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REVIEW ARTICLE

Potential applications of algae in biochemical and bioenergy sector

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Abstract

Algae have gained substantial importance as the most promising potential green fuel source across the globe and is on growing demand due to their antioxidant, anticancer, antiviral, antihypertensive, cholesterol reducing and thickening properties. Therefore, it has vast range of application in medicines, pharmaceutical, cosmetics, paper and nutraceutical industries. In this work, the remarkable ability of algae to convert CO_2 and other toxic compounds in atmosphere to potential biofuels, foods, feeds and high-value bioactive compounds is reviewed. Algae produce approximately 50% of the earth's oxygen using its photosynthetic activity, thus acting as a potent tool to mitigate the effects of air pollution. Further, the applicability of algae as a desirable energy source has also been discussed, as they have the potential to serve as an effective alternative to intermittent renewable energy; and also, to combustion-based fossil fuel energy, making them effective for advanced biofuel conversions. This work also evaluates the current applications of algae and the implications of it as a potential substrate for bioplastic, natural alternative to inks and for making paper besides high-value products. In addition, the scope for integrated biorefinery approach is also briefly explored in terms of economic aspects at the industrial scale, as such energy conversion mechanisms are directly linked with sustainability, thus providing a positive overall energy outlook.

Keywords Microalgae · Biomass · Biofuels · Bioactive compounds · Biorefinery · Sustainability

Introduction

With growing concerns for depleting fossil fuel reserves and CO_2 emissions which is soaring at a rate of 1.2% each year (36 billion tonnes each year as of 2019); global warming continues to accelerate, having consequences such as melting of polar ice caps and rise in sea levels (Friedlingstein et al. 2020; Raj and Singh 2012). These emissions also serve as prime culprit for degrading air quality worldwide. World energy reserves are very limited. Coal will last another 70 years, natural gas 40 years and oil 30 years (https://mahb.stanford.edu/library-item/fossil-fuels-run/). Hence, the demand for sustainable, renewable and carbon–neutral energy rises incessantly thereby flourishing the biofuel industry. Apparently, the need for biofuel production give rise to the most controversial conflict of food versus fuel as the arable land is limited and its use for the cultivation of energy crops can lead to inflation of food prices and its scarcity especially in less developed countries (Ahmed 2020; Kurien and Srivastava 2018). Rising world population demands an increase in supply of food, fuel and fresh water along with sustainable management of resources.

Algae are a primitive polyphyletic, noncohesive assemblage of oxygen-producing photosynthetic organisms that first appeared 3 billion years ago in the ocean and are the primary producers that form the base of the food chain in the aquatic ecosystem (Ebenezer et al. 2012). They are the fast-growing bio factories utilizing sunlight, CO_2 and nutrient water (wastewater) in an open pond or a closed photobioreactor on nonarable land, including saline soil land or even desert land (Mutanda et al. 2011). In comparison with other biofuels, microalgae can accumulate 20% to 80% of the dry weight of lipid than traditional oil crops, which can accrue not more than 5% of dry weight (Raj et al. 2012). Besides, algae have the benefit of metabolic flexibility

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that is by regulating the cultivation conditions, the desired biochemical composition of the biomass can be obtained (Treves et al. 2017). Another advantage is water requirement for algae cultivation is much less as compared to crops, especially in the case of a photobioreactor, i.e., for production of 1 L of biofuel from energy crops, at least 3000 L of water is required. In the case of microalgae, 1 L of biofuel with 50% lipids demands 10 to 20 L taking into consideration the stoichiometric needs to fix 1 mol CO_2 from 1 mol water during photosynthesis and the cells contain up to 85% of the water in themselves (Rosillo-Calle 2012). Therefore, algae are the most promising candidate for biofuel production.

Every year a large number of people suffer from severe malnutrition, oxidative stress-related diseases and several chronic infections. Apart from serving the energy sector, algae are the immense source of several important metabolites such as flavonoids, terpenes, phenols, carbohydrates, PUFA, pigments, omega-3 and vitamins and are known as complete food, leading their way in the nutraceutical industry (Torres-Tiji et al. 2020). Algae are used for centuries to cure a number of ailments and are quite common in the medical industry with its extensive therapeutic properties like anticancer, antiviral, anti-inflammatory and antioxidants (Fernando et al. 2020). This review highlights the significant applications of algae in food, animal feed, medicine, aquaculture, cosmetics, fertilizers and bioremediation. Figure 1 provides an overview of all the potential applications of microalgae. It also addresses the challenges of global concern like air pollution, water sanitation, plastic havoc and deforestation. The excessive consumption of toxic petroleum-based products can be replaced with algae driven solutions like biofuels, bio-inks, bioplastics and paper in foreseeable future. Despite large number of lucrative services, limitations always persist. The drawbacks of the current technology are the culture instability, high investment cost for closed setups, high demand for auxiliary energy for biomass production and the processing costs for finished product development (Su et al. 2017). Therefore, making algae biofuels far from commercial reality. The only key to sustainable production is the use of residual nutrient sources and nutrient recycling. The usage of wastewater such as secondary effluent from the STP (Sewage Treatment Plant) for microalgae cultivation and simultaneous product production is another solution for sustainable management of resources (Arora et al. 2021). Producing various co-products along with biofuel while utilizing the waste and harnessing the environment is the best possible strategy to adapt to the integrated bio-refinery system to offset the cost and upgrade the system. The possibility to integrate various high value products with biofuel production is also discussed. Therefore, there is an uncompromised need for commercial growth and exploitation of algae-based products in both energy as well as utility applications (Sharma and Sharma 2017). The present review is designed to elucidate the current applications of algae in biochemical and bioenergy sectors and to highlight the possible areas for further research and development.

Microalgae cultivation

Microalgae are commonly found photosynthetic organisms, but for commercial biomass production it must be cultured separately providing optimum conditions for maximum growth of microalgae. Algae use CO_2 as inorganic carbon source to synthesize organic compounds and release O_2 as a by-product in photosynthesis in the presence of sunlight as described in the equation:

$$CO_2 + H_2O \longrightarrow [CH_2O] + O_2 \quad (1)$$

Where $[CH_2O]$ is the smallest carbohydrate unit which requires auxiliary energy for such metabolic reactions. Furthermore, referring to stochiometric demands 1.83 kg of CO_2 is required per each kg of dry algal biomass obtained. The amount of CO_2 fixed varies for different compound accumulation in algae. For instance, for high stored starch content (50%) and lipid content (50%), 1.65 kg. kg^{-1} and 2.33 kg. kg^{-1} of CO_2 are needed respectively (Sudhakar et al. 2011). Photo-conversion efficiency (PCE) is the conversion efficiency of light into biomass i.e., the ratio of the LHV (lower heating value) of dry algae biomass to the perceived sunlight on the surface area for the algae cultivation. Higher the PCE under sunlight, higher will be the biomass yield (Tredici 2010). Photosynthetic active radiation (PAR) is only 1% of total sunlight and is used for photosynthesis which consist of photons in the wavelength range between 400 and 700 nm resulting in 55% loss of incident radiation.

Factors affecting cultivation

Different algal strains have different doubling time and different optimum conditions for maximum growth, also depending upon the desired use of the biomass. Algal growth depends upon many factors including light duration and intensity, temperature, wavelength of light used, nutrients, mixing, pH and salinity as shown in Fig. 2. One evaluation showed that 16 h light/8 h dark is the most appropriate for the growth (Carvalho et al. 2011), while another study experimentally proved that an aerated culture of microalgae under 12,000 lx intensities for 12 h of daylight produced much higher yield exceeding the limit will result in photo oxidation and growth inhibition (Mata et al. 2010). According to the study, most of the microalgae species show optimum growth in 200–400 μmol m^{-2} s^{-1} of light intensity (Schuurmans et al. 2015). The study conducted by Ahmet Cetin reported that the highest growth and amount of protein

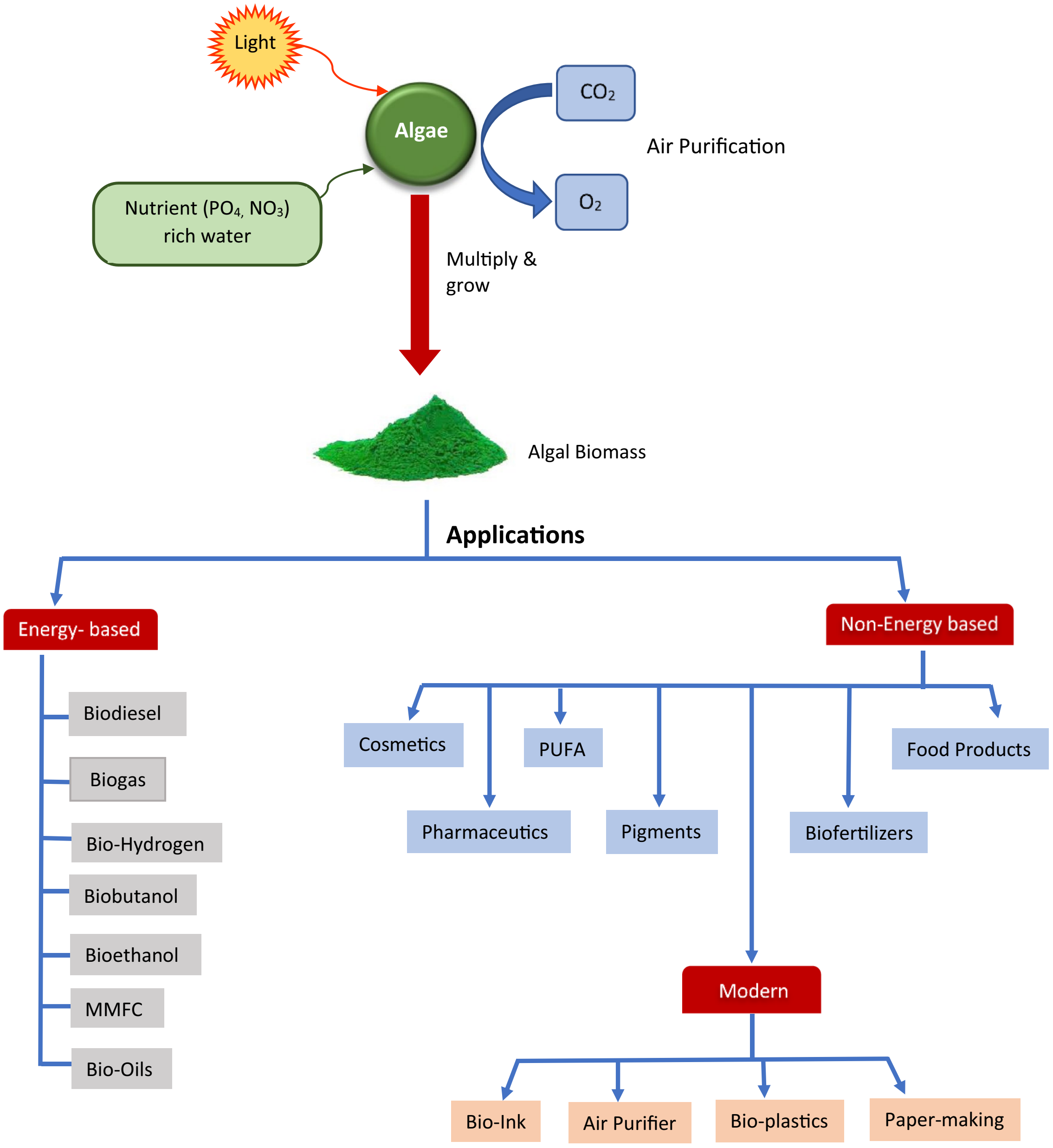

Fig. 1 Current applications of algae in bioenergy and biochemical sectors. The figure illustrates microalgae cultivation and the produced biomass having energy and non-energy-based applications. Many modern applications are also evolving thereby making microalgae more versatile (Sharma and Sharma 2017). *MMFC* Microalgae-microbial fuel cell, *PUFA* Poly-unsaturated fatty acid

in *Chlorella vulgaris* were in cultures illuminated with red light whereas *Tetraselmis* sp. and *Nannochloropsis* sp. grow better in the blue light (Kendirlioğlu Şimşek at el. 2017; Teo et al. 2014). Furthermore, the optimum temperature range for most algal species is 20–30 °C however species like *Chaetoceros* and *Anacystis nidulans* can tolerate temperature up to 40 °C and algae growing in hot spring near temperature 80 °C (Singh et al. 2015; Patel et al. 2019). The main mechanism behind the temperature is the key enzyme ribulose-1,5-bisphosphate (Rubisco) with dual activity as an oxygenase and as a carboxylase that affect the process of photosynthesis. Moving on to the most basic requirement of algae is the nutritional need. Among all nitrogen, phosphorus and carbon are the macronutrients essential for algal growth even though the micronutrients like K, Co, Zn, Mg, Mo, Mn, B and Fe are also needed but in trace amounts and can influence many enzymatic functions responsible for growth in microalgae (Gardner-Dale et al. 2017). Microalgae culture must ensure continuous mixing to enable not only nutrient dissolution and light penetration but also efficient gaseous exchange among all the cells of algae (Show et al. 2017). Microalgae are pH sensitive organisms that grow well

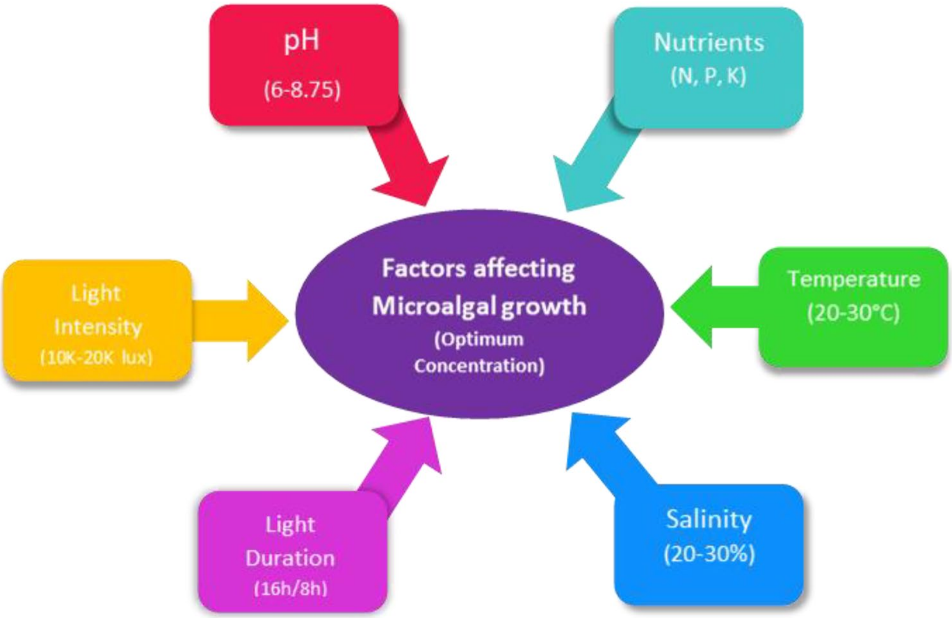

Fig. 2 Various factors affecting microalga growth (Show et al. 2017)

in the range of 6–8.76 but *C. vulgaris* show maximum productivity at pH 9–10 as per the studies (Daliry et al. 2017). Presently, researchers are exploring many new approaches such as combining stress factors, the addition of salts, flue gases and phytohormones and co-culturing with other microorganisms to achieve maximum possible outcome in terms of algal growth (Salama et al. 2018).

Different cultivation systems

Approximately 9000 tonnes of algal biomass are produced per day all over the globe in many different methods for their promising applications in almost all the sectors. Traditionally, microalgae are cultured in the open ponds which are stagnant water around 20 to 30 cm deep and its expected upper performance is 0.5% PCE (Photo-conversion efficiency) which is not better than terrestrial plants. When CO_2 is supplied along with proper mixing PCE increases to 2%. However, such raceway ponds pose a high risk of contamination, inadequate sunlight, CO_2 availability and high rate of evaporation per ground area (1 to 3 m^3 m^{-2}) (Schlagermann et al. 2012). In order to eliminate these drawbacks, the attention of research focus on closed photo bioreactors which separate the culture from environment by transparent walls of polyethylene, PVC or glass with long shelf lives varying in thickness. Such packed containers are very helpful for suitable monitoring by studying growth kinetics of the algae and providing optimum conditions for growth. Also, it renders sterilize environment preferred for maintaining quality and safety standards for high value products in case of integrated product production.

As closed PBRs enable low light conditions (diluted light) i.e., small fractions of the full sunlight can pass through the surface. This offers light intensity close to the optimal efficiency to reach PCE of 5% and under well controlled conditions to 8%, many biofuel manufacturers shift to flat plate reactors from tubular as it requires less cost and energy input (Norsker et al. 2011). On the other hand, membrane PBR offers higher harvesting rate than growth rate with additional filters preventing the wash out but they have some disadvantages too which limits their mass use for cultivation purposes (Marbelia et al. 2014). Alternatively, solid state PBRs also received increasing attention due to high productivity, low water requirement, ease in harvesting and can be arranged in multiple layers to increase the illuminated area (Shi et al. 2014). With recent advances, companies like Solix Biofuels cultivates algae in submerged plastic bags as additional temperature control is not required because the surrounding water act as a temperature buffer (https://lsu.edu/ces/conferences/altenergy2009/ae2009_defoort.pdf). Moreover, this third-generation reactor concept (3G) also reduces the construction costs. Solix are heading for fourth-generation reactor such as membrane aeration. Abomohra et al. performed pilot scale study using 20 plastic bag PBRs made of polyethylene and revealed good yield (Abomohra et al. 2014). Prevailing studies shows plastic PBRs can also be designed to improve the rate of mixing and overcome mass transfer limitations by efficient utilization of ocean waves (Huang et al. 2017; Mark et al. 2019).

Apart from photoautotrophic culturing of microalgae in different PBR setups, heterotrophic cultivation is proved to be an efficient large-scale system for industrial product production. It overcome the light harvesting, CO_2 fixation and energy conversion limitations by rendering carbon sufficient medium (Sun et al. 2018). Such systems provide relatively volumetric growth conditions and higher biomass productivities than photoautotrophic setups. The use of fed-batch cultures and membrane recycle systems offers higher cell density that also aid in reducing downstream processing costs (Morales-Sánchez et al. 2017). This utilizes the process of respiration metabolism to harness energy using carbon substrates like glucose, glycerol and acetate. Glucose is more frequently used as it provides high energy per mole when compared to other sources but found to be more expensive (Mark et al. 2019). Researchers are shifting towards wastewater and agricultural residues like starch hydrolysates, waste molasses and rice straws as an economical carbon options for production of low-value commodities (Sibi 2015). Likewise, a study showed faster growth and increased lipid production in microalgae UM258 and UM268 using oil crop biomass residues as a low-cost culture media (Wang et al. 2013).

After culturing of algal biomass, next step is harvesting to separate microalgae from the used media by different methods like sedimentation, filtration, centrifugation and flocculation. Followed by dewatering or drying by either of spray drying, drum drying or freeze drying. This convert the algae paste into dried powder which is easy to sell as raw material for different product production. Several methods are deployed to break open the cell wall for isolation of metabolites of interest such as mechanical methods (bead mills, high-speed homogenisation, microwave and ultrasound assisted methods) and nonmechanical routes (organic solvents, osmotic shock, enzymatic, acid and base reactions) depending open the desired product. For integrated biorefinery approach, extraction is the very crucial step which is performed in a sequential manner. Proteins should be extracted first to maintain its functionality and quality via centrifugation, chromatography techniques and ultrafiltration. Then lipids are extracted from lyophilized biomass using solvents or mixture of solvents (ethanol, hexane) for biodiesel production. Finally, polysaccharides are extracted from cell wall and starch residues from lipid and protein extraction. This can be used for making biobutanol and ethanol via fermentation processes. However, integrated extraction of compounds is not an easy step without damaging other valuable biomolecules which hereby pose a significant downside of this process (Rösch et al. 2019). Therefore, critical research and analysis are required in this area to make it more feasible and scalable possibility in near future.

Applications of algae

Algae has enormous advantages not only as quenchers of carbon dioxides as 1 kg of algae biomass is capable to fix approximately 1.8 kg of carbon dioxide and it also produces variety of secondary metabolites that help it survive in challenging environments (Sudhakar et al. 2011). These natural compounds are beneficial to humans as they contain anticancerous, antiviral, antibacterial and antioxidant properties (Tredici 2010). The cost of cultivation of algae is very high, this makes it very important to produce various co-products along with biofuels to make this process zero-waste, frugal and sustainable.

Algal biofuels

Biofuel is any solid, liquid or gaseous fuel that is produced from renewable sources like plant biomass through contemporary biological processes. Based on the origin and production technology involved, biofuels are categorized as first, second and third-generation biofuels. First or conventional biofuels are obtained from sugars and vegetable oils found in agricultural crops; thereby, limiting the food supply. Second-generation biofuels are derived from lignocellulosic biomass of agricultural waste, food residues and specially grown energy crops like jatropha, rapeseed and switchgrass through series of physical and chemical treatment. However, this is also not a sustainable choice as there is a trade-off between food and fuel to produce sufficient biofuel (Ramirez et al. 2015; Bindra et al. 2017). Therefore, most of the biofuel industry has shifted its focus from agricultural feedstock to algae cultivation due to its rapid doubling time, wastewater treatment, all year production, no land issues and oil content that can be controlled by adopting different cultivation methods in comparison to arable crops.

These third-generation biofuels are under extensive research to increase the biomass production and biofuel yield to make it a cost-competitive fuel. Recent trends of no economic profits from algae biofuels despite the enormous monetary support from government and few private institutions has only resulted in pilot and industrial scale demonstrations (Su et al. 2017). Many commercial scale producers are now shifted to high-value chains for survival. Still algae biofuels have remained the potential future for green energy synthesis worldwide due to its versatile sustainable benefits. According to the Energy Independence and Security Act, > 10% of current petroleum will be substituted with biofuels with over half quantity coming from microalgae in the US by 2022 (https://arstechnica.com/science/news/2011/04/modeling-the-use-of-algal-biofuels.ars). With technological advancements, many new approaches are coming up for better culture managements, scalable system designs and integrated production (Su et al. 2017). Extensive research is going on to circumvent the cost barrier and to bring biofuels to commerce.

Biodiesel

Biodiesel is a plant or animal oil-based diesel fuel chiefly consisting of a long chain alkyl ester (Mondal et al. 2017). The production of biodiesel from microalgae lipids is fairly easy but the main challenge lies in the high lipid productivity and FAME composition of the energy fluid which determines the quality. Lipid productivity screens microalgae for large scale setups and FAME composition determines the biodiesel properties (Lanjeka and Deshmukh 2016). Apart from the composition analysis, prediction of thermochemical properties like cetane number, density, iodine number, viscosity, heating value and oxidation stability is also very imperative. The species selection can be further refined by evaluating vapor pressure, latent heat of vaporization and vapour viscosity which is critical for spray and combustion modeling (Deshmukh et al. 2019). There are some international property standards like ASTM D6715 and EN 14,214 and the biofuel that qualifies these standards are considered of high quality. One such study estimated all the above-mentioned criteria and screened out *Chlorococcum* sp. to be most suitable among the other five species belonging to Trebouxiophyceae, Chlorophyceae and Cyanophyceae (Deshmukh et al. 2019) . Conventionally, biofuel is produced through ex situ method in which oil is being extracted through Soxhlet extraction using solvents like hexane after harvesting, drying and crushing algae and then transesterification is performed which make the procedure more expensive and cumbersome (Nguyen et al. 2020). With recent advances, there is no need to follow the long protocol and the biomass can directly be trans-esterified (Bindra et al. 2017). Algae such as *Chlorella vulgaris* and *Chlorella protothecoides* can possibly contain 80% of total lipids that can be converted into alkyl esters by the process known as transesterification in which these lipids mainly triacyclglycerols are converted into eco-friendly and more effective biodiesel from renewable raw material with the help of alkali catalyst as they are 400 times faster than acid catalyst (Gulyurt et al. 2016). During the process, crude oil in the presence of suitable catalyst reacts with an alcohol mostly methanol to form fatty acid methyl esters (FAME) along with crude glycerol as the final product.

As shown in Fig. 3, recent trends are focussing on pretreatment of biomass before conversion to biofuel, study showed energy-efficient microwave pretreatment requiring lowest specific energy (5 MJ/kg) as compared to ultrasound pretreatment (Ha et al. 2020). A study performed by Ehimen et al. on dried *Chlorella* biomass was subjected to in situ transesterification and attained 90% biodiesel yield in 4 h at a reaction temperature of 60 °C, a methanol to lipid molar ratio of 315:1, a H_2SO_4 concentration of 0.04 mol (Ehimen et al. 2010). Further, one study demonstrated a significant reduction in emission for CO_2 (of around 55%) as compared to petrodiesel using biodiesel produced from *C. pyrenoidosa* culture grown in dairy wastewater and rice straw hydrolysate (Bindra and Kulshrestha 2019). The only limitation in this approach are high processing and production cost.

To combat such issues, evaluations on model microalgae such as *Synechocystis* PCC 6803 and *Chlamydomonas reinhardtii* due to availability of their genetic sequence using latest approaches like CRISPR Cas9 and transcriptional factor engineering techniques for modifications of algal strains that will dramatically increase the biomass yield and induce enhanced TAG/lipid biosynthesis while few others focus on applying lipidomics and transcriptomics to manipulate acetyl-CoA synthetase in microalgae (Konstantinos V et al. 2019; Arif et al. 2020). The recent advancement in science also found that nano-particles incorporation with algae cultivation, biofuel conversion techniques and applications have amplified the net yield significantly (Hossain et al. 2019).

Bio-oils

Bio oil also known as pyrolysis oil or bio crude oil. These oils are produced by thermo-chemical conversion of biomass at elevated temperature of about 350–530 °C in the absence of oxygen into oil that can be used as a substitute of petroleum. Bio-oil can be produced by pyrolysis and hydro-thermal liquefaction (HTL) techniques. For liquefaction, the wet biomass is maintained at higher pressure of 10 MPa and lower temperature of around 300 °C. Large number of microalgae contribute to bio-oil production from their biomass like *Spirulina* (Jena and Das 2011; Barbosa et al. 2020), *Scenedesmus* (Bordoloi et al. 2016), *Dunaliella* (Chen et al. 2012) and *Desmodesmus* (Felix et al. 2019) yield around 35–41%, 24–45%, 64.68% and 72.62% respectively and when it comes to macroalgae the production yield increases to 79% after hydrothermal liquefaction from *Laminaria saccharin* although *Oedogonium* and *Cladophora* only yield 20–26% (Bach et al. 2014; Neveux et al. 2014). To enhance the bio-oil production advanced pyrolytic techniques like catalytic pyrolysis that reduce nitrogenates and oxygenates, co-pyrolysis that show great enhancement in yield, hydropyrolysis with production of up to 48 MJ/kg i.e., 50 wt.% and microwave-assisted pyrolysis that can shorten the processing time by advanced heating and also favours bio-syngas production by ameliorating the yield by 84 wt.% using both micro and macro algae (Lee et al. 2020). An overview of the production process is shown in Fig. 4.

According to the recent study on *Dunaliella* sp. showed that average bio-oil produced was 11.81% (w/w) obtained after lipid extraction by HTL process at 350 °C at 200 bar in residence time of 60 min. (Shahi et al. 2020). HTL process is more effective than pyrolysis as it can process wet biomass and can save the additional cost required for drying. Likewise, the oil produced through HTL has twice the energy density than the other one. Studies also reported that fast

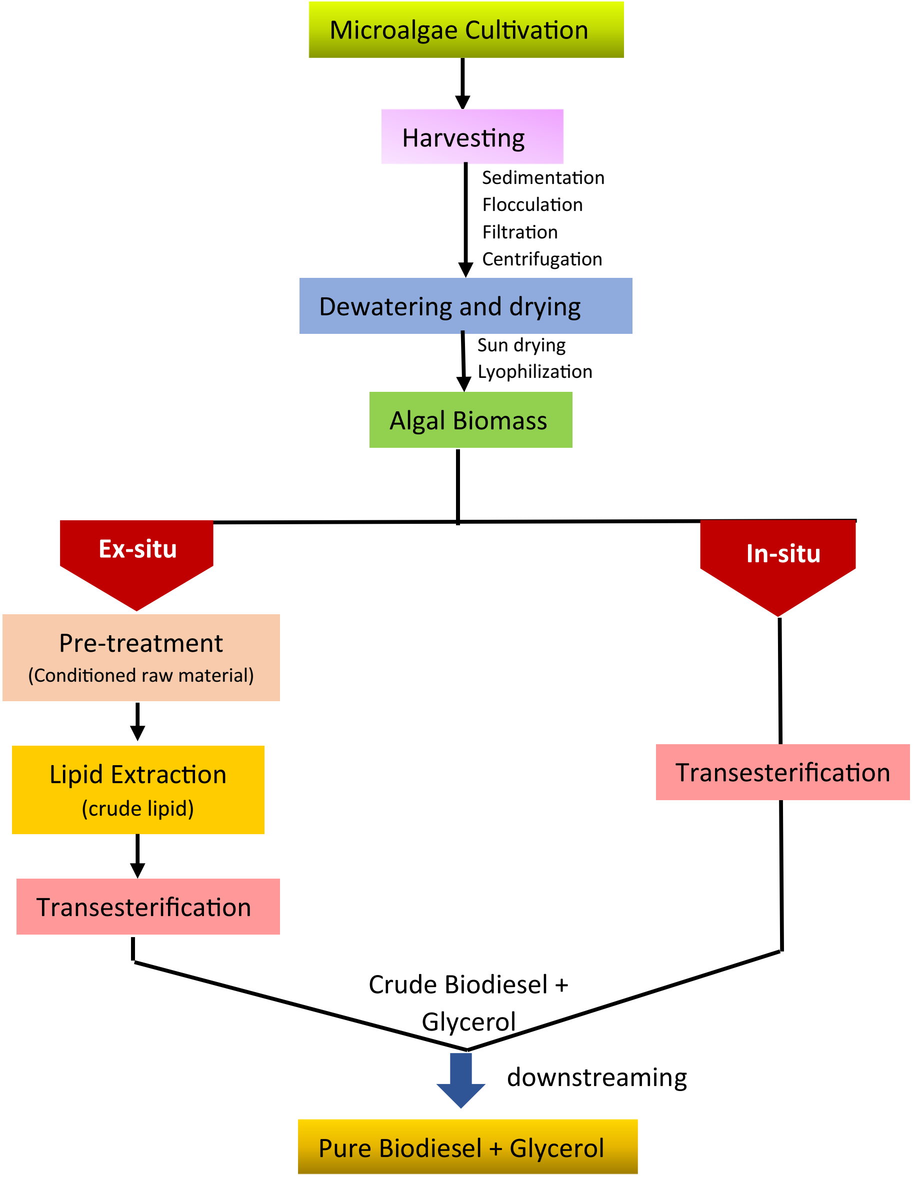

Fig. 3 Schematic representation of steps involved in Biodiesel production from both conventional ex situ and recent in situ transesterification. Microalgae are first cultivated and then harvested and processed via different methods into a dry biomass. Followed by ex situ transesterification which requires pretreatment and then lipid extraction before the final step, whereas in situ transesterification follow direct transesterification (Bindra and Kulshrestha 2019)

pyrolysis and HTL processes delivered 78.7% and 89.8% energy recovery ratios respectively from *Chlorella* (Yang et al. 2017). Along with oil, side products include gases, residual solids and aqueous phase products are generated. Bio-crude oil upgrading must be performed after the processing to improve it with respect to fuel properties and stability (Kazemi et al. 2019).

Hydrocarbons

Few algae such as *Botryococcus braunii* produce long-chained liquid hydrocarbons known as *botryococcenes* after their nondestructive extraction called milking which can then be used as fuel for internal combustion engines. *Botryococcenes* are triterpene instead of lipids and hence do not require transesterification. They can also be used as a feedstock for hydrocracking to produce octane and kerosene in an oil refinery. There are three races of *Botryococcus* strain, i.e., race A, B and L which contain 61%, 86% and 8% dry weight, respectively (Qin 2010). Approx. 35% of photosynthetic carbon is produced in the form of oil in B-race (Melis et al. 2019). According to the study, thermodynamic feasibility and carbon balance solely rely on the hydrocarbon contents of *B. braunii* in the milking process and it consumes more CO_2 than it produces (Sofia et al. 2016). The research aims to elucidate that *botryococcenes* extraction is less toxic and more efficient when *B. braunii* is recirculated through a column system containing *n*-dodecane (Mehta et al. 2019). The only hurdle in the commercial production of *botryococcenes* is the slow growth of the algae which can be escalated by the combined effort of genetic engineering, ecology and molecular biology. For instance, *botryococcenes* producing

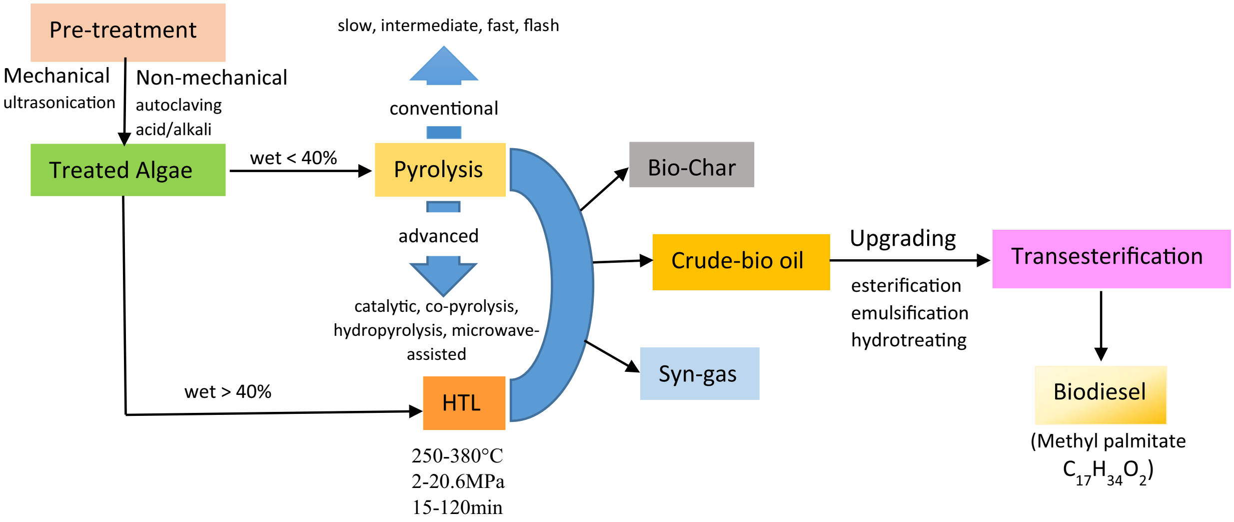

Fig. 4 Bio-oil production process. Algal biomass is pretreated to produce bio-oil by pyrolysis (in case of dryer biomass) and hydrothermal liquation (HTL) (for more wetter biomass) along with different byproducts (bio-char and syn-gas). The crude oil produced, needs upgrading to offer better quality which can further be trans-esterified to obtain biodiesel (Lee et al. 2020)

genes were incorporated into *E. coli* to optimize the production recently (Chacko et al. 2019).

Biohydrogen

Biohydrogen is hydrogen produced from biomass as a renewable resource. It has a huge potential to serve as a clean transport fuel in coming future as it is a strong contender in terms of energy rich pollution free fuel (Patel et al. 2017). Hydrogen has a highest heating value of 142 MJ/kg which makes it compatible for combustion and it can also be used to generate electricity directly from a fuel cell (Nagarajan et al. 2017). However, it still needs a lot of research in this area as its large-scale production is not feasible because of high production cost and low biomass concentration. Hydrogen is usually produced by steam reforming of natural gas, methanol, coal and ethanol for industrial use covering 42% of the global demand, but this thermochemical conversion generates large amount of greenhouse gases (Nagarajan et al. 2017). Another method is by water splitting but it incurs huge electricity costs. Therefore, it poses a need for an alternative and more sustainable method. It has been noted that few species of algae can produce considerable amount hydrogen gas when exposed to environmental stresses like pH alteration, nutrient deprivation and temperature elevation (Saifuddin and Priatharsini 2016). There are three main pathways for biological production of hydrogen: adenosine triphosphate or ATP driven pathway, direct and indirect photolysis. In these processes, photosynthesis and splitting of water into hydrogen and oxygen are interconnected to each other and result in increment in cost as separation of hydrogen and oxygen is a crucial process and also hydrogenase enzyme used is very sensitive to oxygen, thereby favouring indirect process. Starch present in a cell wall of algae can be converted into hydrogen under anaerobic and sulphur limited environment.

Cyanobacteria is found to be the major producer of biohydrogen through biological processes and hydrogenase and nitrogenase enzymes are served to be an effective catalyst in this process (Guan and Savage 2012). At nitrogen deficient conditions, nitrogenase also act as ATP powered hydrogenase and synthesise hydrogen at a greater rate (Nagarajan et al. 2017). Also, waste effluents rich in carbohydrate containing organic acids can be integrated with fermentative hydrogen production (Prakash et al. 2018). Pretreatment are used to increase the yield as it will break open the cell wall to facilitate proper digestion of algal feedstock (Wang and Yin 2018). As per recent studies, pretreatment of macroalgae like *Ulva reticulata* by acidic hydrogen peroxide (H_2O_2) induced microwave pretreatment (AHMW) can improve the biohydrogen production, producing maximum of 92.5 ml H/g COD (Kumar et al. 2019). Another study shows that maximum hydrogen production was achieved by *Clostridium butyricum* when it was fed to a continuous fixed bed reactor (C-FBR) with acid algae hydrolysate containing 1.5 g 5-HMF/L and 15 g hexose/L hexose was partially packed with hybrid-immobilized beads contributing to a hydrogen yield (HY) of 2.3 mol H_2/mol hexose (Anburajan et al. 2019). The limitations to scale up the biohydrogen production for commercialization can be overcome by genetic and metabolic pathway engineering of hydrogenase and nitrogenase enzymes. The newly discovered micro-RNA mediated hydrogen production is gaining considerable attention nowadays (Anwar et al. 2019). Recent sustainable measures are shifting the biohydrogen industry towards an integrated biorefinery approach considering the fact that only 30–40% of the biomass is utilized for hydrogen production and remaining potential feedstock gets wasted. The residual biomass can be used for the recovery of different metabolic products like ethanol, butyric acid, PHA and biodiesel (Rajesh Banu et al. 2021). One such approach is the integration of anaerobic digestion (AD) with dark fermentation (DF). Large amount of CO_2 is produced along with CH_4 in AD which require separate purification steps. This can be compensated with subsequent DF of AD effluent as microalgae can consume the CO_2 and utilize the COD, VFAs, TN and TP present in the effluent for its growth mixotrophically. Microalgae can simultaneously convert the waste products into H_2 and oil-rich biomass for further processing into biofuel. However, there are various parameters that needs to be taken care of like maintaining pH, temperature, light irradiance and sterility or finding tolerant microalgal strain (Chen et al. 2018). The present study could aid in shaping the future of sustainable waste biorefining along with hydrogen production.

Biogas

Biogas is a mixture of gases chiefly consist of methane produced by anaerobic breakdown of organic matter such as carbohydrate, lipids and protein by methanogens in absence of oxygen. Microalgae produces about 50–70% of methane, 30–45% of CO_2, $<2\%$ of H_2 and $<3.5\%$ of H_2S by anaerobic digestion (AD) which is much higher than the terrestrial plants (Allen et al. 2015; Vanegas et al. 2013; Tiwari et al. 2015). Scientist investigated the thermal pretreatment of microalgae *Chlorella* species for augmentation of biomethane production resulted in the high methane yield (322 mL g^{-1} VS_{add}), which was 108% higher than the untreated algae while another study depicted that combined utilization microwave (MW) pretreatment of *Enteromorpha* biomass and metal nanoparticles incorporation gives much higher (53.60 ml/gTS) energy yield (Wang et al. 2017; Zaidi et al. 2019). Figure 5 represents the biogas production mechanism using microalgae.

Algae with highest dry weight, specific growth rate and maximum productivity are analysed for biochemical methane potential (BMP) by Perendeci et al. and showed 308, 293, 242, 229 and 230 $mLCH_4/gVS$ BMP values for *Desertifilum tharense*, *Phormidium animale, Chlorella* sp., *Anabeana variabilis* and *Chlorophyta* uncultured. After applying the Pearson correlation and principal component analysis (PCA) to the extracts, it was proved that glucose, Kjeldahl nitrogen and chlorophyll content of algal species are positively correlated with BPM (Perendeci et al. 2019). Apart from pretreatment, adding low grade crude glycerine produced during biodiesel production can be utilised to increase the methane yield by 9.5%, 14.3% and 14.6% for 5%wt, 10%wt and 15%wt of control glycerine in the slurry respectively (Robra et al. 2010). But the major factors that bottlenecks the energy return on energy invested (EROI) are harvesting, concentrating the algae, pretreatment aimed at improvement of biomethane conversion yield besides the operational cost (Collet et al. 2015; Wang et al. 2015). Therefore, it is preferred to amalgamate various processes like wastewater treatment, biofuel and fertilizer production etc. with biogas production that aim to zero waste circular economy (Milledge et al. 2019).

Bioethanol

Bioethanol is an ethyl alcohol produced by microbial fermentation of biomass containing carbohydrate or starch and can act as a fuel that can be added to gasoline at some

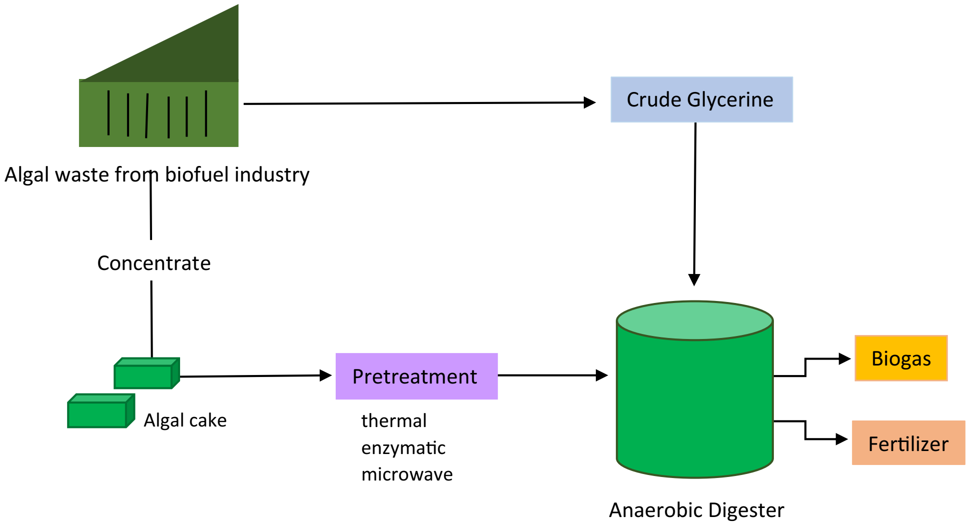

Fig. 5 The figure depicts the biogas production using microalgae. The residual algal biomass and the byproduct (crude glycerin) from the biofuel industry can be reutilized for biogas generation after pretreatment. Further waste generated can be recovered as a fertilizer (Wang et al. 2017)

specific percentage to reduce the dependency on nonrenewable options. In Brazil, bioethanol is commercially used as mixture of ethanol and petroleum in 86% of sold cars (Walker 2010). Amongst all algae, brown algae are considered to be the most potent feedstock for bioethanol production because it is easy to cultivate and rich in carbohydrate (Chaudhary et al. 2014).

After pretreatment of microalgae with acid, alkali, enzymes or yeast, *Saccharomyces cerevisiae* is added for fermentation to convert the sugar into ethanol (Hossain et al. 2019). A study optimized the bioethanol production from brown seaweeds (*Ascophylum nodosum* and *Laminaria digitate*) by pretreatment using hydrolyzation by dilute sulphuric acid and enzymes and obtain high amount of fermentable sugars predominantly glucose and rhamnose (Hossain et al. 2019).

Yoza and Masutani investigated that acid pretreatment can release up to 49% sugars while enzymatic hydrolysis can release only 20% sugar based on the dry weight of algal biomass (Yoza and Masutani 2013). However, combining sonication and enzymatic hydrolysis pretreatment improved total reducing sugar (TRS) productivity by fourfold (280.5 mg g^{-1}) (Eldalatony et al. 2016). Another study revealed that pH, sugar concentration and inoculum size have significant effect on the yield but if yeast is immobilized it will show great increase in conversion efficiency (El Sayed and Ibrahim 2016). Scientist are currently working to use industrial waste algae rather than culturing the specific strain for bioethanol production and obtained the highest yield of 11.6 g of ethanol/ g of algae from *Euchema Spinosum* (Alfonsín V et al. 2019). But still, there are challenges for bioethanol with low vapour pressure, energy density and flame luminosity. Recently, researches are being carried to increase the bioethanol production using genetic engineering by modifying the genes involved in carbohydrate biosynthesis (Torney et al. 2007).

Biobutanol

Biobutanol is made using the same process like bioethanol but it is more preferred as it can offer the ameliorated version of bioethanol. The bacteria responsible for biobutanol production, mostly *Clostridium* sp. by anaerobic ABE (Acetone, Butanol and Ethanol) fermentation will also digest cellulose apart from starch and sugar from the algal cell wall thereby presenting a more economical option but it will in the same time inhibit fermentation and limit the yield and productivity of biobutanol (Gao and Orr 2016).

Butanol is also produced by the fermentation of green algae *Ulva lactuca* also known as sea lettuce by *Clostridium* strains, but butanol yield is lower up to 0.16 g butanol g^{-1} as compared to pretreated with hot water along with enzymatic hydrolysis by cellulases for ABE production with a yield of 0.35 g ABE g^{-1} sugar (van der Wal et al 2013). Apart from *Clostridium acetobutylicum*, microbes such as *E. coli* has showed his competency by concentrating the production through genetic modifications (Phillips 2020). Recent studies show increment of carbohydrate percentage from 23.9 to 34.2% when *Chlorella zofingiensis* was grown in 25% wastewater, 80 µM Indole-3-acetic acid and 4% of hydrogen peroxide pretreatment that produces 0.084 g of bio-butanol/ g of microalgal biomass which corresponds to an increase of 35% (Oney 2020). Moreover, another research on growth conditions investigates that when *Nannochloropsis gaditana* was grown under different light intensities and in different acid hydrolysis treatment, it yielded the highest carbohydrate productivity (21.3%) and bio-butanol production (2.9 g/L) under 3% HCL at 160 µmolm^{-2} s^{-1} of light intensity (Onay 2020a, b).

Bioelectricity: mMFC (microalgae–Microbial Fuel Cell)

Microbial interaction with waste matter to decompose it is known as bioremediation, these approaches can be used for the generation of bioelectricity, as well. Bioelectricity is the energy harnessed by capturing electrons produced during the metabolism of the living organism while microbial fuel cell is an innovative technology to produce electricity using microbial activity in which chemical energy is converted into electrical via microbes that act as a catalyst (Bose et al. 2018a, b, c). Such processes can be used to harness energy from relatively inaccessible locations such as ocean floor and other remote areas, the bioelectricity generated can be used to power low energy devices for rudimentary data transmission (Bose et al. 2018a, b, c). This technology is in the research and development phase and can be used as a renewable and profitable option for electricity production.

microalgae–Microbial Fuel Cell (mMFC) is a state -of the-art technology that can be applied for wastewater treatment along with bioelectricity generation. Wastewater is used in MFC for removal of chemical oxygen demand (COD) and partial removal of phosphorus and nitrogen by anaerobic bacteria by catabolism in anodic chamber. On the other hand, the treated water can then be used for microalgae cultivation in cathodic chamber to further remove remaining nitrogen and phosphorus from the water along with heavy metals as reported by the study valorising the removal up to 99% and 97% respectively (Bose et al. 2018a, b, c). Bioelectricity generation is positively correlated to dissolved oxygen (DO) levels in cathode (Ling et al. 2019). Electrons generated by the breakdown travels through the anode to cathode, where O_2 produced by photosynthesis act as acceptor while protons travel across the nafion 117 proton exchange membrane (PEM) to avoid short circuiting as shown in the Fig. 6. It is reported that O_2 generated in biocathode is much higher

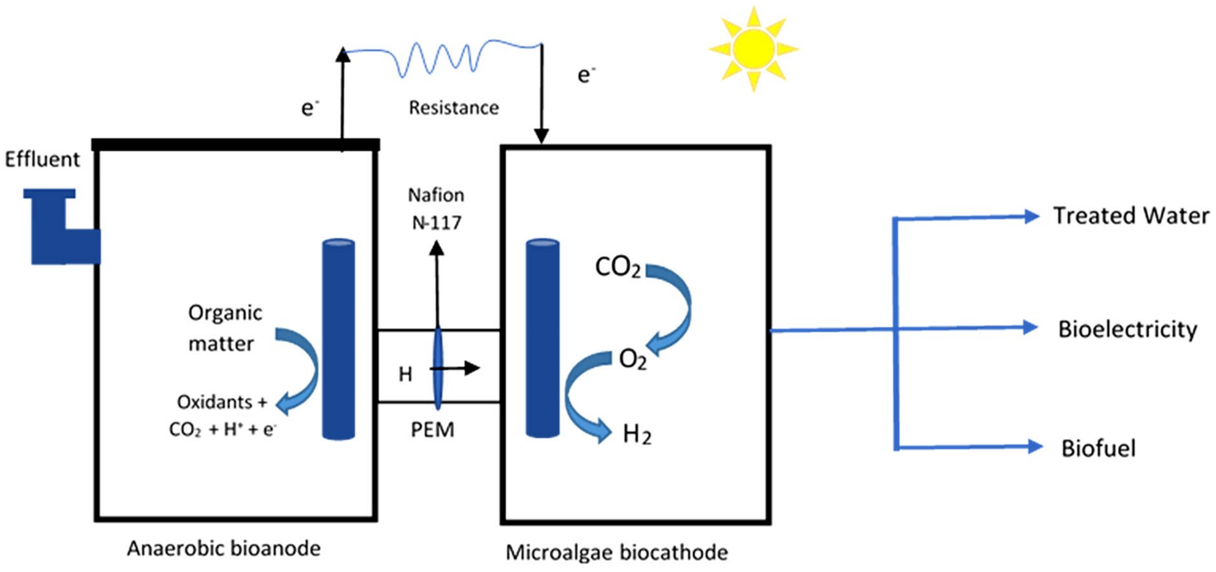

Fig. 6 Configuration of the microbial fuel cell (MFC) and its applications. *PEG* Proton exchange membrane

than in MFC with synthetic air cathode however accumulation of microalgae, light exposure and approaching death phase, all result in low stability of the system. According to the recent study, working voltage of 50% submerged carbon cloth cathode (in order to prevent O_2 limitation) is significantly higher ($p < 0.05$) than partially or fully submerged electrodes (Ling et al. 2019).

The study performed by a group of researchers in Indonesia depicted the power generation using tapioca wastewater and microalgae cultivation to be 30 mW/m^2 with 2:2 ratio of electrode while 18 mW/m^2 with 1:1 ratio of electrode (Hadiyanto et al. 2019). Furthermore, the latest research elucidates the possibility of bioelectricity generation and bioremediation with the algae biocatholyte during the treatment of kitchen wastewater in the MFC by *Synechococcus* sp. and *Chlorococcum* sp. with generation of power density of 41.5 mW/m^2 and 30.2 mW/m^2 respectively (Samsudeen et al. 2019). Above researches anticipate that sufficient amount of bioelectricity can be produced using microalgae Microbial Fuel Cell (mMFC) for scale up and commercialization with further research and development.

Biochemical applications of algae

Algae has enormous applications other than in energy sector but many are still not discovered due to vast untapped potential of this natural resource. Although, there are many algae-based products available in a market in the form of food, cosmetics, alginates, tablets. Still this field need more extensive research.

Cosmetics

Algae both micro and macro are amply utilized in the field of cosmeceuticals due to its antioxidant, moisturizing, antiaging and antitanning properties. It is mostly used in the form of extracts to eliminate chances of contamination in industries that are formulated into skin sensitizers, sunscreens, thickening agents, antiwrinkle creams, hair care products and moisturizers (Joshi et al. 2018). Microalgae species *Arthrospira* and *Chlorella* are most exploited strains in skin care industry by companies like LVMH (Moët Hennessy Louis Vuitton), Daniel Jouvance, Carnac having their own microalgae cultivation plants. The rationale behind such valuable properties of algae is their adaptation to unfavourable environment which lead to the synthesis of secondary metabolites that protect algae from challenging conditions like high UV radiations. Such as amino acid sporopollenin, mycosporine and scytonemin act as guardians that allow passage to visible light but block these harmful radiations are inviting commercial attention (Cardozo et al. 2007).

Usually species like *Chondrus crispus, Ascophylum nodosum, Alaria esculenta, Spirulina platensis, Nannochloropsis oculata, Chlorella vulgaris* and *Dunaliella salina are* used in cosmetics like Spirulina whitening facial mask by Ferenes Cosmetics for enhancing skin complexion and diminishing wrinkles (Schuurmans et al. 2015).

Pharmaceuticals

A large part of pharmaceutical industry is dependent upon wide range of bioactive compounds and extracts isolated from natural sources like living plants and animals

(Atanasov et al. 2015). A great shift has been observed in recent years from such costly to produce pharma proteins, vaccines and nutrients from such sources to rapidly flourishing algae population (Chu et al. 2019). Due to the competition faced by these aquatic producers with consumers growing in the same niche, they come up with great genetic potentials to survive and thrive by synthesizing diverse secondary metabolites. Such precious bioactive compounds can be examined for their properties and screened for their application in biotechnology, nutraceuticals and pharmacology sector (Joshi et al. 2018). Likewise, algae have several vaccine antigens that can be exploited to create recombinant algal fusion proteins that can enhance antigenicity for orally delivered vaccines (Specht 2014). Algae are also identified as vitamin precursors source for riboflavin, thiamine, ascorbic acids and tocopherol. Some unicellular microalgae like *Chlorella vulgaris, Chlamydomonas pyrenoidosa* are known for their antibacterial properties against both gram positive and negative bacteria while *Ochromonas* sp., *Prymnesium parvum* can produce some valuable toxins for pharmaceutics (Jayshree and Jayshree 2016; Katircioglu et al. 2006). Furthermore, many strains of algae show antiviral, antifungal and anticancerous activities. An overview of such applications for algae is presented in Table 1.

Anticancer activity Nowadays with changing habits, children are more adaptable to junks, smoking and alcohol consumption leading to cancer development as a common case. Malfunctioning of neo plastic cells is conventionally controlled by chemotherapy and radiotherapy which have dreadful side effects too and still chances of recovery is very less (Abd et al. 2019). Microalgae such as *Poteriochromonas malhamensis* are known to produce some useful biologically active compounds like chlorosulpholipid that show anticancerous properties by inhibiting the enzymatic activity of protein tyrosine kinase (Shalaby 2011).

Cryptophycin, a metabolite isolated from *Nostoc* is proved to show high anticancer activity while scytonemin, a protein serine inhibitor screened from *Stigonema* tend to offer antiproliferative and anti-inflammatory properties (Stevenson et al. 2002; Eggen and Georg 2002). Another study shows antimalignant activity of fucoxanthin against HL-60 cells by induction of apoptosis (Hosokawa et al. 1999). Recently scientists have investigated the anticancer effects of eight algal meroterpenoids (AMTs, 1–8) isolated from the brown seaweed *Cystoseira usneoides* and are found successful in inhibiting the growth of HT-29 malignant cells and were less toxic towards noncancer colon cells by reducing phosphorylation levels in protein kinase B (AKT) in colon carcinoma cells (Zbakh et al 2020).

Value-added compounds

Over 30,000 discovered species of algae, only few of them are known to harness potential value-added compounds. They can be notable lipid compound produced by marine microalgae explicitly *Dunaliella* sp., *Chlorella* sp., *Spirulina* sp. producer of polyunsaturated fatty acid (PUFA), while *Phaeodactylum tricornutum* and *Odontella aurita* are rich source of eicosapentaenoic acid (EPA) and *Schizochytrium* sp. for docosahexaenoic acid (DHA) (Sathasivam et al. 2019; Hamilton et al. 2016; Souza et al. 2019). These lipid

Table 1 Various properties of algae and its applications in real world

Applications	Properties	Algae	References
Cosmetics	Antioxidant, Moisturizing, Anti-aging, UV-screening or Anti-tanning properties	*Arthrospira, Chondrus crispus, Ascophylum nodosum, Alaria esculenta, Spirulina platensis, Nannochloropsis oculata, Chlorella vulgaris* and *Dunaliella salina*	Joshi et al. (2018)
Pharmaceuticals	Anti-bacterial properties	*Chlorella vulgaris, Chlamydomonas pyrenoidosa*	Jayshree and Jayshree (2016)
	Useful toxin production	*Ochromonas* sp., *Prymnesium parvum*	Katircioglu et al. (2006)
	Chlorosulpholipid production anticancerous property	*Poteriochromonas malhamensis*	Shalaby (2011)
	Cryptophycin production anticancerous property	*Nostoc*	Eggen and Georg (2002)
	Scytonemin protein antiproliferative and anti-inflammatory properties	*Stigonema*	Rastogi et al. (2015)
Nutraceuticals	Production of value-added compounds	*Dunaliella salina, Spirulina platensis, Chlorella vulgaris*	Subudhi (2017)
Pigments	Production of phycobiliproteins and fluorescence properties	*Spirulina* and *Porphyridium*	Sonani (2016)
	Production of β-Carotene	*Dunaliella salina*	Harvey and Ben-Amotz (2020)
	Astaxanthin production	*Haematococcus pluvialis*	Li et al. (2020)

compounds are studied to prevent number of diseases for instance cancer, asthma, skin and kidney disorders, cardiovascular disorders and neural illnesses like schizophrenia and depression.

Polyunsaturated fatty acids (PUFA) Polyunsaturated fatty acids (PUFA) are highly beneficial fatty acid for human health as it can reduce the incidence of thrombosis, bad cholesterol and blood pressure and at the same time can improve the blood lipid levels and heart rate, thereby lowering the risk of heart diseases. They are synthesised by microalgae and are consumed and accumulated by fishes such as Omega-3 are said to be obtained from salmon fish. Instead, it can directly be obtained from the algae source without any foul fish odour and in more purified form (https://www.wur.nl/en/newsarticle/Algae-our-original-omega-3-source.htm). The recent study showed feasibility of converting wastewater-originated waste grease (WG) to PUFA-rich *O. danica* algae culture and PUFA were synthesized and enriched to up to 67% of intracellular FAs, from the original 15% PUFA content in WG (Xiao et al. 2019). Therefore, extraction and purification of PUFA from algal strains is largely demanded by the growing and emerging population of world.

Pigments

Algae are rich in natural pigments that show many exceptional properties and these pigments are categorized into two major group i.e., tetrapyrroles and isoprenoids based on their chemical structures. Chlorophyll and phycobiliproteins (PBP) belong to the tetrapyrrole group while carotenoids fall under isoprenoid group. Except photosynthetic primary pigment chlorophyll, it harnesses some utmost beneficial pigments like phycobiliproteins, β-carotene, lutein, zeaxanthin and canthaxanthin etc. β-carotene isolated from microalgae *Dunaliella* is a precursor of vitamin A (retinol), which along with lutein can prolong vision in people suffering from retinitis pigmentosa (RP) whereas zeaxanthin and canthaxanthin has pharmacological applications (von Lintig 2018). Instead astaxanthin extracted from microalgae *Haematococcus pluvialis* is a super antioxidant used in aquaculture practices and gives characteristic red colour to fishes on consumption (Saha et al. 2018). The slow growth of algae and the cost of extraction are the only constraints to this approach. Therefore, recent researches are focussed on advanced metabolic and genetic engineering techniques that together are used to develop microalgae to increase production of various secondary metabolites (Saini et al 2019).

Phycobiliproteins Phycobiliproteins are group of water-soluble coloured proteins found in cyanobacteria and some algae like rhodophytes, cryptophytes and glaucocystophytes. They are accessory proteins that capture light energy which is then passed to chlorophyll for photosynthesis. They are of three types: phycocyanin, allophycocyanin and phycoerythrin varied only in their spectral properties.

Spirulina and *Porphyridium* are two most exploited algal strains for phycobiliprotein production for the use as cosmetic products, natural dyes and as diagnostic markers in biomedical research due to its fluorescence property (Kuddus et al. 2013). The amount of this protein produced chiefly depends upon the environmental parameters like intensity and spectral quality of light. For instance, *Spirulina platensis* when grown at various light intensities, the amount of phycocyanin varies from 11 to 12.7% dry weight (Chu et al. 2002). Current studies on extraction of phycobiliprotein (PBP) pigments from red algae *Gracilaria gracilis* was optimized using maceration method yielding maximum of 3.6 mg phycoerythrin/g biomass under the optimal conditions among other methods like ultrasound-assisted extraction, high pressure-assisted extraction and freeze–thaw which are not that efficient (Pereira et al. 2020).

β-Carotene β-Carotene is a strongly coloured organic pigment found copiously in plants, fruits and especially in carrots and colourful vegetables. It is a type of carotenoid with antineoplastic and antioxidant activities. Studies have found that natural β-carotene can induce apoptosis and can also triggers the release of natural killer cell, lymphocytes and monocytes, thereby enhancing the immune response during tumour progression as reported by Jayappriyan et al. in case of prostate cancer (Jayappriyan et al. 2013). Hence, the demand for natural β-carotene from microalgae is ever increasing (Novoveská et al. 2019). It also acts as an important precursor of vitamin A and can improve vision. The study reported *C. reinhardtii* culture when illuminated for 24 h a day for 10 days with irradiance 149 μmol m^{-2} s^{-1} producing 0.87 mg/g lutein, and 0.64 mg/g β-carotene, as a maximum value of accumulating carotenoids in 2.15 g/L biomass yield (El-Mekkawi et al. 2019). β-Carotene has many uses like orange dye, food additive, colouring agent in food industry and as a vitamin C supplement despite the fact that it is nonphoto stable and will not be an ideal food colourant due to bleaching of colour on heating (Pénicaud et al. 2011). Microalgae *Dunaliella salina* is long been cultivated for β-carotene extraction by countries like USA, Australia, Israel and China due to the presence of both cis and trans isomers in natural β-carotene and will be more effective as an antioxidant (Hu et al. 2008; Khoo et al. 2011).

Astaxanthin Astaxanthin is a keto-carotenoid with remarkably high antioxidant properties and can act as a scavenger of harmful free-radicals that are produced due to many reasons such as environmental pollution or high intake of junk food etc. Freshwater green algae *Haematococcus pluvialis* is the richest source of astaxanthin and is commercially exploited worldwide. When *H. pluvialis* is exposed to unfavourable stressful conditions like nutrient deprivation and high light and pH conditions, it convert the chlorophyll into red coloured astaxanthin pigment for survival and develop a thick-wall around it in its resting stage (Shah et al. 2016). When *C. zofingiensis* undergoes with nitrogen deprivation it was observed that the primary carotenoids particularly lutein and β-carotene decreased, while the secondary carotenoids increased considerably, with astaxanthin and canthaxanthin being the most increased ones (Zhang et al. 2019). The carotenogenesis pathways were reconstructed.

This pigment is generally used as food colourant, feed additive in poultry business. Currently, astaxanthin is sold in the form of gelatin encapsulated nutraceuticals due to its antioxidant, anti-aging effects and for the treatment of eye disease, metabolic and neurogenerative disorders etc. (Ambati et al. 2014; Khalid et al. 2014). The only drawback which restricts the mass commercialisation of natural astaxanthin is the very slow growth of *H. pluvialis*. Many studies are performed all over the world to overcome the challenge and found that Cd nanoparticles are good inducer of astaxanthin moreover ethyl acetate and 2-methyltehydrofuran are found to be an excellent solvent and can recover > 80% of astaxanthin in 30 min (Cheng et al. 2018; Samorì et al. 2019). Recent findings proved that even almond oil is able to extract astaxanthin and keep *H. pluvialis* alive, without affecting the algal photosynthetic activity, providing the possibility to milk and regeneratively cultivate *H. pluvialis* and avoid an uneconomical loss of biomass (Gomez-Zavaglia et al. 2019). The biosynthesis for the production is shown in Fig. 7.

Biofertilizer

Biofertilizer is a compound that has nutrients to replenish the soil's fertility and promote the growth of plants and algae. After utilizing the oil for biodiesel production, carbohydrates for bioethanol production and other bioactive compounds for nutraceutics or pharmaceutics, the remaining nitrogen rich biomass is best suitable for fertilizer which leads to total consumption of cultivated algae and make it a part of zero waste circular economy.

Almost all the cyanobacteria can efficiently fix atmospheric nitrogen and are the important builder of soil's fertility. Both microalgae and macroalgae are rich in organic compounds that will stimulate germination, flowering and increase leaf and stem growth and at the same time prevent any infestation of pest from roots, thereby acting as a biological protectant against plant disease (Castro et al 2020). Furthermore, algae will help recovers physio-chemical properties and maintains the pH and salinity of soil. Blue green algae like *Nostoc, Anabaena* and *Tolypothrixare* help in fixing atmospheric nitrogen and hence, aids in gradual build-up of soils nitrogen and carbon content (Singh et al. 2016). The study reports improved and sustainable cultivation of rice crop (cv. IR36) by valorising de-oiled microalgal biomass waste (DOMBW) of *Scenedesmus* sp. as an eco-friendly and efficient fertilizer as the consortia of algal biomass with chemical fertilizer ($MA_{50} + CF_{50}$) showed the highest performance in terms of plant height, tiller number, biomass and grain yield. Even the harvest revealed maximum plant dry weight, panicle weight and 1000-grain weight with this combination in comparison to other treatments (Nayak et al. 2019).

However, a life cycle assessment (LCA) study of microalgae biomass (12%) with conventional fertilizer compared to triple superphosphate fertilizer has shown about 75% more environmental impact in climate change and terrestrial ecotoxicity. The major cause of disruption to the environment is the energy used for algae cultivation and biomass processing (Chandra et al 2014). Although this route is environmentally not sound, the cumulative benefits microalgae hold make this process sustainable and viable. Further research is encouraged to optimize the production process. Moreover, the integration of other value-added product is the subsequent solution to overcome the challenges.

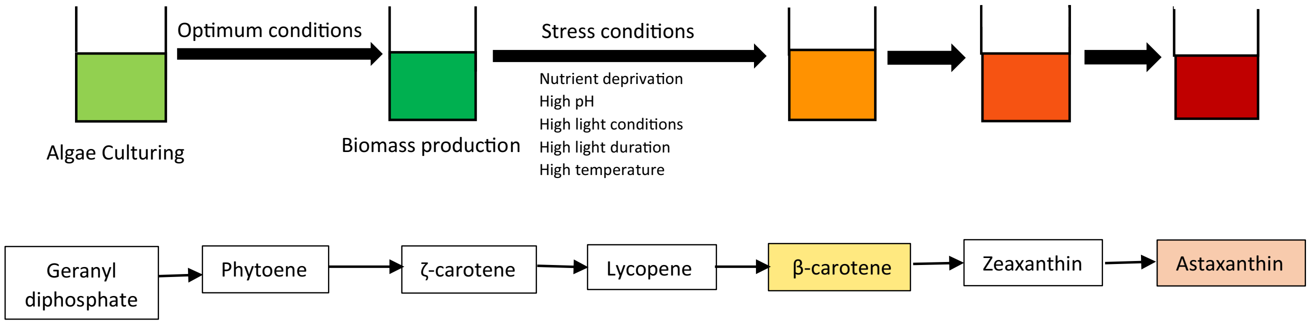

Fig. 7 Process for biosynthesis of β-carotene and astaxanthin as the main carotenoids

Bioplastics

Plastic has done great harm to the environment in both terrestrial and marine life; this poses the need for a biodegradable solution. Bioplastics are any polymeric material derived from a natural living renewable source and can be biodegradable, which can effectively take over the parts of petroleum made plastics. Microbial systems like bacteria (sole producers), yeast systems, microalgae and transgenic plants are the known producers of PHA. Algae are a potentially rich source of polysaccharides (> 60%), proteins (60%) and lipids (> 50%) that can serve as an operative raw material for bioplastic production (Chandra et al. 2019; Nakanishi et al. 2013). Currently, there are many polymeric bio-derived plastic forms available in the market. The most common are Polyhydroxyalkanoates (PHA), Polylactic acid (PLA), starch, cellulose and protein-based plastics. The major applications of bioplastics are in packaging industry, then textile (11%), followed by automobiles and consumer goods (7%) (https://www.european-bioplastics.org/market/). Other important applications include drug delivery, tissue engineering, biocontrol agents, artificial organs etc. are also the major industries for PHA (Kalia et al. 2021).

Both alginate and carrageenan are commercial sources from macroalgae as it renders excellent hydrophobicity and biocompatibility. Companies like Cereplast are harnessing seaweeds for agar production used as a biopolymer and the left out crude glycerol from biofuel production can be utilized here as a plasticizer for plastic production (Jariyasakoolroj et al. 2019). But due to the expensiveness of agar, starch is generally the preferred biopolymer. Mathiot et al. performed a screening of ten algal species. They found that *Chlamydomonas reinhardtii* 11-32A strain displayed a maximum starch-to-biomass ratio of 49% (w/w), 460 h after being switched to a sulphur-deprived medium and it undergoes direct plasticization by twin-screw extrusion (Mathiot et al. 2019).

Microalgae synthesize PHA in the form of intracellular granules under nutrient deprived and carbon-rich physiochemical conditions. Algal metabolism converts the biomolecules into acetyl-CoA and then direct it for PHA biosynthesis through three different pathways typically acetoacetyl-CoA, TCA cycle and fatty acid metabolism pathways. Moreover, research on *Microcystis* sp. revealed the highest concentration of PHB (Polyhydroxybutyrate) among other algae in high-rate algal pond and showed a plasticizing capacity of 530%, which is much higher than blank (Abdo and Ali 2019). On the other hand, carbohydrate-laden algae like *Nannochlorum* sp., *Scenedesmus* sp. are exploited to produce PLA by directly converting carbs to lactic acid under anaerobic dark fermentation conditions (Aikawa et al. 2014). The major constraint to the bioplastic ventures is its cost; conversely, by increasing biomass yield and integrating different value-added products, efforts can be rationalized. Culture optimization, usage of low-cost waste carbon sources like sewage and industrial wastes and finally, strain development, can escalate the technology to rising heights (Zhang et al. 2019). Advanced developments include metabolic engineering by directing flux towards PHA accumulation, but the best suitable method is the integrated biorefinery approach (Tharani and Ananthasubramanian 2020; Patel et al. 2016). Nowadays, algae-based bioplastics are common though not produced in large extent and many nations are adopting this eco-friendly strategy to promote sustainable development and put an end to the plastic menace.

Bio-ink

Ink is a liquid that contains pigments or dyes mixed with petroleum products to give a colour to a surface to make images and designs or write something. It is basically made up of 80% petroleum products that are extracted from underground wells by first clearing off large vegetation that covers the area, thereby by massive devastation and the other 20% are pigments that are usually carbon black mined in deep mines and are really toxic. These synthetic inks are found to be carcinogenic and nonbiodegradable (https://energycentral.com/c/ec/ink-waste-environmental-impact-printer-cartridges).

Algae on the other hand are well known to produce oils and are infused with living pigments. Therefore, different algae with variety of colourful pigments are best suitable for natural ink production as showed by Scott Fulbright, CEO and cofounder of Living Inks (https://livingink.co). Such inks are not only natural, nontoxic, cheap and biodegradable but also are the finest sustainable solutions to traditional inks.

Papermaking

Algae being abundant source of cellulose, hemi-cellulose and absence of lignin in their cell wall make them best suitable raw material for paper manufacturing (Mukherjee and Keshri 2018). *Gelidium amansi* and *Gelidium corneum* are the two most exploited red algae for papermaking as they contain rhizoidal filaments, reddish cortical cells and medullary cells filled with mucilaginous carbohydrates that are processed to make bleached pulps. These pulps are anticipated to produce paper with high brightness (Seo et al. 2010).

Ververis et al. used a mixture of algae from wastewater treatment plant and used it as 10% of pulp mix. As a result, mechanical strength of paper increased with 45% less material cost (Ververis et al. 2007). According to Dr. Phang, University Malaya the paper making process form seaweeds in much more environmentally friendly than from wood pulp as it requires more time and temperature due to presence of lignin in wood (https://www.algaeindustrymagazine.com/making-paper-seaweed/). Therefore, this concept can be implemented worldwide to reduce the dependency on wood pulp and at the same time can solve environmental issues concerned with global warming and deforestation.

Air quality monitoring

Nowadays, with increasing technology and population, air pollution is becoming a threat to human life (Kurien and Srivastava 2020). According to WHO air quality improvement is a major task as poor air quality can cause number of respiratory ailments and upto 7 million death in a year. Therefore, it is a leading cause of concern (Kurien and Srivastava 2018).

Algae are photosynthetic aquatic organisms and can utilize carbon dioxide for photosynthesis and in turn produce around 50% of world's oxygen (Chapman et al. 2013). They are grown in a large bioreactor, where favourable conditions are provided for their optimum growth and such photobioreactors are placed near traffic or pollution source and can act as air purifiers by reducing CO_2, NO_x, PM_{10} and $PM_{2.5}$. The study on microalgae film-based air purifier obtained good results for O_2 production and particulate removal but experience pH and moisture maintenance issues (Lu et al. 2019). According to Australian company Cloud collective that has setup an urban algae farm on Geneva highway, the biomass produced during purification could be used to create biodiesel, green electricity, medication, cosmetic products and even food (https://www.sciencealert.com/this-algae-farm-eats-highway-pollution). It is an ongoing research topic all over the world and similar research is been carried out at Shoolini University in Solan district of Himachal Pradesh, India to ameliorate this technology and make it a fugal option for poor section of the society (https://news.shooliniuniversity.com/indoor-green-purifier-to-tackle-air-pollution/). The obtained biomass after air quality monitoring can be used for additional revenue source by its conversion into bioenergy production (Bhatia et al. 2019).

Food products

Large number of algae derived compounds are used in food industry like agar, alginates and carrageenan. In past few years, the use of such compounds is considerably increased due to their thickening and gelling properties (Bhattacharjee 2016).

Agar

Agar is a gelatinous substance present as the scaffolding structure in the cell wall of red algae species namely *Gelidium* and *Gracilaria* and is released on boiling. It is made up of binary mixture of linear polysaccharide agarose and heterogeneous smaller molecules of agaropectin. Agar has wide range of applications like frozen food, candies, fruit juices, bakery icings, dessert gels and meringues etc. in food industry. Besides, it can be used in adhesives, paper sizing, textile printing, impressions and casting and most importantly for biological culture media preparation (Leet 2001)(http://portal.nceas.ucsb.edu/working_group/science-frameworks-for-ebm/background-information/CA_Living_Marine_Resources_statusreport.pdf/index.html). The study investigated that the agar properties are significantly affected by the extraction procedure while soaking treatment is found to be safe.

The report suggested simple protocols based on the combination of sonification and the hot water treatment can reduce fourfolds of the extraction time without affecting the yield and properties (Martinez-Sanz et al. 2019). Recently the study found that H_2O_2 (peroxide)–assisted enzymatic extraction preserve great amount of sulphate and resulted in high yield (16.08%) due to effective degradation of cellulose but on the other hand decreases thermal stability (Chen et al. 2020). When parameters are studied independently, it was found that yield mostly depend upon extraction temperature whereas time contribute significantly to viscosity. Furthermore, it can be used as a laxative, suppositories, capsules and tablets in pharmaceuticals.

Carrageenan

It is a group of water soluble linear sulphated polysaccharides extracted from red seaweed, *Chondrus crispus* also known as Irish moss. It is widely used as a food additive to thicken, emulsify and preserve food. It is used to improve the texture of ice cream, yogurt, soy milk, cottage cheese, jams, jellies, salad dressings, meat products and other processed foods. Carrageenan also has numerous pharmaceutical applications due to its antitumor, antiviral and anticoagulant properties. Another research on red algae *Kappaphycus alvarezii* suggested extraction using 2.5% KCl, yielded 34.3% carrageenan and finally concluded that higher the KCl concentration resulted in the increase of the carrageenan yield, sulphate and ash content and the decrease in moisture, gel strength and acid-insoluble ash components (Manuhara et al. 2016).

Alginate

Alginate or alginic acid is a polysaccharide present in the form of divalent salts of alginic acid and act as the structural component in the cell wall of brown algae. It is hydrophilic and forms a gelatinous gum when hydrated. Alginate yield can be increased by 44% (w/w of algae) having a molecular weight of 730 kDa with sequential extraction using acid treatment (Lorbeer et al.2015). It is used in the form of sodium alginate to act as an impression-casting material in dentistry, life casting and prosthetics and when combined with bicarbonate, it used in a preparation of Gaviscon which is used to inhibit reflex in pharmaceuticals (Corvaglia et al. 2011). The study on *Sargassum* sp. showed 96% removal Congo red dye by alginate dose (10–60 mg/L), calcium dose (1–6 g/L) and initial dye concentration (50–250 mg/L) at pH 4 and thus valorising the coagulation potential of alginate (Vijayaraghavan and Shanthakumar 2016). Apart from this, it is used in textile industry for cotton yarn sizing and also to smooth texture and to prevent the formation of ice in ice creams due to its chelating ability for highly viscous solutions.

Aquaculture feed

Algae are conventionally used as a feed for aquaculture purposes to culture several fishes like larvae, juvenile fish and finfish. Even the zooplankton need microalgae for their growth that can later be served to hatch carnivorous fishes (Das et al. 2012). Algae like *Tetraselmis, Pavlova, Phaeodactylum, Nannochloropsis, Skeletonema, Thalassiosira* and predominantly *Chlorella* and *Spirulina* in the form of mixtures are used for making aquaculture feed while algae which are high in protein like *Hypneacervicornis* and *Cryptonemiacrenulata* are regarded as a shrimp diet (Sirakov et al. 2015). Study used 1000L outdoor floating photobioreactor to cultivate *Isochrysis zhangjiangensis* and obtained produced the highest cell density of 17.8×10^6 cel L^{-1} and highest biomass productivity of 0.115 g L^{-1} d^{-1} for use as seed inoculums and feed for large area aquaculture water bodies at minimal cost (Zhu et al. 2019).

Selection of particular algae for aquaculture feed depends upon cell size, nutritional composition, growth rate, pigmentation and digestibility. The study found chrysophyte *Boekelovia hooglandii* exhibited a relatively high biomass productivity of 0.52 g L^{-1} day^{-1} dry weight (dw) with growth rate and nutritional profile [protein, lipid (34 fatty acids), carbohydrate and pigment (7 pigments)] to be suitable as aquaculture feed for bivalve larvae and juvenile oysters (Ruffell et al. 2017). Many fishes like wild salmon acquire their red colour due to consumption of astaxanthin producing green algae. Therefore, cultured salmon fishes are needed to be supplied with astaxanthin to sustain the market value.

Current trends and economic aspects for integrated product production

Algae has a profitable global market with its versatile product range targeting various market segments. Market trends show enormous demand for high value commodities i.e., cosmetics, healthcare and food additive sectors (Ruiz et al. 2016). However, algal biofuels being a great alternative for fossil fuels still gives very low returns on investment making the energy sector least profitable. Considering this scenario, various studies and experiments have been carried out to amalgamate different products production along with biofuels to decrease the relative cost incurred and achieve higher returns. Although, there are no such commercial setups yet based on the integrated biorefinery approach since it is still a budding ideology (Rösch et al. 2019). However, there are a few pilot scale case studies and industrial designs that can provide pioneering information and the possible outcomes of this approach.

One of the case studies is carried out in Ethiopian sugarcane-processing factory, Mathura sugar in joint association with ethanol production factory. This integrated model produces biodiesel, upgraded biogas and biofertilizer with 188 tons/year, 1,974,882 m^3/year and 42 tons/year of production capacities respectively. It uses the wastewater effluent from both the industries to cultivate microalgae in the PBR setup using flue gas and undergo pretreatment cell disruption process for lipid extraction (lipid constitute 30% of total biomass) using ethanol as a solvent. The lipid extracted algae, sludge from primary treatment plant and vinasse (by-product from ethanol plant) is further used as a raw material for biogas generation along with 1% (v/v) of crude glycerol generated in transesterification. The supernatant from the anaerobic digestor is recycled back to the pond as a nutrient for the growing algae and the bottom product is utilized for biofertilizer production. The biogas produced is then upgraded to be used as transportation fuel. The results showed positive energy balance ratio of about 4.8, making it an energy efficient strategy in an environment-friendly manner (Zewdie and Ali 2020).

MIRACLES (Multi-product Integrated biorefinery of Algae: from Carbon dioxide and Light Energy to high-value Specialties) project report stated incredible commercial potential for 10,000 tonne integrated microalgae biorefinery in contrast to single product production plant of same size. It studied 3 different multi-products (MPs) scenarios as depicted in Table 2. It is analysed that the biorefinery cost in MP3 is minimum as compared to other two scenarios. IRR (Internal Rate of Return) of both single product and multiproduct case was calculated and it was found that MP show an interesting base case IRR of about 8–18% which is much higher and profitable comparatively. Furthermore, the major cost is attributed to cultivation and harvesting processes (about 60–85%), which needs to be reduced. Energy-saving measures, further optimisation of processes and use of green solvents is required for enhancing biomass productivities and facilitating environment-friendly extraction for complete exploitation of algal resource (https://cordis.europa.eu/proje

Table 2 Cost analysis for different multi-product production scenarios (https://cordis.europa.eu/project/id/613588/reporting)

	MP1[c]	MP2	MP3
Algal species	*Nannochloropsis gaditana*	*Isochrysis galbana*	*Nannochloropsis gaditana*
Products yield	Pigment extract-24% Peptides-18% Proteins-7% Residue-34%	Pigment extract-27% Peptides-54% Residue-15% Lipid residue-3%	Peptides-30% EPA65 oil-25% Colored residue-30% Lipid residue-15%
Cost structure			
CAPEX[a]	€220 million	€130 million	€90 million
OPEX[b]	€5.400/T[d]	€5.800/T	€7.900/T
Biomass value	€10.900/T biomass	€12.000/T biomass	€10.300/T biomass

[a]Capital expenditure

[b]Operational expenditurev

[c]Multi-product scenarios

[d]Tonne

ct/id/613588/reporting). Thus, integrated approach is found to be much more lucrative, energy-saving and has low environmental impact.

Challenges and perspectives

Algae render great potential to circumvent the present energy crisis worldwide and serve as a promising feedstock for cosmetics, pharmaceutics and the food industry. Despite the enormous versatile prospect, it holds for the future, many challenges persist for large-scale setups. Major commercialization constraints include contamination and evaporation in case of open pond culturing, slow growth of algae followed by low biomass yield, huge operational cost in terms of nutrients supply, PBRs, harvesting, dewatering, extraction and downstream processing cost. These energy and labour-intensive procedures make low-value products like biofuels economically inviable and limited to use.

There are many possible solutions to this problem. To surmount the applicable cost, the best strategy is to amalgamate different product production i.e., integrating low-value products with high-value streams. Microalgae grown for biofuel production in sterilized environments can be utilized for nonlipid product extractions in cosmetics and pharmaceutics. Moreover, low-cost sources like industrial or municipal wastewater can be utilized after secondary treatment to earn environmental credits and can simultaneously use for bioelectricity generation (Arora et al. 2021). The obtained biomass can be extracted for bio-oil synthesis and by-products like syn-gas and bio-char can add supplementary income. Succeeded by transesterification for biodiesel development, the obtained crude glycerol could catalyse the biogas generation in anaerobic conditions; and the leftover residue is best suited as a biofertilizer. This integrated biorefinery concept offers a collaborative, sustainable, frugal and zero-waste circular economy.

Furthermore, another way is the live extraction of secondary metabolites without harvesting, dewatering and extracting the product of interest i.e., milking of microalgae. Botryococcenes, high-value compounds like β-carotene, phycobiliproteins. Are being continuously removed from the culture using biocompatible organic solvents. Recent experiments in Japan reported the successful implementation of chemical-free extraction procedure on *Tolypothrix* using cell homogenization to further cut the cost (https://www.sciencetimes.com/articles/26408/20200710/milking-algae-produce-eco-friendly-biofuel.htm). This technique is still in its infancy and needs more research and experimentation.

With the advancement in biotechnology, many intricate transformational systems like genetic and molecular engineering are becoming more accessible and fortuitous for desirable algal strain development. Undoubtedly, these expensive and longstanding solutions are confined to a few academic institutions and private sector labs. Besides, intensive research is going on for creating immobilized, hybrid and co-culturing microalgal systems to further reduce the harvesting expenses. Incorporating metal nanoparticles in algae technologies is still a budding option. Furthermore, selection of microalgae strains based on the rapid doubling time, high lipid content, simultaneous air quality monitoring, water purification or having high tolerance to toxic wastewater systems and potential for soil reclamation are some of the noteworthy characters for future research and economical biorefinery industry. Therefore, algae hold sustainable and lucrative business prospects provided the extensive efforts and research required to meet the demanding challenges are addressed and taken care of.

Conclusion

With aggravating pollution levels and substantial climate change, algae are the indispensable need for the present generation for sustainable living. Algae are a diverse group of photosynthetic organisms that can sequent CO_2 and replenish the atmosphere with fresh O_2. During the process, it can absorb different pollutants for their metabolic processes and in turn produce a variety of secondary metabolites depending upon the controlled environment. Algae is provided with optimum growth conditions for maximum biomass yield, followed by harvesting and dewatering. The dried biomass is then pretreated and treated for the extraction and production of the particular desired product, respectively. Algae containing high lipid content with low water requirements and rapid doubling time has been utilized for bioenergy purposes and are termed as third-generation biofuels, such as biodiesel, bioethanol, biohydrogen and in terms of mMFC for bioelectricity generation. However, algae biofuels are still striving to meet commercial scale production in terms of poor economics. The future lies in integrated product production by supplementing biofuels with high value commodities and therefore, balancing the total cost sustainably.

Algae has huge potential for humans with limitless applications in almost every sector from power generation to food; further it can be cultured in wastewater in open ponds to different PBRs for commercial utilization. Algae are enriched with various bioactive compounds, vitamins and minerals that can boost health, act as an energy source, food derivative, curb air pollution; find applications in cosmetics and can provide raw material for paper, plastic and ink production industries. Advanced techniques like synthetic biology, genome sequencing, genome editing and strain development are providing the necessary thrust to algae powered industries. Some challenges in cultivation and exploitation of algae include the shortcomings like culture instability, high production costs, extraction methods, purification costs and low yield, which can be controlled by strategic integration of different products leading to zero wastage and prudent management of resources. The concept of integrated biorefinery has huge business potential and has valorised different industrial wastewater into multiple bioproduct of commercial value. Deep and intensive research in phycology should be encouraged to explore more untapped application in near future and to devise new techniques for more economical processing and fractionization of biomass as well as to promote an eco-friendly, zero waste and sustainable algae-based bio-economy, which is the foundation for the next stage of sustainable development.

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