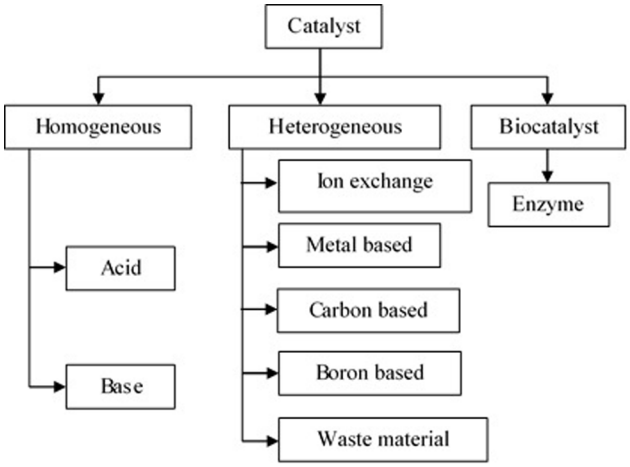

Fig. 5. Different types of catalyst used for biodiesel production.

$$\text{Total FAMEs}\,(\%, \text{w/w}) = \frac{\text{Total weight of FAME(g)}}{\text{Dry Biomass(g)}} \times 100\% \quad (10)$$

Fatty acid methyl conversion was found in the reaction's GC peak area and was obtained through transesterification and conversion as in Eq. (11) given below.

$$\text{FAME conversion yeild} = \frac{\text{Total peak area of FAME}}{\text{Total peak area of the products}} \times 100\% \quad (11)$$

6.5. Hydrothermal liquefaction

The process of converting biomass to liquid and gas by maintaining temperature between 200 and 600 °C at 5 to 40 Mpa keeps the aqueous media in a supercritical phase called Hydrothermal Liquefaction. Waste and lignin products are used in HTL process, but bio oil production is less than in pyrolysis. However, quality through HTL is better than in pyrolysis due to less oxygen content in the reaction. The advantages of this process include high heat transfer rate and reaction at 90% moisture content [121,99,122]. Crude from HTL can be directly used for transportation after removing oxygen [82]. To check its suitability for transportation, biodiesel is checked for it properties against ASTM standards. Some properties of microalgae biodiesel compared to other oil seeds is given in (Table 6).

7. Analysis and characteristics

Analysis of the biodiesel confirms conversion of raw oil to biodiesel. There are various techniques are followed to study the characteristics of the biodiesel such as Fourier transform infrared spectrometry, Nuclear magnetic resonance and Gas chromatography and mass spectrometry. The in-depth study of these characterization techniques are presented below.

7.1. Fourier transform infrared spectrometry

Analysis of the biodiesel confirms conversion of raw oil to biodiesel. Many analyses checked components of transesterification. Fourier transform infrared spectroscopy (FTIR) resolves components quality in the sample. The principle behind FTIR is that when the spectrum is passed through molecules, the latter absorb light where bonds exist. This is used to identify functional groups and samples band width through stretching vibrations. The sample is amalgamated with KBr to form pellets after pressing it with discs. This results in each spectrum detecting 32-scan interferogram with 4 cm^{-1} resolutions in atmospheric temperature [94]. Microalgae esters have two-carbonyl group band wavenumbers at 1750–1730 cm^{-1} and 1300–1000 cm^{-1} and stretching vibration occurs at 1475–1350, 1350–1150 and 722 cm^{-1}. The wavenumber of 3100–3500 cm^{-1} is of the (OH) hydroxyl group [126]. A band of 1237 cm^{-1} reveals that the C-O group stretching esters and carboxylic acid [127]. Fatty acid composition was determined using a Gas chromatogram analysis. The 80% fatty acid present in *N. oculata* extracted oil were palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids. Myristic (C14:0), palmitoleic (C16:1), palmitolenic (C16:2), hirigonic (C16:3) or stearic (C18:0) acids are other fatty acids present in the oil in a less peak area. Arachidic (C20:0) and behenic (C22:0) acids were also present in a small quantity in algae oil [128]. The large amount of oleic acid (1.2 mg g^{-1} dwt.) and palmitic acid (1.3 mg g^{-1} dwt.) were determined in *S. obliquus* YSR01 and *N. pusilla* YSR02. High content of oleic acid was declared to have better fuel performance,

Table 5
Types of pyrolysis and optimum parameter conditions.

Pyrolysis Types	Species	Output	Conditions	References
Pressurized entrained-flow pyrolysis of microalgae	Chlorella vulgaris	CO, CO_2, CH_4, H_2	900 °C & 4 MPa	[106]
Pressurized entrained-flow pyrolysis of microalgae	Chlorella vulgaris	Bio-char	2 Mpa	[107]
Catalytic pyrolysis	Chlorella vulgaris	Bio-oil	300–600 °C	[108]
Microwave-assisted fast co-pyrolysis	Microalgae	Bio-oil	800 W to 1000 W	[109–111]
Microwave pyrolysis	Chlorella and Spirulina	nitrogen-containing compounds inbio-oil	Fe_3O_4	[112]
	Scenedesmus almeriensis	Hydrogen	800 °C	[113]
catalytic and non-catalytic pyrolysis	Chlamydomonas reinhardtii	hydrocarbon	hydrotalcite catalyst	[114]
Solar pyrolysis	Algae biomass	Bio-oil, pyrolytic gas and bio-char	Parabolic scheffler dish, Rotating pyrolysis reactor	[115]
Slow pyrolysis and Fast pyrolysis	Algae Biomass	Bio-char, Bio-oil	Below 400 °C and 400–600 °C	[116]
Co-pyrolysis	microalgae and coal	Tar	600 to 850 °C,	[117]
	microalgae and waste tyre	Bio-oil	330 °C Supercritical ethanol	[118]

Table 6
Comparison of microalgae biodiesel with other oilseed feedstock.

Properties	Microalgae biodiesel [123]	Soybean biodiesel [123]	Jatropha curcus [124]	Ceba pentandra [124]	Chicken biodiesel [125]	Diesel fuel [123]	ASTM method [123]	ASTM limits [123]
Carbon residue (wt%)	0.018	0.019	–	–	–	0.15–0.35	D4530	0.050
Flash point (%)	149	122	125.5	120.5	177	38–52	D93	93
Total glycerin (wt%)	0.169	0.161	–	–	–	–	D6584	0.240
Free glycerin (wt%)	0.006	0.05	–	–	–	–	D6584	0.02
Water and sediment (wt %)	0.005	3.07	–	–	0.04	0.05 max	D2709	0.05
Sulfur, total (ppm)	8.43	3.07	–	–	–	38	D5453	15
Cetane number (%)	71.67	50	–	–	–	40–45	D613	47
Cloud point (°C)	−16	2	2	4	–	–	D2500	–
Solidifying point (°C)	−12	–	–	–	–	−50 to 10	–	–
Sulfated ash (ppm)	0.008	0.011	–	–	–	0.05	D874	0.020
Copper strip corrosion (scale 1–4)	1	1	1a	1a	1a	3	D130	3 max
Acid number (mg KOH/g)	0.01	0.07	0.46	0.51	0.4	0.5 max	D664	0.50
Kinematic viscosity at 40 °C(mm^2/s)	11–35.4	4.5	4.5	4.7	4.9	1.9 – 4.1	D445	1.9–6.0
Cold filter plugging point (°C)	126	185	1	1	–	−6.7 max	D6217b	360 max
Phosphorous (ppm)	1	2	–	–	–	0.1	D4951	10 max
H/C ratio	1.81	1.81	–	–	–	1.81	–	–
Density (kg/L)	0.864	0.838	0.846	0.876	0.926	0.830	–	–
Heating value (mJ/kg)	37–41	–	39.46	39.46	39.71	40–45	–	–

combustion heat, cold filter plugging point, ignition quality, oxidation stability, viscosity and lubricity [129]. Good biodiesel combines (C16:1), (C18:1) and Tetradecenoic acid (C14:0) which have low oxidative stability with high Cetane number and good cold flow property [130].

The FTIR band for algal species is stated in (Table 7) [11]. For pure lipids the FTIR spectra shows absorption bands as CH_3 and CH_2 (3025–2954 cm^{-1}) with the most predominant band being ester C = O (1746–1654 cm^{-1}). In phospholipids, hydroxyl and phosphate groups were formed in the (1,200 – 500 cm^{-1}) absorption band [11]. Large Bands were between 3200 cm^{-1} and 3600 cm^{-1} representing availability of water, alcohol, amides and amines while the 2800 cm^{-1} and 2900 cm^{-1} strong bands represent aliphatic C–H stretching. Amide carbonyl stretching or C = C appeared in 1660 cm^{-1} with the medium strength band at 1550 cm^{-1} being of aromatic compounds [131–133].

Table 7
FTIR Functional groups of algal species.

Band (cm^{-1})	Functional groups of algal species
1740	(C = O) - ester groups, - (lipids and fatty acids)
1655	(C = O) – amides - proteins (amide I)
1545	(N–H) amides - proteins (amide II)
1455	(CH_2) and (CH_3) - methyl from proteins
1380	(CH_2) and (CH_3) - methyl and (C–O) stretching of COO–
1240	(>P = O) - phosphorus compounds
1000	(C–O–C) - polysaccharides

7.2. NMR (Nuclear magnetic resonance)

Nuclear Magnetic Resonance is a promising technique to find the conversion rate of methyl esters from oil, i.e.; the measure of the degree of unsaturation of fatty acid present in the sample. The sample used are 5–10% (w/w) in Deuterated chloroform ($CDCl_3$) and the tetramethylsilane (TMS) was taken as internal reference as per the experimental conditions: spectral width, 3600 Hz (0.0–12.0 ppm); spectral size 16 K, digital resolution 0.2197 ppm/point, 18 μs, 90° pulse, relaxation delay of 10 s and the number of scans being 64. For bio oil, data was acquired from 500 MHz, 90 ° pulse angle and 8000 Hz Sweep width [134].

The fatty acid methoxy groups are found at 3.6 ppm (R) while signals observed by methylenic hydrogen is at 0.5 – 2.8 ppm. Therefore, methyl esters can be calculated using Equation below (12) [120].

$$\%ME = \frac{(R - r)}{E} \times 100 \quad (12)$$

where, r is area per hydrogen and E the integral area of α CH_2 at 2.3 ppm.

7.3. Gas chromatography and mass spectrometry

Gas chromatography and mass spectrometry (GCMS) are used to identify compounds Hydrocarbons are important in fuel composition transportation applications with especially aromatic hydrocarbons being predominant industrial fuel additives to increase the Octane number [60]. Fatty acid profile was found using the Perkin Elmer Clarus 500 Gas Chromatography mass spectrometer with a split automatic injector consisting of a column with an inner diameter of 0.25 mm, length 60 m and a coating of 0.25 μm. The column was heated to 120 °C and increased to 240 °C at 20 °C/min with the line being kept constant for 13 min [135]. The overall Peak data of algae biodiesel is shown in (Table 8).

Molecular weight of compounds was identified by GCMS. From the above table, major compounds found in the algal sample are pentadecane at 4.87 and 5.65% area n-tetracosane, n-dodecane, 2,7,10-trimethyldodecane, n-docosane, n-tetradecane, n-octadecane, n-hexadecane and n-undecane [130]. Thermogravimetric analysis detects the pyrolytic characteristics of algae in three processes through dehydration, devolatilization and decomposition at temperatures between 50 and 900 °C. Weight loss occurs at the first stage due to moisture removal, while second stage releases volatile matter and third stage achieves sample decomposition between 600 and 900 °C. Due to less volatile matter in algae spirulina sp. produces high bio-char than rice husk, corn hob, eucalyptus, sawdust, palm shell and coconut shell which also produce bio-oil [136]. CHNS analysis was carried out in the CHNS analyzer for various feedstocks as shown in (Table 9). The carbon content of spirulina and chlorella biodiesel was higher than biomass while Sulphur content was less in biodiesel compared to biomass which leads to reduced pollution.

Table 8
GC–MS peak data for algae biodiesel.

Molecular Formula	Compounds	Retention time (min)	Area (%)
$C_{12}H_{24}$	Cyclohexane	5.202	0.35
$C_7H_{12}O_2$	Heptanedial	5.617	0.21
$C_7H_{16}O_2S$	sulfone, butyl isopropyl	5.710	0.34
C_6H_{14}	Hexane	5.783	0.45
$C_7H_{12}O_2$	Heptanedial	5.867	0.44
C_6H_{14}	2,2-dimethylbutane	6.027	0.32
$C_{11}H_{24}$	Hexane	6.759	3.47
$C_4H_9Cl_3$	Trichlorosilane	8.254	0.83
$C_4H_9Cl_3$	Trichlorosilane	8.514	0.73
$C_{12}H_{26}$	dodecane	9.570	3.74
C_8H_{18}	Octane	9.980	2.35
$C_5H_{11}NO_2$	trimethylglycine	11.509	0.37
$C_6H_{12}O$	2-Hexanone	11.721	0.28
$C_9H_{20}O$	3-Nonanol	11.793	0.82
$C_{11}H_{10}$	methylnaphthalene	12.410	0.51
$C_{12}H_{26}$	dodecane	12.634	4.32
$C_7H_{12}O_2$	allyl isobutyrate	14.610	0.41
$C_8H_{16}O$	cyclooctanol	14.820	0.24
$C_{12}H_{26}$	docosane	15.021	0.92
$C_{15}H_{32}$	pentadecane	15.738	5.35
$C_{12}H_{14}$	1,1-dimethyl-1,2-dihydronaphthalene	16.340	0.22
$C_{15}H_{32}$	pentadecane	17.634	1.83
$C_6H_{10}O_3$	Ethyl acetoacetate	17.888	0.25
$C_{15}H_{32}$	pentadecane	18.769	6.16
$C_5H_{11}NO_2$	Amino Acid	20.638	0.34
$C_6H_{12}O$	4-methyl-3-pentanone	20.845	0.20
$C_5H_{10}O$	Methyl isopropyl ketone	21.477	0.37
$C_{15}H_{32}$	pentadecane	21.692	5.65
$C_{12}H_{26}$	docosane	23.079	0.57
$C_8H_6O_4$	Phthalic acid	23.139	0.62
$C_{15}H_{32}$	Pentadecane	24.482	5.89
$C_{24}H_{50}$	n-tetradecane	24.652	3.85
$C_{15}H_{32}$	pentadecane	27.147	4.87
$C_{12}H_{26}$	n-docosane	27.400	1.61
$C_{24}H_{50}$	n-tetracosane	29.689	4.22
$C_{22}H_{44}O_2$	eicosyl acetate	31.451	0.56
$C_{16}H_{32}O_2$	hexadecanoic acid	31.978	3.64
$C_{24}H_{50}$	n-tetracosane	32.123	3.87
$C_{18}H_{38}$	n-octadecane	34.448	3.23
$C_{20}H_{40}O$	*trans*-phytol	34.747	1.62
$C_6H_8Cl_2$	5,6-dichlorohexene	35.814	0.41
$C_5H_{10}O$	Methyl isopropyl ketone	36.097	0.45
$C_{16}H_{34}$	n-hexadecane	36.680	2.84
$C_{12}H_{26}$	n-docosane	40.866	6.28
$C_{24}H_{38}O_4$	Bis (2-ethylhexyl) phthalate	43.952	13.33

8. Optimization of microalgal biodiesel production

After oil is extracted from microalgae, biodiesel production is undertaken. To produce maximum biodiesel yield, operating variables are important to optimize parameters involved in biodiesel production. In microalgae reaction temperature, catalyst, speed rate, molar ratio, water content and reaction time are important variables. The results are found using a combinational effect through optimization tools. Some potential microalgae with their optimized parameters for biodiesel production are reviewed and listed in (Table 10). Literature reported that several prediction models were used to obtain optimum parametric conditions. Response surface methodology-based Box-Behnken design was applied to optimize biodiesel production [137,138].

9. Other applications

Microalgae growth still needs high capital cost due to addition of fertilizers. By using waste water as a growth medium, overall cost is reduced and has the advantage of purifying wastewater. Once algae have grown, oil is extracted for biodiesel production. Extracted algae biomass is used for various application as discussed below. Using nutrients from wastewater, lipids in *Botryoccocus strains* meets conventional fuel standards. Water in the medium meets European directives for water supply [141,142,35]. In addition to the lipid, microalgae can be used as a source for many coproducts like astaxanthins, xanthophylls, β-carotenes, omega 3-fatty acids, polysaccharides and proteins. It is also used as bioplastics, food, colorants and pharmaceuticals. Algenol is a leading producer of food, biofertilizers, colorants and patented directly in ethanol technology [143–145,26]. 2,6-dichloro-1,4-benzoquinone produced from chlorinated green algae chlorella vulgaris can be used as precursors in disinfected water [146,147].

Algae can also be used in anti-inflectional, anti-aging and skin tumor treatment. Recent research reported that algae pigments can focus on health, skin care and therapy [115]. Cyanobacteria remove odors from hazardous metabolites and enhance water recyclability [148,149]. Marine microalgae contain bioactive compounds like polyphenols, fatty acids, proteins while sulfated polysaccharides are used for anti-inflammation. Consumption of these bioactive compounds prevents pathological diseases [150,151]. Arachidonic acid and docosahexaenoic

Table 9
CHNS for different algae species.

Various Algae types	Energy content MJ/kg	C	H	N	S	O
Pond water algae – (biomass)	20.05	46.09	6.22	9.70	0.64	37.35
Sp. biomass	22	48.10	6.97	10.14	0.66	34.13
Chl. biomass	24	51.33	7.90	9.80	0.59	30.38
Pond water algae oil	22	59.94	11.57	0.11	0.31	28.37
Sp. oil	35	66.73	12.40	0.50	0.16	20.21
Pond water algae - (biodiesel)	30	71.49	11.00	0.31	0.19	17.01
Sp. biodiesel	38	78.44	12.04	0.20	0.08	9.23

Table 10
Some potential microalgae optimized parameters for biodiesel production.

Species	Temp °C	Catalyst (Oil basis)	Speed rate (rpm)	Molar ratio (solvent:oil)	Water content %	Reaction time (hr)	Yield (%)
Nannochloropsis Spe-cies (biomass)	65	Mg-Zr oxide 1.65:1	–	1569:1	–	4	60
Nannochloropsis Species (Oil) [139]	65	Mg-Zr oxide 1.65:1	–	592:1	–	4	47
Chl. vulgaris [139]	60	NaOH 0.15:1	380	600:1	–	1.25	78
Algae biomass [139]	65	H_2SO_4 0.678:1	150	308:1	8	2	80
Algae biomass [139]	65	H_2SO_4 0.678:1	150	308:1	1	2	86
Chlorella vulgaris [139]	–	NaOH 0.15:1	–	600:1	–	1.15	77.6
Algae biomass [139]	65	H_2SO_4 0.797:1	150	308:1	0.2	2	96
Chl. pyrenoidosa [139]	60		–	–	–	4	7.8
L. starkeyi [139]	70	H_2SO_4 0.093:1	–	868:1	–	20	97
M. isabellina [139]	70	H_2SO_4 0.093:1	–	868:1	–	20	91
R. toruloides [139]	70	H_2SO_4 0.093:1	–	868:1	–	20	98
Chaetoceros gracilis [139]	80	H_2SO_4 0.158:1	–	988:1	–	0.33	82
	80	H_2SO_4 0.158:1	–	1,977:1	100	0.33	67
	80	H_2SO_4 0.158:1	–	3,460:1	400	0.33	57
Chlorella sorokiniana [139]	80	H_2SO_4 0.158:1	–	1,831:1	–	0.33	77
Chlorella vulgaris [139]	60	H_2SO_4 0.35:1	380	600:1	–	20	97
Synechococcus elongatus [139]	80	H_2SO_4 0.158:1	500	2,354:1	–	0.33	40
Jatropha curcas [139]	30	NaOH 2.4:1	300	400: 1	–	0.5	88
Rapeseed [139]	30	NaOH 2.1:1	200	600 :1	6.7 wt%	1	85
Sunflower [140]	20	NaOH 0.5:1	–	101:1	4.6%	0.2	98
Soybean [140]	23	NaOH 2:1	–	543:1	–	8	84
Botryococcus braunii [123]	350	HCl	600	30:1	–	–	30–40
Chaetoceros calcitrans [123]	50	KOH	–	6:1	–	50 min	85
Chlamydomonas sp. [123]	360	Titania	–	32:1	–	30 s	80–85
Chlorella protothecoides[123]	30	H_2SO_4	–	56:1	–	4 hr	63
	38	Lipase from Candidia sp.		3:1		12 hr	98.15
	65	KOH		10:1		5–20 min	97.34
Chlorella sp. *and Dunaliella tertiolecta* [123]	360	Titania		32:1		30 s	80–85
Dunaliella salina [123]	30	2% H_2SO_4		32:1		2–4 hr	40
Monoraphidium minutum [123]	38	HCl, NaOH		6:1		30 min	68
Nannochloropsis sp. [123]	60	KOH		6:1		0.5 – 2 hr	89.7
	260	Acetyl chloride,		4:1		10–30 min	85
Nannochloropsis oculate [123]	360	CaO, MgO				10 min	97.5
Spirulina platensis [123]	40	Scenedesmus dimorphus Lipase		3:1		24 hr	44.8
Chl. pyrenoidosa [139]	90	H_2SO_4 0.234:1	–	154:1	–	2	95

acid in algae produce phospholipids used for brain membrane growth [152].

Algae seaweed is used as agar, hydrocolloids alginate and carrageen and as a viscosity modifier in pharmaceuticals and food. Algae are used for numerous health benefits to control the effects of cholesterol, heart issues, osteoporosis and cancer. Microalgae are a protein factory and have a higher value than other vegetables. Table 11 shows the different species and their applications in human health [19,155]. Recently, marine brown algae are used for the production of biopolymers for the applications of tissue engineering. Alginates and fucoidans are the basic polymers derived from the marine brown algae hydro jelling and bioactive sulfates, respectively. These can be done win the molecular structure modifications by controlled degradation, cross-linking and oxidation [156]. Additionally, carrageenan is a linear structured contains polysaccharides derived from red seaweed, it is widely exploited as hydrophilic and anionic properties. Also used in emulsifying, stabilizing agents, gelling, and base materials packaging and pharmaceuticals industries [157]. Colored dye wastewater results to the environmental wastewater with the penalty of carcinogenic disease. Removing the dye using the conventional methods such as including oxidation, biodegradation, photo-catalyst, precipitation, and reverse osmosis are expensive. Thus, Nano cellulose produced from the algae has surface functionalization, better binding affinity, multiple applications to adsorb and desorb contaminants, renewable and biodegradable, high surface area,

Table 11
Microalgae as a substrate for human benefits application.

Species	Galenic form	Remedies
Spi*rulina* sp.	Sterols, clionasterol's and plasminogen	Cardiovascular disease
Spi*rulina* sp. *Donaliela, Hematococcus, Nannochloropsis, Tetraselmis, Cyanobacteria Haematococcus pluvialis*	astaxanthins, xanthophylls, β-carotenes, omega 3-fatty acids, proteins and polysaccharides	Antioxidant agent [140] Feed additives [153]
Muriellopsis sp.	Lutein	Degenerative disease
Spirulina (Arthrospira) and Chlorella, Dunaliella sp.	Bioactive component molecules	Edible algae
S. platensis and Spirulina maxima	Immune system Phytoplankton	Viral and cancer treatment, cakes and breads [154]
Chlorella	Ethionine	Increases hemoglobin, it maintains level of the sugar, used for hypo cholesterolemic
Chlorella pyrenoidosa, cllipsoidea	Glucose	Immune stimulatory activity
Chlorella sp.	Plankton	Ice cream, soups and sauce.
Scenedesus sp.	Nutritional value	Desserts and fruit puddings
Fucus vesiculosus	Polar solvents	Reduce appearance for dark Circles [140]
Phaeodactylum trycornutum, Thalassiosira pseudonama, N. Oculata, H. pluvialis	Polyunsaturated fatty acids, eicosapentaenoic, docosahexaenoic and arachidonic.	Rheumatism, skin disease and inflammation in gastrointestinal tract.

high mechanical properties, and good surface tension, which favors the wetting of nanocellulose and its stability in water. The efficiency of nanocellulose derived from algae as an adsorbents reported more than the functionalized material [158]. Overall microalgae can be used for agriculture, aquaculture, anti-microbial, cytotoxic, anti-oxidant, anti-nociceptive, anti-ulcer, anti-diarrheal, anti-hyperlipidemic, anti-acetylcholinesterase and anti-inflammatory activities [159].

10. Scope for the future work

The future work can be elaborately progressed on reviewing the native algal species in all over the world. The zonal wise and country wise algal species, structure of algal species, lipid content of algal species, different conversion technique and genetic studies of different species could be done intensively. The use of the algal biomass in fuel and cosmetics is conventional, the further studies can be extended towards the use of algae biomass and algae oil for the treatment of COVID-19 well appreciated. Despite, there are numerous advantages stated above, the development of algae in industrial scale in terms of rationales, techniques and industrializations is far behind. Also, the algae based biofuels are complicated than the other conventional biofuels, 25–70% of energy required for the cultivation and harvesting and for post processing requires 15–30%. The cost of variation in strains, techniques, constituents, and biotransformation process varies independently. Therefore, the minimization of the energy input and reduce the cost of biofuel is still under challenging for the engineering and R&D integration.

11. Conclusion

Biofuel from microalgae is considered as the best alternative to fossil fuel. Microalgae can also be used for applications in the fields of health, water, energy and materials.

1. In contrast, microalgae research and development are expansive which affects the global market in algae products caused by a declining crude oil price. To reduce cost of small and large-scale systems, cheap resources like CO_2 from flue gas and wastewater as nutrient rich fertilizer is used in simple culture designs with greenhouses for biomass production.
2. Biodiesel from microalgae is still a dream and required much research to develop a technology for biosynthesis of lipids. TAG metabolism and cell cycle should be evaluated genetically to improve biodiesel production. For this, genetic manipulation is needed to enhance TAG production.
3. Harvesting methods is not commercialized for biomass separation and consumes 70% of the total cost. Common methods used are centrifugation and flocculation. Energy efficient algae harvesting methods are resorted to in a bid to reduce production cost. As of now, Soxhlet extraction is the best method to extract oil from algae as it consumes excess ethanol, methanol, hexane and chloroform. Some studies reveal that ethanol is a good solvent due to its lipolysis nature and being less polar with TAG.
4. The extracted oil is *trans*-esterified by alcohol with the help of a catalyst for biodiesel production was also discussed. Steps like isolating strains, cultivation, growth, harvesting, oil extraction, biodiesel conversion and characterization are also briefly discussed in this paper.

CRediT authorship contribution statement

C.N. Kowthaman: Writing – review & editing, Data curation, Formal analysis. **P. Senthil Kumar:** Conceptualization, Validation, Supervision. **V. Arul Mozhi Selvan:** Investigation, Formal analysis. **D. Ganesh:** Conceptualization, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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