



## Utilization of algae for biofuel, bio-products and bio-remediation

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### ABSTRACT

Algae are considered as a promising source for biofuel and bio-products. Algae contain carbohydrates, lipids, proteins and their photosynthetic and lipid accumulating potential makes them a suitable candidate for bioenergy. Algal biomass is used in the production of biofuels like biodiesel, bioethanol, biobutanol, and biohydrogen etc. Therefore, this review provides an overview on the usage of algal biomass in multiple applications like fuel, food and environment. Initially, evolution of first and second generation biofuel feedstocks and their limitations have been described. Later, advantages of third generation biofuel feedstock i.e., algae over first and second generation feedstock were discussed comprehensively. Apart from bioenergy production from algae, industrially important co-products or value added products extracted from microalgae were addressed. Various microalgae utilized for the extraction of omega-3 fatty acids were listed. Eventually, environmental application of microalgae namely wastewater treatment and CO<sub>2</sub> sequestration were presented.

### 1. Introduction

Unsustainability and continuous exhausting of non-renewable fossil fuels have initiated importance of renewable fuel sources (Chiappe et al., 2016). In addition, increasing concerns about global warming due to fossil fuel consumption and increasing fuel price had led to explore alternative and renewable energy (Mathimani et al., 2015; Subsamran et al., 2018). At this juncture, biofuel came to limelight to avert green-house gaseous emissions and to substitute fossil fuel. Biofuel is a non-toxic, alternative, renewable, eco-friendly fuel produced from various feed stocks (Chi et al., 2018; J. Gupta et al., 2016; Sharma et al., 2018). Among the feedstocks, microalgae show immense potential in replacing fossil fuels to ensure energy security, and to obviate the environmental hazards across the globe. This could be due to the high biomass producing capability of microalgae over terrestrial crops, and also microalgae require ~49–132 times less area compared to soybean crops (Chisti, 2007).

In addition to fuel production from microalgae, various non-energy products or high value- products can also be extracted from microalgae, which are considered to be commercially valuable (MubarakAli et al., 2012; Trivedi et al., 2015). Microalgae comprise of three major macromolecules namely carbohydrate, protein, lipid, and therefore they have long been employed as a promising biomaterial for the production of various industrially important co-products. Based on the processing

methods, different products could be obtained from the microalgal biomass. The complete or whole microalgal biomass can be transformed into various bio-products through pertinent conversion techniques. Recent past, biorefinery approach has been practiced to transform the microalgal biomass into fuels, products, and other value-added compounds (Chew et al., 2017). Further, microalgae can be used to treat waste water through phycoremediation and mitigate CO<sub>2</sub> from the atmosphere to maintain environmental sustainability. It is essential to provide a comprehensive view on utilization of algae in various sectors like fuel, food and environment. Considering the vast potential of algae, production of fuel or food products from various algal biomasses needs to be tabulated to choose the pertinent algal species. Therefore, this review is attempted to focus broadly on the biofuel, bio-products and bioremediation aspects. Initially, utilization of different feedstock for biofuel production over the generations will be discussed and several valued-added products extracted from algae will be summarized. Eventually, insights on the environmental benefits of algae will be described.

### 2. Biofuel

Biofuel is defined as the renewable, alternative green fuel produced from various biological sources. Biofuel includes biodiesel, biohydrogen, bioethanol, and biogas. Depends on the feedstock used, the

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evolution of biofuel is classified into three generations as first, second, and third generation biofuel feedstocks (Baldev et al., 2018; Prabakar et al., 2018). First generation feedstocks (FGF's) include edible crops such as oil seeds, soybean, wheat, rapeseed, potato, coconut, barley, sunflower, sugarcane, sugar beet, corn and further, corn ethanol in US, sugarcane ethanol in Brazil, and other biofuels elsewhere, have been produced from first generation feedstocks to satisfy the global energy demand between 2000 and 2008 (Alam et al., 2015; de Vries et al., 2010; Fei et al., 2017; Sims et al., 2010). However, the major challenges like food vs fuel controversy, competition for land and water used for food production, increased processing and production price compared to petroleum (Sims et al., 2010). Considering the demerits, FGF could not be a practicable choice to satisfy the global energy need despite being renewable. Therefore second generation biofuel feedstocks (SGF,s) were identified, which includes non-edible crops like, lignocellulosic material, cereal straw, sugar cane bagasse, Cassava, miscanthus, forest residues, municipal solid wastes, vegetative grasses, Jatropha, and wood (Alam et al., 2015; Maity et al., 2014; Sims et al., 2010). At present, utilization of SGF for biofuel production is being done in full swing at different stages. However, biofuel produced from second generation biofuel feedstocks are considered to be expensive as several technical barriers are existing to overcome and pilot scale demonstration facilities are needed (Naik et al., 2010). Second generation biofuel production requires expensive and sophisticated technologies (Alam et al., 2015), and therefore, commercialization remains as a major limitation (Maity et al., 2014).

### 2.1. Microalgae- third generation biofuel feedstock

To overcome food vs fuel dilemma and other shortcomings associated with the utilization of FGF and SGF, third generation feedstocks (TGF's) came into limelight of sustainable biofuel feedstock i.e, algae. Many researchers or research groups are actively engaged in this venture. As a government initiative, National Renewable Energy Laboratory (NREL), U.S.A had launched an R&D program specifically for renewable fuels, which underpins microalgal biodiesel production (Sheehan et al., 1998). The objective of the NREL scheme was to assess the lipid synthesis mechanism in high lipid producing algae and to use the genetic engineering approach for improving microalgal isolates. TGF possesses several advantages viz., less generation time, high photosynthetic efficiency, high growth rate, high lipid productivity, and cheaper nutrients for growth towards sustainable and economically feasible fuel production (Mata et al., 2010; Mathimani et al., 2018; Shimako et al., 2016). Further, replacing first generation feedstock with third generation microalgal feedstock might decrease carbon footprint of the process (Chiappe et al., 2016). Different generations of biofuel feedstock were listed in Table 1. Microalgae are being used by several researchers to produce biofuel as given below. In a recent study, suitability of *Chlorella vulgaris* BDUG 91771 biodiesel-diesel blend to use as a fuel in Kirloskar compression ignition engine has been tested. Based on engine performance and emission profile, biodiesel of *Chlorella vulgaris* BDUG 91771 comply with ASTM D6571 and EN 14214 standards. Further, B40 or B50 biodiesel-diesel blend showed preferable specific fuel consumption, brake thermal efficiency and lowered emissions of carbon monoxide, hydrocarbon, and carbon dioxide (Mathimani et al.,

2017). The microalga *Neochloris aquatica* was studied for biodiesel production by estimating the lipid content and fatty acid composition. The lipid content of freshwater *Neochloris aquatica* grown in artificial pond was 12%, which was calculated through Nile red fluorescence method and analysis of the fatty acid methyl ester of the tested strain showed saturated, monounsaturated and polyunsaturated fatty acids content in the range of 29.15%, 37.95%, and 32.90% respectively (Jaiswar et al., 2017).

Further, *Botryococcus braunii* (*B. braunii*) or *Schizochytrium* sp. comprised of 80% lipid per dry weight, which is higher than oleaginous colza, sunflower crops (Chiappe et al., 2016). The microalga *B. braunii* is able to accumulate hydrocarbons (30,000–42,000 kJ/kg thermal value), in the range of 75–86% and based on the hydrocarbon production, three races of *B. braunii* A, B, and L were documented such as n-alkadienecfcazes/trienes, botryococcenes, and lycopadienes, respectively (Calderón et al., 2018).

In another study, the freshwater cyanobacterium was identified as *Synechococcus elongates* after purification (serial dilution and UV radiation at 280 nm for 10 min), and on day 16 the biomass yield and biomass productivity of the strain was estimated as 1.63 g/L and 0.116 g/L/day respectively. On the other hand, lipid content and lipid productivity of *Synechococcus elongates* was 18.5% and 0.02 g/L/day respectively (Mashayekhi et al., 2017). Though microalgae are advantageous over other feedstocks, still certain expensive bottlenecks need to be resolved to make a competitive algal biofuel industry (Chiappe et al., 2016).

### 3. Bio-products

With reference to the non-fuel products, industrial significance of microalgae is enormous. As shown in Table 2, algal biomass can be utilized for several industrially important co-products or valued added products like pigments, livestock feed, organic fertilizer, cosmetics, chemicals, food, nutraceuticals, and even for electricity generation through combusting (Chew et al., 2017; Spolaore et al., 2006). Among the major components in microalgae, proteins occupy 50–70% of the microalgae composition and they are mainly used in human or animal nutrition (Chew et al., 2017). Proteins and pigments of algal strains have substantial potential for several medical and pharmaceutical uses (Chew et al., 2017). Algal natural pigments carotenoids, chlorophylls and phycobiliproteins are the precursors of vitamins in food and animal feed, colouring agents, biomaterials, cosmetics, and pharmaceutical (Chew et al., 2017). Commercially viable pigments were obtained from various microalgae like astaxanthin from *Haematococcus pluvialis* and  $\beta$ -carotene from *Dunaliella salina* (Trivedi et al., 2015). Commercially viable carotenoids are extracted from algae and they are used as poultry and fish feed or as natural food colorants or in cosmetic industry (Spolaore et al., 2006). On the other hand, the major photosynthetic pigments in cyanobacterial strains are phycobiliproteins, which are used as natural dyes in food and pharmaceutical industry. Phycobilins are important colouring pigments used in cosmetic and food industries. Further, phycobiliproteins are used in health sectors as antioxidant, anticancer, antiviral, anti-inflammatory, anti-allergic, and neuro protective material (Chen et al., 2013; Spolaore et al., 2006).

Phycocyanin are also used significantly to impart colour to foods,

**Table 1**  
Different generations of biofuel feedstocks (Hemaiswarya et al., 2012; Maity et al., 2014; Saravanan et al., 2018).

Generation of biofuel feedstocks	Feedstocks used	Disadvantages
First generation	Wheat, barley, corn, Potato, sugarcane, beet, soybeans, coconut, sunflower, rapeseed	Food vs fuel controversy, land and water usage. High processing and production price
Second generation	Lignocellulosic material, Cassava, Jatropha, Miscanthus-grass, aquatic biomass, water hyacinth and other non-edible biomass.	Technical constraints, need of expensive and sophisticated equipment.
Third generation	Algae	Expensive

**Table 2**

Value added products obtained from algal strains (Mobin and Alam, 2017; Pulz and Gross, 2004; Winwood, 2013).

S.No	Products	Strains	Applications
1.	Phycocyanin, protein, vitamin B <sub>12</sub> , biomass	<i>Arthrospira</i> , <i>Spirulina</i> sp.	Health, cosmetics, antioxidant capsule
2.	Biomass, carbohydrate	<i>Chlorella</i> sp.	Animal nutrition, health drinks, food supplement, feed surrogates
3.	Carotenoids, $\beta$ -carotene	<i>Dunaliella salina</i>	Health, food supplement, feed
4.	Carotenoids, astaxanthin	<i>Haematococcus pluvialis</i>	Health, pharmaceuticals, feed additives
5.	Lipids, fatty acids	<i>Phaeodactylum tricornutum</i>	Nutrition, biofuel
6.	Biomass, EPA	<i>Nannochloropsis oculata</i> , <i>Nannochloropsis</i> sp.	Nutrition, feed for larvae and juvenile marine fish
7.	Polysaccharides	<i>Porphyridium cruentum</i>	Pharmaceuticals, cosmetics
8.	EPA	<i>Phaeodactylum tricornutum</i> , <i>Nannochloropsis</i> , <i>Nitzschia</i>	Food supplement, nutrition

which includes the colouring of fermented milk products, soft drinks, alcoholic drinks, desserts, sweet cake decoration, ice creams, chewing gum, milk shakes and in fish feeds (Suganya et al., 2016). Astaxanthin pigment is considered as potent antioxidants as it has strong anti-aging, anti-inflammatory, sun proofing, and immune system boosting characteristics, which are beneficial in food, feed and nutraceutical applications (Cheng et al., 2016). The pigment carotenoids are widely present in prokaryotic and eukaryotic strains, and they are used as light energy harvesters, photoprotectants, antioxidants. The halophilic *Dunaliella parva* strain is a rich source of natural carotenoids known as  $\beta$ -carotene, which are better antioxidants used for human health and for various industrial applications. In order to increase the carotenoids content through genetic engineering approach, phytoene synthase gene from cyanobacterium *Synechocystis* sp. PCC 6803 was cloned into *Dunaliella parva* strain. The transformed *Dunaliella parva* cells revealed higher carotenoids content of 3.8 mg g<sup>-1</sup> dry weight compared to wild-type cells (2.5 mg g<sup>-1</sup>). Further, *Dunaliella parva* exposed to two different stresses i.e., 30 ppm poly ethylene glycol and 60 ppm CaCl<sub>2</sub> improved the carotenoid content (Ismail et al., 2018). A photosynthetic microalga *Dunaliella salina*, was found to accumulate lipids, vitamins,  $\beta$ -carotene, which can be used for various applications (Morowvat and Ghaseemi, 2016).

Various phenolic compounds and flavonoids extracted from algae were served as antioxidants (Wells et al., 2017). Further, non-proteinaceous amino acid (not an essential amino acid) taurine is prevalent in marine red algae (~1–1.3 g /100 g dry weight), which lowers cholesterol level in the bloodstream (Wells et al., 2017). Various species of green alga *Chlorella*, and the filamentous cyanobacterium *Arthrospira platensis* encompass 70% protein and further, they possess amino acid profile, which has all essential amino acids (cannot synthesize and must obtain from foods) required for human health (Wells et al., 2017). High-value products like carbohydrates, lipids, and proteins, starch, cellulose and polyunsaturated fatty acids (PUFAs), pigments, natural colorants antioxidants, animal feed, pharmaceuticals, fertilizer were obtained from algae (Trivedi et al., 2015; Yen et al., 2013). Among the microalgal strains, *Chlorella* is commonly used as a food supplement in various countries (China, Japan, Europe and US) with an anticipated production at about 2000 t/year (Batista et al., 2013). Carbohydrate component of many algae composed of starch, glucose, cellulose and of these molecules, glucose or starch is utilized for bioethanol and biohydrogen, whereas polysaccharides are used as storage, protective and structural material (Aikawa et al., 2012; John et al., 2011). Further, alginate (polysaccharide) present in brown algae has been directly consumed as human food as it has various beneficial effects i.e., absorb toxins, alter the colonic bacterial profiles, reduce cholesterol uptake, and produce short chain fatty acids (Brownlee et al., 2005). In concern with fatty acids, polyunsaturated fatty acids (PUFA) are most commonly used nutrition to prevent many cardiac disorders and in this connection, microalgae are able to synthesize PUFAs, which are imperative for human health (Trivedi et al., 2015; Wang et al., 2015). With reference to PUFA, certain microalgae *Nannochloropsis*, *Tetraselmis*, *Isochrysis*, *Thalassiosira* and *Chaetoceros* produce valuable health supplements i.e., docosahexaenoic acid (DHA), eicosapentaenoic acid

(EPA), which are long chain fatty acids (Chew et al., 2017). The tubular photobioreactors grown diatom *Phaeodactylum tricornutum* (Chrismadha and Borowitzka, 1994) or co-cultivation of *Odontella aurita* with *Chondrus crispus* in raceway ponds in France has been used for the production of polyunsaturated fatty acids (Wells et al., 2017). Essential fatty acids DHA and EPA extracted from microalgae are the primary sources for zooplankton, fish, and other multicellular organisms (Legeżyńska et al., 2014). DHA derived from algae is supplemented in various nutritional products such as infant formula, infant foods, dairy, bakery, eggs, and non-alcoholic beverages since cardioprotective effect of DHA was observed in certain reports. Cardioprotective effect of DHA extracted from *Cryptocodinium cohnii* and *Schizochytrium* sp. was observed (Barclay et al., 2010; Li et al., 2009).

Several brown macroalgal (*Alaria esculenta*, *Ascophyllum nodosum*, etc.) and red algal species (*Chondrus crispus*, *Meristotheca papulosa*, etc.) produce phenolic compounds (Tibbetts et al., 2016). The extracts of *Arthrospira* and *Chlorella* are mainly used in skin care market as anti-aging creams, anti-irritant in peelers, sun protection cream, hair care products, emollient, refreshing or regenerating product (Trivedi et al., 2015). Among the algal strain, *Spirulina*, *Chlorella* and *Dunaliella* are most commonly exploited in food industry as they yield several products including tablets and capsules. Further, these algae are widely utilized as a supplement to biscuits, ice cream, noodles, candies, breads, and also used as a food additive to improve the nutritive and health values (Mobin and Alam, 2017).

#### 4. Bioremediation

Phycoremediation of waste water and CO<sub>2</sub> sequestration using microalgae are the key environmental benefits of utilizing algae. Particularly, *Chlorella* sp. exhibits a potential to treat wastewater coupled with the simultaneous accumulation of lipids for biofuel applications (S.K. Gupta et al., 2016). Microalgae are capable of removing nutrients from wastewater and concurrently able to generate biomass for biofuel (Chen et al., 2015). Wastewater can be used as cost-effective medium for microalgal cultivation due to the abundance and enrichment of nutrients (Chen et al., 2015). The nitrogen and phosphorus concentration in municipal wastewater is 10–100 mg L<sup>-1</sup> and in agricultural effluent it was 1000 mg L<sup>-1</sup> (de la Noüe et al., 1992). Lipid content of the wastewater-grown microalgae was found to increase remarkably from 10% to 25–30%, which can further be utilized for biodiesel production (Chen et al., 2015). Waste water treatment using microalgae to remove nutrients is done by various researchers. *Chlamydomonas reinhardtii* was able to remove 55.8 mg L<sup>-1</sup> d<sup>-1</sup> nitrogen and 17.4 mg L<sup>-1</sup> d<sup>-1</sup> phosphorus from municipal wastewater. The higher biomass productivity and lipid content of the strain was observed as 2.0 g L<sup>-1</sup> d<sup>-1</sup> and 25.25% respectively (Kong et al., 2010). *Chlorella* sp. grown in raw concentrate was found to remove ammonia, TN, TP by 93.9%, 89.1%, and 80.9% respectively from raw concentrate (Li et al., 2011). Many microalgae can be grown in nutrient rich agricultural wastewater for biofuel production. Notably, green microalga *Chlorella* sp. cultivated in dairy manure wastewater was able to reach a fatty acid productivity of 0.23 g m<sup>-2</sup> d<sup>-1</sup> (Johnson and Wen, 2010).

Further, piggery wastewater grown *Chlorella zofingiensis* was found to reveal a lipid productivity of  $110.56 \text{ mg L}^{-1} \text{ d}^{-1}$  where initial chemical oxygen demand was diluted to  $1900 \text{ mg L}^{-1}$  (Zhu et al., 2013).

Many avenues of research are being exercised to decrease  $\text{CO}_2$  concentration from commercial, industrial and residential sources (Singh and Singh, 2014). Sequester carbon dioxide from ambient air and industrial flue gas could reduce the global warming consequences. Microalgae are capable of fixing carbon dioxide from the atmosphere, or from industrial flue gases or  $\text{CO}_2$  or from soluble carbonates like  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  (Wang et al., 2008). Various studies have been carried out by different researchers on  $\text{CO}_2$  sequestration using microalgae. The marine microalga *Chlorococcum littorale* was found to tolerate higher  $\text{CO}_2$  concentration up to 40% (Iwasaki et al., 1998). Three-stage serial tubular photobioreactor grown *Scenedesmus obliquus* and *Spirulina* sp. were shown to yield higher biomass concentration under  $\text{CO}_2$  purging. Both *Scenedesmus obliquus*, and *Spirulina* sp. were observed to withstand 6% and 12% (v/v) carbon dioxide concentration (De Moraes and Costa, 2007). The microalga *Monoruphidium minutum* was efficiently generated considerable amount of biomass by utilizing flue gas containing higher concentration of  $\text{CO}_2$ , and sulfur oxide, and nitrogen oxide (Zeiler et al., 1995). It is reported that some *Chlorella* sp. could withstand up to 40%  $\text{CO}_2$  (Chang and Yang, 2003). In a research study, among the microalgae *Scenedesmus obliquus*, *Scenedesmus armatus* and *Scenedesmus bernadii*, the high lipid producer *S. obliquus* was chosen to evaluate its growth in response to  $\text{CO}_2$ . The culture was grown in 3 L tubular photo-bioreactors with 16:8 light-dark cycle at 5%, 10%, and 15%  $\text{CO}_2$  concentration. Data indicated that culture could tolerate upto 15%  $\text{CO}_2$  and higher biomass of  $2.3 \text{ g L}^{-1}$  was obtained at 15%  $\text{CO}_2$  supply (Kaewkannetra et al., 2012). Growth profile of *Botryococcus braunii* at 2–20%  $\text{CO}_2$  concentration with 0.2 vvm aeration rate was investigated. Based on the results, it is reported that, hydrocarbon content and size of microalgal colony is increased with an increase  $\text{CO}_2$  supply and maximum biomass of  $2.31 \text{ g L}^{-1}$  was observed on day 25 at 20%  $\text{CO}_2$  (Ge et al., 2011).

$\text{CO}_2$  fixation rate of *Dunaliella tertiolecta* SAD-13.86, *Chlorella vulgaris* LEB-104, *Spirulina platensis* LEB-52 and *Botryococcus braunii* SAG-30.81 were investigated. Among the strains tested, *B. braunii* showed higher  $\text{CO}_2$  fixation rate of  $496.98 \text{ mg L}^{-1} \text{ d}^{-1}$ , while *S. platensis*, *D. tertiolecta* and *C. vulgaris* presented 318.61, 272.4 and  $251.64 \text{ mg L}^{-1} \text{ d}^{-1}$ , respectively (Sydney et al., 2010). In a study, growth rate and  $\text{CO}_2$  sequestration ability of *Synechococcus elongates* was investigated by purging 2%, 5% and 10% air at 32, 52 and  $72 \text{ mL min}^{-1}$  flow rate. Results revealed that maximum growth was seen at  $72 \text{ mL min}^{-1}$  with 2%  $\text{CO}_2$  whereas higher  $\text{CO}_2$  removal efficiency (100%) was attained at  $32 \text{ mL min}^{-1}$  input flow with 2%  $\text{CO}_2$  (Mashayekhi et al., 2017).

## 5. Conclusion

Algae are found abundantly in variety of environment (fresh and marine water) and have rapid doubling rate. Microalgae are used for bioenergy and bio-products production. Microalgal species can also use nutrient from waste water and dwell efficiently, therefore usage of high cost raw materials or chemical as nutrients can be easily replaced, which will also reduce the cost of production. This approach would provide a much sustainable, cleaner, ecofriendly and energy efficient technology for biodiesel production. But the commercialization of microalgal biodiesel is possible by establishing cost-effective methods for extraction and production. Apart from their role in bioenergy production, algae have also been employed in bioremediation of waste water, removal of toxic heavy metals, pesticides and  $\text{CO}_2$  sequestration. Systemic use of algae to generate biomass for value added products will promote the establishment of biorefinery approach. Further, industrial applications of bio-products obtained from algal species highlighted in this review would open a new gateway for the utilization of algal species towards the betterment of human health and nutrition.

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