Renewable and Sustainable Energy Reviews 94 (2018) 49–68

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Production of biofuels from microalgae - A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae

Manoj Kumar Enamala[a], Swapnika Enamala[b], Murthy Chavali[c], Jagadish Donepudi[d], Rajasri Yadavalli[e], Bhulakshmi Kolapalli[a], Tirumala Vasu Aradhyula[f], Jeevitha Velpuri[g], Chandrasekhar Kuppam[h,*]

[a] Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
[b] GSL Medical College, Rajahmundry, Andhra Pradesh, India
[c] MCETRC, 20-26-136, Chiravuru, Tenali, 522201 Guntur, Andhra Pradesh, India
[d] Mechanical Engineering Department, Narasaraopeta Engineering College, Narasaraopet, Guntur, Andhra Pradesh, India
[e] Sreenidhi Institute of Science and Technology, Yamnampet, Ghatkesar, Telangana, India
[f] Department of Mechanical Engineering, National Chung Cheng University, Taiwan
[g] Environmental Science and Technology, JNTUH, Hyderabad, Telangana, India
[h] Green Processing, Bioremediation and Alternative Energies Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Keywords:
Bioenergy
Algae biomass
Harvesting procedures
Lipid
Biodiesel
Third generation biofuel

ABSTRACT

The concern regarding alternate sources of energy is mounting day-by-day due to the effect of pollution that is damaging the environment. Algae are a diverse group of aquatic organisms have an efficiency and ability in mitigating carbon dioxide emissions and produce oil with a high productivity which has a lot of potential applications in producing biofuel, otherwise known as the third-generation biofuel. These third generation biofuels are the best alternative to the present situation since they have the perspective to eliminate most of the ecological problems created by the use of conventional fossil fuels. These organisms are responsible for closely 50% of the photosynthesis process taking place on the planet and are distributed predominantly in many of the aquatic systems. The huge interest in utilizing these organisms as a potential source of energy lies in converting the primary as well as secondary metabolites into useful products. Algae are considered to be the most prominent resource for the upcoming generations as the most suitable and sustainable feedstock. The key process limitations in microalgal biofuel production are inexpensive and effective harvesting of biomass and extraction of lipids. The major objective of this article is to provide a comprehensive review on various methods of both biomass harvesting and lipid extraction from microalgae available, so far, besides to discuss their advantages and disadvantages. This article also deals with various conditions that are favourable for lipid accumulation as well as the yield from different species.

1. Introduction

The energy crisis is increasing globally due to the heavy industrial development and exponentially growing population. Sources like petrol, diesel, natural gas, coal which were considered to be the basic sources for fuelling the life are getting exhausted due to extensive usage [1,2]. Moreover, these fossil fuels release a lot of toxic and harmful gases into the atmosphere and pollute the environment which is the major disadvantage [3,4]. The greenhouse gas (GHG) levels in the environment have increased at an alarming rate in the post-industrialization era by 25% of the total [5]. Natural causes, as well as human activities, have been mentioned as the major causes of this rise in temperature leading to global warming [1]. The major contributors include carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and other fluoro-hydrocarbons. Among them, the major pollutant which damages the environment is CO_2 [1,6]. The above-mentioned gasses are

Abbreviations: GHG, Greenhouse gas; N_2O, Nitrous oxide; TCA, Tricarboxylic acid; ETC, Electron transport chain; ATP, Adenosine triphosphate; Glu-6-P, Glucose-6-phosphate; PPP, Pentose phosphate pathway; NH_4^+, Ammonium; NH_3, Ammonia; O-U, Ornithine-urea; WC, Water column; WB, Water bodies; $NaSO_4$, Sodium sulphate; DIC, Dissolved inorganic carbon; $FeCl_3$, Ferric chloride; Fe_2SO_{43}, Ferric sulphate; $CaOH_2$, Calcium hydroxide; $MgOH_2$, Magnesium hydroxide; R-NH_2, Amine groups; Al_2SO_{43}, Aluminium sulphate; bio-CH_4, Bio-methane; bio-H_2, Biohydrogen; FA, Fatty acids

* Corresponding author.
E-mail address: chandrasekhar.kuppam@tdt.edu.vn (C. Kuppam).

https://doi.org/10.1016/j.rser.2018.05.012
Received 9 May 2017; Received in revised form 12 May 2018; Accepted 13 May 2018

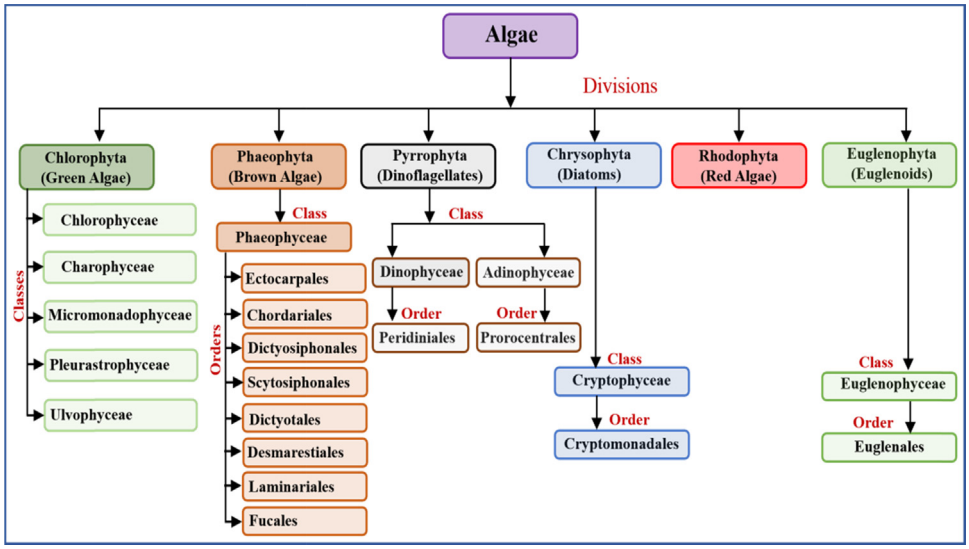

Fig. 1. Classification of algae explained in a simple way.

present in the atmosphere at a normal rate but due to the emissions from the vehicles, their concentration has increased over the past few decades [7]. Owing to all this, there has been a change in climatic conditions over the globe, which has become a topic of debate [8]. At this time, replacing fossil fuels with other alternative sources especially those that benefit the environment is the best solution [1,4]. These microalgae sources act as solar driven energy cell factories and are capable of converting CO_2 to oxygen (O_2) and thus reducing the toxic substances and chemicals in the environment. Hence these organisms are very promising in this aspect [9].

The working machinery of these organisms is the same as that of the plants, as both are photosynthetic. These utilize the sunlight from the atmosphere for the photosynthesis process and other essential nutrients from the surroundings for their growth [10]. There are also many food crops available which are used for the production of fuel apart from algae. Much of the study is being carried out on industrial production of biodiesel from plant sources. Apart from soya bean oil, jatropha, left-over cooking oil, canola, corn, and animal fats etc., are also being tried as fuel sources [11]. However, these sources must also meet the requirement for the food for human beings. Upon extensive usage of these sources for oil production, there may arise a scarcity in providing food for human beings [11]. Production of biofuels from the plant sources was criticized by many scientific communities as well as local farmers and the general public since the growth of these plants needs an extensive usage of land, leading to a crisis in food grain production.

The biofuels are divided into three generations depending on the source from which they are obtained [12].

- First generation biofuels derived from plant sources.
- Second generation biofuels derived from agricultural wastes, lumber wastes etc.
- Third generation biofuels derived from microalgae.

Researchers have turned their interest towards fuel production from one of the oldest living creatures on the earth, microalgae. These are utilized not only in producing fuels but also in capturing the CO_2 from the atmosphere which helps in cleaning the environment and producing better air to breathe [13,14]. There are two different classes of algae known as macroalgae and microalgae. These photosynthetic organisms are mainly found in aquatic habitats both freshwater and marine. These are microscopic and have very amazing and fascinating structures [15].

The reasons for algae being the preferred source over plant sources

(a) The microalgae have a high efficiency for photosynthesis with an adaptability to a wide range of light and temperature variations [16].
(b) The microalgae can grow in water with different levels of nutrients and can adjust to the change in the growth characteristics and nutrient uptake ability [16].

These organisms have a larger surface to volume ratio, which enables them to grow very efficiently. Fixation of CO_2 at different water levels is achieved very easily. But the major challenge would be the cultivation of microalgae on a large-scale, harvesting and finally converting into useful fuels which are beneficial for the human society and as well have an economic impact [12].

In this review article, we have discussed the state-of-the-art in biofuel production from microalgae. The distinctiveness of this review is in its coverage of numerous harvesting procedures, extraction methods and parameters which are involved in growth and the lipid extraction techniques. In this paper, we have given a tabular column and illustrated various conditions that favor lipid accumulation as well as the yield from different species. We also discuss various maximum growth rate values, lipid percentage accumulated in their cells of individual species, numerous methods regarding biofuel and co-products recovery, carbon dioxide mitigation and wastewater treatment.

1.1. General characteristics of algae

The green algae and the cyanobacteria together called the blue-green algae consist of a huge group of photosynthetic organisms, the most efficient organisms reported to date. Unlike other microorganisms, these have abundant chlorophyll inside the cells, with a well-defined nucleus, cell wall, and pigments [8].

1.2. Forms of algae

The various forms of algae which exist are:

- Colonial, Capsoid, Coccoid, Palmelloid, Filamentous, Parenchymatous.

The cell walls of diatoms comprise polymerized silica known as a

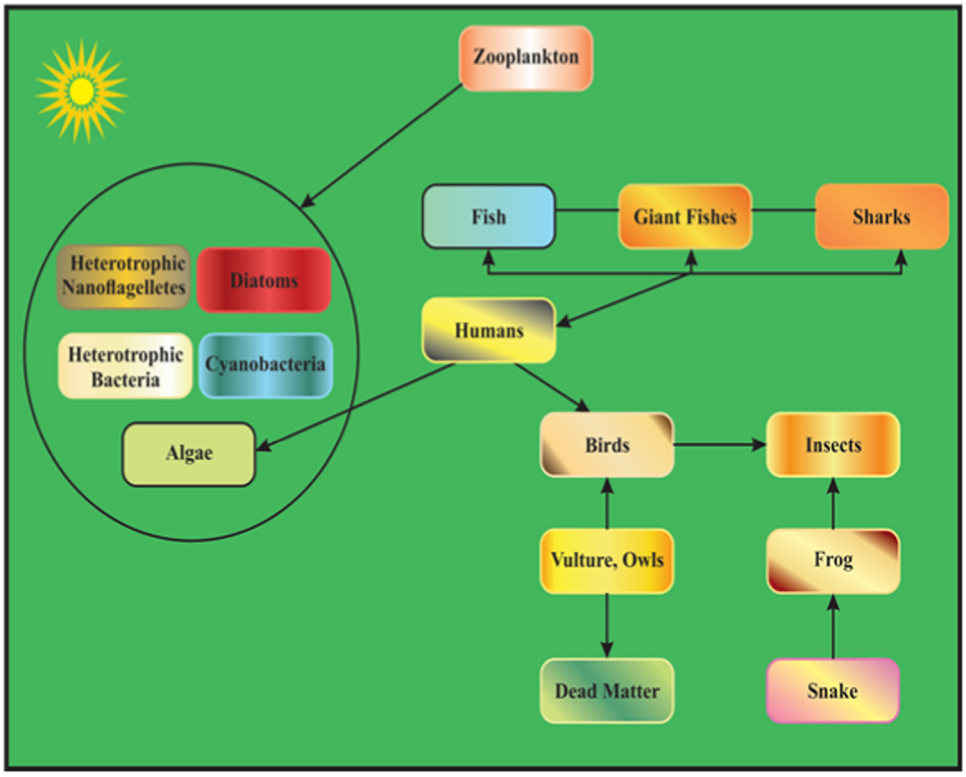

Fig. 2. Food web of algae which shows the relation between humans and other species for its survival.

Table 1
Advantages and disadvantages of microalgae.

Advantages	Disadvantages
• Very short doubling time • Cheap media can be used (including wastewater) • Can be supplied as a food for aquaculture • Absorbs CO_2 as it grows and helps in cleaning of the environment • Can be grown in an non-arable land on a large scale and on small scale can be grown in our own houses • The great decrease of competency for food vs fuel. • New source of fuel can be obtained (capability to produce H_2 from water). • Algae contain a high amount of iron (Fe) which is advantageous if consumed by the pregnant women. • Wastewater can be purified • Contains rich lipid profile which can be used for improving health • Can be grown in open pond cultivation system to several kilometers • They are considered as tough competitors for biofuel production • Solar conversion increases by 10 folds when compared to plants • Cyanobacteria can fix N_2 from the atmosphere	• They can easily grow in any aquariums • When algae are attached to a system it produces methane which gets mixed with the water sources. • Risk in culturing pure cultures of algae due to the bacterial contamination • High temperatures are a serious threat to open pond cultivation system • Due to the large growth of algae on the surface of lakes, ponds they obstruct the light to reach the aquatic plant's fishes and other aquatic species which are deep under water.

frustule. The diatoms often accumulate oils and chrysolaminarin [16]. The green algae are particularly rich in fresh water (Fig. 1). They produce starch as major chief storage compound by the photosynthesis mechanism. However, they can also produce fats and oils. The freshwater green algae *Haematococcus pluvialis* are a freshwater species of *chlorophyte,* which is a chief source of strong antioxidant 'astaxanthin', which is very significant in aquaculture, and cosmetics industry. Hence it is having high commercial importance [16]. The Chrysolaminarin consists of a linear polymer arranged in the chains of $\beta(1-3)$ also $\beta(1-6)$ linked glucose molecules (in 11:1 ratio), which was earlier well-known as leucosin. The Chrysolaminarin is considered as storage polysaccharide as well as the most common biopolymer in the world [17]. It is also utilized as a reserve food by organisms such as *Bacillariophyta* which is similar to the laminarin of brown algae (Fig. 2). The Chrysolaminarin exists as an encapsulated vacuoles when dissolved in water and is stored in the cells [18]. The detailed information regarding advantages and disadvantages of microalgae are provided in Table 1.

2. Metabolism of algae

The metabolic reaction process is almost same in all the photosynthetic organisms. The most important factor is the nutrition uptake from the surroundings through various biochemical and transportation process [17]. The carbon (C) and nitrogen (N) are considered to be the important elements in the photosynthetic metabolic pathways. The major changes which occur during the metabolic pathways are the mass of the cells, volume, densities, protein, chlorophyll, RNA, and vitamin contents [19,20].

2.1. Carbon metabolism

The C metabolism starts with the incorporation of glucose into the algal cells and the addition of phosphate group to hexose which yields glucose-6-phosphate (Glu-6-P), which is easily accessible for the storage, growth, and respiration in the cells [21]. In the darker conditions, algae cannot metabolize glucose because a short supply of energy is expelled through dissimilation of glucose [21]. However, the insufficient amount of the enzyme lactate dehydrogenase will be slowed down this process. Nielsen and Lewin have shown that only Embedded Mayerhof and Pentose phosphate pathway (PPP) have been shown in the algal cells [22]. These algal cells utilize almost the entire glucose present in the environment and only free glucose of about 1% is available. Most of the glucose is converted into oligosaccharides, polysaccharides etc. When compared with other pathways the PPP pathway has a higher flux than compared with the other [22].

2.2. Nitrogen Metabolism

Nitrogen is one of the most plentiful sources available in nature next to C, hydrogen (H_2), and O_2 and is also the main contributor to the dry weight of algae. The metabolism of C and N are interconnected in microalgae [21]. The ammonium (NH_4^+) which is available freely is combined with the inorganic form, which then forms amino acids. These amino acids require a C skeleton which can form keto acids and the energy in the form of ATP molecules will be released, which is necessary for the synthesis of the various amino acids like glutamate, aspartate, glutamine [21]. The metabolism of nitrogen sources is catalyzed by an enzyme known as glutamine synthase. This is considered to have a high affinity towards ammonia and can easily incorporate it into the cells [23].

2.3. Urea metabolism

Some of the algal species utilize only the nitrogen sources which are available. Before it assimilates into the cells it is first converted into ammonia (NH_3) and bicarbonate. The two major enzymes which can utilize urea are urease and urea amidolyase [5]. Most of the species lack this urease enzyme and they metabolize urea by an enzyme known as UALase. The metabolic pathway in which UALase is followed is that the allophanate lyase catalyzes the hydrolysis process of allophanate, which results in hydrolysis process of urea to NH_3 and then to bicarbonate [5]. During the metabolomics analyses, the results indicate that intermediate products in the ornithine-urea (O-U) cycle are mainly exhausted. With the help of the O-U cycle intermediates, a direct relation can be made between both the tricarboxylic acid and the glutamine/glutamate synthase cycles. The O-U cycle consequently signifies an important metabolic pathway for anaplerotic C fixation into nitrogenous compounds which play an important role in the algae growth [24].

3. Biogeochemical role of algae

At present, the working of the biogeochemical cycle is not totally misplaced. They are able to stay detained in one place for an extended period, and this habitation is known as a reservoir [25]. The study of chemical interactions between the atmosphere, hydrosphere (aquatic systems), lithosphere (crustal minerals), and biosphere (living organisms) is called *Biogeochemistry* [26]. In the plants and animals, the C is detained for a moderately small period of time in comparison with the coal deposits [27]. Among the various microorganisms, algae have played a significant function in Earth's biogeochemistry for billions of years and continue to do so today (Fig. 3). Among all the known algal species the cyanobacteria are considered as one of the ancient organism living on Earth's crust and it generates the required amount of oxygen in the Earth. They were also responsible for generating the fossil fuels by the C-rock formation and for generating the reserves for the fossil fuels [28]. When compared with the ancient algae, the modern algae produce half of the oxygen like which the plants and are also responsible for cycling of major elements such as sulfur (S), phosphorous (P), C, N and other trace elements. Hence in nature also these elements play a vital role in various interactions and controlling the atmospheric conditions [26].

Like many of the other microorganism's algae have also played a major role in shaping up with the earth's biogeochemistry and in the coming days these activities would be similar or may be higher and can be compared with the human activities [29]. Moreover 99.9% of the total biomass of the algae is accounted by 6 main elements such as C, N, S, P, O_2, and H_2 plus sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), chlorine (Cl), and silicon (Si) [25]. The leftover elements come about essentially in the form of trace elements for the reason that they are necessary for minor quantities. However, these elements also included into the organic matter and are ultimately used but they are done on a dissimilar time scale [29].

4. Factors involved in the growth of algae

4.1. Cultivation parameters

There are several factors which are required to measure the cultivation of algal biomass. Some of those factors include light (based upon intensity), C source and nutrient sources such as nitrates, phosphates, carbohydrates and other trace elements like manganese, cobalt, zinc, molybdenum etc. [29]. The other parameters involved are the optimal temperature, optimal pH, fine mixing in the photo-reactor, removal of O_2 and uptake of CO_2 in equal proportion [30]. The light, temperature, N, and P have a close association with growth rate and lipid content of the microalgae [31]. Hence these parameters should be maintained and controlled to be effective in reproducing the desired set of results.

4.2. Temperature

Temperature is also considered as a significant factor as well as a problematic parameter to optimize in large-scale outdoor culture systems such as the photo-bioreactor systems and the open pond cultivation system. Daily variations in the temperature can lead to significant decrease in the algal lipid efficiency [29]. These algae also show a decrease in cell volume with an increase in temperature. The optimal growth temperatures generally vary in between 20ºC to 30ºC [32,33]. When the light intensity changes the medium and high temperatures are an environmental aspect which ultimately affects the growth of microalgae [34]. Numerous algal species can withstand the temperatures up to 15ºC lesser than their best, with decreased growth rates, the temperatures higher than few degrees can lead to the death of an organism [33]. However, low evening and low seasonal temperatures significantly reduce the biomass productivity [35].

The acceptance choice of temperature varies with species. In the case of freshwater microalgae, for instance, *Scenedesmus and Chlorella*, are capable of adapting to the temperatures in the range of 5–35°C (ideal temperature range is 25–30°C), which must be brought back into an ideal temperature range in the course of mass cultivation [31]. If the temperature is not maintained optimally, the biochemical pathways inside the cells may lead to damage and there will be no proper accumulation of lipids inside the cells [36]. According to study conducted by Singh et al [37] some species such as *Chlorella*, *Nannochloropsis*, *Neochloris*, *Scenedesmus*, *Spirogyra*, *Chlamydomonas*, *Botrycoccus*, *Haematococcus*, *Ulva* species few red algae, brown algae and blue-green algae can grow in a temperature range of 20ºC-30ºC with the light intensity in the range of 33–400 mmol/m^2/s [38].

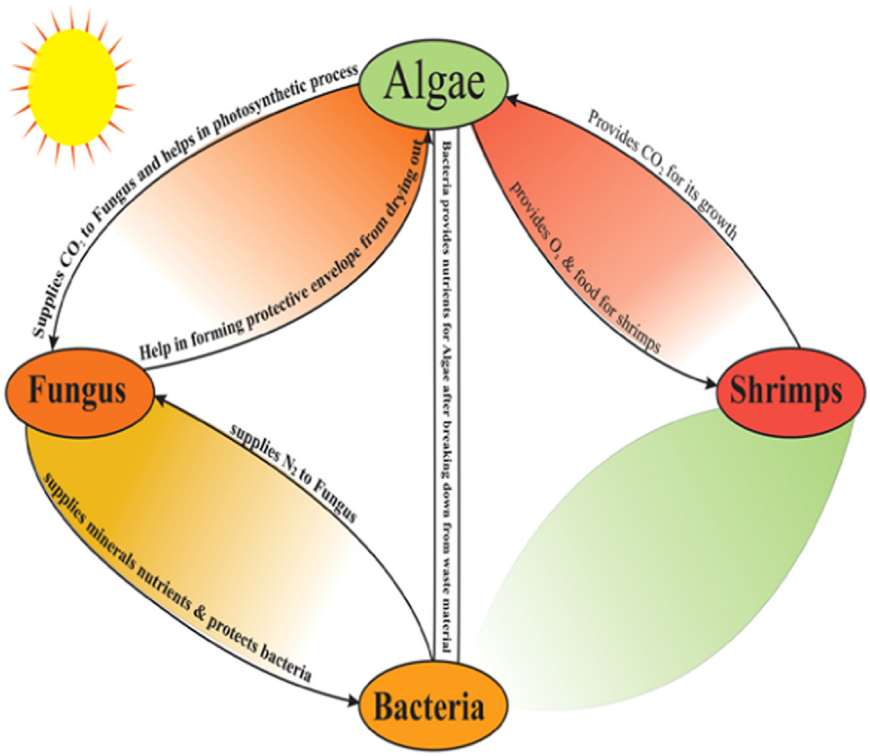

Fig. 3. Relation between algae and other organisms which are benefitted to the environmental process.

4.3. Salinity, nutrients, and pH

The requirements of various factors like salinity, nutrients, and pH are always dependent upon the type of organisms selected. For microalgal growth, the chief nutritional necessities are N and P. Certain diatoms require Si [39]. Salinity also affects the growth of algae. They have their own systems in adjusting the salinity array. In general, seawater microalgae are capable of tolerating higher salinity conditions when compared to the freshwater microalgae. Some studies have shown that algae need optimal salinity for growth. For instance, when the culture is provided with low salinity growth conditions, the situation will be supportive for the growth of algal by the addition of sodium chloride (NaCl) and sodium sulphate ($NaSO_4$). However, high salinity (> 6 g/L) will show the adverse effect and also inhibits the growth rate of microalgae [40].

The pH plays a major role in the growth of algae. Under alkaline conditions, microalgae will easily capture the CO_2 from the atmosphere and yield additional biomass [41]. The pH gradually increases to basic as the algal growth ensues and an instantaneous increase in photosynthesis and aggregation of OH^- ions occurs [42]. Under acidic pH conditions (when the pH is < 5), the mainstream of the dissolved inorganic carbon (DIC) is CO_2. On the other hand, change in pH can also impact the penetrability of the algae cell and the hydronium forms of the inorganic salt, and continuously effect the amalgamation of the inorganic salts [31].

For the growth of algae, nutrients are very important such as C, O_2, H_2, N, K, Mg, Ca, Fe, S, P, and trace minerals. The key nutrients are C, O_2, H_2, N, P, and K. The initial three, namely C, O_2, and H_2 are obtained from water and air and the last three, namely N, P, K have to be taken from the culture medium [29]. Throughout the farming, N, and P turn into the restrictive factors. They together participate in governing the lipid production and growth rate of microalgae. The growth, reproduction and further functional events of microalgae are strongly influenced by the N, which is one among the essential element. The P is one more necessary constituent aimed at the farming of microalgae. The metabolic processes of microalgae will be significantly influenced by the phosphate, hydrogen phosphate. Under nutrient-rich conditions, the mixotrophic Chlorophyceae members will show higher growth rate. On the whole, algae require very tiny quantity of P which is available in the system and can result in ~ 30% of P which is remaining as a residue in the culture [43]. In broad, algal growth has an undesirable association with lipid accumulation. Jacob-Lopes et al suggested that to avoid this concern, N famine cultivation state, and two-step cultivation has to be executed [44]. Prior to the experiment, one should be carefully aware of the various growth parameters which play a significant role in the accumulation of lipids which is the prime factor for increasing the lipid productivity. The optimum conditions which were considered during the experimental procedure that favored lipid accumulation, as well as the yield from different species, are given in Table 2.

4.4. Nutritional mode

The most common mode of nutrition for many algal species is the sunlight, CO_2 from the environment and glucose from the nutrient source. Organisms of this kind are called photoautotrophs [26]. Some species of algae can utilize pure carbon (glucose) for their growth and are called the heterotrophs or the mixotrophic. The main benefit of employing an organic C as the feed is that it reduces the reliance on the light provision, allowing growth of conservative fermenters in the dark. The most favourable growth factors should be maintained, to reach higher cell concentrations as well as to increase the volumetric productivity. The biomass and lipid productivities have been increased in the case of heterotrophic organisms when compared with the autotrophic [45]. Under mixotrophic circumstances, cell number will be amplified very quickly [46]. CO_2 is also one of the controlling factors and the reactant factor in the photosynthesis of microalgae and plants. Increasing CO_2 levels will improve the photosynthetic efficacy which leads to higher biomass yield.

Table 2
Different values of various growth parameters important and required for Algae.

Strain	Habitat	Nutrients	Biomass		Lipid		Ref.
			Biomass production (g/L/day)	yield (g/L)	Lipid production (g/L/day)	Total lipid extracted (wt% of biomass	
P. carterae	Marine water	Modified f/2 medium	0.22	n.a	0.072	n.a	[129]
D. salina	Marine water	j/2 medium	n.a	n.a	0.116	n.a	[129]
Porphyridium cruentum	Marine water	n.a	0.37	n.a	0.034	9.5	[130]
Tetraselmis suecica (F&M-M33)	Marine water	n.a	0.32	n.a	0.027	8.5	[130]
Tetraselmis sp. *(F&M-M34)*	Marine water	n.a	0.3	n.a	0.043	14.7	[130]
Tetraselmis. Suecica (F&M-M35)	Marine water	n.a	0.28	n.a	0.036	12.9	[130]
Phaeodactylum tricornutum (F&M-M40)	Marine water	n.a	0.24	n.a	0.044	18.7	[130]
Nannochloropsis sp. *(F&M-M26)*	Marine water	n.a	0.21	n.a	0.061	29.6	[130]
Nannochloropsis sp. *(F&M-M27)*	Marine water	n.a	0.2	n.a	0.048	24.4	[130]
Nannochloropsis sp. *(F&M-M24)*	Marine water	n.a	0.18	n.a	0.548	30.9	[130]
Nannochloropsis sp. *(F&M-M29)*	Marine water	n.a	0.17	n.a	0.037	21.6	[130]
Ellipsoidion sp. *(F&M-M31)*	Marine water	n.a	0.17	n.a	0.047	27.4	[130]
Nannochloropsis sp. *(F&M-M28)*	Marine water	n.a	0.17	n.a	0.06	35.7	[130]
Nannochloropsis (CS 246)	Marine water	n.a	0.17	n.a	0.049	29.2	[130]
Isochrysis sp.*(CS 177)*	Marine water	n.a	0.17	n.a	0.037	22.4	[130]
Pavlova salina (CS 49)	Marine water	n.a	0.16	n.a	0.049	30.9	[130]
Pavlova lutheri (CS 182)	Marine water	n.a	0.14	n.a	0.052	35.5	[130]
Isochrysis sp. *(F&M-M37)*	Marine water	n.a	0.14	n.a	0.037	27.4	[130]
Skeletonema sp. *(CS 252)*	Marine water	n.a	0.09	n.a	0.027	31.8	[130]
Thalassiosira pseudonana (CS 173)	Marine water	n.a	0.08	n.a	0.017	20.6	[130]
Skeletonema costatum (CS 181)	Marine water	n.a	0.08	n.a	0.017	21.1	[130]
Chaetoceros muelleri (F&M-M43)	Marine water	–	0.07	n.a	0.021	33.6	[130]
Chaetoceros calcitrans (CS 178)	Marine water	n.a	0.04	n.a	0.017	39.8	[130]
Chlorococcum sp. *(UMACC 112)*	Fresh water	n.a	0.28	n.a	0.053	19.3	[130]
Scenedesmus sp.	Fresh water	n.a	0.26	n.a	0.053	21.1	[130]
Chlorella sorokiniana (IAM-212)	Fresh water	n.a	0.23	n.a	0.044	19.3	[130]
Chlorella sp. *(F&M-M48)*	Fresh water	n.a	0.23	n.a	0.042	18.7	[130]
Scenedesmus sp. *(F&M-M19)*	Fresh water	n.a	0.21	n.a	0.04	19.6	[130]
Chlorella vulgaris (F&M-M49)	Fresh water	n.a	0.20	n.a	0.369	18.4	[130]
Scenedesmus quadricauda	Fresh water	n.a	0.19	n.a	0.351	18.4	[130]
Monodus subterraneus (UTEX 151)	Fresh water	n.a	0.19	n.a	0.03	16.1	[130]
Chlorella vulgaris (CCAP 211/11b)	Fresh water	n.a	0.17	n.a	0.032	19.2	[130]
Porphyridium cruentum	Marine water	n.a	1.5	1.90	n.a	n.a	[131]
Chlorella vulgaris	Fresh water	BBM	0.020	3.2	0.002	27	[132]
Aphanothece	Fresh water	BGN	0.031	5.0	0.005	8	[132]
Dunaliella	Marine water	ESAW	0.015	2.4	0.002	17.1	[132]
Phaeodactylum	Marine water	Ukeles	0.0003	0.150	0.000	6.1	[132]
Phormidium		BGN	0.017	2.8	0.002	11.7	[132]
Scenedesmus	Fresh water	BBM	0.027	4.3	0.003	14.1	[132]
Neochloris oleabundans	Fresh water	Bristol medium	0.15	0.09	0.038	56	[133]
Chlorella sp.	Marine water	Walne's nutrient medium	n.a	1.42	0.139	26	[134]
Chlorella vulgaris	Fresh water	Basal medium	0.254	1.69	0.054	38	[135,136]
B. braunii	Fresh water	modified chu13 medium	0.026	n.a	0.005	25.7	[137]
C. vulgaris	Fresh water	BG11 medium	0.104	n.a	0.006	11.9	[137]
Scenedesmus sp.	Fresh water	BG11 medium	0.217	n.a	0.020	11.9	[137]
Scenedesmus obliquus	Fresh water	N-deficient culture medium	0.09	2.0	n.a	35	[133]
Chlorella vulgaris	Fresh water	N-deficient culture medium	0.18	3.0	n.a	40	[133]
Neochloris oleoabundans	Fresh water	N-deficient culture medium,	0.09	2.1	n.a	35	[133]
Spirulina maxima	Fresh water	N-deficient culture nedium	0.21	3.1	n.a	9	[133]
N. oculata (NCTU-3)	Marine water	modified f/2 medium	0.48	n.a	0.142	29.7	[138]
N. oleoabundans	Fresh water	Bristol medium	0.19	1.96	0.004	16.5	[5]
S. obliquus	Fresh water	Bristol medium	0.26	1.87	0.03	12.5	[5]
Chaetoceros muelleri (F&M-M43)	Marine water	n.a	0.07	n.a	0.021	33.6	[139]
Chaetoceros calcitrans (CS 178)	Marine water	n.a	0.04	n.a	0.017	39.8	[139]
P. tricornutum (F&M-M 40)	Marine water	n.a	0.24	n.a	0.044	18.7	[139]
Skeletonomacostatum (CS 181)	Marine water	n.a	0.08	n.a	0.017	21	[139]
Skeletonoma sp.*(CS 252)*	Marine water	n.a	0.09	n.a	0.027	31.8	[139]
Thalassioria pseudonana (CS 173)	Marine water	n.a	0.08	n.a	0.017	20.6	[139]
Chlorella sp. *(F&M-M48)*	Fresh water	n.a	0.23	n.a	0.042	18.7	[139]
Chlorella sorokiniana (IAM-212)	Fresh water	n.a	0.23	n.a	0.044	19.3	[139]
Chlorella vulgaris (CCAP 211/11b)	Fresh water	n.a	0.17	n.a	0.032	19.2	[139]
C. vulgaris (F&M-M49)	Fresh water	n.a	0.20	n.a	0.036	18.4	[139]
Chlorococcum sp. *(UMACC 112)*	Fresh water	n.a	0.28	n.a	0.053	19.3	[139]
Scenedemus quadricauda	n.a	n.a	0.19	n.a	0.035	18.4	[139]
Scenedemus (F&M-M19)	Fresh water	n.a	0.21	n.a	0.040	19.6	[139]
Scenedemus sp. *DM*	Fresh water	n.a	0.26	n.a	0.053	21.1	[139]
T. suecica (F&M-M33)	Marine water	n.a	0.32	n.a	0.027	8.5	[139]
Tetraselmis sp. *(F&M-M34)*	Marine water	n.a	0.30	n.a	0.043	14.7	[139]

(*continued on next page*)

Table 2 (*continued*)

Strain	Habitat	Nutrients	Biomass		Lipid		Ref.
			Biomass production (g/L/day)	yield (g/L)	Lipid production (g/L/day)	Total lipid extracted (wt% of biomass	
T. suecica (F&M-M35)	Marine water	n.a	0.28	n.a	0.036	12.9	[139]
Ellipsoidion sp. *(F&M-M31)*	Marine water	n.a	0.17	n.a	0.047	27.4	[139]
Monodus subterraneus (UTEX 151)	Freshwater	n.a	0.19	n.a	0.030	16.1	[139]
Nannochloropsis sp. *(CS 246)*	Marine water	n.a	0.17	n.a	0.049	29.2	[139]
Nannochloropsis sp. *(F&M-M26)*	Marine water	n.a	0.21	n.a	0.061	29.6	[139]
Nannochloropsis sp. *(F&M-M27)*	Marine water	n.a	0.20	n.a	0.048	24.4	[139]
Nannochloropsis sp. *(F&M-M24)*	Marine water	n.a	0.18	n.a	0.054	30.9	[139]
Nannochloropsis sp. *(F&M-M29)*	Marine water	n.a	0.17	n.a	0.037	21.6	[139]
Nannochloropsis sp. *(F&M-M28)*	Marine water	n.a	0.17	n.a	0.060	35.7	[139]
Isochrysissp. ((T-ISO) CS 177)	Marine water	n.a	0.17	n.a	0.037	22.4	[139]
Isochrysissp. (F&M-M37)	Marine water	n.a	0.14	n.a	0.037	27.4	[139]
Pavlova salina (CS 49)	Marine water	n.a	0.16	n.a	0.049	30.9	[139]
Pavlova lutheri (CS 182)	Marine water	n.a	0.14	n.a	0.050	35.5	[139]
Porphyridium cruentum	Marine water	n.a	0.37	n.a	0.034	9.5	[139]
Chaetoceros muelleri	Marine water	n.a	0.07	n.a	0.021	33.6	[30]
Chaetoceros calcitrans	Marine water	n.a	0.04	n.a	0.017	16.4	[30]
Chlorella emersonii	Fresh water	n.a	0.041	n.a	0.050	25	[30]
Chlorella protothecoides	Fresh water	n.a	7	n.a	1.24	57	[30]
Chlorella sorokiniana	Fresh water	n.a	1.47	n.a	0.044	22	[30]
Chlorella vulgaris	Fresh water	n.a	0.20	n.a	0.04	58	[30]
Chlorella sp.	Fresh water	n.a	2.5	n.a	0.042	48	[30]
Chlorococcum sp.	Fresh water	n.a	0.28	n.a	0.53	19.3	[30]
Dunaliella salina	Marine water	n.a	0.34	n.a	0.116	25	[30]
Ellipsoidion sp.	n.a	n.a	0.17	n.a	0.047	27.4	[30]
Isochrysis sp.	Marine water	n.a	0.17	n.a	0.037	33	[30]
Monodus subterraneus	Fresh water	n.a	0.19	n.a	0.030	16	[30]
Nannochloris sp.	Marine/fresh Water/brackish	n.a	0.51	n.a	0.076	56	[30]
Nannochloropsis oculata	Brackish water	n.a	0.48	n.a	0.142	29.7	[30]
Nannochloropsis sp.	Marine/fresh Water/brackish	n.a	1.43	n.a	0.090	53	[30]
Pavlova salina	Marine water	n.a	0.16	n.a	0.049	30.9	[30]
Pavlova lutheri	Marine water	n.a	0.14	n.a	0.040	35.5	[30]
Phaeodactylum tricornutum	Marine water	n.a	1.9	n.a	0.044	57	[30]
Porphyridium cruentum	Marine water	n.a	1.50	n.a	0.034	60	[30]
Scenedesmus quadricauda	Fresh water	n.a	0.19	n.a	0.035	18.4	[30]
Scenedesmus sp.	Fresh water	n.a	0.26	n.a	0.053	21.1	[30]
Skeletonema sp	Marine water	n.a	0.09	n.a	0.027	31.8	[30]
Skeletonema costatum	Marine water	n.a	0.08	n.a	0.017	51.3	[30]
Thalassiosira pseudonana	Marine water	n.a	0.08	n.a	0.017	20.6	[30]
Tetraselmis suecica	Marine water	n.a	0.32	n.a	0.036	23	[30]
Tetraselmis sp.	Marine water	n.a	0.30	n.a	0.043	14.7	[30]
Scenedesmus sp.	Fresh water	BG 11	0.217	0.003	0.002	n.a	[138]
Botryococcus braunii	Fresh water	Modified chu13	0.026	n.a	0.005	0.005	[138]
Chlorella vulgaris	Fresh water	BG 11	0.104	n.a	n.a	0.020	[138]
Botryococcus sp.	Fresh water	n.a	0.035	n.a	0.011	n.a	[140]
Chlorella vulgaris	Fresh water	n.a	0.074	n.a	0.011	n.a	[140]
Scenedesmus sp	Fresh water	n.a	0.071	n.a	0.009	n.a	[140]
Scenedesmus sp.	Fresh water	50% BG 11	0.11	n.a	0.008	31–33	[141]
Botryococcus braunii	Fresh water	BG 11	n.a	0.037	n.a	13.5	[142]
Chlorella saccharophila	Fresh water	BG 11	n.a	0.002	n.a	18.10	[142]
Dunaliella tertiolecta	Marine water	Modified BG 11	n.a	0.038	n.a	15.20	[142]
Pleurochrysis carterae	Marine water	Modified BG 11	n.a	0.037	n.a	12	[142]
Consortium	Fresh water	BG 11	n.a	0.041	n.a	12.20	[142]
Chlorella Vulgaris	Fresh water	Artificial wastewater medium	n.a	0.69	0.147	42	[143]
Chlorella sp	Fresh water	Tris–acetate–phosphorus	0.92	1.07	0.200	n.a	[41]
Botryococcus braunii	Fresh water	Secondary domestic wastewater	0.034	0.48	n.a	36.14	[13]
T. suecica	Marine water	f/2 media	0.064	0.58	n.a	n.a	[38]
P. tricornutum	Marine water	f/2 media	0.018	0.26	n.a	n.a	[38]
C. calcitrans	Marine water	f/2 media	0.044	0.48	n.a	n.a	[38]
I. galbana	Marine water	f/2 media	0.024	0.57	n.a	n.a	[38]
N. oculata	Marine water	f/2 media	0.020	0.57	n.a	n.a	[38]
Chaetoceros muelleri (F&M-M43)	Marine water	n.a	0.07	n.a	0.021	33.6	[144]
Chaetoceros calcitrans	Marine water	n.a	0.04	n.a	0.017	39.8	[144]
P. tricornutum (F&M-M40)	Marine water	n.a	0.24	n.a	0.044	18.7	[144]
Skeletonema costatum (CS 181)	Marine water	n.a	0.08	n.a	0.017	21.0	[144]
Skeletonema sp. *(CS 252)*	Marine water	n.a	0.09	n.a	0.027	31.8	[144]
Thalassiosira pseudonana (CS 173)	Marine water	n.a	0.08	n.a	0.017	20.6	[144]
Chlorella sp. *(F&M-M48)*	Fresh water	n.a	0.23	n.a	0.042	18.7	[144]

(*continued on next page*)

Table 2 (*continued*)

Strain	Habitat	Nutrients	Biomass		Lipid		Ref.
			Biomass production (g/L/day)	yield (g/L)	Lipid production (g/L/day)	Total lipid extracted (wt% of biomass	
Chlorella sorokiniana (IAM-212)	Fresh water	n.a	0.23	n.a	0.044	19.3	[144]
Chlorella vulgaris (CCAP 211/11b)	Fresh water	n.a	0.17	n.a	0.032	19.2	[144]
C. vulgaris (F&M-M49)	Fresh water	n.a	0.20	n.a	0.036	18.4	[144]
Chlorococcum sp. *(UMACC 112)*	Fresh water	n.a	0.28	n.a	0.053	19.3	[144]
Scenedesmus quadricauda	Fresh water	n.a	0.19	n.a	0.035	18.4	[144]
Scenedesmus (F&M-M19)	Fresh water	n.a	0.21	n.a	0.040	19.6	[144]
Scenedesmus sp. *DM*	Fresh water	n.a	0.26	n.a	0.053	21.1	[144]
Tetraselmis suecica (F&M-M33)	Marine water	n.a	0.32	n.a	0.027	8.5	[144]
Tetraselmis sp. *(F&M-M34)*	Marine water	n.a	0.30	n.a	0.043	14.7	[144]
T. suecica (F&M-M35)	Marine water	n.a	0.28	n.a	0.036	12.9	[144]
Ellipsoidion sp. *(F&M-M31)*	Marine water	n.a	0.17	n.a	0.047	27.4	[144]
Monodus subterraneus	Fresh water	n.a	0.19	n.a	0.030	16.1	[144]
Nannochloropsis sp. *(CS 246)*	Marine water	n.a	0.17	n.a	0.049	29.2	[144]
Nannochloropsis sp. *(F&M-M26)*	Marine water	n.a	0.21	n.a	0.061	29.6	[144]
Nannochloropsis sp. *(F&M-M27)*	Marine water	n.a	0.20	n.a	0.048	24.4	[144]
Nannochloropsis sp. *(F&M-M24)*	Marine water	n.a	0.18	n.a	0.054	30.9	[144]
Nannochloropsis sp. *(F&M-M29)*	Marine water	n.a	0.17	n.a	0.376	21.6	[144]
Nannochloropsis sp. *(F&M-M280*	Marine water	n.a	0.17	n.a	0.060	35.7	[144]
Isochrysis sp. *(T-ISO) CS 177)*	Marine water	n.a	0.17	n.a	0.037	22.4	[144]
Isochrysis sp. *(F&M-M37)*	Marine water	n.a	0.14	n.a	0.037	27.4	[144]
Pavlova salina (CS 49)	Marine water	n.a	0.16	n.a	0.049	30.9	[144]
Pavlova lutheri (CS 182)	Marine water	n.a	0.14	n.a	0.050	35.5	[144]
Porphyridium cruentum	Marine water	n.a	0.37	n.a	0.034	9.5	[144]
Scenedesmus sp. *(LX1)*	Fresh water	BG11 medium	n.a	313[a]	112[b]	n.a	[141]
Chlorella emersonii	Terrestrial	M7 medium	3.7	37.4	n.a	n.a	[145]
Botrycoccus braunii	Fresh water	M7 medium	4.6	36.1	n.a	n.a	[145]
S. obliquus (YSL02)	Fresh water	Bold basal medium	1.84	n.a	0.53	29	[146]
Chla. Pitschmannii (YSL03)	Fresh water	Bold basal medium	1.04	n.a	0.54	51	[146]
C. vulgaris (YSL04)	Fresh water	Bold basal medium	1.65	n.a	0.44	26	[146]
S. obliquus (YSL05)	Fresh water	Bold basal medium	1.71	n.a	0.48	28	[146]
Chla. Mexicana (YSL07)	Fresh water	Bold basal medium	1.53	n.a	0.45	29	[146]
C. vulgaris (2714)	Fresh water	Modified culture medium	0.39	1.17	0.16	40	[147]
Chlorella vulgaris	Fresh water	N11 medium	n.a	0.31	0.171	55	[148]
Chlamydomonas reinhardtii	Fresh water	n.a	2.0	n.a	0.505	25.25	[113]
Scenedesmus obliquus	Fresh water	n.a	0.026	n.a	0.008	31.14	[113]
Botryococcus braunii	Fresh water	n.a	0.345	n.a	0.062	17	[113]
Chlorella vulgaris	Fresh water	Kessler and czygan	0.16	n.a	0.034	30	[149]
Scenedesmus obliquus	Fresh water	Kessler and czygan	0.25	n.a	0.041	60	[149]
Ellipsoidion Parvum	Fresh water	Kessler and czygan	0.09	n.a	0.111	n.a	[149]
C. oleofaciens	n.a	BG11 medium	0.20	n.a	0.035	n.a	[149]
H. pluvialis	Fresh water	BG11 medium	0.06	n.a	0.020	n.a	[149]
B. braunii	Fresh water	BG11 medium	0.03	n.a	0.43	40	[149]
Scenedesmussp	Fresh water	TAP media	0.080	n.a	0.030	n.a	[150]
Chlamydomonas debaryana	Fresh water/ Terrestrial	B3NV media	0.051	n.a	0.005	n.a	[150]
Chlorella sorokiniana-(FGP5)	Fresh water	n.a	0.030	n.a	0.003	n.a	[150]
Nannochloropsis sp	Marine/ Freshwater	n.a	0.21	n.a	0.061	29.6	[151]
Chlorella vulgaris	Fresh water	Thin stillage (TS)	2.5	9.8	1.1	43	[152]
Chlorella vulgaris	Fresh water	Soy whey (SW)	1.6	6.3	0.2	11	[152]
Chlorella vulgaris	Fresh water	Modified basal medium	2.0	8.0	0.6	27	[152]
B. braunii (AP103)	Fresh water	Modified chu13 medium	0.114	1.8	n.a	19	[153]
T. variabilis	Brackish water	BG110	0.040	0.020	n.a	12.1	[154]
A. augstumalis	n.a	BG110	0.017	0.008	n.a	3.2	[154]
P. autumnale	n.a	Modified BG11	0.055	0.027	n.a	2.4	[154]
Nannochloropsis Oculata	Marine water	n.a	0.004	0.002	n.a	15.1	[154]
Spirulina sp	Fresh water	Zarrouk's medium	1.37	n.a	0.66	20	[155]
Chlorella sp.	Fresh water	BBM medium	1.65	n.a	0.74	26	[155]
Amphora sp.	Marine water	n.a	0.16	n.a	0.037	24	[156]
Chlorella vulgaris	Fresh water	n.a	0.46	n.a	0.079	17.3	[156]
Chlorella salina	Marine water	n.a	0.17	n.a	0.0182	11	[156]
Chlorella protothecoides	Terrestrial	n.a	0.25	n.a	0.045	18	[156]
Chlorella emersonii	Terrestrial	n.a	0.29	n.a	0.054	18.6	[156]
Scenedesmus sp.	Fresh water	n.a	0.10	n.a	0.015	16	[156]
Ankistrodesmus sp.	n.a	n.a	0.09	n.a	0.015	17.5	[156]
Chlamydomonas reinhardtii	Fresh water	n.a	0.05	n.a	0.009	18.9	[156]
D. salina (Shariati)	Marine water	n.a	0.05	n.a	0.010	18.9	[156]
Dunaliellasp	Marine water	n.a	0.12	n.a	0.025	22	[156]
D. salina (UTEX 200)	Marine water	n.a	0.15	n.a	0.036	24	[156]
Chlorella pyrenoidosa	Fresh water	Bold's basal medium	0.106	n.a	0.019	29.68	[157]

(*continued on next page*)

Table 2 (*continued*)

Strain	Habitat	Nutrients	Biomass		Lipid		Ref.
			Biomass production (g/L/day)	yield (g/L)	Lipid production (g/L/day)	Total lipid extracted (wt% of biomass	
Isochrysis galbana	Marine	Enriched artificial seawater with f/2 medium	0.51	1.51	0.070	16.47	[158]
Kirchneriella lunaris	Fresh water	BG11 medium	0.293	n.a	0.008	n.a	[159]
Selenastrum capricornutum	Fresh water	BG11 medium	0.097	n.a	0.006	n.a	[159]
Staursatrum sp.	Fresh water	BG11 medium	0.078	n.a	0.000	n.a	[159]
Chlorella vulgaris	Fresh water	BG11 medium	0.225	n.a	0.007	n.a	[159]
Scenedesmus obliqnus	Fresh water	BG11 medium	0.206	n.a	0.006	n.a	[159]
Navicula sp.	Fresh water	D1 medium	0.071	n.a	0.003	n.a	[159]
Phaeodactylum tricornutum	Fresh water	f/2 medium	0.256	n.a	0.026	61.43	[159]
Batrachospermum Sirodotia	Fresh water	BG11 medium	0.049	n.a	0.001	n.a	[159]
Lyngbya kuetzingii	Fresh water	BG11 medium	0.234	n.a	0.007	n.a	[159]
Isochrysis sphacrica	Fresh water	f/2 medium	0.255	n.a	0.008	n.a	[159]
Microcystis aeruginosa (NPCD-1)	Fresh water	n.a	0.046	13.1	0.013	28	[160]
Synechococcus sp. *(PCC7942)*	Marine water	n.a	0.052	n.a	0.014	26.9	[160]
Trichormus sp. *(CENA77)*	Soil, subglacial	n.a	0.030	n.a	0.007	23.7	[160]
Chlorella protothecoides	Terrestrial	Bristol's and Wu's Media culture medium	8.7	43.3	4.32	42.3	[161]
Ettlia sp. *(YC001)*	Fresh water	BG11 Agar medium	0.19	3.10	0.080	42	[162]
Aurantiochytrium sp. *(KRS101)*	Marine water	Defined medium	6.69	31.8	n.a	38.1	[163]
Chlorella protothecoides	Terrestrial	Basal culture medium		0.5	0.015	27	[164]
Endogenous Chlorella sp	n.a	Brewery wastewater	n.a	2.7	0.052	23	[14]
Chlorella vulgaris (UTEX-265)	Fresh water	TAP medium	n.a	3.5	0.108	42	[14]
Ettliatexensis	Fresh water	Bold's basal medium	0.92	0.459	0.322	35	[15]
Synechococcus sp. *(PCC7942)*	Marine water	BG-11 liquid medium	0.124	n.a	0.35	29	[165]
Chlorella sp. *(KMN1)*	Marine water	Bold's basal medium	0.022	0.96	0.26	27.11	[166]
Chlorella sp. *(KMN2)*	Marine water	Bold's basal medium	0.016	0.79	0.24	31.52	[166]
Chlorella sp. *(KMN3)*	Marine water	Bold's basal medium	0.043	1.59	0.31	20.27	[166]
Scenedesmus sp. *(KMN4)*	Marine water	Bold's basal medium	0.023	0.92	0.25	28.63	[166]
Monoraphidium sp. *(KMN5)*	Marine water	Bold's basal medium	0.013	0.65	0.23	34.93	[166]
Chlorococcum sp. *(IMMTCC-1)*	Fresh water	Bold basal medium	n.a	n.a	2.095	8.7	[167]
Chlorella sp. *(IMMTCC-2)*	Fresh water	Bold basal medium	n.a	n.a	4.071	22.7	[167]
Scenedesmus sp. *(IMMTCC-3)*	Fresh water	Bold basal medium	n.a	n.a	2.429	11.04	[167]
Scenedesmus sp. *(IMMTCC-7)*	Fresh water	Bold basal medium	n.a	n.a	2.786	14.6	[167]
Chlorella sp. *(IMMTCC-8)*	Fresh water	Bold basal medium	n.a	n.a	1.167	6.1	[167]
Chlorella sp. *(IMMTCC-9)*	Fresh water	Bold basal medium	n.a	n.a	0.857	4.9	[167]
Micractinium sp. *(ME05)*	Fresh water	BG-11 medium	0.47	0.93	0.05	10.7	[168]
H. tetrachotoma (ME03)	n.a	BG-11 medium	0.04	0.31	0.01	8.7	[168]
Scenedesmus sp. *(ME02)*	Fresh water	BG-11 medium	0.06	0.12	0.004	12.3	[168]
Ettlia sp.	Fresh water	Sugar factory wastewater	n.a	8.02	0.96	42	[46]
I. galbana	Marine water	f/2 medium	n.a	0.001	0.013	21	[36]
P. tricornutum	Marine water	f/2 medium	n.a	0.001	0.017	28	[36]

Note: n.a – not available.
[a] – per g biomass.
[b] – per g lipid.

5. Techniques involved in the processing of algae

5.1. Harvesting techniques

Harvesting is defined as a sequence of process for eliminating water content from the growth culture of algae with the help of various downstream techniques available [47]. It can be also defined as diluting the concentrated microalgal culture or suspension to slurry or paste. Keeping in mind the cost of extraction, the downstream processes must be reduced for an efficient extraction process [48]. The basic process for harvesting is the collection of individual algae cells or the medium upon which they are grown which are analyzed [23]. Generally, the most widespread harvesting processes include screening, coagulation, flocculation, flotation, sedimentation, filtration, and centrifugation [23]. On the other hand, there are also other techniques like electrophoresis, electroflotation, and ultrasound which are of less importance [49]. Hence the selection of harvesting procedure should be based on energy efficiency and cost factor.

An efficient harvesting technique should take into consideration various parameters of algae like size and density, in order to achieve a higher yield of biomass, with less operating cost [50]. However, while designing an efficient harvesting technique a few points should be given importance.

- The choice of microalgae and the desired products
- A complete cell separation process for efficient recycling, that contributes to the low cost of down streaming processing.
- The chosen technique should have minimal impact on the further processes [51].

The harvesting process involves two steps

a) **Bulk harvesting**: A bulk suspension of biomass undergoes sedimentation.
b) **Thickening**: This process is done to separate the biomass and to concentrate the slurry matter with the help of filtration and centrifugation.

Many of the current harvesting techniques have numerous drawbacks which have an impact on the cost and quality of the products.

Harvesting is a challenging task when biofuel production is done on a commercial scale [52]. The reason for high cost for biofuel production is mainly due to these harvesting techniques. They account for about 20–30% of the total cost of algal biomass [52]. Though there are many harvesting techniques, till date no specific technique has been recommended for filamentous algae owing to their structural organisation.

5.1.1. Centrifugation

Centrifugation is a method in which separation of two immiscible liquids takes place with the help of centripetal force. The particle size and density are two crucial factors in a centrifugation process. So much research was done on centrifugation techniques an interesting result was found [34]. Heasman et al [49] stated that 90–100% harvesting effectiveness can be attained via centrifugation. Sim et al [53] reported that centrifugation is the most efficient technique but highly costly when used for production on a commercial scale. Generally, this type of technique is applied for the production of secondary metabolites [52]. When the algal culture is separated by centrifugation, high gravitational force and shear stresses are applied in the process that might damage the cell structure.

The various types of centrifugation techniques or systems available are as follows:

- Hydro-cyclone
- Solid bowl decanter
- Nozzle type
- Solid ejecting disc

5.1.2. Flocculation

It is a method in which scattered units are collected together to form huge units and made to settle down. This method is an extensively used technique in diverse activities ranging from brewing to wastewater treatment and mining etc. [54]. In recent years' various flocculation techniques have been explored ranging from chemical flocculation to the latest bio-flocculation. In chemical flocculation process, several multivalent metal particles like ferric chloride ($FeCl_3$), ferric sulphate ($Fe_2(SO_4)_3$), aluminium chloride and aluminium sulphate which is commonly known as alums are present which interfere with the harvesting procedures [23,55]. During the process, these particles stay on the surface of the biomass and interfere with the extraction of lipids. When compared with the synthetic flocculants, natural flocculants are safer to act together with the negative surface of the cells [56]. A good example for flocculant is chitosan which is very effective but works only at very acidic pH. Therefore, cationic starch which is independent of pH and charge is considered as a substitute for chitosan [57].

5.1.2.1. Bioflocculation. This process occurs in the lakes or ponds spontaneously because of the extracellular polymer substance. However, this particular mechanism is poorly understood and a lot of extensive research needs to be carried out [58]. This method is cost and energy efficient alternative harvesting method. This is usually used in the process of treating wastewaters [55]. Flocculation can also be influenced by the rapid increase in the pH, temperature or nutrient depletion, and changes in dissolved oxygen. Generally, these techniques are not used for pre-harvesting. When this process of flocculation is carried along with the mixed bacterial source then an extra energy should be invested in supplying nutrients otherwise there could be contamination [56,58].

5.1.2.2. Auto flocculation. This process automatically occurs at basic pH due to CO_2 depletion. The major precipitates which are formed in the auto-flocculation process are Ca and Mg precipitates [58]. Since these Ca surfaces are positively charged they can interact easily with the negatively charged surfaces of the algal cells which results in the reserves for the phosphate sources and making the surfaces of the algal cells active. Hence microalgae can be used in the treatment of wastewater for the removal of excess phosphate [59]. When the pH is high flocculation is caused due to the formation of inorganic precipitates, thus after harvesting biomass consists of the excess amount of minerals. This process is performed by the addition of metallic salts, an alkaline compound, or polyelectrolytes. The alkaline compounds such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$), or magnesium hydroxide ($Mg(OH)_2$) cause biomass accumulation [60]. However, the flocculation is reliant on the microalgae cell density logarithm. It is not linearly related to the quantity of algae biomass [61]. During the process, the pH possibly will vary and will influence the further downstream of the biomass processing.

The metal ions like Mg^{2+} and Ca^{2+} play an important role in flocculation process after increasing the pH [58]. During the growth of the species in the medium, it was found that the negatively charged bodies were hydrolysed into positive precipitates by the process of sweeping flocculation. In contrast to an important role in flocculation process by increasing pH, there is also a possibility that the ions have played a crucial part in decreasing pH [62].

The mechanism of flocculation is dependent upon the physico-chemical properties of microalgae cells. Since the surface of the microalgae cells is negatively charged, the zeta potential of microalgae is explored during the flocculation process [58]. The zeta potentials show a quick rise from a range of pH 6.5–4.0 and also the equivalent flocculation efficacies also increase to the highest with the drop in pH [60]. When the pH is greater than 6.0, the surface charge of the microalgae cells is subjected to the neutral amine groups ($R\text{-}NH_2$) and the negatively charged ions (carboxylate) [60]. The carboxylate ions would accept the protons (H^+), at that moment the surface charge is reduced and the algae cells become unstable and are clotted to form big flocks [55]. The flocculation mechanisms are at the maximum when the surface charge of the algae cells is completely neutralized. As soon as the pH values drop from 4.0 to 1.5, the zeta potential constantly increases with equivalent flocculation [60]. In the exponential growth phase, the biomass is high whereas in lag phase it remains low [62]. In stationary phase as well as the exponential phase the cells form clumps and clusters. Since the surfaces of the cells are neutralized the heavier cells settle easily when compared to the single cells. Hence the flocculation efficiencies are greater with the rise in biomass concentration [62]. Some of the prominent flocculants and their optimum pH are listed in Table 3. The Flocculation process varies with different inorganic and organic salts and is classified based on their chemical composition.

5.1.2.3. Inorganic flocculation. As we know that the cells of the microalgae are negatively charged and the ions in the chemicals interact with these, hence disrupting the algae cells resulting in successful harvesting [60]. The flocculation of the microalgae occurs at a considerably low pH. The flocculants with high charge density are considered to be the best flocculants. Among this Alum is the best flocculant in operation during the wastewater treatment, but the only disadvantage is that it may hinder the impurities during the lipid extraction stage [55,56]. To avoid this problem any negatively charged

Table 3
Some of the prominent flocculants and their optimum pH.

S. No	Flocculant	Type of ion	Optimal pH	Water system	References
1.	Alum	Polyvalent Metal Ion	5.3–5.6	Wastewater system	[169]
2.	Lime Treatment Precipitation	Positively charged metal hydroxide precipitates	10.5–11.5	Wastewater systems	[170] [169]

surfaces such as Iron or Aluminium are added to the surface of the microalgal cells to neutralise the medium [63].

5.1.2.4. Organic flocculation. The organic flocculants are otherwise known as polyelectrolytes. The strength of these polymers is dependent on certain properties like the charge of the cells, pH, the concentration of biomass. The high concentration of biomass will help in the flocculation process. Mixing of the biomass at low concentrations brings the cells together, but if the shear forces are high it can disrupt the flocs [63]. Apart from these the functional charges on the algal cell walls play an important role in the formation of the negative charge centers on the cell surface. The size of the floc in an organic sedimentation should be greater than 100 μm for efficient flocculation. Pushparaj et al [64] have shown that the aluminium sulphate ($Al_2(SO_4)_3$) was effective, meant for successful flocculation for algal harvesting. The most successful flocculants for the revival of algal species are the cationic flocculants since they would be attracted to the cells of algae which are negatively charged. Chitosan is considered as the best biodegradable bioflocculant which is used in the water purification process. Since this Chitosan is too expensive, the economically feasible polymeric flocculants are generally used in flocculating the algae species [63].

5.1.2.5. Combined flocculation. The combined flocculation process involves the combination of two or more different flocculants. Sukenik et al [65] studied that marine algae have found two existing different methods for inducing two different flocculation methods. Different low concentrations of chitosans in combination with inorganic flocculants like ($FeCl_3$, Alum etc.) are used. During the experiment, flocculant dosages were reduced. The Ferric chloride dosage was reduced from 100 mg/L to 50 mg/L without/with addition (2.5 mg/L) of chitosan. This was observed in species of *Isochrysis galbana*. In the same way for *C. stigmatophora*, the concentration was also reduced to 50% upon addition of $FeCl_3$. Chitosan played a key role in decreasing the algal flocculation. When Chitosan in combination with $FeCl_3$ did not improve algal removal. The reason for this is that Chitosan molecules fail to bridge between these particles at a high ionic strength which reduces the charge, which also reduces the effectiveness dose [65].

5.1.2.6. Electrolytic process. In this process, the movement of microalgae is subject to the movement of charges from the anode to cathode and they form aggregates [66,67]. This process basically involves three different mechanisms:

- Coagulants production by the electrolytic oxidation of the sacrificial electrode.
- Deterioration of particulate suspension and breaking of the emulsion.
- Destabilized phase accumulation which can form flocs.

Based on the existing literature [66], it was shown that when there is electrolytic flocculation applied there is an approximately 80–95% of algae is removed [67].

5.1.3. Gravity sedimentation

Technically this is a method where the heavier algal particles settle down to the bottom. Hence we can easily detect the lighter and the heavier algal particles separately. The major factors which decide and influence the gravity sedimentation are the radius and density of the algal cells. This technique is generally used in separating algae in wastewater applications. Edzwald [68] revealed the fact that the low mass microalgal units do not resolve well and are not efficiently separated by the settling process. Some of the important factors which affect the settling of algal particles are

- Height
- Time of settling
- The specific gravity suspension and
- The specific gravity of particles [69].

5.1.4. Filtration

It is a mechanical or physical process which is used for separating solids from liquids or gasses by interposing a medium through which fluid can only pass [23]. For this process filters of particular pore size called micro, strainers are used [54,70]. These are used as rotating filters with frequent backwash. This filtration technique is usually used in filtering largely sized algae such as filamentous species. This process is not appropriate for microalgae like *Chlorella*, *Dunaliella*, *Scenedesmus* etc. Several kinds of filtration processes, such as vacuum filtration, micro-filtration, ultra-filtration, dead-end filtration, pressure filtration, and tangential flow filtration (TFF) [71]. According to the recent studies, it is shown that TFF and pressure filtration can be used effectively since they consume less energy. The major drawback of this filtration is the frequent change of filters and membranes which makes the process economically not viable [70].

5.1.5. Microstrainers

Microstrainers are widely used to separate algae from water streams of reservoirs before treating the water. Typically, a centrifugal micro strainer consists of a sealed tubular container with a tubular screen attached, with together revolving about an empty tube making a shared central axis [50]. The solid-liquid mixture is passed through the tube and is operated centrifugally. The mesh size range is 15–64 μm which allow only very fine particles. The suspended particles which are very large in size cannot pass through, remain on its external side.

The major advantages of these micro strainers micro strainers are:

- The simplicity of manufacturing and functioning
- Ease of operation.
- Low investment.
- Low energy consumption
- High filtration ratios.

The major problems encountered while using these micro strainers are:

- Solids are not removed completely.
- Handling of solids is difficult during fluctuations.
- Algae and bacterial films forms on the surface of the micro fabric.

During the filtration process if the concentration of algae is very high then these concentrated algae can block the screen whereas the smaller algae can be run off through the screen very easily [50]. Petruvski et al [71] have recovered a maximum of 70–90% of the freshwater algae through the tangential flow filtration. The main benefit of this filtration process is that it retains the structure, motility, and properties of the collected algae.

5.1.6. Electrophoresis process

It is defined as the movement of charged particles from one end to the other end under the influence of an electric field, which pushes the charged algae to shift out of the tray. This technique is another method which efficiently utilizes and separates the algae without adding any chemicals. The major advantages of this technique are environmental compatibility, adaptability, energy effectiveness, safety, selectivity and price effectiveness [72].

The key factors for separation of the particles in the electrophoresis include:

- Sample
- Electric Field

- Medium
- Buffer

Generally, the algal particles are negatively charged. Throughout the process when a tray of electrophoresis consists of the growth medium an electric current is passed. Pearsall et al. [73] conducted a comparable kind of experiment and proved that the effects of electrophoresis are complex due to its fluid motion. During the fluid movement, it appears that the algal cell and liquid activity can be adequately strong which can pressurize the fluid movement or lead the performance of the fluid. Sandbank et al. [74] have shown that extensive variety of microalgae species were harvested by the electroflotation method with 5% of solids harvested in algae.

5.1.7. Ultra-Sonication

It is defined as an act of employing energy associated with the vibration of matter (well known as sound energy) to disturb particles in a sample. The frequency of > 20 kHz is generally used. Bosma et al. [75] successfully applied this technique to demonstrate that the ultrasonication method is an advantageous method. In this, the efficiencies were higher than 90% and the flow rate varied between 4 and 6 L/day. Li et al. [41] have shown that ultrasound waves could improve the removal by the coagulation of one of the toxic algae namely *Microcystis aeruginosa*. The major process involved in the destruction of algal cells in this process is the ultrasonic irradiation of gas vacuoles. The algal cells perform as “nuclei” for acoustic cavitation and fall down in the course of “bubble crush” period, which resulted in the settlement. Collection and absorption of microalgae biomass cost highly to the overall procedure of cultivation. Therefore, much more resourceful and profitable harvesting technologies have to be developed to improve the commercial as well as for the industrial capacity [76].

5.2. Lipid extraction techniques

In recent years, microalgae are treated as the most potential and valuable feedstock for the source of biofuels. Various products such as bio-ethanol, biodiesel, bio-butanol, bio-methane (bio-CH_4), jet fuel, biohydrogen (bio-H_2), and thermo chemical transformation products such as bio-oil, biocrude, and syngas are feasibly produced from microalgae [23]. Globally huge amount of study is being carried out in improving the lipid yield of microalgae. This lipid extraction is considered as one of the most important processes for extracting biodiesel from microalgae. In the development of algal lipids, there are many techniques available for the extraction of algae. We will discuss those techniques in brief with more emphasis on the cost-effective methods.

In practical, the extraction of lipids from the algal sources has been divided into two major parts like

a) Lipid extraction by chemicals and solvents.
b) Lipid extraction by a mechanical process.

5.2.1. Chemical method of extraction

5.2.1.1. Folch method. This is the most popular extraction technique, in which solvents are used to homogenize the cells [77]. Several organic solvents or mixture of different solvents have been recommended to selectively extract lipids from microalgae. The most common combination used is chloroform and methanol in the ratio of 2:1 to a final volume twenty times (10 g sample in the 200 ml of solvent mixture) the volume of the algae cell for the extraction of lipids [78]. After the addition of sample to the solvent mixture, the whole mixture is exposed to gentle agitation (around 20–25 min) followed by centrifugation (2000 rpm) or filtration (with a funnel and folded filter paper) to recover the liquid phase [78]. In some conditions, dichloromethane also used instead of chloroform [79]. Fast and easy handling of huge quantity of samples is the key advantage of this method. Various other advantages and disadvantages are listed in the Table 4.

5.2.1.2. Bligh and Dyer method. This technique is quite similar to the folch method but majorly varies in the ratio of solvents to the tissues [80]. In this method, the proteins from the sample are precipitated between the two lipid phases, both lipid extraction and partitioning are accomplished simultaneously. The combination of the solvent can also change based on the polarity of the lipids present in the species of algae. Apart from the main technique by the Bligh and Dyer, many researchers globally have also modified depending on the situation and the condition of the laboratories. The most common method used for modification was done by the [81]. They have shown that they have replaced water with 1 M NaCl so that it can evade attachment of acidic-lipids to denatured-lipids [82]. From the previous extraction procedures, it was also known that by adding a salt solution, the yield of lipid improves with a shorter separation time. In the same way, adding 0.5% of CH_3COOH to the water-phase improved the retrieval of acidic-phospholipids. Among the above processes, Hajra [81] method was found to be the most efficient process which has been demonstrated till now.

Regarding various advantages and disadvantages of the above methods, many results were discussed for Folch and Bligh & Dyer methods. Many of them published results, which were neither validated nor described. Many researchers even indicated the modifications which were made. However, the research conducted by Iverson [83] have shown that Bligh and Dyer's procedure is more effective and reliable. The results obtained from Bligh and Dyer had identical lipid samples when compared with the Folch method where the tissue sample lipid was taken (< 2%). After many experiments conducted by many researchers, they have concluded that both the methods employed gave or produced accurate results out of which some of them have used high sample/solvent ratio, which could not alter the lipid content significantly. If the samples contained a greater amount of lipid then in such samples an increase solvent/sample ratio is necessary for quantitative lipid evaluation [83]. Various other advantages and disadvantages are listed in the Table 4.

Later many researchers have tried to modify the solvents used in the procedure of Bligh and Dyer. Considering the Chloroform solvent as a toxic substance [84] were the first to substitute (or) change the solvent system to hexane-isopropanol. This result showed the lower efficiency of extracted substance (lipid). Many years later after a careful examination of the solvent few researchers replaced the chloroform-methanol to heptane and ethyl acetate mixture [85]. In the point of environmental protection, these hazardous chemical combinations should be replaced by bio solvents [77]. The basic method involves the addition of 1 M NaCl instead of water which helps in preventing the binding of acidic lipids to denatured lipids. Furthermore, the addition of 0.2 M phosphoric acid and hydrochloric acid. Similarly, the addition of 0.5% acetic acid to water increases the recovery of acidic phospholipids. Finally, they have concluded that Hajras method was the most efficient procedure for extraction.

5.2.2. Mechanical method of extraction

5.2.2.1. Expeller press. The expeller press is considered as the oldest way for extraction of oil from algae. This is easy yet efficient mechanical crushing process. The main principle involved in this technique is, by applying high pressure the oil content in the dried or wet biomass is out by breaking the cells. The application of the pressure should not be that much high, otherwise, the lipid quality, as well as the quantity, gets decreased [84]. The expeller press works in the following way; A screw type machine was used which presses the dried biomass. The dried biomass entered through one side and the product was exited

Table 4
Advantages and disadvantages of Folch and Bligh & Dyer methods.

Method	Advantages	Disadvantages
Folch Method	• It is a standard method. • Well established method to determine lipids.	• Adverse effects of chloroform on the environment. • It is a laborious process/method.
Bligh and Dyer Method	• Standard and simple method. • It can determine total lipids. • Samples can be analyzed directly without pre drying.	• Adverse effects of chloroform on the environment. • It is a laborious process/method.

from the other side of the press. Continuous pressure and friction were applied for compressing the algae dried biomass. Upon applying the high pressure, the oil flows through the small openings which allow only the oil to flow through instead of allowing other components. During the application of a high-pressure temperature of 60–100ºC is reached [84].

5.2.2.2. Bead beating. Bead beating method is another technique where the algal cells are broken by the high-speed mechanism techniques [86]. The most common instruments used in this technique are the vibrating containers and agitated beads [87]. In the primary method (vibrating or shaking containers), the cells get damaged by shaking in the complete container. Another technique followed in the bead beating is the agitated bead method where the entire culture gets agitated [86]. During this process, there is a lot of generation of heat which makes the instrument to release more heat. To reduce such heat, the instrument is surrounded by the cooling jackets, hence it protects the sensitive biomolecules during the process. Lee et al. [87] have shown that the material of the bead inside the vessel also affects the lipid productivity of the oil produced. Hence he had shown that optimal size for the cells of the algae as well as the beads are in the size of 0.5 mm can increase their hardness and density [87].

5.2.2.3. Ultra-sonication extraction. This is an alternative method for the extraction which can be easily used. This process is furthermore economical as well eco-friendly because it can be finished within a shorter phase of time with high reproducibility [86]. The Ultrasonication process involves the passing of sound waves which propagate into the liquid which results in the alternating low and high pressure generated or created. During this process, the bubbles generated attain a specific size and collapse violently. This process is known as cavitation [88]. During the cavitation process, microbubbles are produced hence an ultrasound is applied which breaks the cell components of the algae. The key benefit of the ultra-sonication would be that it utilizes low temperature in breaking the cell walls when compared with the heat generating equipment's like autoclave, microwave oven etc. [88]. Further, this technique does not involve the chemicals or any other kind of stuff which in general are to be removed later. The removal of these components is again a burden involving high cost as well as high labour. One of the recent developments in the field of ultrasound extraction is the "ultrasound assisted extraction". Generally, a frequency of 20–100 kHz is used for common ultrasound processing. This frequency can be used to produce high acoustic pressure or acoustic cavitation. Different algal species differ in both their resistance to disruption and cell size [86].

5.2.2.4. Microwave extraction. The microwave extraction was first demonstrated by Ganzler et al. [89]. They established a new method for extracting the lipids from the food, algae, seeds etc. The application process of this technique is quite simple when compared with other techniques. Amarni et al. [90] have explained the methodology of microwave extraction after a long time since Ganzler et al. [89] had explained. In this process, a polar material is hosted in an electric field which is oscillating very rapidly and produces microwaves which generate heat from the frictional forces which arise from the inter and intramolecular movements. During this heating process, the vapour is generated inside the vessel which results in the disruption of the cell. This will, in turn, enhance or open up the cell membranes of the organisms with the electroporation effect. This technique involves quick generation of heat and high pressure inside the vessel resulting in a good class of the extracts.

Hemwwimon et al. [91] and Šoštarič et al. [92] have revealed that microwaves are the best pick nowadays due to short response period, less operational expenses, and capable extraction of algal oils. The revival time of biodiesel from the reaction blend is very less compared with the long process involved in the heating method which takes approximately 6 h for completion of oil extraction [93].

5.2.2.5. Electroporation technique. Hu et al. [94] explained that the lipid extraction efficiency from the cell walls of algae did not affect the composition and quality of extracted products such as fatty acids (FA). From the algal biomass, maximum of 92% of the total lipid was extracted in a single electroporation treatment. On the other hand, only 62% was managed without the electroporation technique.

5.2.2.6. Osmotic technique. This is the best and effective technique when compared to other extraction techniques. This technique can disturb both the exterior as well as the interior of the algal cells in a very fast and effective way [95]. Generally, in an osmotic press, there are two different techniques which can be incorporated for extraction of lipids.

a) Hyperosmotic
b) Hypo-osmotic.

Basically hyperosmotic implies the concentration of the salt is higher outside the cells which cause damage to the cells. Conversely hypo-osmotic refers to the condition when the concentration of the salt content is low on the exterior and the fluid can flow easily into the cells to balance the osmotic pressure and the cells burst open if the stress is very high [95]. Among these two techniques, hypo-osmotic is frequently used by many researchers to extract the inside components of the cell. According to researchers, the osmotic pressure technique is the most simple, easy and most efficient way to extract the lipids from the microalgae species [96]. Several freshwater strains like *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. have been tested and were found successful, whereas research has to be conducted on the marine species and also this technique has yet to be tested in the pilot scale, where it is found to be unsuccessful [86].

5.2.2.7. Enzymatic assisted extraction. The cell wall structure of Algae is easily affected by the process of enzymolysis. It is a novel method of extraction of lipids from the walls of algal species. The enzymes like cellulose as well as trypsin are added to microalgal biomass [86]. The function of these enzymes is to break the tough cell wall of the algae and extract the cellular components of them. The major disadvantage of this technique is that it should be conducted at a very low temperature and the process is also very costly [97]. The extraction process can combine several enzymes which can degrade efficiently. The research group had conducted an experiment where they had extracted oil from Scenedesmus species with the help of enzyme assisted hydrolysis [86].

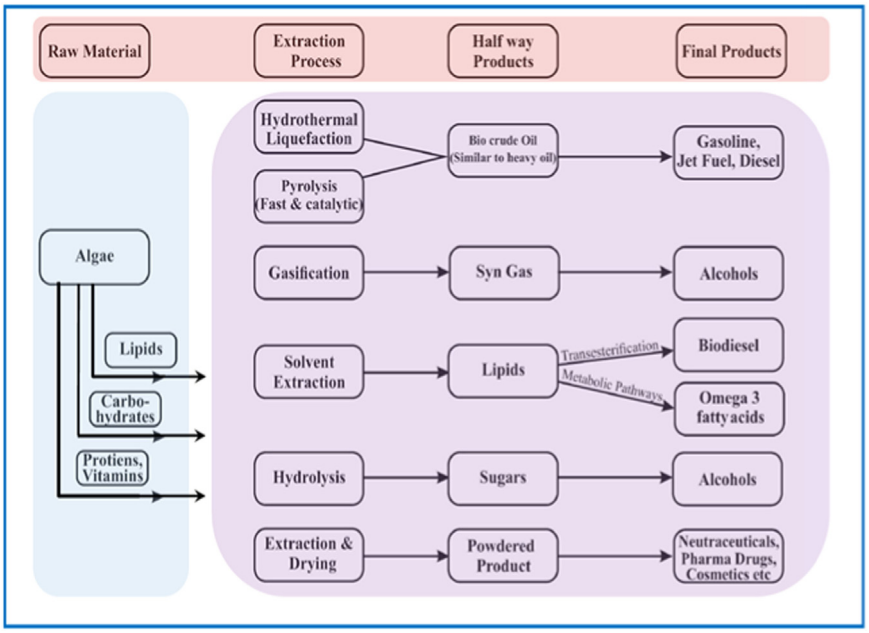

Fig. 4. Different Products of algae through various extraction processes.

5.2.2.8. Lipid extraction by single step procedure. Axelsson and Gentili [77] have proposed a method in which the algal material was made into a paste and was suspended in 1:2 ratio of methanol and chloroform. Later the tube was shaken vigorously up to the entire biomass was suspended in the solvent medium, 0.73% water and sodium chloride mixture were added to form a ratio of1:2:0.8 system of methanol:chloroform: water respectively [77]. This technique was effective in detecting that the one-step process which was found suitable for extraction of total lipid and this process can be applied in the selection of algae for qualitative and quantitative analyses of total FAs. Various extraction methods for different products have been illustrated in Fig. 4.

The three main criteria which are considered in extraction steps in improving the FA profile:

- Direct trans-methylation of lipids.
- Preliminary extraction steps were eliminated
- Fatty acid methyl esters (FAME) were generated by single step derivatization which helped to denature the protein fraction.

6. Applications of microalgae

The usage of microalgae by humans was dated some 2000 years ago by the Chinese people during the famine situation in their country. Nowadays there is a variety of commercial as well as industrial applications of the algae (Fig. 5). With the genetic modification of the genes in potent organisms, we can achieve new products [98]. Other than biofuel, microalgae have also been proven to produce important products like pigments, polyunsaturated FAs, antioxidants, carbohydrates, pharmaceuticals, natural colourants for cosmetics lipids, proteins and too as an animal feed [12,23]. Algae is considered to be one of the most potential sources in each and every application of life sciences, some of which include biofuel, bio fertilizer, bioelectricity, essential food supplement, stabilizers, pollution control, wastewater treatment, biohydrogen etc. [23,99,100].

6.1. Fuel sources

There is much amount of research done and still going on the biofuel production from algae. Many researchers are utilizing this opportunity to increase the lipid inside the tiny algae for conversion of oil and utilizing for biofuel production [99]. Researchers also concentrate on the nutrient medium which is being used for growth culture of algae [23]. The most researched species to date are *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. Now researchers also shifted concentration on the locally available species and those which do not have the lipid content. Genetic engineering and molecular biotechnology techniques are also being applied to such kind of species for increasing the lipid productivity. By doing this the production of biofuel on a large scale would be much easier since there will be a mixed consortium of algal species in the container or tank [23].

6.2. Pollution control

Nowadays pollution is a major threat from the harmful gasses emitted from the exhaust of vehicles as well as from the chimneys and various power plants. These gasses are responsible for heating up the surface as well damaging the Ozone layer which makes the sun rays to directly pass through the atmosphere and causes severe damage to human health [1,9]. Along with many plant species, algae also take part in a significant role in cleaning the earth's atmosphere by absorbing the CO_2 for their growth. Microalgae function similar to plants and are capable of photosynthesis. Using the energy from the sunlight they turn CO_2 and water into sugar. Algae have a special enzyme in their cells with an ability to detoxify the nitric acid which helps the dangerous nitrate to get reduced and thus cleans the environment [101].

6.3. Wastewater treatment

Nowadays along with pollution, wastewater released into many water bodies is also a major concern [102–106]. The water is not being purified properly and many human beings are affected by consuming the partially purified water [107–112]. There are many techniques involved in cleaning the wastewater with the help of algae. The most prominent technique is the CO_2 enhanced wastewater ponds, stabilization ponds, anaerobic ponds etc. Research in this particular area has proved that algae can survive the high level of toxic compounds in the wastewater [113]. These toxic substances are utilized by algae for their growth purpose and in turn, these magnificent species have shown that biomass and lipid content in the algal cells is increased, thereby giving

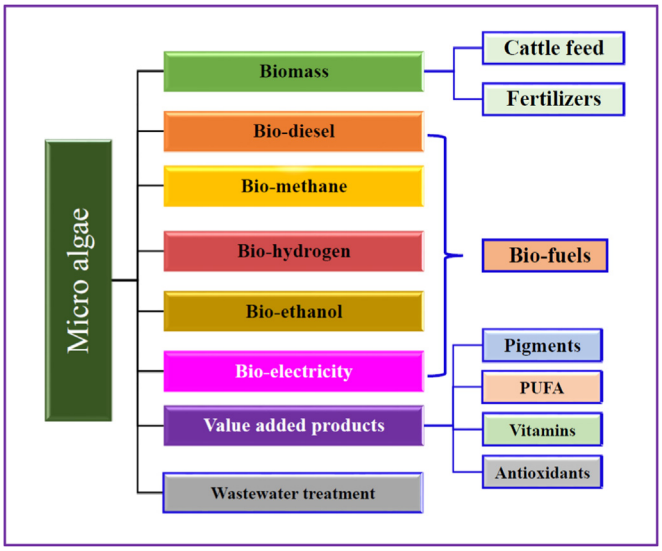

Fig. 5. Schematic illustration of various potential applications of algae.

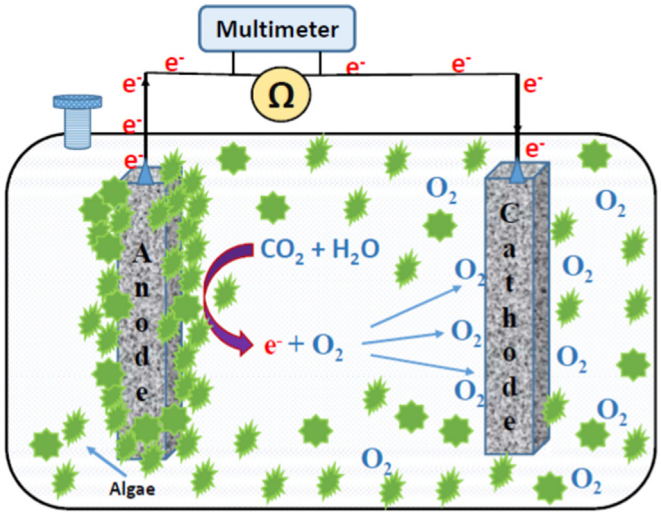

Fig. 6. Schematic design of single chamber microalgae catalyzed MFC.

dual benefits. Further research should be done for the improvisation of the techniques used in the treatment of wastewater [114,115]. Once the wastewater treatment infrastructure is developed in a full-fledged manner, it provides an opportunity for biofilm technologies which is also another key factor in the treatment of wastewater [116]. The biomass formed for the duration of the treatment of wastewater could be used as a potential source for the human as well as animal feed.

6.4. Bioelectricity

Nowadays there is a great crisis of power supply affecting the daily activity. More power is needed to empower gas turbines in generating the required current [117]. Due to the continuous generation of current from these sources, there is a heavy noise pollution and a lot of heat generation during the process. Since these microalgae are very efficient converters of solar power they can be used in producing the electricity through the biotic system as well as enhancing biomass [9]. Since it is still an emerging process more exploration needs to be carried out in the field of bioelectricity to make a full-fledged algal-based microbial fuel cell system to cut short the power crisis for the generations to come [9,118–120] (Fig. 6).

6.5. Algal biohydrogen

Bio-H_2 is a potential alternative source of energy. Researchers have considered microalgae as the easily and cheaply available source for the production of bio-H_2 [1]. Hans Graffon and his research team from Germany have observed that the species *Chlamydomonas reinhardtii* which they were studying had frequently switched from the production of O_2 into H_2 [121]. Later after some research, it was seen that the enzyme known as hydrogenase is responsible for the production of this bio-H_2. When the algal species are deprived of S they exchange on the formation of H_2 from O_2 [122]. A less number of species are identified for producing the bio-H_2 [123].

6.6. Algal biogas

The major obstacle in generating the biogas from algal source is the feedstock related issues and also that most of them have seasonal variation and needs to adjust to the ecological conditions [32]. The algal biogas production has not been much explored and the various factors which could be considered are the pre-treatment of algal biomass [31]. It is also essential to investigate the effects of diverse pre-treatments under most favourable parameters.

6.7. Biomaterials and bioproducts

The macro-algae have their own kind of gel-formation and have the capacity of dissolving in water because of which they are used in industries. Agar is a by-product of macro-algae, which has glutamic acid which is a valuable chemical inside the cells [124].

6.8. Algae in cosmetics

The extracts from microalgae are being used in cosmetic products like anti-ageing and anti-irritants. They are also found in sunscreen lotions and hair care products. Few classic examples of commercially accessible products are Arthospira which are rich in protein and are responsible for the repair of early skin ageing, tightening effect and avoid stretch marks formation [124]. *Chlorella vulgaris* contains collagen which can stimulate collagen synthesis in the skin, which supports the rebirth of the soft-tissue and reduce wrinkle formation. The component from *D. Salina* stimulates the cell propagation and enhances the energy metabolism of the skin tissue [124].

6.9. Algae in human nutrition

Nowadays algae are being manufactured in the form of tablets, gums, capsules, syrups etc. Since they have a wide variety of chemical composition they can be used in a variety of food products like pasta, snacks, gums, and drinks [23]. The four promising strains which are used for this purpose are *Arthrospira, Chlorella, D. salina* and *Aphanizomenon flos-aquae*. The major reason for which *Arthrospira* is being used in a human diet is that it has an excellent nutritive value [124].

6.10. Generation of stable isotopes by microalgae

Since algae are known to possess phototrophic growth capability, they can be used for generation of precise molecules/substances which are collectively labelled with ^{13}C, ^{15}N or ^{2}H. The compounds from the above reaction can be utilized in generating many different products [125]. Generally, drugs are developed depending on the interaction in the middle of the receptor and ligand. The drug manufacturers study the interaction between the molecules among themselves and design the chemical structure of the drug. Hence the complex formation between these two is really advantageous in designing the drug.

Table 5
Different kinds of components synthesized by microalgae.

S.No	Components	Products	Applications
	Pigments/carotenoids	B-carotene	Beta-carotene is a red-orange pigment, used as a precursor for vitamin-A (retinol).
		Astaxanthin	Astaxanthin is a reddish pigment used for treating Alzheimer's disease.
		Lutein and Zeaxanthin	Lutein and Zeaxanthin are yellow to red pigments used to prevent eye diseases.
		Canthaxanthin	It is a keto-carotenoid pigment which is widely used as lipid-soluble antioxidant.
		Chlorophyll	Chlorophyll is a green coloured pigment which is used as a food additive (colorant).
		Phycocyanin	It is a pigment-protein which is used as an antioxidant and anti-inflammatory agent.
	Polyunsaturated fatty acids (PUFA)	Docosahexaenoic acid (DHA) (C22:6)	DHA is a long-chain omega-3 fatty acid which is essential for visual and neurological development
		Eicosapentaenoic acid (EPA) (C20:5)	EPA is a long-chain omega-3 fatty acid. It is a part of healthy diet (recommended to reduce the risk of heart disease).
		Arachidonic acid (AA) (C20:4)	AA is omega-6 fatty acid which is a precursor molecule for the synthesis of both the prostaglandins and leukotrienes in humans.
		γ-Linolenic acid (GAL) (C18:3)	GAL is omega-6 fatty acids used for the treatment of diabetics.
	Vitamins	Vitamin-A (retinol)	Fat soluble and strong antioxidant. It is essential for immune function, vision, reproduction, and cellular communication
		Vitamin-B1 (thiamine)	It is a part of an enzyme which help the body convert food into fuel.
		Vitamin-B6 (pyridoxine)	It is a part of an enzyme which help the body for protein metabolism and to generate red blood cells.
		Vitamin-B12 (cobalamin)	It is a part of an enzyme which is vital for nerve function.
		Vitamin-C (ascorbic acid)	It is a part of enzyme required for protein metabolism and iron absorption.
		Vitamin-E	It functions as antioxidant.
		Biotin	It is a part of enzyme needed for energy generation.
		Vitamin-B2 (riboflavin)	It is essential for energy generation.
		Vitamin-3 (nicotinic acid)	It is used to prevent and treat of pellagra.
		Vitamin-B5 (pantothenate)	It is required for the synthesis of coenzyme-A (CoA).
		Vitamin-9 (folic acid)	It is a used to prevent and treat liver deseases, ulcerative colitis.
	Antioxidants	Catalases, polyphenols, superoxide dismutase, tocopherols	These are used as antioxidant supplementation.
	Others	Antimicrobial, antifungal, antiviral agents, toxins, amino acids, proteins, sterols.	These are having several medical, pharmaceutical, agriculture and industrial applications.

6.11. Breath test diagnostics

The family of microalgae may offer a means to build up a series of tests which are related to the gastrointestinal tract which are not only minimally invasive but also simple to quantify the sum of ^{13}C in breath CO_2 [126]. These diagnostics tests are used as a replacement therapy for the high-risk procedures (endoscopies and colonoscopies) [126]. These 13C breath tests include the evaluation of the^{13}C:^{12}C ratio present in breath CO_2 when ingestion of a nutrient or supplementarymealhaving^{13}C [127]. Some of the precise cases include the usage of labelled-galactose to observer liver function and the usage of labelled-xylose to conclude the range of microbial/bacteriological overgrowth of the small intestine. Different kinds of components synthesized by microalgae are listed in Table 5.

6.12. High-value molecules

Pure molecules can be found in high concentration in many valuable products like fatty acids, pigments and sterols (Table 6). Since the plants and higher animals lack the system to generate or synthesize polyunsaturated FAs [124]. Thus these FAs must be generated from the food which we consume. Generally, fish and fish oils are taken to have these FAs in the body, but due to the safety regulations and rules for the accumulation of toxins [23].

6.13. Small molecules

Since many years' macro-algae has been employed for the production of alginates, carrageenans or agars, which are either located in cell walls or within the cells and serve as storing materials [23]. One of the distinctive features of marine algae in the presence of abundant sulphated-polysaccharides in their cell walls. Another product from the macro-algae which can be considered is the hydrocolloids. These can be found in various red and brown seaweeds [124].

6.13.1. Ulvans

These are the compounds extracted from the family Ulvales generally belonging to the genera *Ulva* and *Enteromorpha*. These are extracted from water which contains a cation chelator from the cells of green weeds [128]. The structure of these ulvans consists of various reiterating arrangements of rhamnose, glucuronic acid, iduronic acid, xylose, and sulphate [128].

Table 6
UV Screening compounds from microalgae.

S. No	UV-screening compound	Species of algae
1.	Sporopollenin	*Characium Terrestre*, *Coelastrum microporum*, *Enallax coelastroides*, *Scenedesmus Sp.*, *Scotiella chlorelloidea*, *Scotiellopsis Rubescens*, *Spongiochloris Spongiosa*, *Dunaliella salina* and *Chlorella Fusca*
2.	Scytonemin	*Chlorogloeopsis* Sp., *Calothrix* Sp., *Scytonema* Sp., *Rivularia* Sp., and *Nostoc commune Lyngbya* cf. *aestuarii Chroococcidiopsis* Sp., *Nostoc Punctiforme*
3.	Mycosporine-Like Amino Acids	*Ankistrodesmus spiralis*, *Chlorella minutissima*, *Chlorella sorokiniana*, *Dunaliella tertiolecta*, *Scotiella Chlorelloidea*, *Isochrysis* Sp., *Pavlova gyrans*, *Corethron criophilum*, *Thalassiosira tumida*, *Porosirapseudo Denticulata*, *Stellarimamicrotrias*, *Thalassiosira weissflogii*, *Alexandrium catenella*

7. Conclusion

Since the atmospheric pollution is rising alarmingly due to increase in atmospheric CO_2 concentration, resulting in the depletion of fossil fuel reserves causing hectic situations bringing fossil fuels to the wedge of depletion. This review provides the existing technical viability for the production of biofuels from microalgae as a renewable energy resource. Besides, numerous harvesting procedures, extraction methods and parameters which are involved in growth and the lipid extraction procedures were also discussed in detail. Utilization of the algae biomass is not only advantageous for CO_2 fixation in the atmosphere but also marks for the production of biodiesel which is environmental friendly as well as also safe for human beings. Hence the biodiesel produced from the algal source plays an important role in maintaining the environment clean, can be used for transportation and will show less impact in the growth of biodiesel crops which have high impact in growing vegetable crops which can be used for human welfare.

- Production of algal biodiesel necessitates huge-scale cultivation and harvesting systems, considering some of the challenges such as the reduction of the cost per unit area.
- In near future, the major challenges for the researchers would be the improvement of lipid profiles of certain important strains which have high lipid productivity through the genetic engineering techniques.
- Efforts must be invested in the reduction of the harvesting technique procedures as discussed in the previous section, which is significant in obtaining the desired amount of biomass needed for biofuel production and in determining the lipid content.
- The FA profiling is also an important factor in the determination of the suitable species for the production of biofuel.

Acknowledgement

Authors would like to acknowledge the financial assistance from Ton Duc Thang University, Ho Chi Minh City, Vietnam. Authors also like to acknowledge Ravi Vaghela, Suraghavi Bommareddy, G. Pavan Kumar for their kind help and valuable suggestion to prepare tables.

References

[1] Chandrasekhar K, Lee Y-J, Lee D-W. Biohydrogen production: strategies to improve process efficiency through microbial routes. Int J Mol Sci Int J Mol Sci 2015;16:8266–93. http://dx.doi.org/10.3390/ijms16048266.
[2] Kumar G, Sivagurunathan P, Pugazhendhi A, Thi NBD, Zhen G, Chandrasekhar K, et al. A comprehensive overview on light independent fermentative hydrogen production from wastewater feedstock and possible integrative options. Energy Convers Manag 2017;141:390–402. http://dx.doi.org/10.1016/j.enconman.2016.09.087.
[3] Kumar EM, Jagadish D, Kumar RB. A note on algae as potential source fer alternate fuels – Biodiesel. Int J PharmTech Res 2014;6:1783–93.
[4] Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS. A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alex Eng J 2016;55:427–43. http://dx.doi.org/10.1016/j.aej.2015.10.008.
[5] Da Silva TL, Reis A, Medeiros R, Oliveira AC, Gouveia L. Oil production towards biofuel from autotrophic microalgae semicontinuous cultivations monitored by flow cytometry. Appl Biochem Biotechnol 2009;159:568–78. http://dx.doi.org/10.1007/s12010-008-8443-5.
[6] Mohan SV, Chandrasekhar K, Chiranjeevi P, Babu PS. Biohydrogen production from wastewater. Biohydrogen 2013:223–57. http://dx.doi.org/10.1016/B978-0-444-59555-3.00010-6.
[7] Baral SS, Singh K, Sharma P. The potential of sustainable algal biofuel production using CO_2 from thermal power plant in India. Renew Sustain Energy Rev 2015;49:1061–74. http://dx.doi.org/10.1016/j.rser.2015.04.181.
[8] Singh RN, Sharma S. Development of suitable photobioreactor for algae production - A review. Renew Sustain Energy Rev 2012;16:2347–53. http://dx.doi.org/10.1016/j.rser.2012.01.026.
[9] Saratale RG, Kuppam C, Mudhoo A, Saratale GD, Periyasamy S, Zhen G, et al. Bioelectrochemical systems using microalgae – a concise research update. Chemosphere 2017;177:35–43. http://dx.doi.org/10.1016/j.chemosphere.2017.02.132.
[10] Prommuak C, Pavasant P, Quitain AT, Goto M, Shotipruk A. Microalgal lipid extraction and evaluation of single-step biodiesel production. Eng J 2012;16:157–66. http://dx.doi.org/10.4186/ej.2012.16.5.157.
[11] Xin L, Hong-Ying H, Yu-ping Z. Growth and lipid accumulation properties of a freshwater microalga Scenedesmus sp. under different cultivation temperature. Bioresour Technol 2011;102:3098–102. http://dx.doi.org/10.1016/j.biortech.2010.10.055.
[12] Trivedi J, Aila M, Bangwal DP, Kaul S, Garg MO. Algae-based biorefinery - how to make sense? Renew Sustain Energy Rev 2015;47:295–307. http://dx.doi.org/10.1016/j.rser.2015.03.052.
[13] Sydney EB, da Silva TE, Tokarski A, Novak AC, de Carvalho JC, Wojciechowski AL, et al. Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. Appl Energy 2011;88:3291–4. http://dx.doi.org/10.1016/j.apenergy.2010.11.024.
[14] Farooq W, Lee YC, Ryu BG, Kim BH, Kim HS, Choi YE, et al. Two-stage cultivation of two Chlorella sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. Bioresour Technol 2013;132:230–8. http://dx.doi.org/10.1016/j.biortech.2013.01.034.
[15] Isleten-Hosoglu M, Ayyildiz-Tamis D, Zengin G, Elibol M. Enhanced growth and lipid accumulation by a new Ettlia texensis isolate under optimized photo-heterotrophic condition. Bioresour Technol 2013;131:258–65. http://dx.doi.org/10.1016/j.biortech.2012.12.070.
[16] Nascimento IA, Marques SSI, Cabanelas ITD, Pereira SA, Druzian JI, de Souza CO, et al. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. Bioenergy Res 2013;6:1–13. http://dx.doi.org/10.1007/s12155-012-9222-2.
[17] Miazek K, Kratky L, Sulc R, Jirout T, Aguedo M, Richel A, Goffin D. Effect of organic solvents on microalgae growth, metabolism and industrial bioproduct extraction : a review. Int J Mol Sci 2017;18(7).
[18] Beattie A, Hirst EL, Percival E. The metabolism of the chrysophyceae. Biochem J 1961:531.
[19] Martinez F, Ascaso C, Orus MI. Morphometric and stereologic analysis of chlorella-vulgaris under heterotrophic growth-conditions. Ann Bot 1991;67:239–45.
[20] Endo H, Nakajima K, Chino R, Shirota M. Growth characteristics and cellular components of *chlorella regularis*, heterotrophic fast growing strain. Agric Biol Chem 1974;38:9–18. http://dx.doi.org/10.1080/00021369.1974.10861121.
[21] Goncalves EC, Wilkie AC, Kirst M, Rathinasabapathi B. Metabolic regulation of triacylglycerol accumulation in the green algae: identification of potential targets for engineering to improve oil yield. Plant Biotechnol J 2016;14:1649–60. http://dx.doi.org/10.1111/pbi.12523.
[22] Neilson AH, Lewin RA. The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. Phycologia 1974;13:227–64.
[23] Brennan L, Owende P. Biofuels from microalgae–a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–77. http://dx.doi.org/10.1016/j.rser.2009.10.009.
[24] Allen AE, Dupont CL, Oborník M, Horák A, Nunes-Nesi A, McCrow JP, et al. Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. TL - 473. Nature 2011;473:203–7. http://dx.doi.org/10.1038/nature10074.
[25] Pancost RD, Badger MPS, Reinfelder J. Biogeochemistry: ancient algae crossed a threshold. Nature 2013;500:532–3. http://dx.doi.org/10.1038/500532a.
[26] Graham LE, Wilcox LW. Algae. 2000.
[27] Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS. Algae-bacteria interactions: evolution, ecology and emerging applications. Biotechnol Adv 2016;34:14–29. http://dx.doi.org/10.1016/j.biotechadv.2015.12.003.
[28] Holloway JM, Dahlgren RA. Nitrogen in rock: occurrences and biogeochemical implications. Glob Biogeochem Cycles 2002;16(65):1–17. http://dx.doi.org/10.1029/2002GB001862.
[29] WEHR JD. Algae: anatomy, biochemistry, and biotechnology by Barsanti, L. & Gualtieri, P. J Phycol 2007;43:412–4. http://dx.doi.org/10.1111/j.1529-8817.2007.00335.x.
[30] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14:217–32. http://dx.doi.org/10.1016/j.rser.2009.07.020.
[31] Zhu J, Rong J, Zong B. Factors in mass cultivation of microalgae for biodiesel. Chin J Catal 2013;34:80–100. http://dx.doi.org/10.1016/S1872-2067(11)60497-X.
[32] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008;26:126–31. http://dx.doi.org/10.1016/j.tibtech.2007.12.002.
[33] Venkata Subhash G, Rohit MV, Devi MP, Swamy YV, Venkata Mohan S. Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. Bioresour Technol 2014;169:789–93. http://dx.doi.org/10.1016/j.biortech.2014.07.019.
[34] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy 2010;87:38–46. http://dx.doi.org/10.1016/j.apenergy.2009.06.016.
[35] Griffiths MJ, Dicks RG, Richardson C, Harrison STL. Advantages and challenges of microalgae as a source of oil for biodiesel. Biodiesel - Feed Process Technol 2011:178–200. http://dx.doi.org/10.5772/1094.
[36] Kurpan Nogueira DP, Silva AF, Ara??jo OQF, Chaloub RM. Impact of temperature and light intensity on triacylglycerol accumulation in marine microalgae. Biomass-Bioenergy 2015;72:280–7. http://dx.doi.org/10.1016/j.biombioe.2014.10.017.
[37] Singh SP, Singh P. Effect of temperature and light on the growth of algae species: a review. Renew Sustain Energy Rev 2015;50:431–44. http://dx.doi.org/10.1016/j.rser.2015.05.024.
[38] Lee S-J, Go S, Jeong G-T, Kim S-K. Oil production from five marine microalgae for the production of biodiesel. Biotechnol Bioprocess Eng 2011;16:561–6. http://dx.doi.org/10.1007/s12257-010-0360-0.
[39] Anderson D, Morel F. Copper sensitivity of Gonyaulax tamarensis. Limnol

Oceanogr 1978;23:283–95.
[40] Liu ZY, Wang GC, Zhou BC. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour Technol 2008;99:4717–22. http://dx.doi.org/10.1016/j.biortech.2007.09.073.
[41] Li Y, Chen YF, Chen P, Min M, Zhou W, Martinez B, et al. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 2011;102:5138–44. http://dx.doi.org/10.1016/j.biortech.2011.01.091.
[42] Mahapatra SS, Sahu M, Patel RK, Panda BN. Prediction of water quality using principal component analysis. Water Qual Expo Heal 2012;4:93–104. http://dx.doi.org/10.1007/s12403-012-0068-9.
[43] Ramachandra TV, Durga Madhab M, Shilpi S, Joshi NV. Algal biofuel from urban wastewater in India: scope and challenges. Renew Sustain Energy Rev 2013;21:767–77. http://dx.doi.org/10.1016/j.rser.2012.12.029.
[44] Jacob-Lopes E, Scoparo CHG. Lacerda LMCF, Franco TT. Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors. Chem Eng Process Process Intensif 2009;48:306–10. http://dx.doi.org/10.1016/j.cep.2008.04.007.
[45] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. J Biotechnol 2006;126:499–507. http://dx.doi.org/10.1016/j.jbiotec.2006.05.002.
[46] Moon M, Kim CW, Park WK, Yoo G, Choi YE, Yang JW. Mixotrophic growth with acetate or volatile fatty acids maximizes growth and lipid production in Chlamydomonas reinhardtii. Algal Res 2013;2:352–7. http://dx.doi.org/10.1016/j.algal.2013.09.003.
[47] Show KY, Lee DJ. Algal biomass harvesting. Elsevier B.V; 2013. http://dx.doi.org/10.1016/B978-0-444-59558-4.00005-X.
[48] Wang B, Li Y, Wu N, Lan CQ. CO_2 bio-mitigation using microalgae. Appl Microbiol Biotechnol 2008;79:707–18. http://dx.doi.org/10.1007/s00253-008-1518-y.
[49] Heasman M, Diemar J, O'Connor W, Sushames T, Foulkes L. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs - a summary. Aquac Res 2000;31:637–59. http://dx.doi.org/10.1046/j.1365-2109.2000.00492.x.
[50] Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 2011;102:71–81. http://dx.doi.org/10.1016/j.biortech.2010.06.159.
[51] Kim J, Yoo G, Lee H, Lim J, Kim K, Kim CW, et al. NU SC. Biotechnol Adv 2013. http://dx.doi.org/10.1016/j.biotechadv.2013.04.006.
[52] Singh M, Shukla R, Das K. Harvesting of microalgal biomass. Biotechnol Appl Micro Biodiesel Value-Added Prod 2013:77–88.
[53] Sim TS, Goh A, Becker EW. Comparison of centrifugation, dissolved air flotation and drum filtration techniques for harvesting sewage-grown algae. Biomass 1988;16:51–62. http://dx.doi.org/10.1016/0144-4565(88)90015-7.
[54] Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 2003;20:491–515. http://dx.doi.org/10.1016/S0734-9750(02)00050-2.
[55] Papazi A, Makridis P, Divanach P. Harvesting Chlorella minutissima using cell coagulants. J Appl Phycol 2010;22:349–55. http://dx.doi.org/10.1007/s10811-009-9465-2.
[56] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 2008;1:20–43. http://dx.doi.org/10.1007/s12155-008-9008-8.
[57] Vandamme D, Foubert I, Muylaert K. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol 2013;31:233–9. http://dx.doi.org/10.1016/j.tibtech.2012.12.005.
[58] Salim S, Bosma R, Vermuë MH, Wijffels RH. Harvesting of microalgae by bioflocculation. J Appl Phycol 2011;23:849–55. http://dx.doi.org/10.1007/s10811-010-9591-x.
[59] Lee AK, Lewis DM, Ashman PJ. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. J Appl Phycol 2009;21:559–67. http://dx.doi.org/10.1007/s10811-008-9391-8.
[60] Ummalyma SB, Gnansounou E, Sukumaran RK, Sindhu R, Pandey A, Sahoo D. Bioflocculation: an alternative strategy for harvesting of microalgae - An overview. Bioresour Technol 2017. http://dx.doi.org/10.1016/j.biortech.2017.02.097.
[61] Schlesinger A, Eisenstadt D, Bar-Gil A, Carmely H, Einbinder S, Gressel J. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Biotechnol Adv 2012;30:1023–30. http://dx.doi.org/10.1016/j.biotechadv.2012.01.011.
[62] Ren H-Y, Liu B-F, Ma C, Zhao L, Ren N-Q. A new lipid-rich microalga Scenedesmus sp. strain R-16 isolated using Nile red staining: effects of carbon and nitrogen sources and initial pH on the biomass and lipid production. Biotechnol Biofuels 2013;6:143. http://dx.doi.org/10.1186/1754-6834-6-143.
[63] Pragya N, Pandey KK, Sahoo PK. A review on harvesting , oil extraction and biofuels production technologies from microalgae. Renew Sustain Energy Rev 2013;24:159–71. http://dx.doi.org/10.1016/j.rser.2013.03.034.
[64] Pushparaj B, Pelosi E, Torzillo G, Materassi R. Microbial biomass recovery using a synthetic cationic polymer. Bioresour Technol 1993;43:59–62. http://dx.doi.org/10.1016/0960-8524(93)90083-N.
[65] Sukenik A, Bilanovic D, Shelef G. Flocculation of microalgae in brackish and sea waters. Biomass 1988;15:187–99. http://dx.doi.org/10.1016/0144-4565(88)90084-4.
[66] Poelman E, De Pauw N, Jeurissen B. Potential of electrolytic flocculation for recovery of micro-algae. Resour Conserv Recycl 1997;19:1–10. http://dx.doi.org/10.1016/S0921-3449(96)01156-1.
[67] Vandamme D, Pontes SCV, Goiris K, Foubert I, Pinoy LJJ, Muylaert K. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Biotechnol Bioeng 2011;108:2320–9. http://dx.doi.org/10.1002/bit.23199.
[68] Edzwald J. Coagulation in ,drinking water treatment: particles, organcs and coagulants; 2016.
[69] Robinson CD. Some factors influencing sedimentation. Ind Eng Chem 1926;18:869–71. http://dx.doi.org/10.1021/ie50200a036.
[70] Mohn FH. Experiences and strategies in the recovery of biomass from mass cultures of microalgae. Algal Biomass 1980:471–547.
[71] Petru??evski B, Bolier G, Van Breemen AN, Alaerts GJ. Tangential flow filtration: a method to concentrate freshwater algae. Water Res 1995;29:1419–24. http://dx.doi.org/10.1016/0043-1354(94)00269-D.
[72] Mollah MYA, Morkovsky P, Gomes JAG, Kesmez M, Parga J, Cocke DL. Fundamentals, present and future perspectives of electrocoagulation. J Hazard Mater 2004;114:199–210. http://dx.doi.org/10.1016/j.jhazmat.2004.08.009.
[73] Pearsall RV, Connelly RL, Fountain ME, Hearn CS, Werst MD, Hebner RE, et al. Electrically dewatering microalgae. IEEE Trans Dielectr Electr Insul 2011;18:1578–83. http://dx.doi.org/10.1109/TDEI.2011.6032827.
[74] Sandbank E, Shelef G, Wachs AM. Improved electroflotation for the removal of suspended solids from algal pond effluents. Water Res 1974;8:587–92. http://dx.doi.org/10.1016/0043-1354(74)90116-X.
[75] Bosma R, Van Spronsen WA, Tramper J, Wijffels RH. Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol 2003;15:143–53. http://dx.doi.org/10.1023/A:1023807011027.
[76] Yang Z, Kong F, Yang Z, Zhang M, Yu Y, Qian S. Benefits and costs of the grazer-induced colony formation in Microcystis aeruginosa. Ann Limnol Int J Limnol 2009;45:203–8. http://dx.doi.org/10.1051/limn/2009020.
[77] Axelsson M, Gentili F. A single-step method for rapid extraction of total lipids from green microalgae. PLoS One 2014:9. http://dx.doi.org/10.1371/journal.pone.0089643.
[78] FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509. http://dx.doi.org/10.1371/journal.pone.0020510.
[79] Nanton DA, Vegusdal A, R??r?? AMB, Ruyter B, Baeverfjord G, Torstensen BE. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (Salmo salar) fed fish oil and vegetable oil. Aquaculture 2007;265:230–43. http://dx.doi.org/10.1016/j.aquaculture.2006.03.053.
[80] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7. http://dx.doi.org/10.1139/o59-099.
[81] Hajra aK. On extraction of acyl and alkyl dihydroxyacetone phosphate from incubation mixtures. Lipids 1974;9:502–5.
[82] Jensen SK. Improved Bligh and Dyer extraction procedure. Technology 2008;20:280–1. http://dx.doi.org/10.1002/lite.200800074.
[83] Iverson SJ, Iverson SJ, Lang SLC, Cooper MH. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 2001. http://dx.doi.org/10.1007/s11745-001-0843-0.
[84] Radin NS. Lipid extraction of tissues with a low-toxicity solvent. Anal Biochem 1978;426:420–6.
[85] Sheng J, Vannela R, Rittmann BE. Evaluation of methods to extract and quantify lipids from Synechocystis PCC 6803. Bioresour Technol 2011;102:1697–703. http://dx.doi.org/10.1016/j.biortech.2010.08.007.
[86] Ranjith Kumar R, Hanumantha Rao P, Arumugam M. Lipid extraction methods from microalgae: a comprehensive review. Front Energy Res 2015;2:1–9. http://dx.doi.org/10.3389/fenrg.2014.00061.
[87] Lee AK, Lewis DM, Ashman PJ. Disruption of microalgal cells for the extraction of lipids for biofuels: processes and specific energy requirements. Biomass- Bioenergy 2012;46:89–101. http://dx.doi.org/10.1016/j.biombioe.2012.06.034.
[88] Suslick KS, Flannigan DJ. Inside a collapsing bubble: sonoluminescence and the conditions during cavitation. Annu Rev Phys Chem 2008;59:659–83. http://dx.doi.org/10.1146/annurev.physchem.59.032607.093739.
[89] Ganzler K, Salgó A, Valkó K. Microwave extraction. J Chromatogr A 1986;371:299–306. http://dx.doi.org/10.1016/S0021-9673(01)94714-4.
[90] Amarni F, Kadi H. Kinetics study of microwave-assisted solvent extraction of oil from olive cake using hexane. Comparison with the conventional extraction. Innov Food Sci Emerg Technol 2010;11:322–7. http://dx.doi.org/10.1016/j.ifset.2010.01.002.
[91] Hemwimon S, Pavasant P, Shotipruk A. Microwave-assisted extraction of anti-oxidative anthraquinones from roots of Morinda citrifolia. Sep Purif Technol 2007;54:44–50. http://dx.doi.org/10.1016/j.seppur.2006.08.014.
[92] Šoštarič M, Klinar D, Bricelj M, Golob J, Berovič M, Likozar B. Growth, lipid extraction and thermal degradation of the microalga Chlorella vulgaris. N Biotechnol 2012;29:325–31. http://dx.doi.org/10.1016/j.nbt.2011.12.002.
[93] Refaat aa, Attia NK, Sibak H a, Sheltawy ST, ElDiwani GI. Production optimization and quality assessment of biodiesel from waste vegetable oil. Int J Environ Sci Technol 2007;5:75–82. http://dx.doi.org/10.1007/BF03325999.
[94] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 2008;54:621–39. http://dx.doi.org/10.1111/j.1365-313X.2008.03492.x.
[95] Adam F, Abert-Vian M, Peltier G, Chemat F. "Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour Technol 2012;114:457–65. http://dx.doi.org/10.1016/j.biortech.2012.02.096.
[96] Yoo G, Park WK, Kim CW, Choi YE, Yang JW. Direct lipid extraction from wet Chlamydomonas reinhardtii biomass using osmotic shock. Bioresour Technol 2012;123:717–22. http://dx.doi.org/10.1016/j.biortech.2012.07.102.
[97] Liang K, Zhang Q, Cong W. Enzyme-assisted aqueous extraction of lipid from

microalgae. J Agric Food Chem 2012;60:11771–6. http://dx.doi.org/10.1021/jf302836v.
[98] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng 2006;101:87–96. http://dx.doi.org/10.1263/jbb.101.87.
[99] Maity JP, Bundschuh J, Chen C-Y, Bhattacharya P. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: present and future perspectives – a mini review. Energy 2014;78:104–13. http://dx.doi.org/10.1016/j.energy.2014.04.003.
[100] Sambusiti C, Bellucci M, Zabaniotou A, Beneduce L, Monlau F. Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: a comprehensive review. Renew Sustain Energy Rev 2015;44:20–36. http://dx.doi.org/10.1016/j.rser.2014.12.013.
[101] Christenson L, Sims R. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 2011;29:686–702. http://dx.doi.org/10.1016/j.biotechadv.2011.05.015.
[102] Mohan SV, Chandrasekhar K. Solid phase microbial fuel cell (SMFC) for harnessing bioelectricity from composite food waste fermentation: influence of electrode assembly and buffering capacity. Bioresour Technol 2011;102:7077–85. http://dx.doi.org/10.1016/j.biortech.2011.04.039.
[103] Mohan SV, Chandrasekhar K. Self-induced bio-potential and graphite electron accepting conditions enhances petroleum sludge degradation in bio-electrochemical system with simultaneous power generation. Bioresour Technol 2011;102:9532–41. http://dx.doi.org/10.1016/j.biortech.2011.07.038.
[104] Chandrasekhar K, Venkata Mohan S. Bio-electrochemical remediation of real field petroleum sludge as an electron donor with simultaneous power generation facilitates biotransformation of PAH: effect of substrate concentration. Bioresour Technol 2012;110:517–25. http://dx.doi.org/10.1016/j.biortech.2012.01.128.
[105] Reddy MV, Chandrasekhar K, Mohan SV. Influence of carbohydrates and proteins concentration on fermentative hydrogen production using canteen based waste under acidophilic microenvironment. J Biotechnol 2011;155:387–95. http://dx.doi.org/10.1016/j.jbiotec.2011.07.030.
[106] Pandit S, Chandrasekhar K, Kakarla R, Kadier A, Jeevitha V. Basic principles of microbial fuel cell: technical challenges and economic Feasibility. Microb. Appl Vol 1 Bioremediation Bioenergy 2017:165–88. http://dx.doi.org/10.1007/978-3-319-52666-9_8.
[107] Kiran Kumar A, Venkateswar Reddy M, Chandrasekhar K, Srikanth S, Venkata Mohan S. Endocrine disruptive estrogens role in electron transfer: bio-electrochemical remediation with microbial mediated electrogenesis. Bioresour Technol 2012;104:547–56. http://dx.doi.org/10.1016/j.biortech.2011.10.037.
[108] Venkata Mohan SR, Devi MP, Reddy M, Chandrasekhar K, Juwarkar A, Sarma PN. Bioremediation of petroleum sludge under anaerobic microenvironment: Influence of biostimulation and bioaugmentation. Environ Eng Manag J 2011;10:1609–16.
[109] Reddy MV, Devi MP, Chandrasekhar K, Goud RK, Mohan SV. Aerobic remediation of petroleum sludge through soil supplementation: microbial community analysis. J Hazard Mater 2011;197:80–7. http://dx.doi.org/10.1016/j.jhazmat.2011.09.061.
[110] Chandrasekhar K, Venkata Mohan S. Induced catabolic bio-electrohydrolysis of complex food waste by regulating external resistance for enhancing acidogenic biohydrogen production. Bioresour Technol 2014;165:372–82. http://dx.doi.org/10.1016/j.biortech.2014.02.073.
[111] Chandrasekhar K, Venkata Mohan S. Bio-electrohydrolysis as a pretreatment strategy to catabolize complex food waste in closed circuitry: function of electron flux to enhance acidogenic biohydrogen production. Int J Hydrog Energy 2014;39:11411–22. http://dx.doi.org/10.1016/j.ijhydene.2014.05.035.
[112] Deval AS, Parikh HA, Kadier A, Chandrasekhar K, Bhagwat AM, Dikshit AK. Sequential microbial activities mediated bioelectricity production from distillery wastewater using bio-electrochemical system with simultaneous waste remediation. Int J Hydrog Energy 2017;42:1130–41. http://dx.doi.org/10.1016/j.ijhydene.2016.11.114.
[113] Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 2011;102:17–25. http://dx.doi.org/10.1016/j.biortech.2010.06.035.
[114] Gómez-Villa H, Voltolina D, Nieves M, Piña P. Biomass production and nutrient budget in outdoor cultures of Scenedesmus obliquus (Chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatl??N, Sinaloa, Mexico. Vie Milieu 2005;55:121–6.
[115] Sawayama S, Inoue S, Dote Y, Yokoyama SY. CO_2 fixation and oil production through microalga. Energy Convers Manag 1995;36:729–31. http://dx.doi.org/10.1016/0196-8904(95)00108-P.
[116] Hodaifa G, Martínez ME, Sánchez S. Use of industrial wastewater from olive-oil extraction for biomass production of Scenedesmus obliquus. Bioresour Technol 2008;99:1111–7. http://dx.doi.org/10.1016/j.biortech.2007.02.020.
[117] Chandrasekhar K, Amulya K, Mohan SV. Solid phase bio-electrofermentation of food waste to harvest value-added products associated with waste remediation. Waste Manag 2015;45:57–65. http://dx.doi.org/10.1016/j.wasman.2015.06.001.
[118] Kakarla R, Kim JR, Jeon B, Min B. Enhanced performance of an air-cathode microbial fuel cell with oxygen supply from an externally connected algal bioreactor. Bioresour Technol 2015. http://dx.doi.org/10.1016/j.biortech.2015.06.062.
[119] Kakarla R, Min B. Evaluation of microbial fuel cell operation using algae as an oxygen supplier : carbon paper cathode vs . carbon brush cathode. Bioprocess Biosyst Eng 2014. http://dx.doi.org/10.1007/s00449-014-1223-4.
[120] Kakarla R, Min B. Photoautotrophic microalgae Scenedesmus obliquus attached on a cathode as oxygen producers for microbial fuel cell (MFC) operation. Int J Hydrog Energy 2014;39:10275–83. http://dx.doi.org/10.1016/j.ijhydene.2014.04.158.
[121] Melis A, Happe T. Hydrogen production . Green algae as a source of energy. Plant Physiol 2001;127:740–8. http://dx.doi.org/10.1104/pp.010498.740.
[122] Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, et al. Microalgae: a green source of renewable H2. Trends Biotechnol 2000;18:506–11. http://dx.doi.org/10.1016/S0167-7799(00)01511-0.
[123] Jung KW, Kim DH, Shin Hang-Sik HS. Fermentative hydrogen production from Laminaria japonica and optimization of thermal pretreatment conditions. Bioresour Technol 2011;102:2745–50. http://dx.doi.org/10.1016/j.biortech.2010.11.042.
[124] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 2004;65:635–48. http://dx.doi.org/10.1007/s00253-004-1647-x.
[125] Acién Fernández FG, Fernández Sevilla JM, Egorova-Zachernyuk TA, Molina Grima E. Cost-effective production of 13C, 15N stable isotope-labelled biomass from phototrophic microalgae for various biotechnological applications. Biomol Eng 2005;22:193–200. http://dx.doi.org/10.1016/j.bioeng.2005.09.002.
[126] Radmer RJ, Parker BC. Commercial applications of algae: opportunities and constraints. J Appl Phycol 1994;6:93–8. http://dx.doi.org/10.1007/BF02186062.
[127] Weaver LT. Stable isotope breath tests. [Review] [27 refs]. Nutrition 1998;14:826–9.
[128] Lahaye M, Robic A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules 2007;8:24–30.
[129] Moheimani NR, Borowitzka MA. Limits to productivity of the alga Pleurochrysis carterae (haptophyta) grown in outdoor raceway ponds. Biotechnol Bioeng 2007;96:27–36. http://dx.doi.org/10.1002/bit.21169.
[130] Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–12. http://dx.doi.org/10.1002/bit.22033.
[131] Ugwu CU, Aoyagi H, Uchiyama H. Photobioreactors for mass cultivation of algae. Bioresour Technol 2008;99:4021–8. http://dx.doi.org/10.1016/j.biortech.2007.01.046.
[132] Richardson JW, Outlaw JL, Allison M. The economics of microalgae oil. AgBioForum 2010;13:119–30. http://dx.doi.org/10.1002/jctb.2338.
[133] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. J Ind Microbiol Biotechnol 2009;36:269–74. http://dx.doi.org/10.1007/s10295-008-0495-6.
[134] Hsieh CH, Wu WT. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresour Technol 2009;100:3921–6. http://dx.doi.org/10.1016/j.biortech.2009.03.019.
[135] Cheng Y, Lu Y, Gao C, Wu Q. Alga-based biodiesel production and optimization using sugar cane as the feedstock. Energy Fuels 2009;23:4166–73. http://dx.doi.org/10.1021/ef9003818.
[136] Cheng Y, Zhou W, Gao C, Lan K, Gao Y, Wu Q. Biodiesel production from Jerusalem artichoke (Helianthus tuberosus L.) tuber by heterotrophic microalgae Chlorella protothecoides. J Chem Technol Biotechnol 2009;84:777–81. http://dx.doi.org/10.1002/jctb.2111.
[137] Yoo Chan, Jun So-Young, Lee Jae-Yon, Chi-Yong Ahn H-MO. Selection of microalgae for lipid production under high levels carbon dioxide. 101. Elsevier Ltd; 2010. p. S71–4. http://dx.doi.org/10.1016/j.biortech.2009.03.030.
[138] Chiu SY, Kao CY, Tsai MT, Ong SC, Chen CH, Lin CS. Lipid accumulation and CO_2 utilization of Nannochloropsis oculata in response to CO_2 aeration. Bioresour Technol 2009;100:833–8. http://dx.doi.org/10.1016/j.biortech.2008.06.061.
[139] Khan SA, Rashmi, Hussain MZ, Prasad S, Banerjee UC. Prospects of biodiesel production from microalgae in India. Renew Sustain Energy Rev 2009;13:2361–72. http://dx.doi.org/10.1016/j.rser.2009.04.005.
[140] Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM. Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 2010;101:S75–7. http://dx.doi.org/10.1016/j.biortech.2009.03.058.
[141] Xin L, Hong-ying H, Jia Y. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, Scenedesmus sp. LX1, growing in secondary effluent. N Biotechnol 2010;27:59–63. http://dx.doi.org/10.1016/j.nbt.2009.11.006.
[142] Chinnasamy S, Bhatnagar A, Hunt RW, Das KC. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol 2010;101:3097–105. http://dx.doi.org/10.1016/j.biortech.2009.12.026.
[143] Feng Y, Li C, Zhang D. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. Bioresour Technol 2011;102:101–5. http://dx.doi.org/10.1016/j.biortech.2010.06.016.
[144] Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: a review. Renew Sustain Energy Rev 2011;15:584–93. http://dx.doi.org/10.1016/j.rser.2010.09.018.
[145] Carvalho J, Ribeiro a, Castro J, Vilarinho C, Castro F. Biodiesel production by microalgae and macroalgae from north littoral Portuguese coast 2011:5–10.
[146] Abou-Shanab RAI, Matter IA, Kim SN, Oh YK, Choi J, Jeon BH. Characterization and identification of lipid-producing microalgae species isolated from a freshwater lake. Biomass- Bioenergy 2011;35:3079–85. http://dx.doi.org/10.1016/j.biombioe.2011.04.021.
[147] Heredia-Arroyo T, Wei W, Ruan R, Hu B. Mixotrophic cultivation of Chlorella vulgaris and its potential application for the oil accumulation from non-sugar materials. Biomass- Bioenergy 2011;35:2245–53. http://dx.doi.org/10.1016/j.biombioe.2011.02.036.
[148] Mallick N, Mandal S, Singh AK, Bishai M, Dash A. Green microalga Chlorella vulgaris as a potential feedstock for biodiesel. J Chem Technol Biotechnol 2012;87:137–45. http://dx.doi.org/10.1002/jctb.2694.
[149] Abomohra AEF, Wagner M, El-Sheekh M, Hanelt D. Lipid and total fatty acid

productivity in photoautotrophic fresh water microalgae: screening studies towards biodiesel production. J Appl Phycol 2013;25:931–6. http://dx.doi.org/10.1007/s10811-012-9917-y.
[150] Park KC, Whitney C, McNichol JC, Dickinson KE, MacQuarrie S, Skrupski BP, et al. Mixotrophic and photoautotrophic cultivation of 14 microalgae isolates from Saskatchewan, Canada: potential applications for wastewater remediation for biofuel production. J Appl Phycol 2012;24:339–48. http://dx.doi.org/10.1007/s10811-011-9772-2.
[151] Georgianna DR, Mayfield SP. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature 2012;488:329–35. http://dx.doi.org/10.1038/nature11479.
[152] Mitra D, van Leeuwen J (Hans), Lamsal B. Heterotrophic/mixotrophic cultivation of oleaginous Chlorella vulgaris on industrial co-products. Algal Res 2012;1:40–8. http://dx.doi.org/10.1016/j.algal.2012.03.002.
[153] Ashokkumar V, Rengasamy R. Mass culture of Botryococcus braunii Kutz. under open raceway pond for biofuel production. Bioresour Technol 2012;104:394–9. http://dx.doi.org/10.1016/j.biortech.2011.10.093.
[154] Bruno L, Di Pippo F, Antonaroli S, Gismondi A, Valentini C, Albertano P. Characterization of biofilm-forming cyanobacteria for biomass and lipid production. J Appl Microbiol 2012;113:1052–64. http://dx.doi.org/10.1111/j.1365-2672.2012.05416.x.
[155] Anitha S, Narayanan JS. Isolation and identification of microalgal strains and evaluation of their fatty acid profiles for biodiesel production. Int J Pharm Biol Arch 2012;3:939–44.
[156] Talebi AF, Mohtashami SK, Tabatabaei M, Tohidfar M, Bagheri A, Zeinalabedini M, et al. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. Algal Res 2013;2:258–67. http://dx.doi.org/10.1016/j.algal.2013.04.003.
[157] Shekh AY, Shrivastava P, Krishnamurthi K, Mudliar SN, Devi SS, Kanade GS, et al. Stress-induced lipids are unsuitable as a direct biodiesel feedstock: a case study with Chlorella pyrenoidosa. Bioresour Technol 2013;138:382–6. http://dx.doi.org/10.1016/j.biortech.2013.04.010.
[158] Picardo MC, De Medeiros JL, Araújo QF, Chaloub RM. Effects of CO_2 enrichment and nutrients supply intermittency on batch cultures of Isochrysis galbana. Bioresour Technol 2013;143:242–50. http://dx.doi.org/10.1016/j.biortech.2013.05.113.
[159] Song M, Pei H, Hu W, Ma G. Evaluation of the potential of 10 microalgal strains for biodiesel production. Bioresour Technol 2013;141:245–51. http://dx.doi.org/10.1016/j.biortech.2013.02.024.
[160] Da Rós PCM, Silva CSP, Silva-Stenico ME, Fiore MF, De Castro HF. Assessment of chemical and physico-chemical properties of cyanobacterial lipids for biodiesel production. Mar Drugs 2013;11:2365–81. http://dx.doi.org/10.3390/md11072365.
[161] Cerón-García MC, Macías-Sánchez MD, Sánchez-Mirón A, García-Camacho F, Molina-Grima E. A process for biodiesel production involving the heterotrophic fermentation of Chlorella protothecoides with glycerol as the carbon source. Appl Energy 2013;103:341–9. http://dx.doi.org/10.1016/j.apenergy.2012.09.054.
[162] Yoo C, Choi GG, Kim SC, Oh HM. Ettlia sp. YC001 showing high growth rate and lipid content under high CO_2. Bioresour Technol 2013;127:482–8. http://dx.doi.org/10.1016/j.biortech.2012.09.046.
[163] Ryu BG, Kim K, Kim J, Han JI, Yang JW. Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, Aurantiochytrium sp. KRS101. Bioresour Technol 2013;129:351–9. http://dx.doi.org/10.1016/j.biortech.2012.11.049.
[164] Wen Q, Chen Z, Li P, Duan R, Ren N. Lipid production for biofuels from hydrolyzate of waste activated sludge by heterotrophic Chlorella protothecoides. Bioresour Technol 2013;143:695–8. http://dx.doi.org/10.1016/j.biortech.2013.06.085.
[165] Silva CSP, Silva-Stenico ME, Fiore MF, de Castro HF, Da Rós PCM. Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production. Algal Res 2014;3:1–7. http://dx.doi.org/10.1016/j.algal.2013.11.012.
[166] Tale M, Ghosh S, Kapadnis B, Kale S. Isolation and characterization of microalgae for biodiesel production from Nisargruna biogas plant effluent. Bioresour Technol 2014;169:328–35. http://dx.doi.org/10.1016/j.biortech.2014.06.017.
[167] Nayak M, Jena J, Bhakta S, Rath SS, Sarika C, Rao BVSK, et al. Screening of fresh water microalgae from eastern region of India for sustainable biodiesel production (Yu et al 2012).pdf. Int J Green Energy 2011:669–83. http://dx.doi.org/10.1080/15435075.2011.588764.
[168] Onay M, Sonmez C, Oktem HA, Yucel AM. Thermo-resistant green microalgae for effective biodiesel production: isolation and characterization of unialgal species from geothermal flora of Central Anatolia. Bioresour Technol 2014;169:62–71. http://dx.doi.org/10.1016/j.biortech.2014.06.078.
[169] Nichols RL. Algae from separation oxidation effluents. J (Water Pollut Control Fed) 2016;49:111–9.
[170] Folkman Y, Wachs AM. Removal of algae from stabilization pond effluents by lime treatment. Water Res 1973;7:419–35. http://dx.doi.org/10.1016/0043-1354(73)90024-9.