Development of Sustainable Bioproducts from Microalgae Biomass: Current Status and Future Perspectives

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Population and pollution make notable contributions to introducing novel sophisticated techniques. From vehicles to industries, the release of CO_2 into the atmosphere and wastewater into the running water streams are key concerns. On the other hand, the population is responsible for the rapid manufacturing of all commercial goods. Microalgae are the only answer accessible for the aforementioned difficulties. Similar to plants, microalgae need CO_2 and light to thrive and produce a variety of bioproducts such as carbohydrates, protein, lipids, vitamins, sterols, pigments, and silica. Physical (light, temperature, CO_2, and UV), chemical (nutrient addition or depletion), enzymatic, and metabolic pathway reconfiguration, as well as indoor or outdoor growing, are highly regarded among the several optimization strategies to make desired products. Wastewater pollution is rectified by growing microalgae in nutrient-rich organic water for their growth, which is used to accelerate bioproducts. This review considers the use of bioproducts in food, animal and aquatic feed, fertilizer, biofuel, medicinal and nutraceutical sectors. This paper also provides different optimization strategies, which include physical and chemical means of extraction methods for enhancing bioactive products. Challenges and future recommendations for enhancing target bioproducts are discussed to overcome environmental issues.

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INTRODUCTION

Population growth increases the demand for natural resources, which are employed to produce food, energy, and chemicals. Consequently, it greatly enhances the greenhouse gas emissions in the atmosphere, increasing global temperature (Hussain *et al.* 2021). However, the existing system of producing food and supporting products that cannot meet the current needs of humans. To combat the shortage of the existing and future world, it is imperative to obtain various alternatives or technological innovations that could ramp up production (Rahul *et al.* 2020). Microalgae is an appropriate answer to the aforementioned challenges because of its major advantages. It has a doubling time on average of 26 h, high productivity, and the capacity to withstand food and feed competition (Kawamura *et al.* 2021). Microalgae is ecologically susceptible to manipulation, grows in non-arable land, requires exceptionally small spaces for farming, harnesses greenhouse gases for its growth, and is able to grow in wastewater with minimal nutrients (Odjadjare *et al.* 2015).

Microalgae are broadly classified into Chrorophyceae (green algae), Cyanophyceae (blue-green algae), Xanthophyceae (yellow green algae), Chrysophyceae (golden algae), Bacillariophyceae (diatom), and others (Andrade *et al.* 2018; Pirbazari *et al.* 2019; Yu *et al.* 2017) on the basis of pigments. Microalgae are a complex range of marine and freshwater eukaryotes, including unicellular to colonial forms of tiny algae with sizes ranging from a few micrometers to a few millimeters (Sarpal *et al.* 2015). They have tremendous ecological plasticity in adapting to harsh conditions, such as high temperature, salinity, light, pH, moisture, and nutrients. Similar to plants, microalgae function as a primary producer reliant on the abundantly available light source and assimilate atmospheric carbon dioxide (CO_2) to synthesize carbohydrates (18 to 46%), lipids (12 to 48%), proteins (18 to 46%), and other bioproducts (Tibbetts *et al.* 2014; Williams *et al.* 2019). Its metabolic process responds to the removal of 20% of CO_2 from the atmosphere and 40% of CO_2 from the ocean. According to assessments, about 1.83 kg of CO_2 was used to produce 1 kg of algal biomass (Kumar *et al.* 2011; Li *et al.* 2013). Microalgae can synthesize a variety of goods including food, animal and aquatic feed, fertilizers, nutraceuticals, pharmaceuticals, cosmetics, and alternative bioenergy products (Khan *et al.* 2018). One of the main unprecedented increases in demand is fuel, as fossil fuels are nearly expended. To meet customer demands, crude oil is imported from numerous nations. It also increases the processing costs of producing gasoline and diesel substantially. Microalgae are commonly cultivated for lipid production, which accounts for almost 40% of their total biomass (Raja *et al.* 2018). The generation of a high proportion of lipids by microalgae paved the path for the centralization and restructuring of biodiesel synthesis *via* transesterification. The biodiesel produced from algal resources can be blended with conventional diesel or gasoline at various proposition to run motor vehicles, thereby reducing the consumption and cost of traditional fuel (Melvelle 2012).

The study of genetic architecture also stimulates the production of novel microalgal products *via* insertion, deletion, and translocation of genes. This genetic method of microalgae can help to accumulate the target products to improve economic feasibility (Fu *et al.* 2017). Although there have been many studies reporting on bioactive products, understanding their low-cost production at a commercial scale still needs extensive work. To overcome these issues, microalgae have numerous advantages when compared to other conventional resources (Odjadjare *et al.* 2015). Studies show that microalgae effectively utilize wastewater as a low-cost nutrient source for producing biomass and its target products. Notwithstanding the massive potential applications of microalgae, their improvement is hampered by a myriad of challenges. Therefore, independent research and novel low-cost technologies should be implemented for the enhancement of algal biorefinery on the commercial scale (Mehrabadi *et al.* 2016).

This review article describes the latest technological approaches in microalgal biorefinery for the production of high-value-added bioproducts. In addition, this study also addresses the current challenges and future work on wastewater treatment using microalgae and genetic approaches for enhanced production of various bioproducts.

BIOACTIVE PRODUCTS FROM MICROALGAE BIOMASS

Microalgae are capable of creating numerous bioactive products that act as feedstock for different products (Chew *et al.* 2017). Carbohydrates, proteins, lipids, cellulose, silica, pigments, vitamins, and sterols are essential for a healthy human diet and the treatment of numerous ailments. The residual goods encompass food for humans, biofuels, animal and aquatic feed, cosmetics, nutraceuticals, and pharmaceuticals (Mendoza *et al.* 2013; Prussi *et al.* 2014). Deep seawater (DSW) is used as a novel method for microalgae cultivation for fisheries, aquaculture, and medicine. Plant-growth-promoting bacteria (PGPB), when co-cultured, form a symbiotic relationship with the microalgae for better biomass production (Tan *et al.* 2015). The entire product will be renewable and harmless compared to chemically synthesized products. Different bioproducts produced from the microalgae are illustrated in Fig. 1.

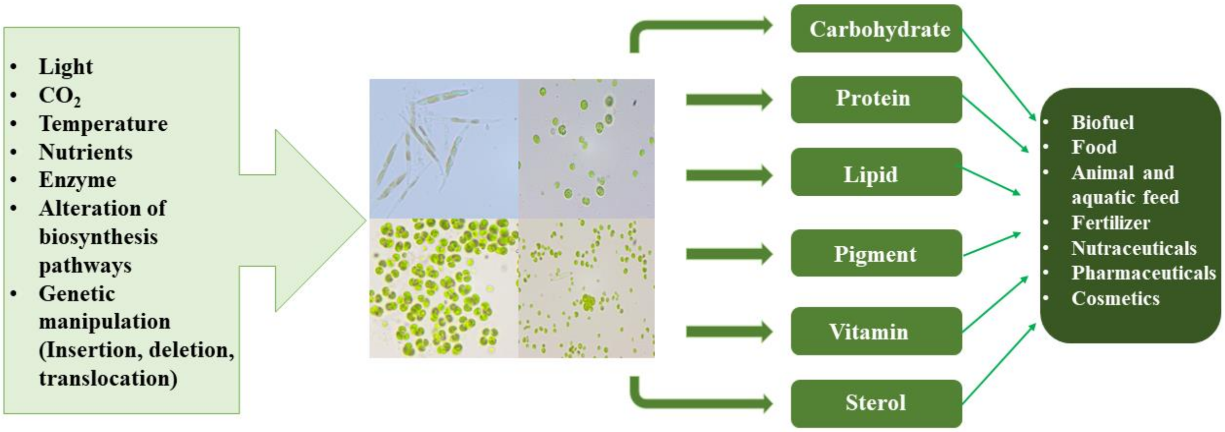

Fig. 1. Illustration of various cultivation conditions used in microalgae to improve diverse bioproducts

Carbohydrates

Microalgal biomass contains a high amount of carbohydrates derived from the photosynthetic process. Carbohydrates are either located in the cell wall as structural components or in the plastids as reserve materials (Chen *et al.* 2013). Most of the microalgae possess starch as a reserve material, while cyanobacteria synthesize glycogen and sucrose as storage products (Gonzalez-Fernandez and Ballesteros 2012; Markou *et al.* 2013). The carbohydrate content in the microalgal cell may vary from species to species. Nevertheless, the composition of carbohydrates could be increased by various environmental stress conditions (Domozych *et al.* 2012).

Carbohydrates from microalgae are utilized for the development and manufacturing of biofuels including bioethanol, biobutanol, biomethane, and biohydrogen (Markou and Georgakakis 2012) (Table 1). These biofuels are employed in running high-performance with low-emission engines (Simas-Rodrigues *et al.* 2015; Quader and Ahmed 2017). Examples of fuels, such as biobutanol, are reported to be produced by *Chlorella vulgaris* and *Spirulina platensis* approximately 21% and 71% *via* fermentation, respectively (Tan *et al.* 2020). *Synechocystis* sp. produced bioethanol at a density of 0.186 g/g (Ashokkumar *et al.* 2019). Further, the production of bioethanol in *Chlorella minutissima* was achieved by increasing carbohydrate up to 60.5% (Menestrino *et al.* 2020). *Scenedesmus obliquus* produced biohydrogen at a volume of 68.9 mL/g (Singh *et al.* 2019). Cuellar-Bermudez *et al.* (2019) proved that *Pseudanabaena* sp. yielded 25.1 mL/g of biomethane. Other possibilities include food thickeners, painkillers, biodegradable materials, and functional foods (De Souza *et al.* 2020).

Table 1. Several Optimization Strategies to Manufacture a High Amount of Carbohydrates to Extract Numerous Bioproducts

Microalgae	Carbohydrate Content (%)	Product	Optimization	References
Chlorella vulgaris FSP-E	59.53	Bioethanol	Light and nitrogen starvation	Condor *et al.* (2022)
Neochloris aquatica CL-M1	50.5	Biobutanol	Phosphorus and nitrogen starvation	Wang *et al.* (2017)
Chlorella sorokiniana SLA-04	20 to 23	Lipid	Calcium and nitrogen starvation	Hanifzadeh *et al.* (2018)
Tribonema minus	26.6	Lipid	Phosphorus and nitrogen starvation	Wang *et al.* (2019)
Monoraphidium QLZ-3	19.1	Biofuel	Phosphorus and nitrogen starvation	Dong *et al.* (2019)
Scenedesmus obliquus BR003	62.5	Biobutanol	Sulphur and phosphorus	Narchonai *et al.* (2020)
Spirulina sp. LEB 18.	63.3	Bioethanol	CO_2 injection	Braga *et al.* (2019)
Scenedesmus obliquus UTEX 393	55.4	Biohydrogen	pH	Singh *et al.* (2019)
Chlamydomonas moewusii	72.8	Starch and lipid	Irradiance	Gifuni *et al.* (2018)
Parachlorella kessleri QWY28	43	Carbohydrate	Temperature	Qu *et al.* (2019)
Chlorella minutissima	60.5	Bioethanol	Magnetic field	Menestrino *et al.* (2020)
Pseudoneochloris marina	53.77	Biofuel	Airlift photobioreactor	Goncalves *et al.* (2019)
Geitlerinema sp. *Coellastrella* sp.	46 56	Biofuel	Semi-continuous photobioreactor	Solis-Salinas *et al.* (2021)
Spirulina platensis LEB-52	35	Bioethanol	Enzyme hydrolysis	Rempel *et al.* (2021)
Chlorella sp.	26	Bioproducts and biofuels	Enzyme	Arora and Philippidis (2021)
Pseudanabaena sp.	23	Biomethane	Anaerobic digestion	Cuellar-Bermudez *et al.* (2019)
Arthrospira platensis	20	Biomethane	Anaerobic digestion	Markou *et al.* (2013)

Polysaccharides

Polysaccharides are also called carbohydrate polymers. They have intricate structures that differ (structurally and biochemically) among the different species of microorganisms. Xylose, glucose, galactose, mannose, and rhamnose are the major components in microalgal polysaccharides (Yi *et al.* 2021; Chanda *et al.* 2019; Bernaerts *et al.* 2018). In microalgae, polysaccharides are primarily formed as part of the cell wall (as structural polymers), involved in various metabolic functions (as energy storage polymers) (Yi *et al.* 2021; Markou and Georgakakis 2012), and also in cellular interaction and protection (as exopolysaccharide) (Morais *et al.* 2022; Prybylski *et al.* 2020).

Microalgae are able to synthesize polysaccharides under various stress conditions (Parwani *et al.* 2021; Colusse *et al.* 2021) such as temperature, light, salinity, and nutrient uptake *etc.* (Costa *et al.* 2021). Hence, standardization of these cultural conditions for microalgae cultivation are imperative to increase the production of polysaccharide

(Colusse *et al.* 2021). Earlier reports showed that white light was more suitable for the production of polysaccharides at levels of about 0.10 and 0.14 g/L in *Porphyridium sordidum* and *Porphyridium purpureum*, respectively (Medina-Cabrera *et al.* 2020). Further, *Chlorella vulgaris* can accumulate about 32.7% of polysaccharides at a light intensity of 65 µmol m^{-2} s^{-1} at 28 °C (Gui *et al.* 2019). About 65 µmol m^{-2} s^{-1} of light was supplied in nutrient media with 1%w/v of glucose helps to produce 1.46 g/L of polysaccharide in *Chlorella* sp. (Cheirsilp *et al.* 2016). In addition to that, the nutrient media supplemented with high salinity (40g/L) was supported the polysaccharide production in *Spirulina* sp. by 1.02g/g of biomass (Chentir *et al.* 2017).

Polysaccharides derived from *Anabaena* sp. CCC 745 have a significant antioxidant and scavenging activity in food industries (Tiwari *et al.* 2019). It has been reported that polysaccharides from *Nostoc* sp., *Phormidium* sp. and *Scytonema arcangeli* can be used as soil-fixing agent in the agricultural fields (Park *et al.* 2017). On the other hand, polysaccharides of *Spirulina platensis* were used as feed for zebra fish growth and development (Rajasekar *et al.* 2019). Utilizing nanotechnology to create polysaccharide-based goods can be applied in the food, health and beauty industries (Morais *et al.* 2022). Moreover, the polysaccharides are being used in the biomedical field for antithrombotic, immunomodulatory, antitumor, anticoagulant, anti-inflammatory, antimutagenic, antiviral, and antioxidant activities, as reported by many authors (Xu *et al.* 2017; Moreira *et al.* 2022; Patil *et al.* 2018; Li *et al.* 2019).

Cellulose

Cellulose is the most abundant sustainable source on Earth. It has enormous potential for producing renewable fuels, bioplastics, and nanomaterials. Cellulose is a linear homopolymer consisting of repeating β-d-glucopyranosyl units connected by 1–4 glycosidic linkages in a diversified arrangement depending on the presence of crystallites and disordered amorphous regions. Cellulose is the major component in the cell wall of plants and algae (Popper *et al.* 2011). In general, the ratio of cellulose Iα/Iβ in algae was found to be 60/40, whereas in plants it was 25/75. Until now, four totally different sub-polymorphs of cellulose such as, cellulose (I), cellulose (II), cellulose (III), and cellulose (IV) have been reported. Hydrogen bond arrangements and polarity in constituting chains are varied between these sub-polymorphs. The most common form of crystalline cellulose (I) was observed in nature, whereas cellulose (II), (III), and (IV) have been synthesized by thermal and chemical treatments. Based on the chain alignment and interchain hydrogen bonding, cellulose allomorphs have distinctive geometries (Zanchetta *et al.* 2021). Regarding micro- and macroalgae, it was reported that microalgae have remarkable flexibility in terms of their mode of cultivation for cellulose production. However, only limited studies are available for increasing the cellulose content in microalgae biomass, which may be the focus of more attention in the future.

From a commercial standpoint, producing higher biomass in a short period of time is essential for the overall yield of cellulose. For instance, *Chlorella* is reported as a fast-growing microalga and consists of a fibrillar layer of polysaccharides that can be secured in certain cases through a resistant algaenan outer layer (Domozych *et al.* 2012; Kroger *et al.* 2018). Another report shows that the cell wall of *Scenedesmus quadricauda* is a trilaminar arrangement in which cellulosic and pectic layers can be distinguished and secured by an algaenan layer (Nemcova 2003). Further, the cell wall of *Oedogonium bharuchae* has two layers that are a mix of cellulose, pectins, and glycoproteins followed by a cellulose-free layer having both extensin and arabinogalactan proteins (Estevez *et al.*

2008). Similarly, the cell wall of *Nannochloropsis* has a special porous inner layer mixed with struts connecting the cell membrane to a porous cellulose-dependent layer covered by an algaenan outer layer (Scholz *et al.* 2014).

Furthermore, *Nannochloropsis* sp. is a well-studied microalga that is known for its great potential in cellulose extraction and production (Hamed *et al.* 2016; Lee *et al.* 2018). However, there is no sufficient information about other microalgal species, and hence, more attention is required to identify potential strains for cellulose production. A high concentration of cellulose was reported in *Chlorella* sp., *Oocystis* sp. (Aguirre and Bassi 2013; Kroger *et al.* 2018), *Scenedesmus* sp., *Coelastrella* sp., *Chlorococcum* sp., *Selenastrum* sp. (Yap *et al.* 2016; Kroger *et al.* 2018), *Chaetosphaeridium* sp., and *Staurastrum* sp., which garners increased interest for further study in detail. Table 2 illustrates the quantification of cellulose content in various microalgal strains. From these, the highest cellulose content (75 wt% dry weight basis) was observed in *Nannochloropsis gaditana* in their cell wall (Scholz *et al.* 2014). In contrast, low cellulose content (15.4 wt%) was reported in *Chlorella pyrenoidosa* (Northcote *et al.* 1960). The variation of cellulose content from species to species can be influenced by many cultural conditions. Currently, genetic engineering technologies are also opening the way to enhance biomass yields with desired products. It was proposed that the biorefinery approach can be used for the successful production of cellulose (Lee *et al.* 2018).

Table 2. Cellulose Content of the Microalgal Feedstock

Name of the Algal Strain	Class	Cellulose (%)	References
Mixed culture of microalgae and cyanobacteria from the wastewater treatment plant	Mixed culture	7.1^a	Ververis *et al.* (2007)
Chlorella vulgaris	Chlorophyceae	10–47.5^a	Aguirre and Bassi (2013)
Nannochloropsis gaditana	Eustigmatophyceae	25^a	Hamed *et al.* (2016)
Chlorella pyrenoidosa	Chlorophyceae	15.4^b	Northcote *et al*. (1960)
Nannochloropsis gaditana	Eustigmatophyceae	75^b	Scholz *et al.* (2014)
Staurastrum sp.	Chlorophyceae	72^b	Gunnison and Alexander (1975)

a. Represents the amount of cellulose in a total dry weight basis; b. Represents the amount of cellulose in a cell wall dry weight basis

Proteins

Proteins are produced from a variety of green vegetables and animal meat, but diatoms can generate them in greater quantities, which allows them to be sold competitively as tablets and pills. For instance, *Tetraselmis suecica* can produce a protein of approximately 12% using bead milling. In general, microalgae generated a protein with outstanding emulsification, frothing, and gelatin properties, a noteworthy breakthrough for the food sector (Garcia *et al.* 2018). *Chlorella thermophile* exhibited an increase of 36% in its protein content and was studied using artificial neural networks-genetic algorithm (ANN-GA) (Sarkar *et al.* 2022). In addition, *Arthrospira platensis* was discovered to account for 110% of the protein production from sugarcane bagasse in solid-state cultivation (Pelizer *et al.* 2015). Microalgal proteins are used in the cosmetic, medicinal, and animal/aquaculture feed industries (Caporgno and Mathys 2018). *Chlorella* has the capability of producing 62% protein content for cosmetics and nutraceutical production through enzyme hydrolysis (Pekkoh *et al.* 2021). Microalgae such as *Botryococcus braunii*, *Chlorella protothecoides*, *Chlorella vulgaris*, *Neochloris oleoabundans,* and *Scenedesmus acutus* can synthesize protein as a nutrition and dietary supplement of approximately 40%, 36.1%, 48.6%, 49.5%, and 53.2% protein content, respectively, using photobioreactors (PBRs) (Tibbetts *et al.* 2015; Baldisserotto *et al.* 2022). In comparison to another optimization method, enzyme hydrolysis helps to extract the maximum amount of protein present in the microalgal cell.

Lipids and Their Derivatives: Eicosapentaenoic Acid, Docosahexaenoic Acid, and Isoprenoids

Lipid productivity in microalgae can be classified into two parts: (i) 14 to 19 carbon atom chains used for the generation of biodiesel because of the unavailability of double bonds in the chain; and (ii) poly-unsaturated fatty acids (PUFAs) (Surendhiran *et al.* 2015). Furthermore, δ-3 fatty acids have many health benefits and can be found in a variety of food products that act against arthritis, asthma, cancer, cardiovascular disease (CVD), inflammatory disorders, depression, and schizophrenia by preventing the production of oxidative stress as antioxidants (Adarme-Vega *et al.* 2012). Lipids are also used as nutritional supplements and feed, antioxidants in beverages and functional foods, pills, capsules, and food additives in candies, gum, pasta, *etc.* (Chen *et al.* 2014).

The PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are necessary for the development of the human brain and the prevention of coronary heart disease (CHD). Additionally, because of their anti-carcinogenic, anti-thrombotic, anti-diabetic, and anti-obesity quality, as well as an immune modulator and in pregnant women for better fetus growth (Echeverria *et al.* 2017), they are commonly utilized in many health drinks. The production of EPA, C20:5 n-3 was 23.6% and DHA, C22:6 n-3 was 36.5% in *Phaeodactylum tricomulum* under the nutrient depletion (Yi *et al.* 2017). The quantity and composition of PUFAs are invariably influenced by growth phases and environmental factors. The lipid's PUFA can also be turned into biodiesel by the transesterification method (Chen *et al.* 2012).

Microalgae can store a substantial amount of lipids with high biological activity. *Phaeodactylum tricornutum* constituted a neutral fraction containing greater than 60% triglycerides (TAGs), the primary component for biodiesel generation under outdoor conditions (Steinrucken *et al.* 2018). It was reported that *Chlorella vulgaris, Scenedesmus* sp., and *Spirogyra* sp., can accumulate 15 to 40% of lipids by their dry weight of biomass (Mata *et al.* 2009; Cai *et al.* 2013). In addition, this fraction of lipid accumulation might be augmented to 70 to 90% by modifying cultivation conditions, such as supplementing KNO_3, as a nitrogen source (Gour *et al.* 2018). Addition of $NaNO_3$ at a concentration of 18.75 mg/L to *Nitzschia* sp. produced a lipid content of around 60 mg/L (Harini *et al.* 2020). Carbon-nitrogen (C/N) ratio, pH, high salinity, temperature, and nitrogen depletion in the medium play a key role in boosting lipid formation (Kwak *et al.* 2016). *Amphora coffeaeformis* RR03 produced a lipid of about 67.15% cultured in an open raceway pond (Rajaram *et al.* 2018). In the semicontinuous mode in the open raceway pond (ORP) *Chlorella vulgaris* UTEX 26 was grown and produced the highest lipid of 6.1 g m^{-2} d^{-1} along with the periodic addition of NH_4HCO_3 and ammonia (Ramirez-Lopez *et al.* 2019). Nitrogen plays a major role in the synthesis of lipids. Excessive addition of nitrogen to the media helps improve the production of the lipid and the products (Table 3).

Table 3. Adaptation of Conditions to Promote Lipid Products for the Production of a Wide Range Bioproducts

Organism	Lipid Content (%)	Product	Optimization	References
Chlorella sp.	34	Bioproducts and Biofuels	Enzyme	Arora and Philippidis (2021)
Neochloris oleoabundans; *Chlorella vulgaris*	16.04 > 20	-	Ionic liquids	Zhou *et al.* (2019)
Chlorella vulgaris	> 19	-	Bio-sourced solvent	Breil *et al.* (2017)
Thrustochytrium sp.	91	Biorefinery	Organic Solvents	Zhang *et al.* (2018)
Ascochloris sp. ADW007	34.98	-	Open raceway pond	Kumar *et al.* (2019)
Tribonema sp.	55.4	-	Intermittent-vacuum stripping (IVS) system	Huo *et al.* (2020)
Chlamydomonas mexicana	33	Biodiesel	-	Abou-Shanab *et al.* (2013)

Pigments

Pigments present in microalgae have a pivotal role in the development of a myriad of bioactive compounds in the form of secondary metabolites. Microalgae are used to produce feed, antibiotics, cosmetics, nutritional food, and economically efficient pigments. It is also used to treat cancers, neurological disorders, and eye ailments (Chew *et al.* 2017). Current research focuses on the exploitation of wastewater sources for pigment synthesis (McClure *et al.* 2018). Algal pigments include phycocyanin, lutein, fucoxanthin, β-carotene, diatoxanthin, diadinoxanthin, and astaxanthin (Sathasivam *et al.* 2017). Phycocyanin fluorescent blue-colored phycobiliprotein acts as an antioxidant and anti-inflammatory, is found in cosmetics, and it also helps treat liver, colon, lung, and breast cancers (Fernandez-Rojas *et al.* 2014; Kumar *et al*. 2014). Among its many health benefits are its antioxidant, anti-inflammatory, and hepatoprotective properties (Lima *et al.* 2018). It is also used in popsicles, chewing gum, confectionery, wasabi, dairy products, and soft drinks (Gattullo *et al.* 2012). Research has shown that extract of phycocyanin used for the production of biscuits along with which wheat flour has higher nutritional properties (Garcia *et al.* 2017). *Spirulina platensis* produced 159.9 mg/g of phycocyanin using a pulsed electric field, a sort of pretreatment method that increases the permeability of algal cells (Martinez *et al.* 2017). Ultrasound-assisted extraction (UAE) of phycocyanin from *Spirulina platenis* yielded around 13.6% of pigment (Hadiyanto and Suttrisnorhadi 2016). Phycoerythrin, a phycobiliprotein was purified and exploited as a fluorescent dye for *Porphyridium marinum* study and produced B-phycoerythrin of about 40 mg/g of dry cell weight (DCW) $NaNO_3$ = 3.4 g/L with the light intensity of 70 µmol photons/m^2/s and metal solution about 1.5 mL/L (Garcouch *et al.* 2018). Biomass from *Porphyra* sp. and *Arthrospira* sp. produced 8.32 mg/g and 8.18 mg/g of phycoerythrin, respectively using UAE (Ardiles *et al.* 2020).

Lutein is one of the two most abundant carotenoids in the human eye (macula and retina). Many individuals consider lutein to be "the eye vitamin." Many microalgae can synthesize lutein pigment. For example, *Chlorella minutissima* MCC-27 contributes to the generation of 17.28 mg/L of lutein based on the study of ANN and particle swarm optimization (PSO) (Dineshkumar *et al.* 2015). In a two-stage fed-batch mixotrophy condition, *Chlorella sorokiniana* FZU60 produced 65.96 mg/L of lutein (Ma *et al.* 2020).

Isoprenoids constitute a significant and vital class of biomolecules. A collection of isoprenoids composes the various pigments. Fucoxanthin, β-carotene, diatoxanthin, and diadinoxanthin are regarded as isoprenoids in this context and found in *Phaeodactylum tricornutum* and *Botryococcus braunii* (Niehaus *et al.* 2011). Fucoxanthin has an allenic link, nine conjugated double bonds, a 5,6-monoepoxide, and several oxygenic functional groups. They possess numerous biological features, including antioxidant, anti-obesity, and anti-inflammatory qualities (Maeda 2015; Zhang *et al.* 2015). Fucoxanthinol, the deacetylated derivative, demonstrated potential in the therapy of numerous cancer cell types and antineoplastic activity (Martin 2015). Fucoxanthin is one of the main chlorophyll a/c complex compounds found predominantly in diatoms, where it functions as a light-harvesting pigment during photosynthesis and growth. For instance, *Isochrysis* sp. in better media after optimization produced 7.5 to 23.3 mg/g (Sun *et al.* 2019). For *Nitzschia* sp. under high silica, the concentration produced was 12 to 32.8 mg/g (Mao *et al.* 2020) and *Tisochrysis lutea* synthesized 2.1 to 79.4 mg/g under single-cell fluorescence (Gao *et al.* 2020). *Phaeodactylum tricornutum* produced approximately 59.2 mg/g of fucoxanthin. Compared to the synthesis of fucoxanthin in an open raceway pond, the growth of microalgae in a controlled environment, such as a PBR, yields a higher product yield (Quader and Ahmed 2017).

Carotenoids are isoprenoid structured lipophilic pigments that are found in non-photosynthetic organisms. They have strong antioxidant properties, thereby protecting the organisms from oxidative and free-radical stress. A range of 0.1 to 0.2% of total dry matter of microalgae may consist of carotenoids. It contains an abundance of colors, including yellow, orange, and red. Carotenoids consist of over 600 colors found in nature (Herrero *et al.* 2013). In the food and pharmaceutical industries, it has expanded applications to lessen the effects of smoking, hypertension, dyslipidemia, diabetes, cancer, cardiovascular disease, and atherosclerosis (Lobo *et al.* 2010; Herrero *et al.* 2013). According to reports, using the modified medium with different concentrations of NaCl, 10% to 14% of β-carotene was recovered from *Dunaliella salina*, which is beneficial for vision and the immune system (Sathasivam and Juntawong 2013).

Xanthophylls, specifically diatoxanthin and diadinoxanthin, are regarded as beneficial chemicals that are diatom-specific (Sathasivam *et al.* 2017). The article demonstrates that numerous diatom species manufacture these pigments efficiently. *Mytilus coruscus* produced approximately 133.97 mg/kg of diatoxanthin and 107.16 mg/kg of diadinoxanthin from its DCW by altering the acetylenic carotenoid pathway and 4-keto oxidative pathway (Li *et al*. 2022a). *Isochrysis zhangjiangensis* also generated 0.75 mg/g and 4.5 mg/g of diatoxanthin and diadinoxanthin, respectively. The researcher also found that increasing light induces the biosynthesis of fatty acids but reduces the formation of fucoxanthin, whereas, in intense light, the cycle of diadinoxanthin to diatoxanthin was also triggered (Li *et al.* 2022b). *Haematococcus pluvialis* produced approximately 4% of its DCW as astaxanthin using different cell disruption methods (Kim *et al.* 2022). It was observed that *Oedocladium carolinianum* produced 24.2 mgL^{-1}day^{-1} of astaxanthin along with the production of lipids in the open and closed PBRs (Wang *et al.* 2022). Possessing medicinal properties, these pigments treat maladies such as diabetes, ageing, cancer, obesity, and stroke (Raposo *et al.* 2013; Lin *et al.* 2016). Based on previous studies, it can be assumed that a consistent light source can enhance the growth of microalgae, leading to a rise in product output. Depending on the different physical and chemical factors and the type of microalgae, the composition of the pigments varies in their biomass.

Silica

Silica forms the cell wall of diatoms, a characteristic feature in the family Bacillariophyceae. They overlap with valves designated epitheca and hypotheca, which mimic a petri dish structure interconnected by a silica girdle band. Utilizing a minuscule amount of energy, silica absorbs diatoms from the external environment *via* silica acid transporters. In addition, the consumption of silica from surface water to sediments is a benefit of diatoms. Because diatoms are the primary and dominant producers in aquatic ecosystems, they deposit a greater quantity of silica in deep water over time (Sun *et al.* 2017). The presence of silica causes the diatoms in diatomaceous earth to settle and settle over years. According to reports, diatomaceous earth could potentially act as an adjuvant for vaccination against chicken infections (Nazmi *et al.* 2017). This silica is primarily obtained from *Coscinodiscus wailesii*, *Cyclotella* sp., and *Chaetoceros* sp. (Esfandyari *et al.* 2020) and is taken advantage of in the biosensor field (antibody conjugation). In addition, it serves as a drug carrier for *Nitzschia palea* (Singh *et al.* 2020) and *Thalassiosira weissflogii* (Cicco *et al.* 2016).

Vitamins

Microalgae are capable of generating and accumulating a wide assortment of vitamins, including pro-vitamin A, some B vitamins (B_1, B_2, B_3, B_5, B_6, B_8, B_9, and B_{12}), vitamin C, and vitamin E (Galasso *et al.* 2019). *Spirulina platensis*, *Isochyris galbana*, *T. suecia*, and *P. cruentum* produced an abundance of vitamin E (tocopherols) about 120.5, 115.5, 159.8, and 184.7 µg/g in continuous cultivation (Lopez-Hernandez *et al.* 2020). *Porphyridium cruentum* generates large quantities of vitamin A of 0.75 mg/g in closed PBR (Santiago-Morales *et al.* 2018). *Dunaliella salina* synthesizes vitamin E, vitamin C, pyridoxine, nicotinic acid, thiamine, riboflavin, and biotin efficiently (Tafreshi and Shariati 2009). In contrast, Tarento *et al.* (2018) investigated the vitamin synthesis of several microalgae. Based on his study, 1 g of cylindrical *Anabaena* powder contains 64% of vitamin B_{12}. Additionally, *Spirulina* has a maximum of 40.9 mg/g of vitamin B_2. Approximately 0.24 mg/g of vitamin B_3 was extensively synthesized by *Chlorella* sp.

Sterols

Phytosterols are sterols, such as sitosterol, campesterol, and stigmasterol, which have diverse pharmacological effects, including anticancer and anti-inflammatory, are exploited as food additives. Several microalgal strains produce phytosterols primarily utilized to reduce cholesterol levels (Luo *et al.* 2015). *Chaetoceros* sp. yielded phytosterols at a concentration of 27.7 mg/g DCW. In similarity, *Pavlova lutheri* produced 22 mg/g of total sterol (Ahmed and Schenk 2017). *Pavlova lutheri*, *Tetraselmis* sp. M8, and *Nannochloropsis* BR2 yield a range of 0.4 to 2.6% sterols (Ahmed *et al.* 2015; Santhosh *et al.* 2016) (Table 4).

Table 4. Bioactive Chemicals from Several Microalgae for the Synthesis of Non-Hazardous Renewable Products

Compounds	Product	Examples	References
PUFA	Docosahexaenoic acid (C22:6), Eicosapentaenoic acid (C20:5), Arachidonic acid (C20:4), Linolenic acid (18:3)	*Chlorella vulgaris*, *Phaeodactylum tricornutum*, *Botryococcus bruanii*, *Amphora* sp., *Nitzschia* sp.	Yi *et al.* (2017); Gour *et al.* (2018); Rajaram *et al.* (2018); Harini *et al.* (2020)
Pigments/ Carotenoids	β-carotene, astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, phycoerythrin, fucoxanthin	*Chlorella vulgaris*, *Coelastrella striolata*, *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Dunaiella salina*, *Muriellopsis* sp.	Koller *et al.* (2014); Hamed (2016)
Vitamins	A, B1, B6, B12, C, E, biotin, riboflavin, nicotinic acid, pantothenate, folic acid	*Cylindrospermus* sp., *Tolypothrixtenus*, *Nostoc muscorum*, *Hapalosiphon fontinalis*, *Nostoc*, *Hapalosihon*	Custodio *et al.* (2012); Xia *et al.* (2014)
Antioxidants	Catalases, polyphenols, superoxide dismutase, tocopherols	*Lyngbya majuscule*, *Chlorellazo fingiensis*, *Coccomyx aonubensis*	Mostafa (2012); Xia *et al.* (2014)
Bioactive compounds	Antimicrobial, antifungal, antiviral, amino acids, proteins, sterols, toxins	*Chlorella vulgaris*, *Phaeodactylum tricornutum*, *Dunaiella salina*, *Muriellopsis* sp.	Markou and Georgakakis (2012); Mostafa (2012)

GENETIC ENGINEERING OF MICROALGAE

Genetic engineering is the process of manipulating genes to mass-produce the desired product. The associated procedures are mostly utilized in chemistry, pharmacology, biochemistry, and biotechnology (Manuel *et al.* 2018). Important steps in genetic engineering investigation include transformation techniques and selection. Genetic engineering uses particle bombardment, glass beads, electroporation, agrobacterium-mediated transformations, direct gene editing, *etc.* for direct gene transfer (Ng *et al.* 2017).

The genomic sequences of *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum* were completely analyzed and sequenced. In microalgae, *Chlamydomonas reinhardtii* is the first model organism (Merchant *et al.* 2007). The comprehensive examination of the order of the genes prepares for the enhancement of the production of various chemicals *via* genetic modifications such as inducible promoters, regulatory elements, and the insertion or exclusion of genes. The transition of the gene into nuclear DNA results in either stable or temporary gene expression for the synthesis of various products (Kao and Ng 2017).

Through CRISPR-mediated phosphoenolpyruvate carboxylase regulation, *Nannochloropsis* sp. has become a new model organism for carbon sequestration and oil production with 94% stability over seven generations (Kao and Ng 2017). It has been reported that suppression of CrPEPC1 by substituting the CRISPRi gene in *Chlamydomonas reinhardtii* CC400 was used for the first time to effectively increase lipid synthesis (Johnson *et al.* 2016). As described by Jester *et al.* (2022), homologous recombination of the antibiotic-resistance (ABR) gene and the gene of interest (GOI) in the genomic DNA of *Spirulina* produced 15% of the targeted product, it can be administered orally without purification. The HMG reductase gene was targeted to alter the critical components that are produced by the diatoms. In *Phaeodactylum tricornutum*, the gene HMG reductase and IDI-SQS are targeted for the expression and overproduction of the triterpenoid biosynthesis pathway (D'Adamo *et al.* 2018).

Ribonuclease (RNA) interference using the PEPC1 gene in *Chlamydomonas reinhardtii* resulted in a 1.5-fold increase in lipid accumulation by electroporation (Ahmad *et al.* 2015). Modified NAB1 gene from *Chlamydomonas reinhardtii* (T541A, T676A) upregulated by glass bead transformation (Beckmann *et al.* 2009). In *Haematococcus pluvialis*, particle bombardment induced the expression of the modified PDS gene (L504R) to boost astaxanthin synthesis by 45% (Steinbrenner and Sandmann 2006). Electroporation and agrobacterium-mediated transformation of the gene by RNA interference, gene-editing employing ZFNs and CRISPR was tested in *Chlamydomonas reinhardtii* to determine the most effective gene transformation (Mini *et al.* 2018).

WASTEWATER AS A NUTRIENT SOURCE FOR PRODUCING BIOACTIVE COMPOUNDS

Microalgae, particularly diatoms, can proliferate under low-light circumstances, which may aid their growth in wastewater. The increase of numerous hazardous greenhouse gases, such as CO_2, methane (CH_4), nitrous oxide (N_2O), and wastewater (Gimpel *et al.* 2013) commensurately accentuates several environmental dangers. To reduce the use of commercial nutrients, wastewater is increasingly and commonly used for algae cultivation (Ho *et al.* 2011). Because of its dual role in treating wastewater and creating biomass, it has attracted considerable interest (Rajkumar *et al.* 2022).

Consequently, microalgae play a vital role in a variety of industries for satisfying test demands without causing harm to the environment or posing a threat to human health. Phycoremediation is the utilization of microalgae in wastewater treatment (Phang *et al.* 2015). Because of their efficient cellular mechanisms and adaptive methodology, microalgae have the capacity to phycoremediate diverse forms of wastewater. They uptake macro-and micronutrients from wastewater to make biomass. When diatoms are grown in wastewater, their biomass can be used to produce a wide range of high-value chemicals with a broad range of applications in bioenergy, medicinal chemistry, food, and nutrition (Olguin 2012). Thus, wastewater can be utilized for the production of desirable items such as renewable fuels, food, fertilizer, pharmaceuticals, cosmeceuticals, PUFAs, and aquaculture. The oxygen produced by photosynthesis can support the growth of heterotrophic aerobic bacteria, hence accelerating the biodegradation of pollutants (Godos *et al.* 2010). Microalgae were cultivated using several forms of wastewater, including municipal, aquaculture, dairy, poultry, *etc.* (Fig. 2)

Approximately 116.2 mg/g of ethanol was produced from 1.4 g/L of biomass containing 38% carbohydrates, 15% proteins, and 22% lipids by growing in dairy effluent (Hemalatha *et al.* 2019). *Tribonema* sp. was cultivated in swine effluent and yielded a lipid concentration of approximately 42.4% (Cheng *et al.* 2020). In *Desmodesmus* sp. PW1, 29.4% of the generated lipids were derived from swine wastewater (Chen *et al.* 2020). *Chlorella sorokiniana* CY-1 cultivated in the wastewater of a palm oil mill yielded 14.43% lipids (Cheah *et al.* 2020).

Chlorella sp. helps anemic consumers boost their haemoglobin (Barrow and Shahidi 2007), whereas *Azolla* and *Anabaena* work as a biofertilizer to increase the nitrogen content of soil (Priyadarshani and Rath 2012). For cosmetics, *Dunaliella salina* strongly affects the energy metabolism of cells to promote their growth (Stolz and Obermayer 2013). Treatment of slaughterhouse wastewater by *Chlorella salina* for the utilization of nitrate and phosphate for the growth of the microalgae was performed in the open raceway pond. The biomass further used for producing different bioactive products (Habibi *et al.* 2018).

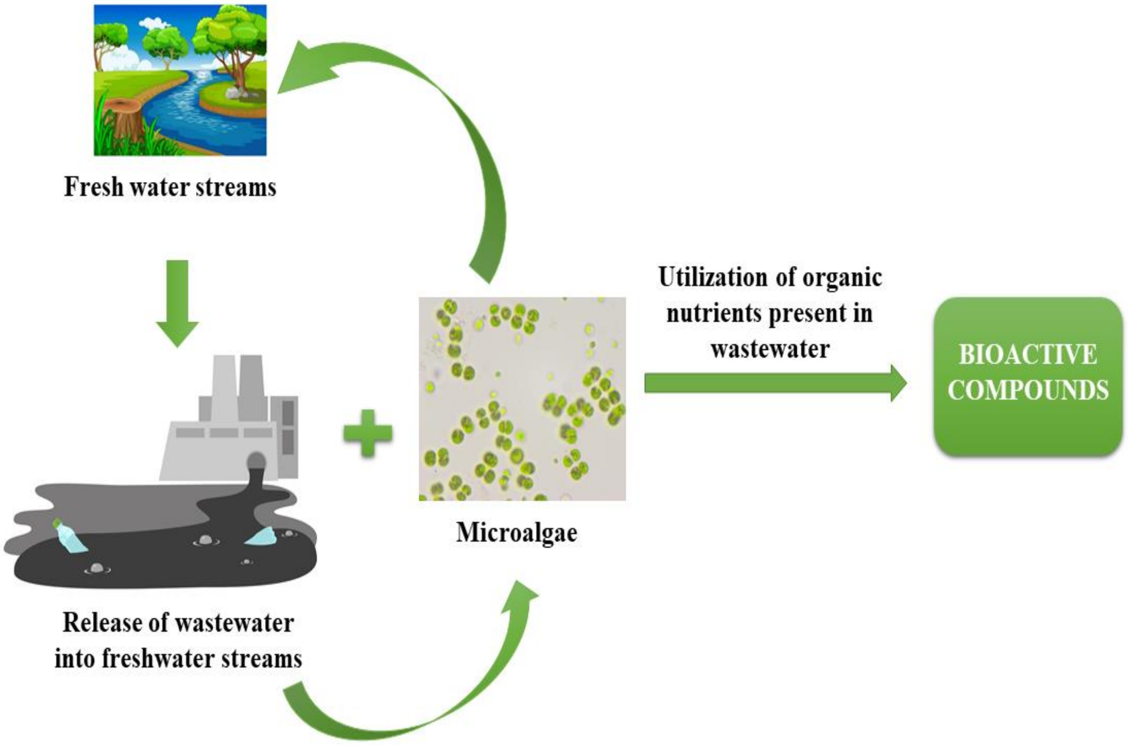

Fig. 2. Exploitation of microalgae for the biodegradation of toxic nutrients from wastewater from different streams to manufacture bioactive products and recycle water

CHALLENGES AND PROSPECTS

Because the products from the microalgae are exposed to the higher demands in various streams such as food, cosmetics, nutraceuticals, and synthesis of the renewable products occurs. Challenges occur when the production cost is comparatively higher than the product cost. Hence the need for alternatives is high before production at a low cost. The necessity of the compounds has increased, and therefore the best technologies have to be implemented for better results.

Existing systems for cultivation and post-harvest processing are unsustainable and unaffordable, as they consume around 40% of the total cost (Gifuni *et al.* 2019). Changes in geometry and fluid mixing pattern, better gas exchange, light penetration, and building material, all of which have advantages and disadvantages, must still be adjusted for production in PBRs. When operating PBRs, the majority of studies prioritize lighting patterns, fluid dynamics, cooling requirements, mixing efficiency, and mass transfer (Sirohi *et al.* 2022a).

There are certain downsides to the large-scale cultivation procedure. Therefore, the creation of open ponds for growth decreases costs and is simple to handle, but there are more variances such as pollution, irregular growth, predator contamination, temperature and light complications (Xu *et al.* 2009).

The creation of large-scale PBRs, such as tubes and containers, with minimal contamination risk would be more beneficial in the future (Wang *et al.* 2012). The PBRs provide numerous advantages over open systems, in terms of reduced pollution and the ability to cultivate monocultures of axenic algae. They provide more places before starting factors including pH, temperature, light, and CO_2 concentration. In addition, water does not evaporate in PBRs. In PBRs, higher cell concentrations are also realistically possible (Sirohi *et al.* 2022b; Udayan *et al.* 2022). The construction cost of the PBRs is higher compared with the open raceway ponds. The production of the product has to be more continuous than batch culturing. For biomass collection and product recovery, several technologies must be updated for the effective collection of products. Environmental and economic studies are needed along with a life cycle assessment for a better yield of the products without loss.

CONCLUDING REMARKS

In comparison to a great number of other chemical and artificially manufactured goods, microalgae are considered as more important while avoiding many of the associated drawbacks. Scientists in multiple fields are studying the potential of microalgae in different products. Microalgae include a variety of species capable of utilizing environmentally harmful compounds, wastewater, and CO_2. Treatment of wastewater is one of the most efficient strategies for the management and production of high-value compounds. Even while it serves as biomass for biofuels, health, cosmetics, and saves the environment from profoundly detrimental repercussions, biodiesel is less economically competitive than biohydrogen and biobutanol.

The high value-added bioproducts, such as astaxanthin, that were derived from microalgal sources have the potential to be applied in the pharmaceutical and nutraceutical industries. In comparison to first and third-generation fuels, intensive research has been conducted on fourth-generation fuels, which includes advanced low-cost technology and genetic modification for enhancing sustainable bioproducts. This has been done in an effort to improve the algal bioeconomy.

There is still a significant amount of cross-disciplinary research and development work that has to be done before more complex uses of algae may be implemented in any industry. To improve the process of acquiring the products, a wide variety of procedures and extraction methodologies need to be developed and put into practice.

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