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Algae as green energy reserve: Technological outlook on biofuel production

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HIGHLIGHTS

- Energy demand and the importance of bioenergy as alternative fuel was addressed.
- Classification of biofuels and significance of microalgae was discussed.
- Bioethanol, biodiesel, biogas, biohydrogen from algae was extensively described.
- Open pond and photo bioreactor based cultivation and its development was reviewed.
- Pretreatment processes, factors affecting algal fuel production was presented.

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ABSTRACT

Depletion of fossil fuel sources and their emissions have triggered a vigorous research in finding alternative and renewable energy sources. In this regard, algae are being exploited as a third generation feedstock for the production of biofuels such as bioethanol, biodiesel, biogas, and biohydrogen. However, algal based biofuel does not reach successful peak due to the higher cost issues in cultivation, harvesting and extraction steps. Therefore, this review presents an extensive detail of deriving biofuels from algal biomass starting from various algae cultivation systems like raceway pond and photobioreactors and its bottlenecks. Evolution of biofuel feedstocks from edible oils to algae have been addressed in the initial section of the manuscript to provide insights on the different generation of biofuel. Different configuration of photobioreactor systems used to reduce contamination risk and improve biomass productivity were extensively discussed. Photobioreactor performance greatly relies on the conditions under which it is operated. Hence, the importance of such conditions alike temperature, light intensity, inoculum size, CO_2, nutrient concentration, and mixing in bioreactor performance have been described. As the lipid is the main component in biodiesel production, several pretreatment methods such as physical, chemical and biological for disrupting cell membrane to extract lipid were comprehensively reviewed and presented. This review article had put forth the recent advancement in the pretreatment methods like hydrothermal processing of algal biomasses using acid or alkali. Eventually, challenges and future dimensions in algal cultivation and pretreatment process were discussed in detail for making an economically viable algal biofuel.

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1. Introduction

Modernization has today led to infinite advents in every field possible. Not only has this made life easier, but has paved a way for more. With this, the consumption of energy in every form has drastically risen and has now reached an alarming level. Major portion of the global consumption of fuel is met by fossil fuels like coal, petroleum products, and natural gas which are non-renewable sources (Mathimani et al., 2017). Shortage of fuels, imbalance between supply and demand of fuels has led to a steep rise in the cost of fuels (Franchino et al., 2013). This hike in fuel prices is predicted to lead catastrophic effects on the leading economies which might lead to overall inflation in every sector. Thus to meet the demands of the global market, alternative energy sources like solar energy, nuclear energy, wind energy, and bioenergy are more sought. Among the renewable energy, biofuel has gained more attention as alternative fuel to fossil fuel and lot of research works are being undertaken to produce sustainable bioenergy for the long run (Anto et al., 2019; Koley et al., 2018; Mathimani et al., 2017).

Biofuels, as the name suggests, are the fuels derived from biological sources like plants, animals, microbes etc., which are biodegradable, non-toxic, and environmentally safe (Vogel et al., 2019; Wu et al., 2016). These contemporary biological sources, in comparison to fuels from geochemical processes, have gained attraction towards sustainable feedstock. However, the combustible form of such feedstock is customized by harnessing the synthesis and accumulation of intracellular compounds such as carbohydrates or lipids. Despite the fact that the biofuels are feasible, only a very small percentage of fuel consumption is accounted for biofuels globally. Certain limitations of plant sources such as land and water consumption, time consuming growth period and food versus fuel debate, microbial sources have become the optimistic solution for biofuel production because of uncomplicated cultivation and production parameters. In fact, microbial sources can be genetically manipulated to produce strains with better fit qualities that lead to improved yields and economic feasibility of the process. Algal strains are one of the commonly worked upon microbial sources for production of both bioethanol and biodiesel. They are easier to be grown in large scale in open ponds, photo-bioreactors or even a simple CSTR system (Hulst, 2012). The requirements for the growth of these algae can be satisfied by wastewater which contains an optimum amount of carbon and some nutrients (Mathiyazhagan and Ganapathi, 2011). Both microalgae and macroalgae have been widely used for their potential towards biofuel production. The extensive work to reduce the gap between the advantages and impracticalities in the production of biofuels from the algal sources is commendable. As algae seem to be the best feedstock for sustainable biofuel production through many researches, emphasizing on the beneficial aspects and constraints of such consistent renewable feedstock is crucial. Therefore, a comprehensive critique on various aspects of biofuel production including the technological renovation and current details in the field of algal energy will be advantageous for many researchers focusing on renewable energy sources.

This review has gathered widespread understanding in the upcoming era of algal energy, thereby accomplishing a broad aspect of the assessment of various parameters in the field of biofuel production using valuable and consistent algal feedstock as the cornerstone. This comprehensive review is based on algae as the central component, featuring its availability, cultivation (open and closed) system, cell membrane disruption, intracellular component (lipids, carbohydrates, etc.) extraction, factors affecting production performances, and challenges and future prospective of biofuel production from algal source. A brief outline of diverse feedstocks that are available for the biofuel production, considering its merits and demerits will be discussed. Various cultivation systems of algae such as open and closed (bioreactors) systems and different pretreatment methods such as physical, chemical and biological ways of disrupting cell membrane for the ease of extracting intracellular components will be explored extensively. In addition, factors that affect the production performances, challenges and future prospective of biofuel in the industrial sector considering its pros and cons will be covered in this review.

2. Energy demand and classification of biofuels (Fig. 1)

2.1. First-generation biofuels

Food based feed stocks such as sugars, starch, corn, rapeseed, sugar beet, sunflower, wheat, barley etc. are the first generation biofuel sources (Table 1). But these feedstocks are not sustainable in situations of rising food demands and “food versus fuel” controversies. Moreover, certain limitations like low return on investment, dependence on fossil fuels for production processes (Doshi et al., 2016), and the higher price of food crops downgrade the economic feasibility for biofuel production from the first generation feedstock. Vegetable oils were the earliest source of first-generation fuels so far reported, which could be used as food as well as to run engines. Dating back to the 19th century, when diesel engines were invented it was first successfully run on vegetable oils. Further, biodiesel are trans-esterified vegetable oils such as canola or hemp oils, animal oil/fats, tallow and waste cooking oils which have long carbon chains resulting in esterified fuels. Vegetable oils when mixed with methanol and sodium hydroxide results in biodiesel and glycerol formation. Glycerides are converted to biodiesel in this way and the glycerol that is produced in this process is an important industrial byproduct (Ullah et al., 2015).

Bio-alcohols are produced by simple fermentation of sugars, starches or cellulosic biomasses from food crops by the application of yeast or others microorganisms forming alcohols which can be used as alcoholic beverages as well as fuels to run engines (Velazquez-lucio et al., 2018). If used as blending fuel, they increase the octane number of the fuel when mixed with conventional fossil fuels such as gasoline which lowers the volatility.

2.2. Second-generation biofuels

The second-generation biofuels have been developed with the objective of overcoming some important limitations of first-generation biofuels i.e. their usage as food as well as on their low return on investment. Therefore, non-edible lignocellulosic feedstocks such as tree biomass, bagasse, Jatropha, agricultural residues, demolition wood, straw, grass etc. are used as starting materials for second generation biofuels. Organic matters from forests litter, wood, leaves, contain more amount of carbohydrates which become raw material for biofuel generation (Ullah et al., 2015) and as they are cheaply available, one can improve the return on investment with less dependence on food crops. But this evacuation of natural organic matter will in turn increase the use of fertilizers and nitrogenous supplements thereby increasing chances of nitrogen oxide emissions and significant biodiversity losses as well (Ullah et al., 2015).

Table 1
Evolution of biofuel feedstocks and their advantages and disadvantages.

Types of feedstock	Biofuels	Advantages	Limitations	References
First generation feedstock (food crops eg. Grains, sugar cane, vegetable oils etc)	Biodiesel, Biobutanol, Bioethanol	• Surplus demand for crops thereby financially assisting agricultural and rural communities • Environment friendly fuel by limiting green-house gas emission into atmosphere	• Food vs fuel controversy (limited feedstock) • Threat to food prices • Requires large arable land area • Production processes require high amounts of energy (growth, harvesting and processing)	(Mohr and Raman, 2015; Naik et al., 2010)
Second generation feedstock (cellulosic energy crops eg. Non-edible crops, agricultural residues, forest residues, forage crops, municipal solid wastes etc)	Biodiesel, Bio-oil, Lignocellulosic ethanol, Syngas, Pyrolysis oil (Biocrude)	• Don't compete food crops and have minimal impact on food prices • Feedstock is less expensive than food crops • Cultivable on marginal and degraded lands • Higher yields of biomass by supplementing lower agri-chemical inputs.	• Requires pre-treatment of biomass • Takes few years for full final feedstock production • Consistent yields of feedstock is quite difficult	(Bhuiya et al., 2016; Carriquiry et al., 2011; Robak and Balcerek, 2018)
Third generation feedstock (Microbial sources eg. Algae, yeast, fungi)	Biodiesel, Bioethanol, Biohydrogen, Biogas, Biomethane	• Ease of cultivation; can use barren lands • Converts CO_2 emissions into useable fuel • Algal biomass per unit area is higher than other feedstocks • Faster growth rate and can grow on sewage, saltwater or industrial wastewater thereby not competing human needs • Completely renewable feedstock for biofuel production	• Production cost of algae based fuel is slightly higher than fuel from other sources • Biodiesel from algal source is less stable than biodiesel from other sources because of the presence of unsaturated oils	(Alam et al., 2015; Singh et al., 2011)

2.3. Third-generation biofuels

Limitations of first- and second-generation biofuels such as low returns on investments, sophisticated techniques for production processes have led the foundation for third generation biofuels. The feedstocks for third generation biofuels are photosynthetic microbes like microalgae, cyanobacteria, and algae. The advantages of algae as biofuel feedstock are multifold. Low area requirements and tolerance of algae to harsh conditions makes them a good choice for biofuel production and algae have the ability to mitigate CO_2 (Liu et al., 2017; Schenk et al., 2008). Algae can grow almost in all types of water like fresh water, seawater, even in industrial waste waters. Considering the growth and oil content, the growth rate of algae is approximately 20–30 times faster than food yielding crops and the oil content of algae is around 30 times more than the conventional first and second generation feed stocks (Ullah et al., 2015). The algae remnants after oil extraction can be used as fertilizers or as fish feed in fish and oyster farms. Since algal based biofuel source is completely biodegradable and virtually Sulphur free, the oil quality is better (Ullah et al., 2015).

3. Algae based biofuels

3.1. Bioethanol potentials of algae

Bioethanol is ethanol or ethyl alcohol derived from a biological source. It can be used as a substitute or an additive to petrol (Nahak et al., 2013). A demand for bioethanol as a transportation fuel is on the rise. Several nations like India, china and Brazil have taken an initiative towards production of bioethanol as a commercial fuel (Lee and Lee, 2016) Bioethanol is preferred not only because of its source, but also on its impact on the environment compared to fossil fuels. It contains lesser amounts of Sulphur compared to petrol thus reducing its harmful emission of greenhouse gases on combustion. It contains about 66% of the energy contained by petrol of the same quantity. Considering its renewable nature, scope for use of bioethanol is high. Bioethanol is manufactured by breaking down of starch or other sugars from first and second generation feedstocks such as corn, lignocellulosic biomass (sugarcane waste), wheat, etc. (John et al., 2011). The drawbacks of second-generation sources are overcome by algae. The debate of 'food versus fuel', use of arable land, and water use are some of them. Algae, a third-generation renewable source, are one of the most sought after sources for the production of bioethanol. Algae have a capability to grow on industrial or municipal waste water. This helps in bioremediation as they consume carbon dioxide, and other nutrients for photosynthesis, thus in turn treating the water (John et al., 2011). Bioethanol can be produced mainly by fermentation of either starch, which is a storage component or by cellulose which is a component of the cell wall (Ullah et al., 2015). Species like *Chlorella vulgaris* can store starch up to 37% of its dry weight. Blue-green algae including Spirogyra species and *Chlorococum* sp. have high levels of reserved polysaccharides in their cell walls (Chaudhary et al., 2014). Different species reserve their food in the form of different components like alginate, mannitol, glucan, galactan and laminarin (John et al., 2011). Algae are considered as beneficial source for bioethanol since they contain a good amount of carbohydrates. Some of the commonly used algae for bioethanol production are *Sargassum, Glacilaria, Prymnesium parvum, Euglena gracilis, Porphyridium, Chlorella, Dunaliella, Chlamydomonas, Scenedesmus,* and *Spirulina* (Chaudhary et al., 2014).

3.2. Biodiesel potentials of algae

Biodiesel, like bioethanol, is also a highly sought-after alternative to fossil fuels. Biodiesel is produced by the transesterification of lipids obtained from algae, to form methyl esters of long chain fatty acids. The length of the chain depends on the source of the lipid. The sources of biodiesel are the oils from palms, soybeans, canola, sunflower, rapeseed, etc. which are more expensive than fossil fuels (Demirbas and Demirbas, 2011). However, these sources too lead to the fuel versus food conflict, high usage of arable land and poor

economy, thus making algae as one of the most feasible sources. Also, from the environmental point of view, biodiesel from algae is more preferable as it has lesser emission of carbon dioxide, NO_x and other greenhouse gases (Scott et al., 2010).

Both microalgae and macroalgae can be used for the production of biodiesel. Some of the most commonly examined species for biodiesel production are *Chlamydomonas reinhardtii, Dunaliella salina, Chlorella* sp., *Botryococcus braunii, Phaeodactylum tricornutum and Thalassiosira pseudonana, Nannochloropsis* and *Isochrysis* sp. (Scott et al., 2010). For production of biodiesel, species with higher weight percentage of lipid content are opted. Some algae have lipid contents as high as 60% of its weight. These lipids are commonly triglycerides or TAGs. They are commonly stored as membrane components, storage products or metabolites. Fatty acids or lipids obtained from algae are generally polyunsaturated, which leads to lower melting points and also instability (Demirbas and Demirbas, 2011). The yield of biodiesel from every batch of algae can be increased by optimizing different parameters. The growth characteristics of the algae can be manipulated such that it leads to more accumulation of fatty acids. This can be done by nitrogen starvation, controlled supply of nutrients and other parameters that effect the lipid formation and accumulation in algae. With respect to this, several studies have been carried out, with varying parameters and comparing the performance of macroalgae and microalgae. Sharif Hossain et al. (2008) studied the performance of *Oedogonium* and *Spirogyra* with respect to amount of biodiesel, biomass produced and the sediments formed. The results show that *Oedogonium* sp. is better for biodiesel production (Sharif Hossain et al., 2008). Demirbas (2008) also conducted a similar study with a macroalga (*Cladophorafracta*) and a microalga (*Chlorella protothecoides*), where the microalgae exhibited better yield (). This could be due to the fast growing nature of microalgae. It can be noted that different microalgal strains have different positive and negative aspects of them. Thus selection has to be made with the practical aspects like economy, ease of use and availability of raw material (Sharif Hossain et al, 2008).

3.3. Biohydrogen potentials of algae

Bio hydrogen could be considered the most upcoming, yet an unchartered area of biofuel production. Biologically produced hydrogen is a renewable, non-polluting and efficient source of energy. Despite of its high energy capacity, which is two to three folds of other non-renewable forms of energy, bio hydrogen is generally not used due to its practical drawbacks (Vassilev and Vassileva, 2016). The production of bio hydrogen is not economical as the yield is very low compared to the investment required. Other major drawbacks include storage and transportation of the non-condensable gas to the required site. Generally, hydrogen is produced by methods like coal gasification or electrolysis of water, but the novel technique of using algal biomass for production of hydrogen is gaining interest (Vassilev and Vassileva, 2016). In algal biomass, bio hydrogen is mainly produced by two different mechanisms (Saladini et al., 2020; Sharma and Arya, 2017). The two major system involved in biohydrogen production are fermentation and photosynthesis. In fermentation, biohydrogen is produced through photofermentation and dark fermentation, while photosynthetic production of biohydrogen occurs via direct biophotolysis and indirect biophotolysis (Saifuddin and Priatharsini, 2016).

Bio photolysis: Bio photolysis occurs in two different mechanisms, direct or indirect. Direct photolysis is the process of breakdown of water molecules into hydrogen and oxygen by the action of an enzyme hydrogenase. The process occurs with an effect of high intensity light energy on a living system (Yu and Takahashi, 2007). Indirect photolysis follows a similar path but the substrate for the process comes from the carbon reserve, wherein energy is reserved in the form of starch, glucose or similar compounds.

Photofermentation: It is the breakdown of an organic substrate to hydrogen and carbon dioxide in the presence of light. It mainly occurs as a part of the TCA cycle, nitrogen being the limiting factor (Sambusiti et al., 2015).

Dark fermentation: The breakdown of complex organic compounds in the absence of sunlight, into simpler monomers which are then converted to low molecular weight organic acids and alcohol is called dark fermentation. Hydrogen is produced along the process, but in very low quantities. Value added by-products like butyric acid, acetic acid, etc. are also produced during the course of the reaction (Sambusiti et al., 2015). One of the major drawbacks of the above-mentioned processes is the evolution of oxygen during the reaction of photosynthesis. The oxygen produced inhibits the activity of the hydrogenase or nitrogenase enzymes halting the process (Shaishav et al., 2013). Hence, in some cases, two or more reactors are used for separation in photosynthetic stage for carbon accumulation and fermentation stage for carbon breakdown (Yu and Takahashi, 2007). The low yield of hydrogen can be improved by the manipulation of culture conditions. It mainly depends on the type of algae used, but the different factors that can be manipulated for a better yield of hydrogen are pH (between 5.2 and 6.0), substrate concentration, feedstock properties, type of bioreactor, etc. (Jankowska et al., 2017).

3.4. Biogas potentials of algae

Biogas refers to a mixture of gases produced by the anaerobic digestion of biomass. This biomass could be agricultural wastes, plant material, sewage, manure, food waste and even algal biomass after the extraction of lipids (Ošlaj and Muršec, 2010). Algal biomass having more lipid content have been found to have more potential to produce biogas and has been theoretically calculated to be around 287–611 L/kg biomass. But this yield of biogas is highly dependent on the algal strain chosen as well as operating temperatures. For example, *Chlamydomonas reinhardtii* produces around 580 L/Kg at temperatures around 28–31 °C whereas *Chlorella-Scenedesmus* produces around 611 L/Kg at temperatures around 45 °C. Moreover, it has also been seen that thermophilic digestion (50–60 °C) yields biogas of around six to ten times more than mesophilic digestion (20–40 °C). Anaerobic digestion occurs by following the four stages as below:

Hydrolysis (Maneein et al., 2018)**:** This is the reaction of breaking down of complex long chain lipids or carbohydrates with water in presence of catalysts such as acids or bases to its corresponding monomers. This break down is also facilitated by exoeznzymes such as cellulosome, proteases etc. produced by fermentative bacteria, protozoa or fungi along with the production of hydrogen.

Acidogenesis (Jankowska et al., 2017)**:** In this stage the soluble monomers are converted to acids such as propionic acids, butyric acids, lactic acids etc. by the reaction of those monomers with hydrogen.

Acetogenesis (Sambusiti et al., 2015)**:** In this stage the mild acids produced in the acidogenesis stage are fed upon by acetogenic bacteria resulting in the formation of acetic acid, CO_2 and H_2. Several bacteria are involved in acetogenesis such as *Syntrophobacter wolinii* (Propionate decomposer), *Syntrophomonos wolfei* (Butyrate decomposer).

Methanogenesis (Sambusiti et al., 2015)**:** This is the last stage of anaerobic digestion where multiple reactions take place resulting in the formation of methane as the major product. In this stage the alcohols and acids produced in the medium are converted to methane in the presence of methanogenic bacteria like

Methanobacterium, Methanobacillus, Methanococcus, or *Methanosarcina*. Among different digestion techniques for biogas production, thermophilic digestion has been observed as best condition for methane production (42–62%).

4. Algae cultivation systems

4.1. Raceway ponds

Algae can be cultivated in raceway ponds, and photo bioreactors (Fig. 2). In raceway pond system, there are two types namely open ponds and covered ponds for the mass cultivation of algae (Table 2).

4.1.1. Open ponds

These are the most economical sites for algal growth which simply consists of natural open ponds which can be both fresh water as well as salt water ponds depending on the algal strain taken for biofuel production. These ponds can be easily scaled up to several hectares but the main disadvantages of such open systems are algae grazers, other algae invasions, fungal growth and contamination of the selected species of microalgae (Ullah et al., 2015).However, around 98% of the total biomass production is achieved using open pond systems (Ullah et al., 2015) and since microalgae growth rates are so high they are capable of producing around 15–20 tons of dried biomass per hectare annually. And around 50–60% of high yielding varieties of algae's dry mass is the oil content which even more economizes the process (Kumar et al., 2015). There are several experimentations on the open raceway pond cultivation of microalgal species. For example, growth of *Chlorella pyrenoidosa* using secondary wastewater effluent in an outdoor open raceway pond has successfully removed excess nutrients and yielded the highest biomass concentration of 1.71 g/L (Dahmani et al., 2016). Similarly, growth of *Dunaliella salina* and *Nannochloroposis* sp. in open raceway pond has obtained biomass productivity of 0.096 g $L^{-1}day^{-1}$ and 0.208 g $L^{-1}day^{-1}$ respectively (Ghorbani et al., 2018). Therefore, open pond system can be considered for obtaining huge biomass for non-edible, non-therapeutic product like biofuel. A comparative assessment of the cost and productivity of the different microalgal cultivation systems is given in Table 3.

4.1.2. Covered ponds

The shortcomings of open ponds are met in covered ponds wherein the other algae invasions and fungal growth are to some extent handled. The huge evaporation losses from open ponds which is a major drawback are also taken care of in these closed ponds (Carvalho et al., 2006). Yet, the drawback in these covered ponds is that since the pond is covered, there is a significant temperature rise and to handle that situation agitation is provided. Many modifications in the configuration of open pond and closed pond have been made for significant improvement in the biomass productivity of selected strains. In a study performed by (Thomas et al., 2015), a thin layer cascade system was built on the roof top and the microalgal cultures were kept in motion on the inclined surface through gravity. The microalgal cultures at the end of the inclined surface gets collected in the tank below the roof and then pumped back to the roof. High evaporation rate on sunny days and volumetric variations on rainy days are properly maintained in the tank which acts as buffering agent. Microalgal species such as *Scenedesmus* sp. (Thomas et al., 2015), *Dunaliella salina* and *Nannochloropsis* sp. (Shenbaga Devi et al., 2012) have been cultured in outdoor systems.

4.2. Photobioreactor

Photobioreactors are man-made or artificial cultivation system which favours the growth of selected strain under optimal conditions such as light, temperature, pH etc. They are different configurations namely tubular, flat plate or vertical column type structures etc. (Table 2), and in the photobioreactors system, the algal cultures are pumped through and recirculated continuously (Carvalho et al., 2006). Since these tubes are made of acrylic or glass materials, they are transparent which aids the photosynthesis and growth of the algae by allowing the natural sunlight to enter.

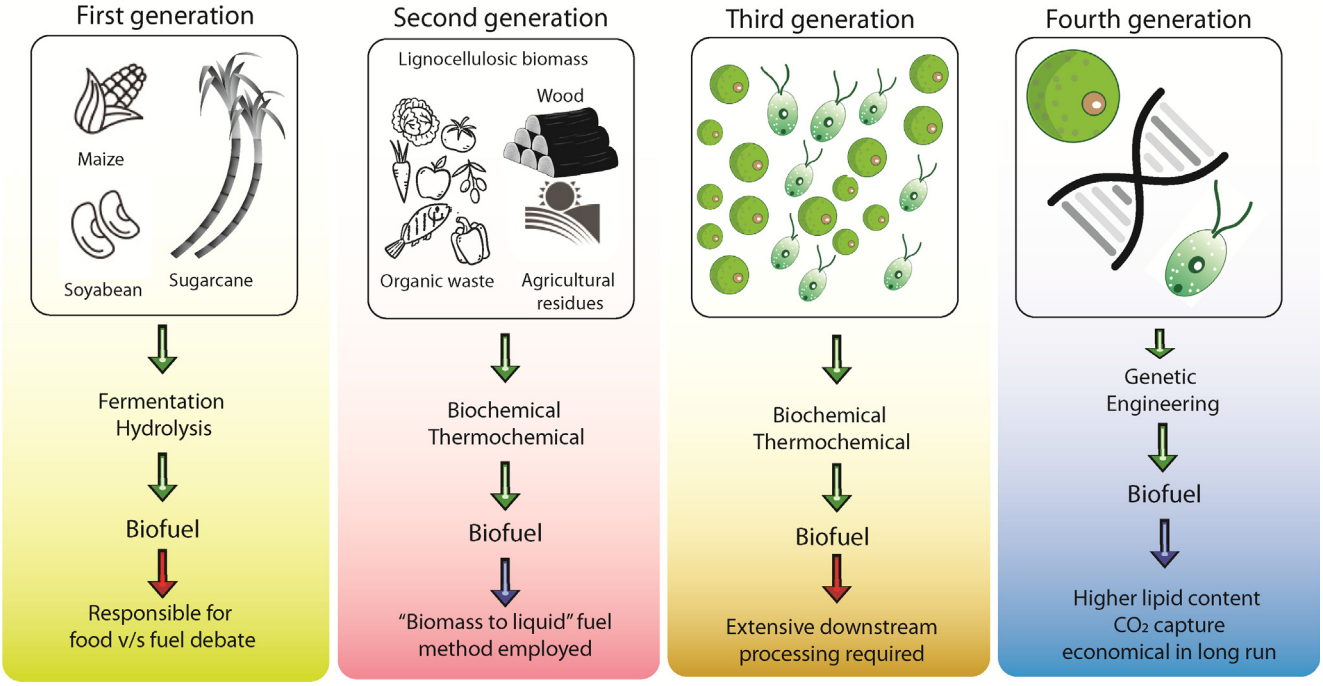

Fig. 1. Generations of biofuels.

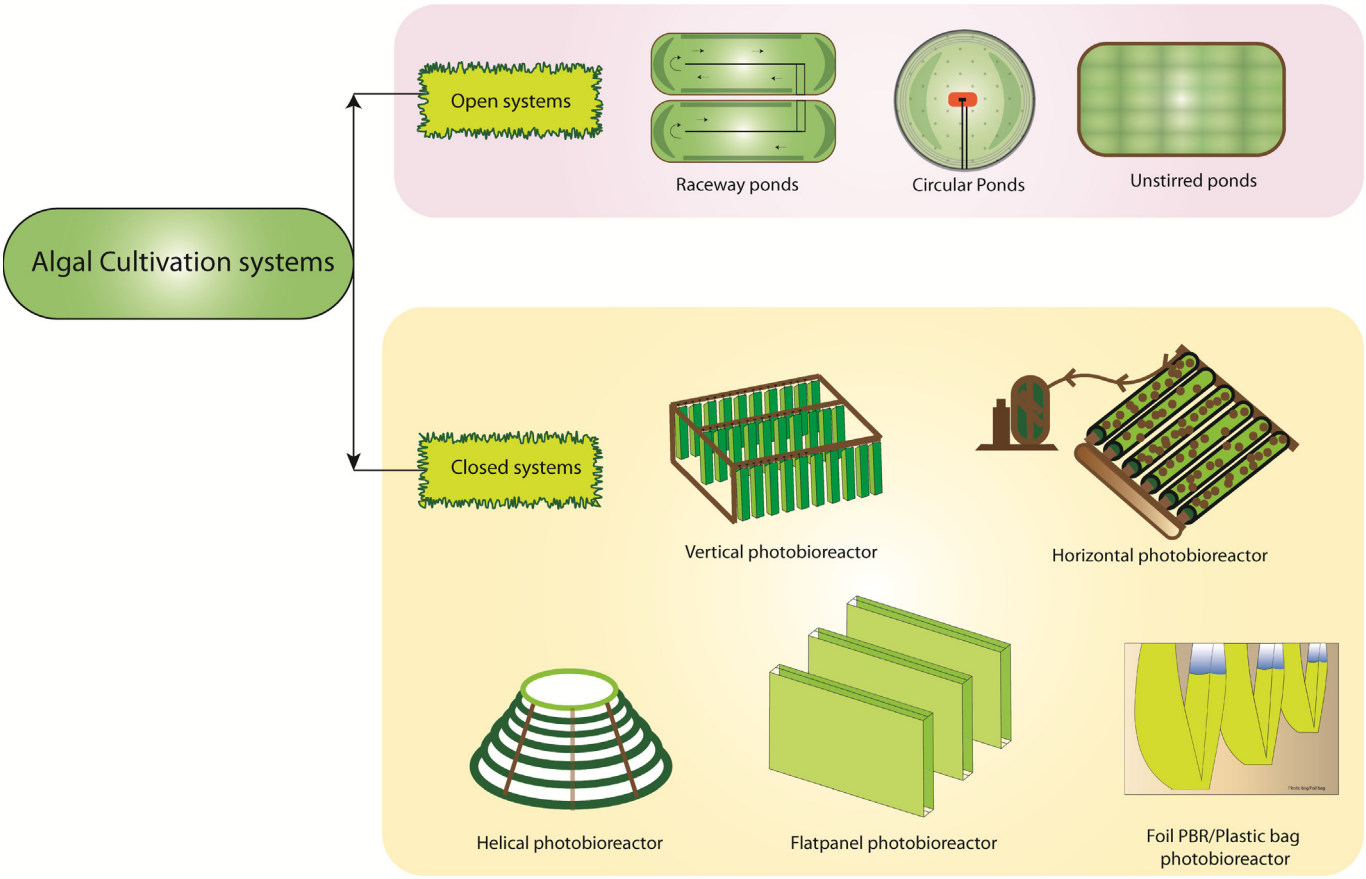

Fig. 2. Different algal cultivation systems.

Photobioreactors can achieve yields up to 100 gms/m^2/hr by the application of light emitting diodes, which replicates the natural sunlight source for indoor photobioreactor and changing the intensity of light can produce very high dark reactions of photosynthesis (Ullah et al., 2015). These PBR's are much costlier than open pond systems but have many advantages of which a few are listed below:

- Fully closed system eliminating the chances of contamination by foreign algal species, fungi or amoeba.
- Evaporation losses are minimal which saves a lot of make-up water needed for open and covered ponds.
- Better heat dissipation systems and nutrient dispersion systems which ensures uniform and controlled growth of algae biomass
- Controlling and monitoring of nutrient levels, CO_2 levels and all other parameters are efficiently done
- Can produce biomass even during night conditions by artificial light emitting diode systems which replicate natural sunlight.

4.2.1. Tubular photo-bioreactors

These are the most common type of photo-bioreactors where the reactors are designed as tubes which are connected in series as parallel arrangement to accommodate the flow volumes. These tubes are generally made of glass or acrylic tubes that are oriented vertically or horizontally and their transparency facilitates the penetration of sunlight for efficient algal growth inside the bioreactor.

4.2.2. Plate photo-bioreactor

These bioreactors are built based on plastic or glass plates. Plates of different design are mounted on stands which can hold a thin layer of culture suspension of algae. The simpler construction of plate photo-bioreactors permits the usage of less expensive plastic materials and thereby made cost effective than tubular photo-bioreactors (Qiang and Richmond, 1996).

4.2.3. Helical photo-bioreactor

These types of photo-bioreactors have a tapered design and have helical coiled structures much similar to tubular PBRs. The tubes are generally made of translucent materials such that biofouling inside the bioreactors does not occur (Carvalho et al., 2006).

4.2.4. Horizontal photo-bioreactor

This has a flat sheet type of configuration with larger surface area to maximize the capturing capacity of incident light on the bioreactor. A rotary pump ensures the proper mixing of the culture broth in a circular motion to the medium (Dogaris et al., 2015).

4.2.5. Foil photo bioreactor

To economize the bioreactors, these types of foiled PBRs have been introduced which are made from low cost PVC or PE sheets which form a bag or vessel type of structure exposing the culture to the sunlight. However, these foil structures degrade over time and have to be replaced.

4.2.6. Tubular bioreactors

These can be further classified into Airlift or bubbling tubular

Table 2
Algal cultivation systems and their merits and demerits.

Cultivation systems	Advantages	Disadvantages	References
Open systems • Circular ponds • Raceway ponds • Unstirred ponds	• Reasonable price for pond construction • Low operating costs • Biomass production from direct sunlight • Agitator/Paddle wheel for constant mixing of algal culture • Ease of maintenance	• Low productivity • Risk of culture contamination • Susceptible to water loss by evaporation • Not suitable for large scale pure culture • Harvesting is difficult and expensive • Not compatible with diverse strain for cultivation i.e. strain specific • Easily affected by atmospheric variations • Poor mass transfer capability that affects biomass production	(Duan and Shi, 2014; Shen et al., 2009)
Closed system (Photobioreactors) • Tubular PBR • Vertical PBR	• Series of parallel tubes connected through loops captures large amount of solar radiation • Less susceptible to contamination • Photosynthetic efficiency is higher • Productivity can be maximized by placing more vertical PBRs than placing a single horizontal PBR	• High light requirements • Growth of algae in the walls of tube blocks light source • Low mass productivity per unit area • Higher areal productivities • Cleaning problems	(Cuaresma et al., 2011; Zhou et al., 2018)
• Horizontal PBR	• Higher volumetric productivity • Higher photon flux densities on the exposed surface penetrates deeper and decrease the dark zone • Inexpensive in terms of construction and less energy is consumed	• Requirement of more space • High energy consumption	(De Vree et al., 2015; Płaczek et al., 2017)
• Helical PBR	• This design provides better spatial distribution of light source and requires minimal space. • Minimizes energy consumption • Capable of high photosynthetic activity by microalgal cells • Effective mass transfer strategies • Scale up is easy	• Useful for cultivation of small volume of microalgal culture • Fouling is observed as a result of blocked tubes with algal biofilm	(Briassoulis et al., 2010; Płaczek et al., 2017)
• Foil PBR/Plastic bag	• Cheap and disposable photobioreactors • Have better surface area to volume ratio • One of the best methods to improve biomass concentration • Temperature can be controlled by immersing these bags in water pool	• Light availability is less (Photolimitation) because of distortion of bags due to gravity • Inadequate mixing and seems to have dark zone • Short lifespan of bags; leakage problems • Not economical for large scale production	(Huang et al., 2017; Liang et al., 2015; Płaczek et al., 2017)
• Flat panel PBR	• High surface area to volume ratio • Light source is spread uniformally and the temperature can be easily maintained by spraying water on the top • High gas-liquid mass transfer rate • Less exposure to contamination by other microbial sources • Biomass and cell concentration increases by exposing low amount of CO_2 • Not affected by external environmental factors	• Scaling up is difficult • Hydrodynamic stress is observed for some algal species • Requires large area for maximal productivity	(Huang et al., 2017; Ojamae, 2011; Płaczek et al., 2017)

bioreactors, Horizontal Tubular reactors, Helical Tubular reactors. These reactors are generally made of transparent materials for sunlight to penetrate, thus helping in photosynthesis. The airlift bioreactors have a dipped pipe through which CO_2 is fed to the system and this creates an agitation in the medium, whereas in the bubbling reactor the same concept is used but with a fitted Sparger at the bottom of the bioreactor through which the CO_2 is fed (Ramnarayan and Sharma, 2015). Horizontal tubular bioreactors comprise of horizontal transparent tubing along with inbuilt gas transfer arrangements. Helical tubular bioreactors have flexible transparent tubing coiled in a helical fashion so as to accommodate greater lengths of tubing in a small space and these bio reactors are quite space efficient (Chen et al., 2011). Scale up of vertical tubular reactors need to consider more working volume and for that pipes

Table 3
Comparative assessment of cost, revenue generated, biomass productivity in large scale algal cultivation systems.

Cultivation systems	Conditions	Productivity (kg m^{-2} d^{-1})	Biomass cost per kg	Biofuel type	Cost of converted product/Revenue generated from product	References
Photobiorectors	Area occupied: 5681 m^2; 6 units considering 132 parallel tubes/unit with the dimension of 80 m length and 0.06 m dia	0.048 (Microalgal biomass)	$2.95	oil	$1.40/L	Chisti (2007)
Open raceway ponds	Area occupied: 7828 m^2; 12 m wide, 82 m long, 0.30 m deep	0.035 (Microalgal biomass)	$3.80	oil	$1.81/L	Chisti (2007)
Closed raceway ponds	Total surface area: 52 m^2; 14 m length, 4 m wide, 0.75 m deep; Capacity: 40,000 L	0.013 (*Scenedesmus obliquus*)	–	Biodiesel	–	Bagchi et al. (2019)
Clsoed system	100 ha scale	Between 0.0084 and 0.014 (Microalgal biomass)	$ 3.83	Biofuel	Lowest revenue per biomass ($ 0.34/kg)	Ruiz et al. (2016)
Open raceway pond	300 kL capacity; 400–1200 μmol m^{-2} s^{-1}, 18–27 °C	11.83 kg d^{-1} *S.obliquus* biomass for digestion	–	Biogas	Revenue generated for selling biogas is $ 793.2/yr	Ansari et al. (2017)
Open raceway pond	Total surface area: 52 m^2, 14 m length, 4 m wide, 0.75 m deep; Capacity: 40,000 L; winter season	0.010 (*Scenedesmus accuminatus*)	–	Biodiesel	–	Koley et al. (2019)

or tubes of bigger diameter are required which again compromises the surface to volume ratio as well as causes a reduction in photosynthetic efficiency. Another major drawback of these bioreactors is that they reflect the sunlight due to the large angles at which they are oriented (Carvalho et al., 2006). However, horizontal tubular bioreactors overcame this problem but due to the high intensity of light penetration, heating is a common drawback of this system. High temperatures inhibit the growth of algae and thus they need additional cooling systems. These cooling systems include air cooling, sprinkling water on the tubes etc. but that again increases the water consumption. Helical tubular bioreactors are a good alternative to Horizontal tube bioreactors. It has a flexible polyethylene tube coiled in a framework, associated with dedicated gas exchange unit and a heat exchange unit, and aslo attached with a centrifugal pump which ensures proper mixing of the culture broth throughout the system (Carvalho et al., 2006). This type of bioreactor is easy to scale up because of the gradient caused by the height of the helical coil. But for a scaled-up design, a powerful centrifugal pump is required which can cause sufficient stress to cause rupture of the algal cells. Therefore, shear resistant algal strains are suitable for this sort of systems. Moreover, artificial illumination can be provided inside the coiled structure to compensate for the large angle towards natural sunlight.

4.2.7. Flat plate bioreactors

These bioreactors are aimed to achieve the maximum efficiency from sunlight. This essentially consists of a number of flat modules connected in series or parallel with an optional degassing unit (Carvalho et al., 2006; Qiang and Richmond, 1996). Thus, these flat plates highly increase the surface to volume ratios. Some basic disadvantages are that the flow control is disrupted in flat plate systems. But since the photosynthetic efficiency is very high, growth rates are moderately high and the oxygen content in the culture increases to a high level and a degasser is thus required which maybe a closed or an open one as has been innovated by many (Ramnarayan and Sharma, 2015).

4.2.8. Fermenter type bioreactors (Carvalho et al., 2006)

These are the conventional fermenter type bioreactors which have this huge disadvantage of a low surface area to volume ratio. Illuminating such systems is also a huge problem. They need internal illumination for a more homogenous distribution of light. CO_2 rich air is sparged from the bottom of the reactor.

4.2.9. Cultivation conditions in photobioreactor

4.2.9.1. Temperature. Typically, microalgae grow in the temperature range of 10–40 °C (Huang et al., 2017). Below this range the growth kinetics are affected while above this range the algae cells die. So, maintaining the temperature inside these reactors are of utmost importance. Open ponds have no temperature control so robust strains of algae can survive there in such temperature fluctuations whereas for temperature sensitive strains, closed systems are feasible where effective temperature control can be exercised.

4.2.9.2. Light intensity. This is the most important parameter for reactor development. Algae can perform photosynthesis in the Photosynthetically Active Radiation range (400–700 nm) (Scott et al., 2010). For Open pond configuration the light intensity goes down with depth from the free surface, hence is undesirable which is well handled in tubular reactors by reducing the thickness of pipes. For more effective light utilization, flat plate bioreactor modules are available. But high intensity of sunlight causes photo oxidation of microalgae which again reduces their productivity.

4.2.9.3. Culture density. This is directly proportional to the growth rate of the microalgae. For open pond bioreactors, low culture density is preferred so that there is uniform distribution of nutrients however in systems such as flat plate or tubular bioreactors, high culture density can be tolerated due to better mass transfer and light distribution is also uniform unlike open pond systems.

4.2.9.4. Carbon dioxide and nutrient concentration. Algae need carbon dioxide to grow and during photosynthesis they produce oxygen. This increases the oxygen content of the water which again starts inhibiting the growth of the algae. This problem is faced in open systems where CO_2 availability is limited and accessible by the algae floating on the surface. To avoid the disadvantages, photo bio reactor systems where CO_2 could be sparged in the culture broth and the close monitoring of nutrients could be done. However, degassing of culture broth is needed at intervals to remove that

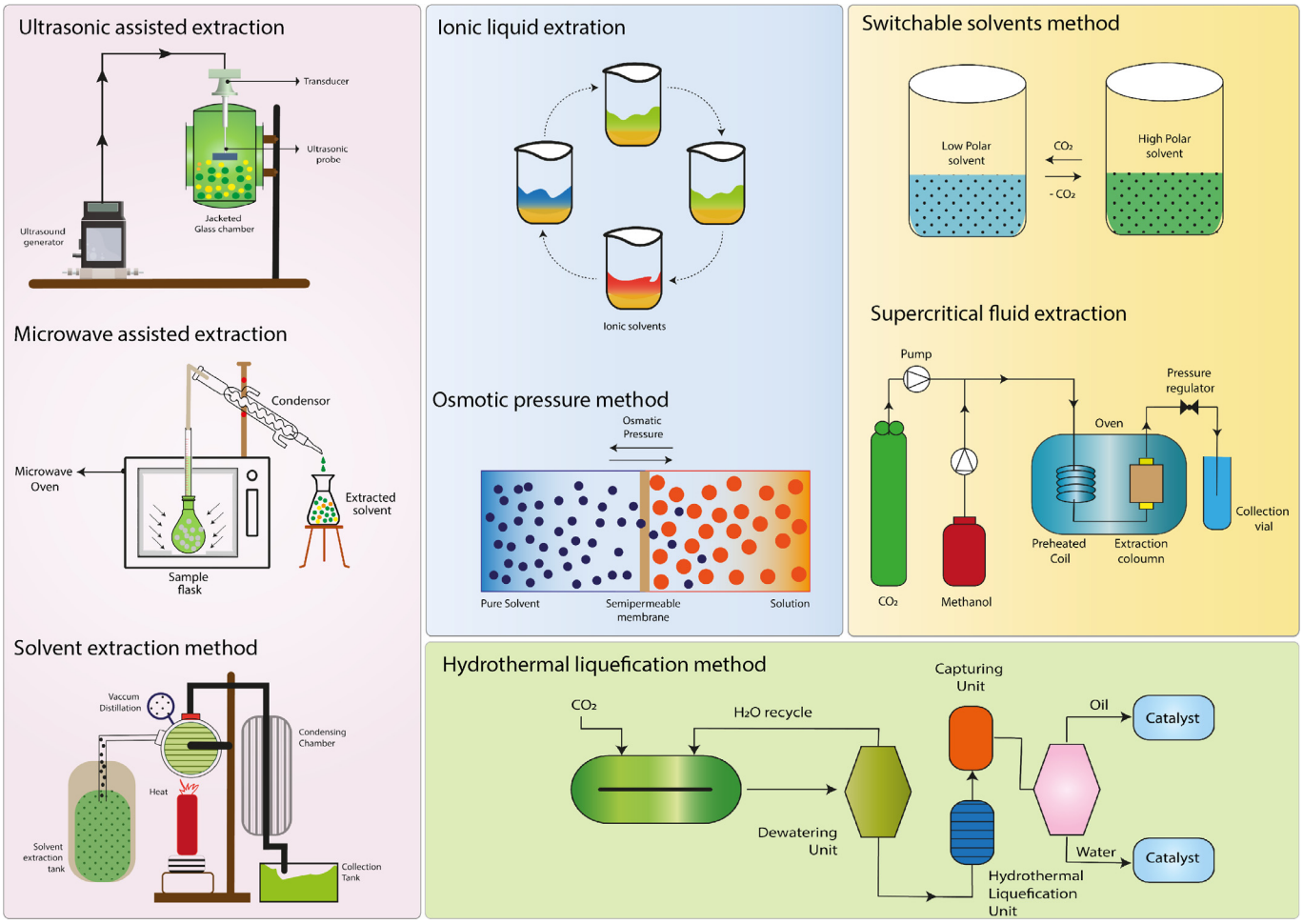

Fig. 3. Different lipid extraction techniques for algae.

accumulated oxygen in the water and keep the growth rate going (Hannon et al., 2010).

4.2.9.5. Mixing. It is one of the most important parameters to ensure uniform growth and nutrient availability of algae. For open ponds, mixing is not there, so the growth can be uneven, whereas in closed systems like flat plate or tubular photo bioreactors, a constant state of mixing is maintained which helps in uniform growth (Qiang and Richmond, 1996). Mixing has its contribution towards the light, CO_2, as well as nutrient availability. Photo bioreactors have sufficient advantages over open pond systems in terms of countering water losses, preventing foreign strain invasion, close and uniform monitoring of the nutrients, light and CO_2 (Carvalho et al., 2006). Moreover, the flexibility of associating a photo bioreactor to a manufacturing unit also improves its techno-economic feasibility when it comes to carbon sequestration. Bioreactors can be Tubular, Flat Plate or Fermenter type. Both tubular and Flat Plate bioreactors are the commercial choice because of their high surface to volume ratios, efficient utilization of sunlight, good mixing efficiencies and high working volume.

5. Types of pretreatments or lipid extraction process in algae fuel production

Algae can be classified into microalgae and macroalgae. Of these two, macroalgae is mostly used for human consumption purposes specifically for their large content of polysaccharides and proteins (Yoo et al., 2015). Microalgae on the other hand are highly preferred for the production of biofuel specifically for their high carbohydrate and lipid content. Another very promising property of microalgae is that their production of lipids and carbohydrates can be drastically increased under biotic or abiotic stress conditions (Yoo et al., 2015). But before using these biomasses for extraction of carbohydrates and lipids a pretreatment step is essential and this step varies based on the feedstocks used for the biofuel production (Fig. 3). The basic objective of pretreatment of algal biomass is to make the raw materials readily available for extraction or for chemical conversion of intracellular compounds such as proteins, carbohydrates, lipids, oligosaccharides, pigments etc. (Velazquez-lucio et al., 2018). Different pretreatment methods being used currently and their limitations are given in Table 4.

5.1. Ultrasound pretreatment

This method involves sound waves travelling through a liquid medium forming areas of compression and rarefaction and as a result creating cavitation, i.e. the formation of bubbles in the liquid medium (Velazquez-lucio et al., 2018). The low pressure inside the bubbles causes them to collapse violently releasing large amounts of energy. This causes variations in pressure and temperature throughout the liquid medium and generates hot spots. The collapsing bubbles have sufficient energy to break the cell walls of microalgae creating micro jets causing the cellular contents to solubilize (Velazquez-lucio et al., 2018). Yet another possible

Table 4
Different pretreatment or lipid extraction techniques for microalgae.

Methods	Microalgae	Advantages	Limitations	References
Ultrasonic-assisted extraction (Ex. 40 KHz, 20 KHz etc.)	*Nannochloropsis oculata*, *Chlorella vulgaris*, *Trichosporon oleaginosus*	• Eco-extraction process • Enhances extraction rate • Reduces time of extraction • Less solvent consumption • Greater penetration into microalgal cell • Possess no effects on fatty acid profile	• Costly approach • Not easy to scale up • Prolonged exposure may create free radicals	(Adam et al., 2012; Kim et al., 2013; Ranjith Kumar et al., 2015; Zhang et al., 2014)
Microwave-assisted extraction (Irradiation power of 300 W, 800 W etc.)	*Nannochloropsis* sp. *Scenedesmus obliquus*	• Efficient heat and mass transfer • Reduced equipment size • Elimination of various sub-processes • Increased production and low solvent usage	• Maintenance cost is higher • Scale up is difficult	(Iqbal and Theegala, 2013; Ranjith Kumar et al., 2015; Zhu et al., 2019)
Solvent extraction method (Ex. Benzene, cyclohexane, acetone, chloroform etc.)	*Chlorella* sp., *Isochrysis galbana*, *Botryococcus braunii*, *Chlorococcum* sp.	• Easy processing and rapid • Efficient and reliable • Some solvents achieve easy solubility of lipids	• Presence of solvent residues after extraction • Some solvents are toxic in nature	(Borowitzka and Moheimani, 2013; Ranjith Kumar et al., 2015)
Ionic liquid extraction (Ex. [Cyano-mim][Br], [Propyl-mim][Br], [Emim][MeSO4] etc.)	*Chlorella vulgaris*, *Neochloris oleoabundans*	• Green solvent • Stable than conventional solvent • Synthetic flexibility; non-volatile • Thermal stability • Non-flammable and less hazardous • Single solvent extraction method	• Solubility of lipid is low • Hydrophobic and water immiscible ionic liquids have lower extraction efficiency • Costly approach • Scale up is difficult	(Jeevan Kumar et al., 2017; Kim et al., 2012; Zhu et al., 2019)
Switchable solvents (Ex. DBU/Octanol system, Secondary amines, Tertiary amines etc)	*Botryococcus braunii*, *Desmodesmus* sp. *N.gaditana* *T.suecica*	• Suited for wet extraction • Reuse of solvents • Efficient extraction • Non-hazardous • Dewatering process is eliminated	• Process intensification have to be studied	(Boyd et al., 2012; Jeevan Kumar et al., 2017; Schuur et al., 2013)
Osmotic pressure (Ex. Induced by NaCl, Sorbitol etc.)	*Chlamydomonas reinhardtii*, *Botryococcus* sp., *Chlorella vulgaris*, *Scenedesmus* sp.	• Cost effective • Economical • Easy and efficient method for lipid extraction • Consumes low energy • Easy to scale up	• Consumes much time • Generates waste salt water	(Byreddy et al., 2015; Ranjith Kumar et al., 2015)
Supercritical fluid extraction (CO_2 is highly selective having density like liquid and viscosity like gas)	*Scenedesmus* sp. *Botryococcus braunii*	• Extraction time is less • Efficient mass transfer • Maximum lipid yield than conventional solvent system • Toxicity level is low • Reduces greenhouse effect by recycling CO_2 • Industrial tool for lipid extraction	• Operational and equipment cost is high • Pre-treatment of biomass is required	(Jeevan Kumar et al., 2017; Taher et al., 2014; Tippelt et al., 2017)
Hydrothermal liquefaction (Thermochemical conversion at subcritical temperature and high pressure)	*Spirulina platensis*, *Nannochloropsis salina*, *Scenedesmus* sp., *Chlorella* sp.	• Energy recovery from biomass to fuel is comparatively high • Consumes energy from feedstock biomass and yield high energy efficiency • Several by-products such as bio-crude, solid waste as fertilizer, processed water are obtained	• Economically not feasible • Equipment and operating cost is high • Scalability issues	(Elliott et al., 2015; Gollakota et al., 2018)
Enzyme-assisted extraction (Ex. Cellulose, neutral protease, alkaline protease, trypsin, Snailase etc)	*Nannochloropsis* sp. *Chlorella vulgaris*, *Scenedesmus dimorphus*	• Cell disruption with minimal damage • Higher lipid recovery • Ease of extraction of neutral lipid bodies	• Types and dosage of enzymes for extraction are high in cost • Better efficiency is obtained when operated at low temperature • Extraction efficiency increases with incubation time • Strongly dependent on pH	(Liang et al., 2012; Ranjith Kumar et al., 2015; Zuorro et al., 2016)

explanation for the ultrasound assisted pretreatment is the formation of microbubbles inside the cells by rapid and continuous compression and decompression of sonic waves, resulting in bursting of microbubbles thereby causing cell wall rupture (Passos et al., 2014a). In case of cyanobacteria, the disruption of gas vesicle is reported when it is exposed to ultrasonication (Tekile et al., 2017). However, the disruption or disintegration of cell wall completely depends on the applied specific energy and the type of microalgae used (Park et al., 2013; Passos et al., 2014a). The ultrasonication units for performing batch operations include horn and bath types which differ from one another in delivering ultrasonic waves to the sample. Horn type ultrasonication unit uses titanium metal horn or probe for creating cavitation whereas bath type ultrasonication unit uses transducers for generating ultrasonic waves (Al hattab and Ghaly, 2015). Application of ultrasonication in microalgal biomass has been increased in the recent times due to its efficiency in biomass solubilization, cell disruption, and in increasing the desired biofuel components. As in case of *Scenedesmus* biomass, ultrasound at 20 Hz with specific supplied energy of 128.9 MJ/kg have resulted in 2 and 3.1 fold increase in methane production and organic matter solubilization respectively with a noticeable disruption of cell wall components than the untreated biomass

(González-Fernández et al., 2012).Similarly, the total energy consumption was 4.79 KJ during sonication pretreatment which yielded 91% of sugars after enzymatic saccharification of *Scenedesmus obliquus* biomass through which the significance of pretreatment on enzyme accessibility to perform hydrolysis is well understood (de Farias Silva et al., 2018). In case of bio-oil production, maximum yield of 28.9% was achieved using ultrasonic-assisted HTL with the sonication parameters of 100 W, 90 s at 250 °C (Saber et al., 2018). Though there are many reports stating the significance of ultrasonic pretreatment of biomass, the major disadvantage of using ultrasonication unit lies in the dissipation of energy with respect to distance (Park et al., 2013).

5.2. Microwave pretreatment

This employs micro waves which are known to heat up water molecules due to the rotation of the dipoles where a polar molecule tries to align in the magnetic field (Velazquez-lucio et al., 2018), eventually vaporizing water molecules and as a result cause disruption of hydrogen bonds in the cells by exerting pressure on the cell walls (Kapoore et al., 2018). It also enhances solubilization of biomass by polarizing the macromolecules, thereby promoting hydrolysis of cellular components and changes in protein conformation (Passos et al., 2014b). Since the cellular contents and the medium is water based, microwaves rupture the cell walls by heating these water molecules. This method efficiently promotes starch digestibility thereby helping in further enzymatic activity. This pretreatment process when applied to microalgal biomass render advantages such as dewatering and biomass thickening which ultimately benefits the lipid extraction (Passos et al., 2014b). Moreover, microwave assisted heating up of cells are controlled by radiation rather than conduction or convection which ease the pretreatment process by rapid increase in temperature (Kapoore et al., 2018). The microwave radiation for pretreatment of microalgal biomass with desired temperature for constant period of time in disintegrating the cell wall aids in subsequent biofuel production process including the extraction and conversion technologies. The microwave asssisted pretreatment of *Botryococcus braunii* has increased the yield of lipids from 18% to 38% at 45 °C for 15 min (Rokicka et al., 2018). Likewise, the optimal microwave conditions for pretreating *Chlorella vulgaris* biomass for syngas production was found to be 750 W for 60s which improved the novel thermochemical conversion technology of chemical looping gasification (Hu et al., 2018). In the recent years, microwave assisted pyrolysis (MAP) is at the peak of conversion of biomass into its corresponding fuels due to its numerous advantages such as i) rapid heating rate ii) low energy input for pyrolysis iii) less activation energy iv) target specification v) automation process (Chen et al., 2019). A latest study on microwave assisted co-pyrolysis of *Chlorella* and tire on 50:50 ratios under N_2 atmosphere gave the highest yield of liquid and solid with desirable chemical compositions consisting of low oxygenate compounds and high hydrocarbon content (Fang et al., 2018). Despites its high maintenance cost and heat generation, it is highly recommended to operate at industrial level (Kapoore et al., 2018).

5.3. Pulse/electric field pretreatments

It is a simple electricity-based technique, also known as electroporation or permeabilization where high electric potentials of the order of 100–300 $kVcm^{-1}$ are applied across cell cultures for a very short duration of time which cause permeations to occur in the cell wall resulting in efficient extraction of vital components such as proteins, carbohydrates or other targeted compounds from the cell (Yoo et al., 2015). The advantage of this method is that it can be used for both low and high cell concentration mediums. When an electric field is applied, the cell wall being negatively charged experiences a dipole moment in the direction of electric field and at reaching a certain threshold electric field, it breaks and perforations are formed (Velazquez-lucio et al., 2018) through which intracellular organic compounds can be extracted. Several advantages of using pulse/electric field pretreatment includes i) application of short electric pulses efficiently rearranges membrane and create pores ii) demands low energy input because of usage of short electric pulses iii) samples are subjected to limited temperature and shear forces iv) does not lead to additional impurities in the pretreatment process v) prevent obnoxious changes in cell membrane (Goettel et al., 2013; Postma et al., 2017). The lipid extraction efficiency of *Ankistrodesmus falcatus* biomass when subjected to pulsed electric field was recorded a high yield of 90% when compared to non-treated biomass (83–88%) (Zbinden et al., 2013). Also, it facilitates the use of low toxicity solvent for extraction leading to low cost and environmental effect which is very well demonstrated in the evaluation study of cell disruption in *Synechocystis* sp. under the pulsed electric intensity of >35 KWh/m^3 (Sheng et al., 2011).

5.4. Mechanical methods

These are the crudest methods widely used at industrial scale for releasing out the cell internals which include bead and ball milling, grinding, high pressure homogenization and cavitation methods (Sambusiti et al., 2015). Bead and ball milling involve attrition forces to work on the cell walls causing their disruption whereas in high pressure homogenization the same concept of attrition is used in liquid medium (Velazquez-lucio et al., 2018). The cell culture is pressurized and driven towards an orifice and this creates high-pressure gradient along the flow of the fluid thus helping in generating viscous shear on the cell walls of microalgae thereby causing disruption. Cavitation methods on the other hand uses throttle valves to maintain this pressure gradient due to which cavitation occurs and bubbles form. When these bubbles collapse, they release large amounts of energy as pressure, waves and heat which helps disrupting the cell wall (Velazquez-lucio et al., 2018). Mechanical pretreatments for microalgal biomass for biodiesel production have been widely reported to successfully aid in lipid extraction process. Similarly, for biogas production, mechanical pretreatment such as ultrasound and microwave have shown promising result in methane yield (Passos et al., 2014c). For macroalgae also, mechanical pretreatment takes place by size reduction (cutting mill, centrifuge mill and ball mill), washing and sonication which subsequently reduces the problems in downstream processing (Maneein et al., 2018).

5.5. Freezing/thawing pretreatments

This method cools down the cell culture to subzero temperatures so that the water inside the cell crystallizes to ice and this transformation readily breaks the cell walls due to increased volume of the ice crystals (Yoo et al., 2015). The underlying mechanism of cell damage by freezing process is explained by different proposed statements. These include i) extracellular constituents namely electrolytes and other solutes which are present in higher concentrations are reported to damage the cells by formation of ice when exposed to water removal through dehydration process ii) flow of water through osmosis via cell membrane also causes cell damage iii) Cell damage by shrinking of cells due to highly concentrated extracellular components iv) slow cooling rate damages the cell by forming large external ice crystals v) rapid cooling rate damages the cell by forming intracellular ice crystals (Taylor and Fletcher, 1998). However, the process faces severe

disadvantages consisting of high energy input, tedious and expensive process, high maintenance cost of pumps, and most importantly cell wall is not completely disrupted but it is weakened.

5.6. Hydrothermal pretreatment

This pretreatment method is an alternative to enzymatic pretreatment and employs heat to breakdown cell walls of the microalgae (Biller and Ross, 2012). It can be done in neutral, acidic as well as alkaline mediums but the disposal of the waste water of acidic and alkaline hydrothermal pretreatments are hazardous and thus this operation in neutral medium is highly preferred. In addition, water can react with numerous solutes because of its disparity in dielectric constants with respect to temperature, particularly under hydrothermal conditions (Okuda et al., 2008). Besides, the downstream contamination is also avoided in neutral medium operations which might otherwise interfere with the microbial growth in the fermentation stages. This method operating at a range of 60–180 °C (Velazquez-lucio et al., 2018) sometimes even going up to 200 °C (Pirwitz et al., 2016) for short residence times of up to 60 min which helps rupturing the cellulosic cell walls and solubilizing various organic compounds resulting in a gelatinous mass. This method might also employ high pressures along with the high temperature which might result in the degradation of certain proteins and other organic compounds. The acid hydrothermal treatment uses Sulphuric and hydrochloric acids which helps degrading the cellulose matrix and hydrolysis of starch into simple sugars (Velazquez-lucio et al., 2018). The alkaline hydrothermal treatment generally uses Sodium Hydroxide which has a solvating effect on the cell wall creating pores in them and helps decreasing the size of starch molecules. In fact, hydrothermal pretreatment is also employed to reduce the N content in bio-oil which is demonstrated in *Nannochloropsis occulata* biomass where the amount of N content was decreased and the carbon content was increased in the resulting bio-oil (Du et al., 2012).A recent study aimed at rectifying the high energy consumption of hydrothermal pretreatment by coupling with solar energy and was considered as alternative energy saving pretreatment process (Xiao et al., 2019).

5.7. Enzymatic pretreatment

This pretreatment method is preferred over others due to its main advantage of being highly specific and efficient without forming any inhibitory byproducts (Al Abdallah et al., 2016). Enzymatic pretreatment totally depends on the composition of microalgal cell wall as the catalytic activity of enzymes act according to the cell wall component which includes cellulose, pectin, hemicellulose, glycoprotein etc (Hom-Diaz et al., 2016). However, the cell wall components vary based on the microalgal strain, the ambience of algae consisting of media growth conditions, nutrient concentration and different phases of algal growth (Gerken et al., 2013). Single enzyme or multi enzyme mix is available for disintegration of cell walls, in which the most common single enzyme available for disruption belongs to carbohydrases which includes amylases, cellulases, pectinases, hemicellulases and the multi enzyme mix or the enzyme cocktail consists of different combinations of lysozyme, protease, laccase and carbohydrases (Magdalena et al., 2018; Passos et al., 2016). Many microalgae and macroalgae upon enzymatic pretreatment are reported to yield higher quantity of biofuel components. For instance, cellulase and the enzyme mix consisting of cellulase, glucohydrolase and xylanase on microalgal biomass showed higher methane yield of 8% and 15% respectively when compared to non-pretreated microalgal biomass (Passos et al., 2016). In fact, the fusion of two or three pretreatment processes enhances the yield and also reduces the cost to a reasonable extent. The mechano-enzymatic (vibro-ball milling and centrifugal milling – Haliatase cocktail) disintegration of two macroalgal species namely, *Ulva lactuca* and *Gelidium sesquipedale* have been reported to yield higher amount of total sugars (13.1 g/100 g total soilds (TS) for *U.lactuca*; 10.8 g/100 g TS for *G.sesquipedale* at enzymatic concentration of 30 g/L; total sugars from enzymatic treatment increased to 126% and 129% after treatement with vibro-ball milling and centrifugal milling) and bioethanol (6 g/100 g TS for *U.lactuca* with only enzymatic treatment; 4 g/100 g TS for *G.sesquipedale* with enzyme and centrifugal milling) (Amamou et al., 2018). The cell wall polysaccharides are hydrolyzed using glucanases and glucosidases to break the cellulose matrix and glycosidic bonds forming glucose and maltose (Cristina et al., 2016). Thus degeneration of cellulose matrix further helps in extraction of different target compounds (Velazquez-lucio et al., 2018). As a matter of fact, enzymatic hydrolysis requires enzymes which are costly and reduces the cost effectiveness of the pretreatment (Khan et al., 2018). All these pretreatment methods are chosen based on the feedstocks used and the biofuel that is desired. For example in bioethanol or bio hydrogen production, chemical and enzymatic pretreatments are required whereas hydrothermal pretreatment would be mostly required for lignocellulosic feedstocks (Yoo et al., 2015).

6. Factors affecting production performance

6.1. Algal strain and medium composition

About 1,00,000 strains of different algae have been identified growing on fresh, saline and terrestrial conditions. However, only a few are used for cultivation of biofuels. These strains are dependent on factors such as light, pH, temperature, salinity, nutrients, and even water circulations (Vassilev and Vassileva, 2016). They have three major components such as lipids (5–60%), proteins (40–60%) and carbohydrates (8–30%) in varying proportions (Kumar et al., 2014). In the case of cultivation medium, a lot of different mediums such as fresh water, saline water, industrial waste water, even dairy farm waste waters are used. Many culture media have been developed considering the sole components required for growth of different species. In fact, the nutrient components in culture media vary for each species belonging to different classes.

The effect of different nutrients such as nitrogen, phosphorus, iron on lipid productivity of *Ankistrodesmus falcatus* was analysed through response surface methodology using Box-Behnken design which showed that the highest lipid productivity of 74 mg L^{-1} d^{-1} was obtained at 750 mg L^{-1}, 0 mg L^{-1}, and 9 mg L^{-1} concentration of nitrogen, phosphorus, and iron respectively (Singh et al., 2015). However in another study carried out by Yang et al. (2014), response surface methodology by Box-Behnken design for lipid production showed that the three important nutrients $NaHCO_3$ at 3 g/L, $NaH_2PO_4 \cdot 2H_2O$ at 15 mg L^{-1}, $NaNO_3$ at 750 mg L^{-1} gave the highest lipid production for *Scenedesmus* sp. The nutritional contents of *Nannochloropsis occulata* such as proteins, carbohydrates, pigments, ascorbic acid were optimized by Plackett-Burman method to find out the most effective nutrient component in the seawater enriched f/2 medium. This study concluded that NO_3 and PO_4 are the important nutrients in growth of *N. occulata*, a 1.5 time increase of NO_3 concentration than f/2 media increased the protein content, whereas decrease of PO_4 and increase of NO_3 increased the carbohydrate content, β-carotene, and ascorbic acid (El-sheekh et al., 2016).

6.2. Light intensity and CO_2 concentration

Light is important for CO_2 assimilation and biomass production. As light intensity varies between algal species, optimizing light intensity is essential for algal cultivation (Mathimani et al., 2018). Various statistical methods have been used to optimize the light intensity for microalgal growth and lipid content. The effect of light intensity on *Ettlia* sp. was studied and the optimal condition was found by experimentation using response surface methodology with central composite face-centered (CCF) design. This study showed the optimal light intensity at 730 $\mu E\, m^{-2}\, s^{-1}$ where the maximum obtained biomass productivity was $28 \pm 1.5\, g\, m^{-2}\, d^{-1}$. However, maximum lipid productivity of $4.2 \pm 0.3\, g\, m^{-2}\, d^{-1}$ was obtained at 500 $\mu E\, m^{-2}\, s^{-1}$ (Kim et al., 2018). Another study involved the use of mathematical modelling based on specific growth rate for finding out the optimal average daily light irradiance for microalgae and cyanobacterial species. This study optimized the average light irradiance to be 208 $\mu E\, m^{-2}\, s^{-1}$ for *C. vulgaris*, 140 $\mu E\, m^{-2}\, s^{-1}$ for *M. aeruginosa*, 258 $\mu E\, m^{-2}\, s^{-1}$ for *P. subcapitata*, and 178 $\mu E\, m^{-2}\, s^{-1}$ for *S. salina* (Gonçalves et al., 2016). To the contrary, *C. vulgaris* was reported to have high biomass and lipid content at 20 $\mu mol\, m^{-2}\, s^{-1}$ (Mathimani et al., 2018). Likewise, several studies have reported the importance of light intensity on microalgal growth substantiating the increase of biomass concentration under optimal light conditions (Ota et al., 2015; Qiang and Richmond, 1996; Xu et al., 2016). Another important factor that influences algal biomass and lipid yield is CO_2 concentration. Optimization of CO_2 concentration in *Ettlia* sp. for maximum biomass and lipid concentration has been experimented using response surface methodology with CCF design which resulted in 8% CO_2 and 7% CO_2 as optimal concentration for maximum biomass ($28 \pm 1.5\, g\, m^{-2}\, d^{-1}$) and lipid ($4.2 \pm 0.3\, g\, m^{-2}\, d^{-1}$) productivity respectively (Kim et al., 2018). In case of *Chlorella vulgaris*, the optimal concentration of CO_2 was found to be 6.5% which was statistically determined through response surface methodology with central composite design (CCD) having $R^2 > 0.90$ (Anjos et al., 2013). However, in a study conducted Kasiri et al. (2015), 22% CO_2 concentration maximized the specific growth rate to 0.310 d^{-1} whereas at 35% CO_2 concentration, maximum CO_2 uptake rate of 63.03 $mg\, L^{-1}\, d^{-1}$ in *Chlorella kessleri* was observed.

6.3. Temperature

In addition to the light intensity, temperature is also an important parameter to have a positive impact on biomass and lipid yield of microalgae. For *Chlorella protothecoides*, maximum lipid productivity of 274.15 $mg\, L^{-1}\, d^{-1}$ was observed under optimal temperature and pH at 28.63 °C and 6.51 respectively which was statistically proven by response surface methodology using Box-Behnken design with smaller p-value ($p < 0.001$) indicating its significance (Binnal and Babu, 2017). In case of higher growth rate, the optimal temperature of 25 °C and 20 °C was reported for *Nannochloropsis occulata* and *Tetraselmis subcordiformis* respectively whereas the neutral lipid concentration was found to be at higher level under 15 °C and 20 °C for *T. subcordiformis* and *N.occulata* respectively (Wei et al., 2014). It is reported that, higher temperatures disrupts the cell metabolism and cease the proliferation of cells through enzyme damage (Renaud et al., 2002).

7. Perspectives and prospects for future research works

Efficiency of biofuel production from algae depends on various physico-chemical and biological factors including irradiance, temperature, mixing, pH, dissolved gases, qualitative and quantitative characteristics of biomass, photosynthetic efficiency. In the case of light irradiance, biggest challenge in an algal growth system is availability of sunlight. This is done by fabricating the system with transparent materials such as Acrylics, plastics, or glass however it will become costly. So, to maintain the cost effectiveness of the establishment it is preferred to go with acrylics type material. It has also to be kept in consideration that algae don't accumulate on the walls of the tubes which might again hinder the transmittance of sunlight. During night hours, artificial illumination helps algae to produce biomass all day round enhancing the overall productivity. Nutrient Solubility is one more factor to be considered in algal growth systems. As the CO_2 is bubbled through the system HCO_3^- ions start forming. Although algal cells can take up these ions but the control of algal culture pH becomes difficult. So, it is widely preferred to administer gaseous CO_2 using a bubbler or a sparger kind of arrangement. Till date more than 20 algal genomes have been fully sequenced and studied thus giving important insights into the machinery and mechanisms involved in the production of biofuels and bioenergy from various algal species in isolation and in consortium. The identification of genes and enzymes involved in the biosynthesis of algal lipids and carbohydrates can then be employed for manipulating the efficiency of biofuel production with the help of up/down-regulation of associated genes or site-directed mutagenesis. Overall, algal biomass can be contemplated as a justifiable and potential source of bioenergy. The recent efforts in sequencing algal genome sequences have facilitated isolation of genes involved in lipid biosynthesis, photosynthesis, anaerobic adaptation, and stress regulation. However, the molecular toolbox required for reliable genetic manipulation of microalgae remains limited to only a few species. Therefore, identification and interpretation of molecular mechanisms underlying various favorable traits in highly oleaginous algal species such as capability of anaerobic fermentation and enhanced oil accumulation under stress conditions are of fundamental and practical importance to algae as a feedstock for the derivation of fuels. Multidisciplinary research needs to be carried out to make algae derived fuels a genuine industrial service. Particularly, biology, biotechnology and engineering can be integrated together with life-cycle assessment for the optimization of bioenergy production from algae. Further, to make the biofuel production process economically viable, cost related to cultivation, harvesting, pretreatment and processing of algal biomass must be reduced for enhancing the sustainability of the process. The valuable co-products such as arachidonic acid, docohexaenoic acid, eicosapentanoic acid, β-carotene, omega-3 fatty acids etc. can make the production process a economically feasible by surmounting the cost hurdles. Moreover, from a commercial perspective, standard or well established innovative technologies in biofuel production process might make better investments and improve the profitability of the process.

8. Conclusion

First and second-generation biofuels are not abundant enough to meet the global requirements. A high dependence on these feedstocks may affect the global carbon cycle as well. In this perspective, algae are photosynthetic living organisms that present a potential emerging feedstock for biofuels production. Algal lipids and carbohydrates can be processed into bioethanol, and biodiesel, respectively through suitable protocols. In this article, the potential of algae as a source for various biofuels such as biodiesel, bioethanol, biohydrogen and biogas were described and their challenges were also highlighted. Various algal cultivation systems like open pond and photobioreactor were extensively discussed and limitations were also given for further research attempts. Eventually, various algal pretreatment processes and factors affecting the production performance were intensively addressed. However,

several R & D works need to be undertaken to overcome the barriers on algal biofuel production systems.

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