10 Third-Generation Hybrid Technology for Algal Biomass Production, Wastewater Treatment, and Greenhouse Gas Mitigation

Ashwani Kumar, Pavithra Acharya, and Vibha Jaiman

Abstract

Greenhouse gas accumulation and climate change impact reduction requires widespread utilization of green technology. However, escalating demand for crops as a food source coupled with the finite availability of arable land makes cultivation of biofuel crops unsustainable. Algal biomass can be grown using non-arable areas such as lakes, oceans, or deserts, thus avoiding the current problem of land use competition with the food supply chain. Third-generation biofuels mainly consist of algal biofuels. However recently, hybrid use of algae for production of biofuels and also treating the wastewater for greenhouse gas reduction is gaining ground. Algae have the potential to produce valuable substances for the food, feed, cosmetic, pharmaceutical, and waste treatment industries. Microalgae mass cultures using solar energy and concentrated CO_2 sources can be used to produce renewable fuels such as methane, ethanol, biodiesel, oils and hydrogen and for other fossil fuel sparing products and processes. Recently developed hybrid technologies include biomass production, wastewater treatment, and GHG mitigation for production of prime products as biofuels. This also helps in atmospheric pollution control such as the reduction of GHG (CO_2 fixation) and bioremediation of wastewater microalgae growth. However, the selection of efficient strain, cultivation systems, microbial metabolism, and biomass production are important steps of viable technology for microalgae-based biodiesel production and phytoremediation. This chapter will

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discuss the latest developments in area of selection, production, and accumulation of target bioenergy carrier's strains, as well as the third-generation biofuels and hybrid technology for development of oil, biodiesel, ethanol, methanol, biogas production, and GHG mitigation.

Keywords

Biodiesel · Hybrid technology · biogas production · GHG mitigation · Ethanol · Microalgae

10.1 Introduction

Anthropogenic carbon dioxide emissions largely from fossil fuels in energy and transportation sectors have raised the concentration of CO_2 from pre-industrial levels of 280 ppm to about 415 ppm today, and they will continue to raise to about 570 ppm by the twenty-second century (Aaron and Tsouris 2005). This will have disastrous consequences on human health, climate, and agriculture (Kumar 2013, 2018; Kumar et al. 2018, 2020). In general, the use of photosynthetic organisms as a feedstock mitigates ever-increasing anthropogenic CO_2 emissions. High photosynthetic carbon sequestration efficiencies and carbon capture percentages of 90% make algae very well suited as a source for biofuels and bioproducts (Demirel 2018b). Algae are capable of eliminating 513 tons of CO_2 and producing up to 100 tons of dry biomass per hectare per year (Bilanovic et al. 2009).

The first generation of biofuels from food crops or their derivatives require farmland or wasteland but irrigation and fertilizer use improved their growth and productivity. Thus, the debate is focussed on food vs. fuel. It also posed a severe threat to food security triggering food versus fuel dilemma (Kumar 2018; Kumar et al. 2018, 2020; Nazneen and Kumar 2014).

The second-generation biofuels derived from lignocellulosic biomass were largely extracted from agricultural waste, lignocellulosic materials, food waste, etc. Although they addressed the above-mentioned problems, their processing technology and biofuel production has not been perfected for large-scale utilization (Anindita and Kumar 2013; Kumar and Gupta 2018).

10.2 Third-Generation Biofuels

Photosynthetic organisms include filamentous and unicellular macro- and microalgae, and cyanobacterial species. Microalgae have the potential to produce valuable substances for the food, feed, cosmetic, pharmaceutical, and waste treatment industries. Commercial culture of microalgae has >40 years history with some of the main species grown being *Spirulina* for health food, *Dunaliella salina* and *Haematococcus pluvialis* for carotenoid production, and several species for aquaculture (Lee 1997; Borowitzka 1999; Carvalho et al. 2006; see review Behera et al.

2019). Biorefinery approach with integrated biology, ecology, and engineering could lead to a feasible algal-based technology for a variety of biofuels and bioproducts (Allen et al. 2018).

The environmental consequence of global warming has led to a paradigm shift towards renewable fuels (Maity et al. 2014). Algal biofuel is an example of third-generation biomass which has the potential to replace fossil fuels and animal feeds (John et al. 2011; Sharma and Singh 2017; Gajraj et al. 2018; Kumar et al. 2018, 2020). Macro and microalgae transform solar energy into the carbon storage products, leading to lipid accumulation, including those that can be transformed into biodiesel, bioethanol, and biomethanol (Kraan 2013; Maity et al. 2014). Maity et al. (2014) reported that microalgae provide mainly TAG (triacylglycerols), a potential source of biofuel at large scale.

Although wastewater treatment and CO_2 removal by microalgae have been studied separately for a long time, there is no detailed information available on combining both processes (Craggs et al. 2011; Li et al. 2017; Kumar 2018; Kumar et al. 2019, 2020).

10.3 Wastewater Treatment

Discharge of untreated/partially treated brewery wastewater leads to environmental problems, such as water scarcity, excessive growth of undesirable microbes that cause loss of aquatic lifeforms (Okolo et al. 2018), and health-related problems in communities around the discharge areas (Norman 1997). Wastewater is a complex mixture of natural organic and inorganic materials, as well as man-made compounds. Different sources of pollutants include "Discharge of either raw or treated sewage from towns and villages; dis-charge from manufacturing or industrial plants; run-off from agricultural land; and leachates from solid waste disposal sites" these sites of pollution have problems so that a solution is sought (Gray 1989; Horan 1990; Showkat and Najar 2019). Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, ammonium salts bicarbonate, and heavy metals (Talbot et al. 1990; Horan 1990; Lim et al. 2010). Scarcity of water, the need for energy and food are forcing us to explore the feasibility of wastewater recycling and resource recovery (De la Noue and De Pauw 1988).

Microbiological composition of sewage wastewater environment is an ideal media for a wide range of microorganisms, especially bacteria, viruses, and protozoa. The majority is harmless and can be used in biological sewage treatment, but sewage also contains pathogenic microorganisms. However algae have been used in hybrid technology which produces biofuels along with removing the heavy metals and pollutants from wastewater. In conventional wastewater treatment system, the removal of biochemical oxygen demands (BOD) suspended solids, nutrients (NO_3^--N, NO_2^--N, NH_4^+-N and PO_4^{3-}-P), coliform bacteria, and toxicity are the main goal for getting purified wastewater (Abdel-Raouf et al. 2012; Lim et al. 2010; Amenorfenyo et al. 2019; Kumar et al. 2020).

10.3.1 Hybrid Technologies

Molazadeh et al. (2019) reviewed microalgae-based CO_2 biofixation, various microalgae cultivation systems, as well as concept of integration of CO_2 biofixation process and wastewater treatment. This helps in photosynthetic biomass production, wastewater treatment, and GHG mitigation. Thus, hybrid technology offers atmospheric pollution control such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Weissman and Goebel 1988; Abdel-Raouf et al. 2012; Maity et al. 2014) (Figs. 10.1 and 10.2). Sharma and Singh (2017) made systematic analyses of energy demand and GHG emission statistics of various nations, as well as all the steps involved in overall process from algal strain selection to biodiesel production. Microalgae including eukaryotic algae and cyanobacteria, as well as macro-algae, have demonstrated to be an environmental-friendly and sustainable alternative to energy-intensive and conventional biological treatment processes (Mohsenpour et al. 2021).

Fig. 10.1 Hybrid technology: Microalgae and wastewater treatment. (*Source*: Abdel-Raouf, N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci*. 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access: Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink)

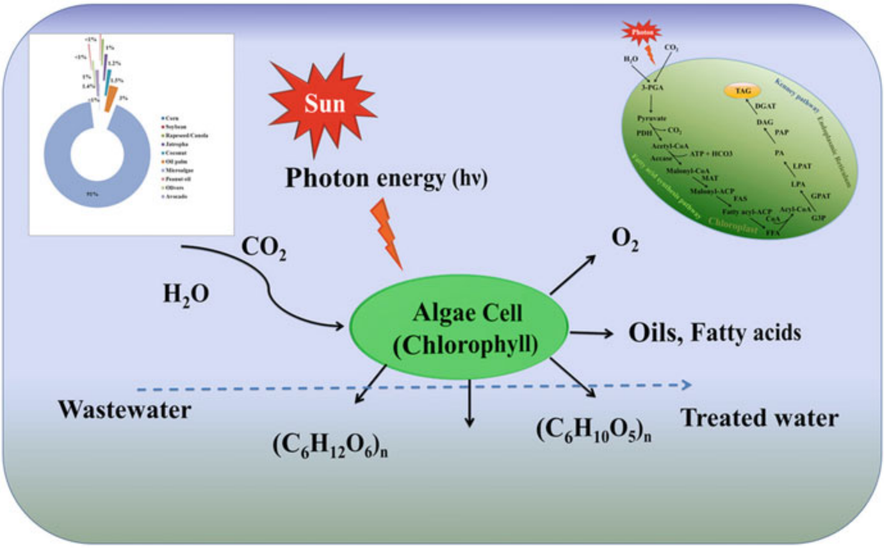

Fig. 10.2 Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions, and wastewater treatment. (*Source*: Maity, J. P., Bundschuh, J., Chen, C.-Y., & Bhattacharya, P. (2014). Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104–113. https://doi.org/10.1016/j.energy.2014.04.003. Reproduced with licence no 5064180534847 dated 8th May 2021)

Currently, algae wastewater treatment and its biomass use are attracting attention worldwide (Simate et al. 2011). Liu et al. (2013) highlighted the use of microalgae to address the problems of wastewater treatment. The current progress of hybrid technologies (biomass production, wastewater treatment, GHG mitigation) for production of prime products as biofuels offer atmospheric pollution control, such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Maity et al. 2014).

Wang et al. (2017) reported reuse of secondary municipal effluent from wastewater treatment plants to alleviate freshwater resource shortage. Wastewater treatment (WWT) for biofuel production provides additional incentives (Milano et al. 2016). This integrated approach (as illustrated in Fig. 10.5) is postulated to provide resource recovery-based monetary benefits, as well as 800–1400 GJ/ha/year energy which can be a source of energy at the community level (Mehrabadi et al. 2015). Furthermore, algae-based strategies for the removal of toxic minerals, such as As, Br, Cd, Hg, Pb, Sc, and Sn ions, have also been reported individually or in combination (Abdel-Raouf et al. 2012; Amenorfenyo et al. 2019). Olajire (2020) reviewed some of these challenges with a focus on key issues: water consumption and waste generation, energy efficiency, emission management, environmental impact of brewing process, and best environmental management for breweries. The potential for remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is well documented (Shi et al. 2007; Abhinandan et al. 2015; Whitton et al. 2015). Abhinandan et al. (2015) studied cultivation of microalgae species namely *Chlorella pyrenoidosa* and *Scenedesmus abundans* in rice mill effluent (i.e., paddy soaked water) for nutrient removal.

10.3.2 Steps of Wastewater Treatment

10.3.2.1 Cultivation

Cultivating microalgae under photosynthetic conditions on wastewater with low cost resources and easy separation of the biomass from the treated wastewater and high value products can be obtained in an eco-friendly manner. It can also be used to decontaminate pesticides and heavy metals. Singh et al. (2016) presented a hypothetical model of cyanobacteria in sustainable agriculture and environmental management (Fig. 10.3). Cyanobacteria could play a potential role in the enhancement of agriculture productivity and mitigation of GHG emissions (Singh 2011). Cyanobacteria are also useful for wastewater treatment and are an effective bio-fertilizer source and have the ability to degrade the various toxic compounds, even the pesticides (Cohen 2006). They also help in ecological restoration of degraded lands (Singh 2014, 2015a, b) (Fig. 10.4). Wastewater treatment, however, can also be organized or categorized by the nature of the treatment process operation being used, for example, physical, chemical, or biological. Li et al. (2020) reported that coupling algal growth on wastewater with hydrothermal liquefaction (HTL) is regarded as an environment-enhancing pathway for biomass amplification, wastewater management, sustainable energy generation, and value-added products generation. They proposed a paradigm shift involving enhanced algal wastewater treatment and bioenergy production for field application (Fig. 10.3).

Cyano bioremediation using cyanobacteria is a green clean tool for decontamination of synthetic pesticides from agro- and aquatic ecosystems (Kumar and Singh 2017) (Figs. 10.4 and 10.5).

Microalgal production inside industrial premises using effluent rich flue gas and wastewater would produce biomass for meeting the ever increasing energy demands, with the added benefits of wastewater treatment (WWT) and emission control (Milano et al. 2016). The algal biomass can be converted into various types of renewable biofuels including bioethanol, biodiesel, biogas, photo biologically produced biohydrogen, and further processing for bio-oil and syngas production through liquefaction and gasification, respectively (Kraan 2013). This integrated approach of microalgal wastewater treatment with resource recovery for maximizing the derivable products is illustrated in Fig. 10.6 (Behera et al. 2019).

10.3.2.2 Physical Treatment

Physical wastewater treatment, a pretreatment stage, has been used generally to reduce suspended solids, as well as grease and oil from wastewater through sedimentation by gravitational force (Jayanti and Narayanan 2004).

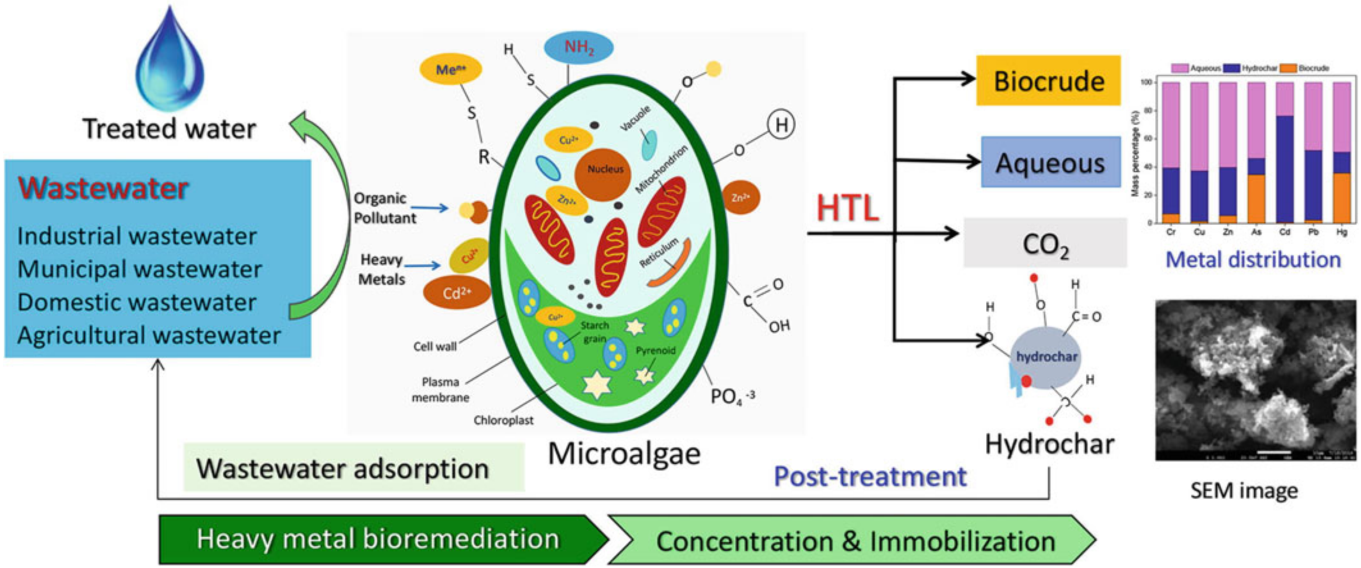

Fig. 10.3 Algal growth on wastewater with hydrothermal liquefaction (HTL). (*Source*: Li H, Watson J, Zhang Y, Lu H, Liu Z. Environment-enhancing process for algal wastewater treatment, heavy metal control, and hydrothermal biofuel production: A critical review. Bioresour Technol. 2020 Feb; 298: 122421. doi: https://doi.org/10.1016/j.biortech.2019.122421. Epub 2019 Nov 14. PMID: 31767428. Licence no 5065350239998 dated 10th May)

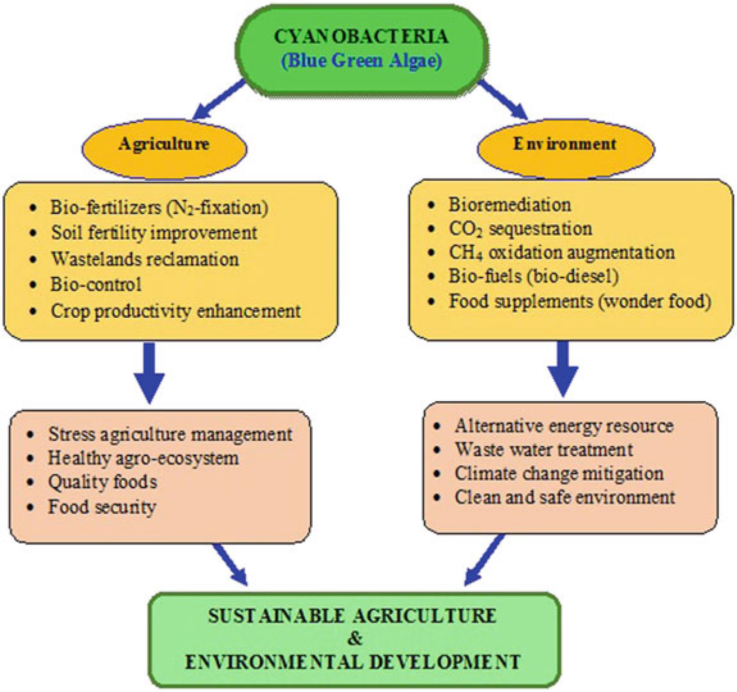

Fig. 10.4 A hypothetical model exhibiting the potential roles of cyanobacteria in sustainable agriculture and environmental management. (Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

10.3.2.3 Chemical Treatment

Chemical treatment processes involve pH adjustment or coagulation/flocculation by adding different chemicals to the effluent to alter its chemistry (Simate et al. 2011). Flocculation involves stirring/agitation of chemically treated effluent to induce coagulation that improves sedimentation performance by increasing particle size, thereby increasing settling efficiency (Simate et al. 2011).

10.3.2.4 Use of Consortia of Bacteria Cyanobacteria/Microalgae

From an environmental friendly perspective, bacteria, cyanobacteria, and microalgae, and the consortia have been largely considered for biological treatment of wastewaters (Perera et al. 2019). Perera et al. (2019) highlighted the use of specific molecular techniques of proteomics, genomics, transcriptomics, metabolomics, and genetic engineering to develop more stable consortia of bacteria and cyanobacteria/microalgae with their improved biotechnological capabilities in wastewater treatment.

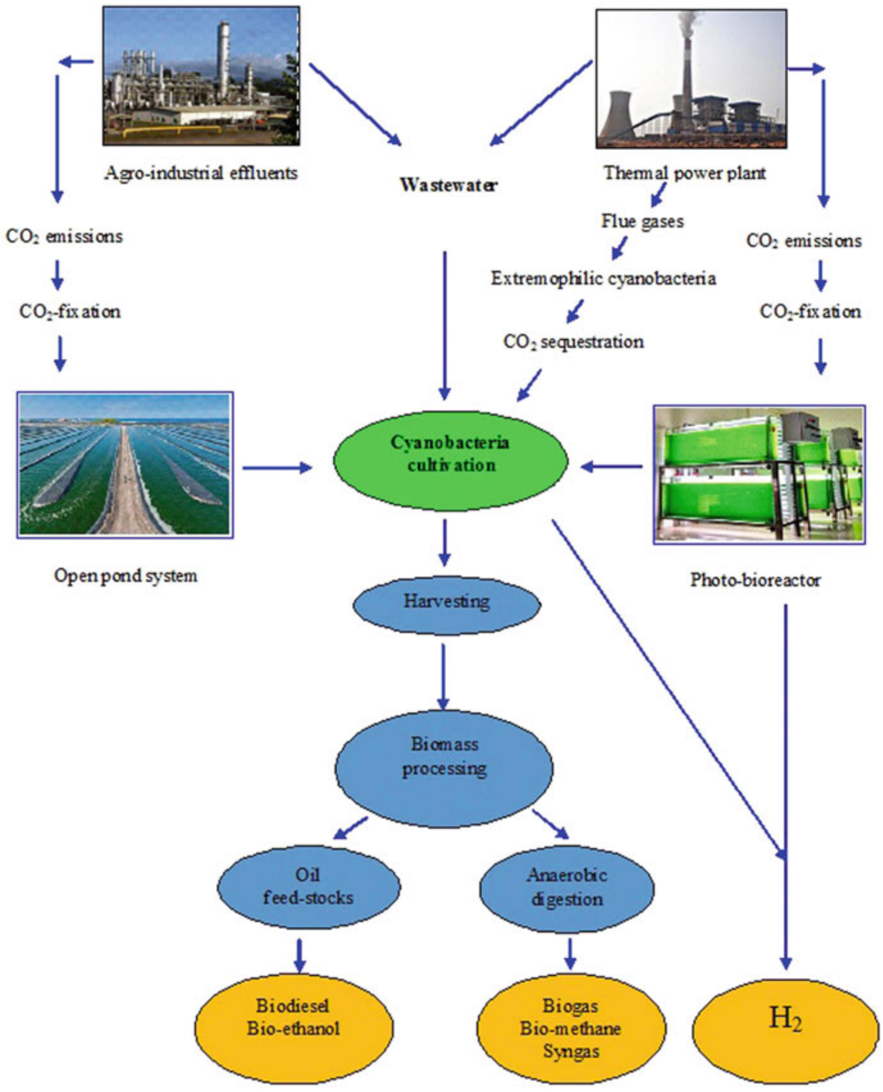

Fig. 10.5 A model showing the technologies used for production of biofuels from the mass cultivation of cyanobacteria. (Source: Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in Microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. Reproduced under CC-BY license which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and the source are credited)

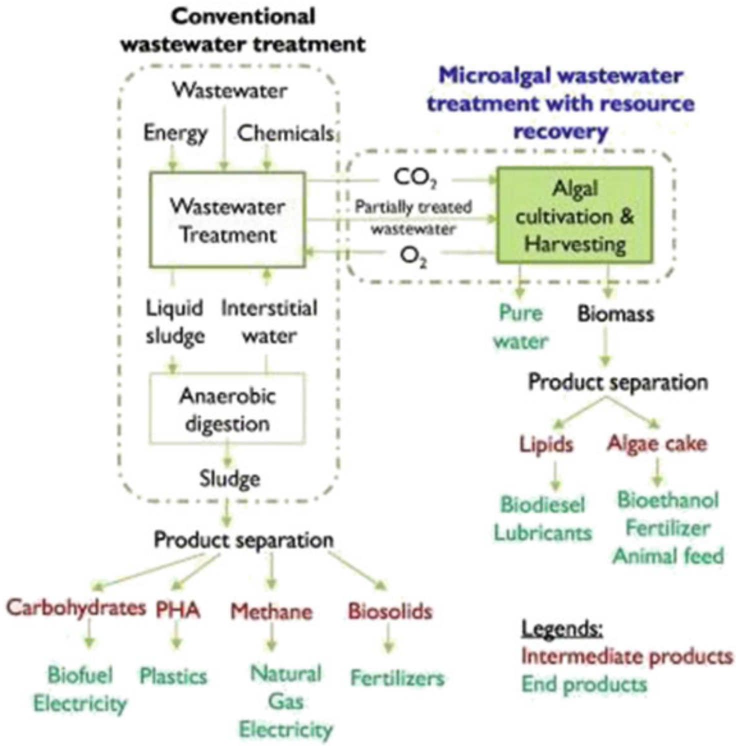

Fig. 10.6 Integration of microalgal wastewater treatment with resource recovery for maximizing the derivable products. (*Source*: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licence number 4906420744566 dated 12th September)

10.3.2.5 Overview of Microalgal Nutrient Remediation Mechanisms

Direct remediation is the most commonly discussed mechanism of remediation and is achieved through interconnected biochemical pathways for the uptake of the target nutrients into the biomass for storage (Seco and Ferrer 2015) or assimilation into nucleic acids and proteins for biomass growth (Cai et al. 2013).

Whitton et al. (2015) described that nutrient remediation with microalgae occurs through one of two pathways. A nitrogen source is required for the synthesis of proteins (Powell et al. 2008) or assimilation into nucleic acids and proteins for biomass growth (Cai et al. 2013; see also Whitton et al. 2015). The microalgae can be utilized for total nitrogen (TN) removal (nitrification and denitrification), with NO_3^- assimilation observed following the near-complete exhaustion of NH_4^+ (Eixler et al. 2006) from the source wastewater. Phosphate, in the preferred form of $(H_2PO_4)^2$ and $(HPO_4)^2$, is transported across the cell membrane via energized transport and assimilated into nucleotides following phosphorylation for the synthesis of ribosomal RNA (Beuckels et al. 2015; see Whitton et al. 2015).

10.4 Algal CO_2 Fixation

According to Benemann and Pedroni (2003), approximately 1.8 tons of CO_2 can be fixed through photosynthesis by producing 1 ton algal biomass in high rate algal pond (HRAP)-based wastewater treatment (see Craggs et al. 2014). Raeesossadati and Ahmadzadeh (2015) reported CO_2 fixation rates of several microalgae and cyanobacteria species under different CO_2 concentrations and culture conditions for biomass production (see also Moheimani et al. 2015). According to Allen et al. (2018), some of the best biofuel microalgae strains are *Nannochloropsis* spp., *Chlorella vulgaris*, *Chlorella minutissima*, *Chlorella protothecoides*, *Botryo-coccus braunii*, *Spirulina platensis*, *Chlorella emersonii*, *Spirulina maxima*, *Phaeodactylum tricornutum*, *Scenedesmus obliquus*, *Chlorococcum* spp., *Dunaliella tertiolecta*, *Crypthecodinium cohnii*, *Dunaliella salina*, *Schizochytrium* spp., *Chlamydomonas reinhardtii*, and *Microcystis aeruginosa* in terms of high growth rate and lipid contents. These algae usually require light, nutrients, and carbon dioxide, to produce high levels of polysaccharides such as starch and cellulose. These polysaccharides can be extracted to fermentable sugars through hydrolysis and further fermentation to bioethanol and separated through distillation (Allen et al. 2018; Rodionova et al. 2017).

10.5 Algal Ccultivation for Biomass Production

High carbon capture percentages of 90% paired with the ability to perform high photosynthetic carbon sequestration efficiencies and harvesting and using the totality of the biomass make algae a suitable source for biofuels and bioproducts (Demirel 2018b).

Microalgae mass cultures can use solar energy for the biofixation of power plant flue gas and other concentrated CO_2 sources into biomass Gajraj et al. (2018). Owing to the presence of low lignin and hemicellulose content in algae in comparison to lignocellulosic biomass, the algal biomass has been considered more suitable for the bioethanol production (Chen et al. 2013).

Macro- and microalgae are a promising new source of biomass that may complement agricultural crops. They are found in diverse environments, some species thriving in freshwater, others in saline conditions and sea water (Benemann 1993; Carlsson et al. 2007; Schenk et al. 2008). As demonstrated here, microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti 2007). There are three types of macroalgae, brown, green, and red algae approximately 40 kg wet biomass/m^2 of gulf-weed (*Sargassum muticum*), compared to 2.3 kg/m^2 and 6.6 kg/m^2 of green laver (*Ulva lactuca*) and agar weed (*Gelidium amansii*), respectively. The brown algae such as sea mustard (*Undaria pinnatifida*) and kelp (*Saccharina japonica*) are one of the promising biomass for biofuel production because cultivation productivity based on area size is the highest among three types of macroalgae (Clarens et al. 2010; Eshaq et al. 2011; Slade and Bauen 2013; Rajkumar et al. 2014; Directive 2009/28/EC). Silambarasan et al. (2021) studied use of *Chlorella* sp., *Scenedesmus* sp., and their consortium for the biorefinery approach. Moreover, deoiled algal biomass (DAB) waste used as a biofertilizer combined with inorganic fertilizer resulted in the greater improvement of Solanum lycopersicum (Silambarasan et al. 2021).

Currently, cultivating microalgae has gained large momentum among researchers due to their photosynthetic rate of CO_2 fixation and its versatile nature to grow in various wastewater systems (Abhinandan and Shanthakumar 2015). However, the efficiency of wastewater treatment differs species to species. Abhinandan and Shanthakumar (2015) reviewed the use of microalgae group *Chlorophyta* for industrial wastewater treatment, domestic wastewater treatment, and nutrient removal.

Several physicochemical factors govern algal growth such as carbon concentration, medium pH, and bubbling depth, on absorption and utilization of supplied CO_2 (Yin et al. 2019). They reported that increasing CO_2 bubbling depth and keeping higher carbon concentration and higher pH in microalgae culture can improve CO_2 absorption ratio, which will optimize the bio fixation of CO_2.

Applications of aquatic ecology to algal cultivation systems, in optimizing nutrients designing and constructing biotic communities, can help to maximize algal biomass yields (Shurin et al. 2013; Bartley et al. 2016; Smith and Mcbride 2015). Algal biofilms in natural ecosystems represent three-dimensional, multispecies, and multi-layered structures which involve consortia of heterotrophic and photoautotrophic prokaryotic and eukaryotic organisms (Berner et al. 2015; Gross et al. 2015).

Some microalgae can be grow under saline conditions in desert zones near the ocean when freshwater supply is not feasible (Mussgnug et al. 2010). According to Demirel (2018a), microalgae can fix CO_2 10–50 times more efficient than other energy plants making them a very well-suited resource for biofuels and bio products.

Kargupta et al. (2015) studied growth of *Chlorella pyrenoidosa* and *Scenedesmus abundans* in a tubular batch photo bioreactor with provision for continuous flow of 10% CO_2 enriched air through the headspace. They reported that CO_2 sequestration and growth rate were comparable at height/diameter ratio of 8 and 16.

10.6 Bioreactors

Whitton et al. (2015) summarized different types of bioreactors with sub-categories, either open to the environment or enclosed, which include suspended and non-suspended systems (see also Christenson and Sims 2011) (Fig. 10.7).

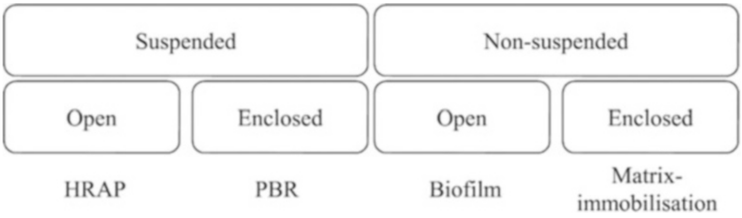

Fig. 10.7 Categories of microalgal bioreactors for wastewater remediation. (*Source*: Whitton, R Francesco Ometto, Marc Pidou, Peter Jarvis, Raffaella Villa & Bruce Jefferson (2015) Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, Environmental Technology Reviews, 4: 133–148, DOI: https://doi.org/10.1080/21622515.2015.1105308. This is an open access article distributed under the terms of the Creative Commons CC BY license)

10.6.1 Suspended Type Bioreactors

10.6.1.1 Suspended Open High Rate Algal Pond (HRAP)

High rate algal ponds (HRAPs) are open shallow raceway ponds about 30–40 cm deep, with a paddle wheel that are used for low energy wastewater treatment (Mehrabadi et al. 2017a, b). It is a raceway-configured open pond mixed via a paddle wheel to circulate the algal culture and prevent settlement where sunlight is the primary method of irradiation, and as such, culture depths of 20–60 cm are typical (García et al. 2017). High rate algal ponds (HRAP) also provide more efficient natural disinfection. HRAP performance can be further enhanced by bubbling CO_2 into the pond during the day to promote algal growth when it is often carbon-limited. Craggs et al. (2014) presented the design and operation and performance of HRAP systems and their application for economical, low-energy upgrade of conventional wastewater treatment ponds combined with energy recovery and biofuel production. Currently, the HRAP-based wastewater treatment is the most economical and environmental approach to produce algal biomass for conversion to biofuels. Kumar et al. (2010) developed technology for efficient use of microalgal CO_2 fixation integrated with wastewater treatment (Kumar et al. 2018; Gajraj et al. 2018). Currently, by producing 1 ton algal biomass in HRAP-based wastewater treatment approximately 1.8 tons of CO_2 can be fixed through photosynthesis (Benemann and Pedroni 2003). However, there is a critical need for a cost-effective alternative upgrade option for pond systems. This can include:

1. Solids are removed and digested in covered anaerobic digester ponds (CADP) anaerobically.
2. Aerobic treatment by sunlight-powered algal growth on the supernatant;
3. Removal of algal growth and subsequent conversion to biofuel; and
4. Further polishing of the treated effluent as required.

The HRAP supports a symbiotic community of microalgae and bacteria for the assimilation of nutrients and organic matter (Park and Craggs 2014). It can be found

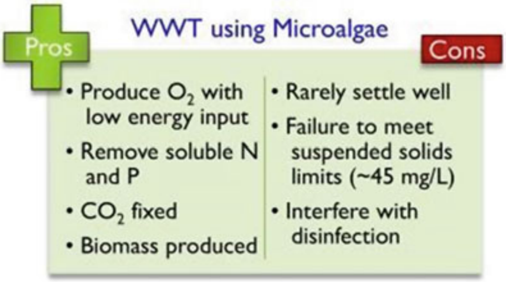

Fig. 10.8 Pros and cons of utilizing microalgae in WWT HRAPs. (*Source*: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licencenumber 4906420744566 dated 12th September)

operational at full scale with a demonstration plant located in New Zealand with individual pond footprints of 1.25 ha (Sutherland et al. 2014). The major drawback in integrating waste water treatment (WWT) with algal cultivation among others (as shown in Fig. 10.8) (Behera et al. 2019) is the low lipid content of the biomass due to the presence of bacteria (lipid content <10%) available in HRAPs that reduces the biomass energy (Mehrabadi et al. 2015).

The concept of industrial symbiosis, with synergistic effects of achieving WWT and biofuel production, is well-known (see Behera et al. 2019). The algal growth rate is dependent on the complex interaction of various environmental, operational, and biological factors. Different parameters influence microalgal growth and associated energy production in WWT HRAPs (Behera et al. 2011) (Fig. 10.9).

10.6.1.2 Suspended Closed Photobioreactor (PBR)

Bioprocess engineers have developed photobioreactors (PBRs) aiming for mass culture of microalgae. Photobioreactors are devices that are optimized to convert light energy into biomass energy and offer the highest levels of experimental control for developing optimal microalgae production systems (Chisti 2007). Photobioreactors (Hulatt et al. 2017) obtained light conversion efficiency up to 0.70 g biomass per mol of PAR for *Nannochloropsis* sp. (Davis et al. 2011; Ehimen et al. 2011).

Allen et al. (2018) reported some possible algal-based processes for biofuel and by-product productions: OP open pond, PBR photobioreactor (Fig. 10.10).

Behera et al. (2019) extensively reviewed the design considerations, mass transfer characteristics, economic and energy consideration for increasing the performance of closed PBRs.

A PBR is an example of a closed, suspended system available in various configurations, including horizontal or vertical tubular photo bioreactor (TPBR) (Fig. 10.11) (Abdel-Raouf et al. 2012; Molina et al. 2001; Hulatt and Thomas

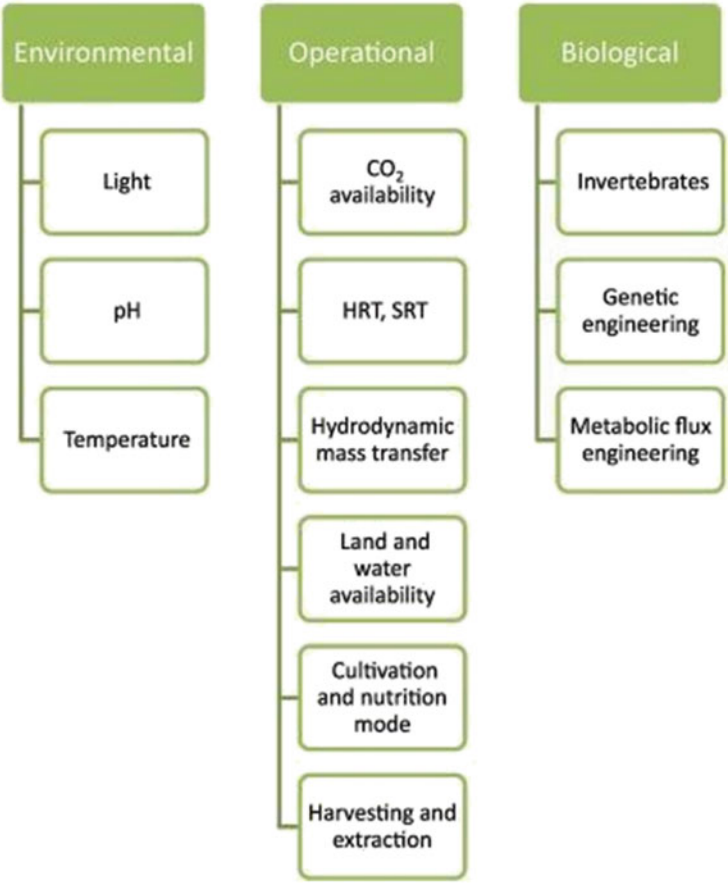

Fig. 10.9 Parameters influencing microalgal growth and associated energy production in WWT HRAPs. (Source: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under licence number 4906420744566 dated 12th September)

2011; Bechet et al. 2013) or flat panel reactors (Hu et al. 1998; Ugwu et al. 2008; Sierra et al. 2009) and bio-film (Blanken et al. 2014).

Flat-plate photo bioreactors with short light path lengths as they have a high surface area to volume ratio and consume less energy than tubular systems (Zou et al. 2000; Jorquera et al. 2010; Vejrazka et al. 2012).

Some species of microalgae synthesize very long chain fatty acids (carbon chains 20+ in length), including eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Guiheneuf and Stengel 2013). *Nannochloropsis* is a genus of robust, oleaginous microalgae that synthesizes EPA during balanced growth and is a promising candidate for commercial applications

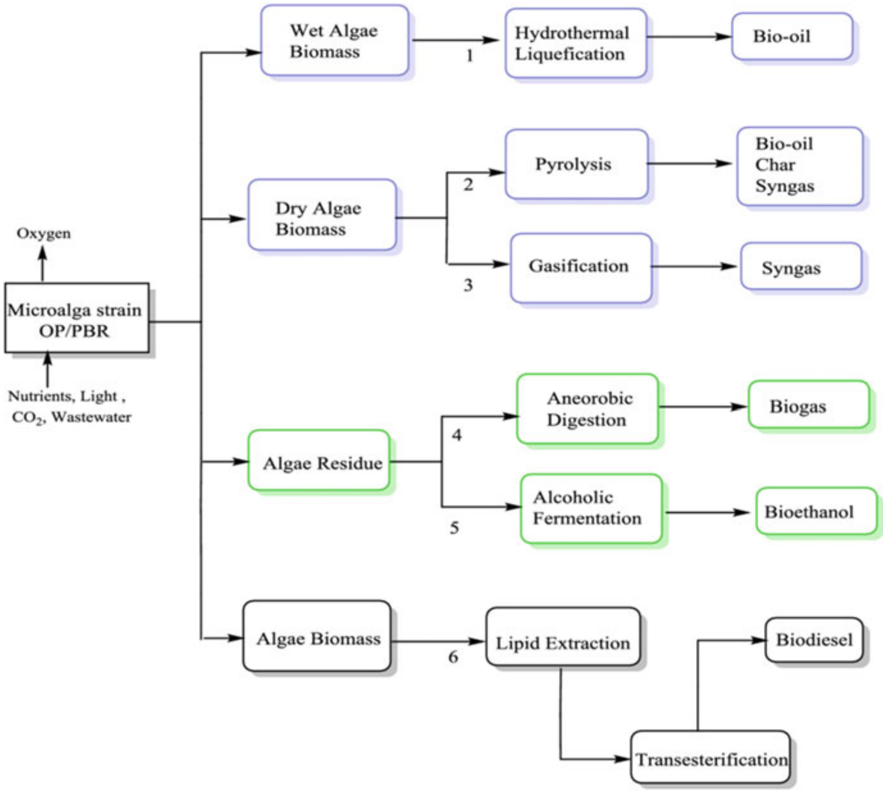

Fig. 10.10 Some possible algal-based processes for biofuel and by-product productions. *OP* open pond, *PBR* photobioreactor. (Source: Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

(Sharma and Schenk 2015). Hulatt et al. (2017) examined changes in the biochemical composition of *Nannochloropsis* sp. cultivated in optimized flat-plate photo bioreactors as a potential feedstock for aqua feeds (Fig. 10.12) (Yustinadiar et al. 2020).

10.6.2 Non-suspended Open Algal Biofilms

Microalgae may grow in suspension, but also in biofilms. Gross et al. (2015) summarizes the state of the art of different algal biofilm systems in terms of their design and operation. Microalgae biofilms represent an alternative to the suspension-based systems and could be used as a production platform for microalgae biomass

Fig. 10.11 Schematic photobioreactor design that follows a horizontal tube. (*Source*: Abdel-Raouf N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci*. 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink)

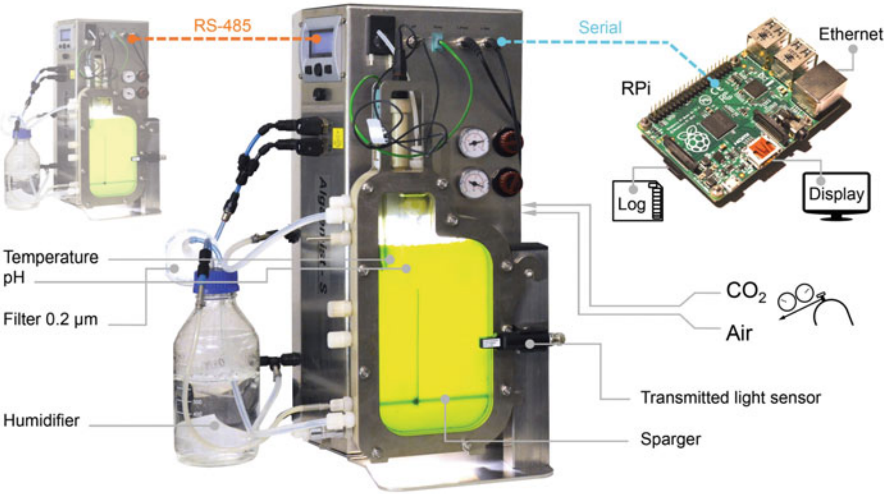

Fig. 10.12 Configuration of the flat-plate photobioreactor systems. Photobioreactors used a 14 mm light path length with illumination by warm white LED lights. Photobioreactors were set up, monitored, and data logged using a custom program running on a Linux single board computer. (This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Hulatt CJ, Wijffels RH, Bolla S, Kiron V (2017) Production of Fatty Acids and Protein by *Nannochloropsis* in Flat-Plate Photobioreactors. PLoS ONE 12(1): e0170440. https://doi.org/10.1371/journal.pone.0170440)

(Blanken et al. 2014). The advantage of algal biofilm systems is that algae can be simply harvested through scraping and thus avoid the expensive harvesting procedures used in suspension-based harvesting, such as flocculation and centrifugation. A biofilm looks as a slimy, green layer and consists of large numbers of microalgae entrapped in a gel-like matrix (Miranda et al. 2017) (Fig. 10.13).

Miranda et al. (2017) isolated and characterized a number of natural microalgal biofilms from freshwater, saline lakes, and marine habitats (Fig. 10.13). Structurally, these biofilms represent complex consortia of unicellular and multicellular, photosynthetic and heterotrophic inhabitants, such as microalgae, bacteria, cyanobacteria, diatoms, and fungi. Symbiotic microalgal–bacterial biofilms can be very attractive for municipal wastewater treatment (Boelee et al. 2014). Boelee et al. (2014) described that microalgae remove nitrogen and phosphorus and simultaneously produce the oxygen that is required for the aerobic, heterotrophic degradation of organic pollutants. However, attempts are on to obtain a balanced system where no additional oxygen is required.

10.6.3 Non-suspended Enclosed Immobilized Cell System

Abdel-Raouf et al. (2012) reported that one of the major problems in the utilization of microalgae for the biological tertiary treatment of wastewater is their recovery from the treated effluent. However, cell immobilization allows high cell density of immobilized cells, improves the product yield and the volumetric productivity of bioreactors. Immobilization appears to offer several advantages in comparison with batch or continuous fermentation where free microorganisms are used.

It has been reported that *Phormidium laminosum* immobilized on polymer foam has the potential to remove nitrate in a continuous flow system with uptake efficiencies above 90% (Sawayama et al. 1998).

Matrix immobilization is a variant of the attachment theme of reactors through the entrapment of living microalgae cells within a natural or artificial resin, e.g., alginate or carrageenan beads (Mallick 2002; Mehta and Gaur 2005). Shi et al. (2007) reported removal of nitrogen and phosphorus from wastewater by two green microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*) using a novel method of algal cell immobilization, the twin-layer system. In the twin-layer system, microalgae are immobilized by self-adhesion on a wet, microporous, ultrathin substrate (the substrate layer) (Fig. 10.14).

10.7 Harvesting

Harvesting of the microalgae requires the separation of a low amount of biomass consisting of small individual cells from a large volume of culture medium. The microalgal cell surface is negatively charged, and thus harvesting typically involves dosing a positively charged metal coagulant to neutralize the surface charge, allowing the cells to aggregate together, creating flocs (Henderson et al. 2008a).

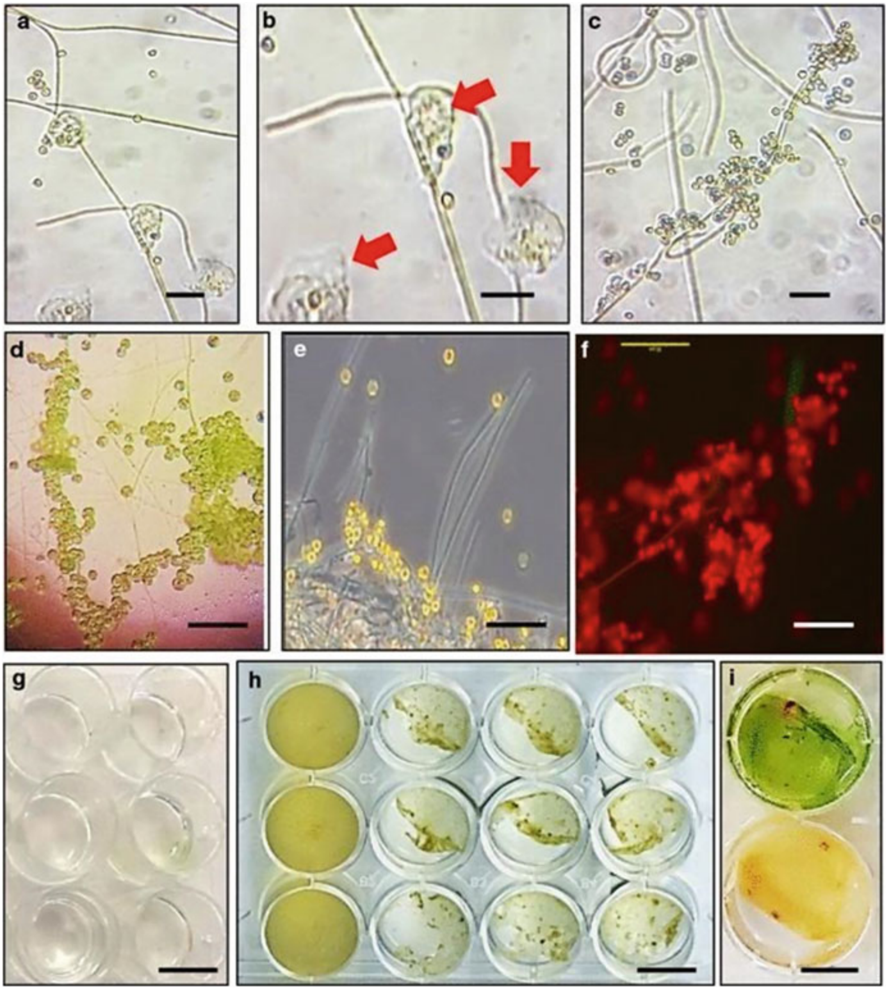

Fig. 10.13 Bio-flocculation of *Isochrysis* sp. cells by BAPS-52-2 filaments. (**a**–**f**) Attachment of *Isochrysis* sp. cells to BAPS-52-2 filaments. Secreted EPS shown by *red arrows*. (**g**) *Isochrysis* cells (*left* wells, controls) and *Isochrysis* cells mixed with BAPS-52-2 filaments (*right* wells) at day 0 and day 10 (**h**). Green pigmentation produced by biofilm produced by monocultured BAPS-52-2 filaments at day 10 ((**i**) *upper* well) and Biofilm #102 at day 10 ((**i**) *bottom* well). *Scale bars* (**a**–**f**) 20 μm; (**g**–**i**) 1 cm. (Source: Miranda, A.F., Ramkumar, N., Andriotis, C. *et al.* Applications of microalgal biofilms for wastewater treatment and bioenergy production. *Biotechnol Biofuels* 10, 120 (2017). https://doi.org/10.1186/s13068-017-0798-9 (http://creativecommons.org/licenses/by/4.0/))

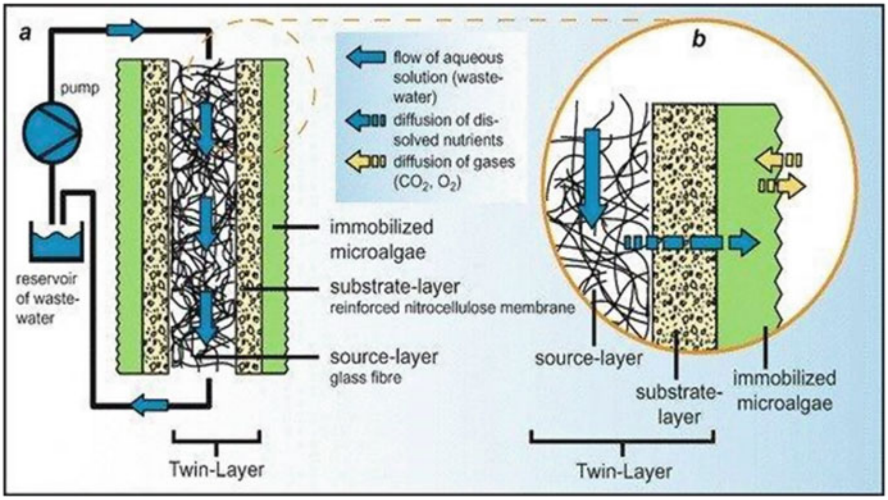

Fig. 10.14 Configuration of twin-layer wastewater treatment system. (*Source*: Shi, J., Podola, B. & Melkonian, M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J Appl Phycol* **19**, 417–423 (2007). https://doi.org/10.1007/s10811-006-9148-1. Reproduced with Licence no 4881430070033 dated 3rd August 2020)

These flocs are removed through filtration, sedimentation, centrifugation, or dissolved in air flotation (DAF) (Henderson et al. 2008b). DAF utilizes the natural tendency of algae to float by raising the biomass to the surface, where it is skimmed off and recovered.

10.7.1 Extraction Methods

Various methods, including autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution, have been tested to identify the most effective cell disruption method. The total lipids from *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were extracted using a mixture of chloroform and methanol (1:1) (Lee et al. 2010; Mercer and Armenta 2011) Harun et al. (2013) presented approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain (Fig. 10.15).

However, unicellular algae are difficult to harvest, resulting in recent research into non-planktonic algae, especially filamentous species such as *Oedogonium* sp. and *Tribonema* sp. (Roberts et al. 2013). For instance, Wang et al. (2013) reported that for the filamentous algae trialled, *Cladophora* sp. was most efficient under low N:P ratio wastewater whilst *Pseudanabaena* sp. was better for removing nitrogen from high N:P ratio wastewater.

Harun et al. (2013) presented approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain (Fig. 10.15).

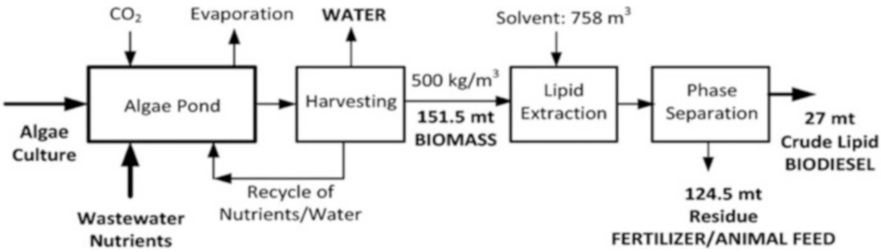

Fig. 10.15 An approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain. (*Source*: Harun R, Doyle M, Gopiraj R, Davidson M, Forde GM, Danquah MK (2013) Process economics and greenhouse gas audit for microalgal biodiesel production, in: advanced biofuels and bioproducts. Springer, New York, pp 709–744): *Source*: Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

10.8 Algal Biofuel Production

Demirbas (2006) investigated microalgal biomass for bio-oil production and found the quality superior to the wood. Microalgal biomass with the diversified biochemical content of lipids, carbohydrates, and proteins can be processed via a variety of thermal and biochemical conversion routes into biodiesel, biohydrogen, biomethane, bio-oil, bio-crude oil, etc. (Behera et al. 2019). Behera et al. (2015) discussed the importance of the algal cell contents, many strategies for product formation through various conversion technologies.

Recently, attempts have been made for bioethanol production through fermentation process using algae as the feed stock (Singh 2011; Nguyen and Vu 2012; Chaudhary et al. 2014; Kumar et al. 2018). Sunil Kumar and Buddolla (2019) reported that many species of microalgae can be induced to accumulate significant quantities of lipids, thus contributing to an elevated oil yield, which is later converted into biodiesel by a process called transesterification. Rodionova et al. (2017) presented a scheme for production of biofuels from the hybrid technology (Fig. 10.16).

10.9 Pyrolysis

Pyrolysis is the thermochemical process involving the application of heat (300–700 °C and higher) at atmospheric pressure under anoxic (the total absence of oxygen) conditions (Kazemi et al. 2019). Porphy and Farid (2012) produced bio-oil from pyrolysis of algae (*Nannochloropsis* sp.) at 300 °C after lipid extraction, which is composed of 50 wt% acetone, 30 wt% methyl ethyl ketone, and 19 wt%

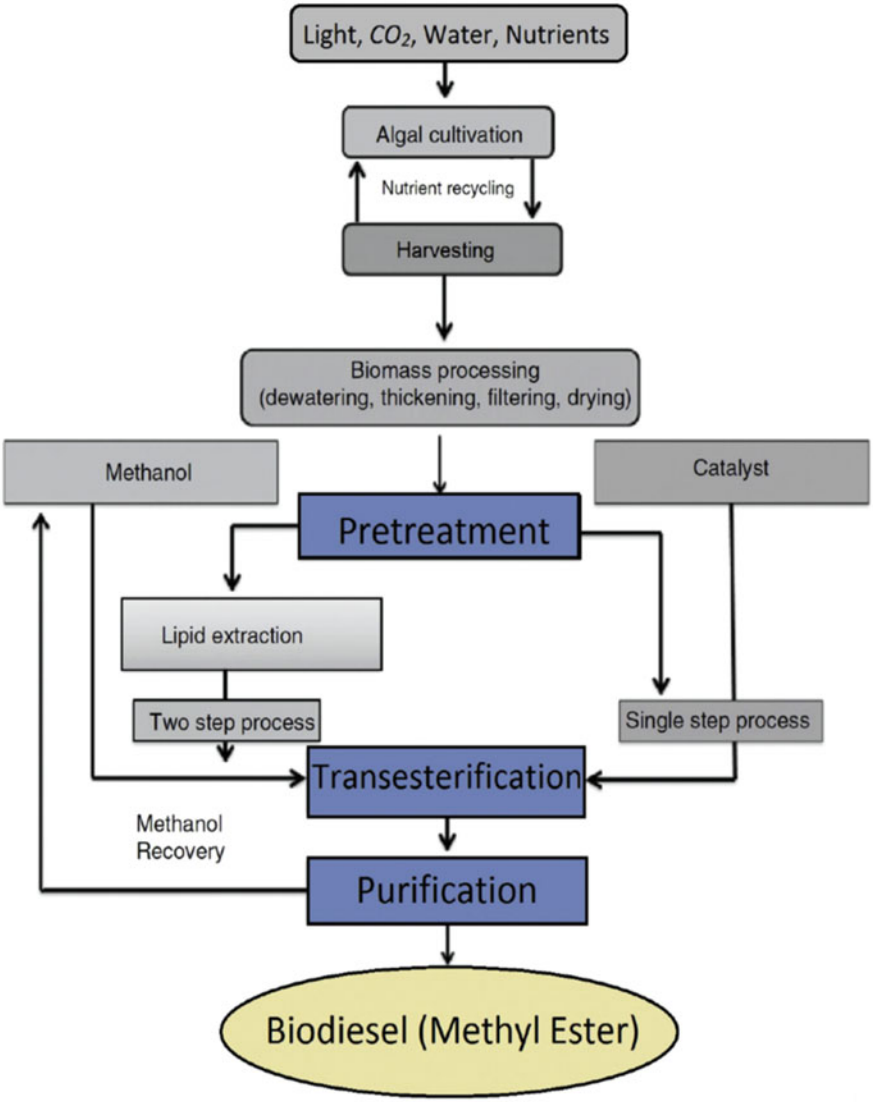

Fig. 10.16 Algal cultivation for biofuel production. (*Source*. Rodionova, M. V, Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., Allakhverdiev, S. I. (2017). Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy*, *42*(12), 8450–8461. https://doi.org/10.1016/j.ijhydene.2016.11.125. License Number 4883421498494 dated Aug 07, 2020)

aromatics, such as pyrazine and pyrrole. Similarly, Choi et al. (2014) carried out pyrolysis study on a species of brown algae *Saccharina japonica* at a temperature of 450 °C and obtained about 47% of bio-oil yield. They reviewed thermochemical conversion of microalgae into bio-crude oil through pyrolysis and hydrothermal liquefaction technologies. Kazemi et al. (2019) presented HTL process of microalgae biomass (Fig. 10.17).

Allen et al. (2018) biorefinery approach with integrated biology, ecology, and engineering has been presented in Fig. 10.18.

10.10 Discussion

Anthropogenic emissions of excessive atmospheric CO_2 is leading to climate change causing cyclones never heard of, like Tauktae which was responsible for large number of deaths in India (https://economictimes.indiatimes.com/news/india/168-deaths-98-tauktae-hit-hospitals-resume-work/articleshow/82809054.cms). The frequency and fury of cyclones has been increasing to devastating levels due to heating of oceans which results in formation of cyclones. The world's largest iceberg, dubbed A-76, has calved from Antarctica. The animation in the link shows a giant slab of ice breaking off from the Ronne Ice Shelf, lying in the Weddell Sea (https://www.nbcnews.com/science/science-news/worlds-largest-iceberg-just-broke-antarctic-ice-shelf-rcna974). This is also outcome of global warming.

The Paris Accord commits to reduction of global emissions to zero by 2050 in order to achieve 1.5 °C (Kumar et al. 2020). Biodiesel derived from oil crops is a potential renewable and carbon-neutral alternative to petroleum fuels (Behera et al. 2015; Kumar 2018; Kumar et al. 2019, 2020). According to Chisti (2007), commercial production of biodiesel or fatty acid methyl ester (FAME) usually involves alkaline-catalysed transesterification of triglycerides found in oilseed crops (mainly rapeseed in Europe and soybean in the United States) with methanol. However, escalating demand for these crops as a food source coupled with the finite availability of arable land makes their cultivation for biofuels unsustainable (Chisti 2007).

Appropriation of the metabolic products of microorganisms as sources of energy and specialized organic compounds represents a growing form of agriculture (Shurin et al. 2013).

Many microalgae species have been adapted to grow efficiently in wastewater. Thus, the cost of production may be decreased due to the simultaneous use of wastewater and cultivation of specific nutrient-rich microalgae. Therefore, microalgae-mediated CO_2 bio-mitigation can be more economic, cost-effective, and eco-friendly, when it is incorporated into a wastewater treatment infrastructure (Kuo et al. 2016; Collotta et al. 2018).

According to Molazadeh et al. (2019), three major methods are being taken into action in order to remove excess CO_2 which is the largest contributor to the greenhouse effect: (1) transforming CO_2 to organic matters through photosynthesis. The use of third-generation biofuels does not require land and water like crops which compete with food crops (Kumar 2018; Kumar et al. 2010, Chap. 3). (2) Using chemical reactions including chemical/physical solvent scrubbing, adsorption, cryogenics, and membranes, (3) The storage of CO_2 emitted underground or into the ocean. According to Miranda et al. (2017), next generation of bioenergy feedstocks should meet key selection criteria: (1) low freshwater requirement; (2) high growth

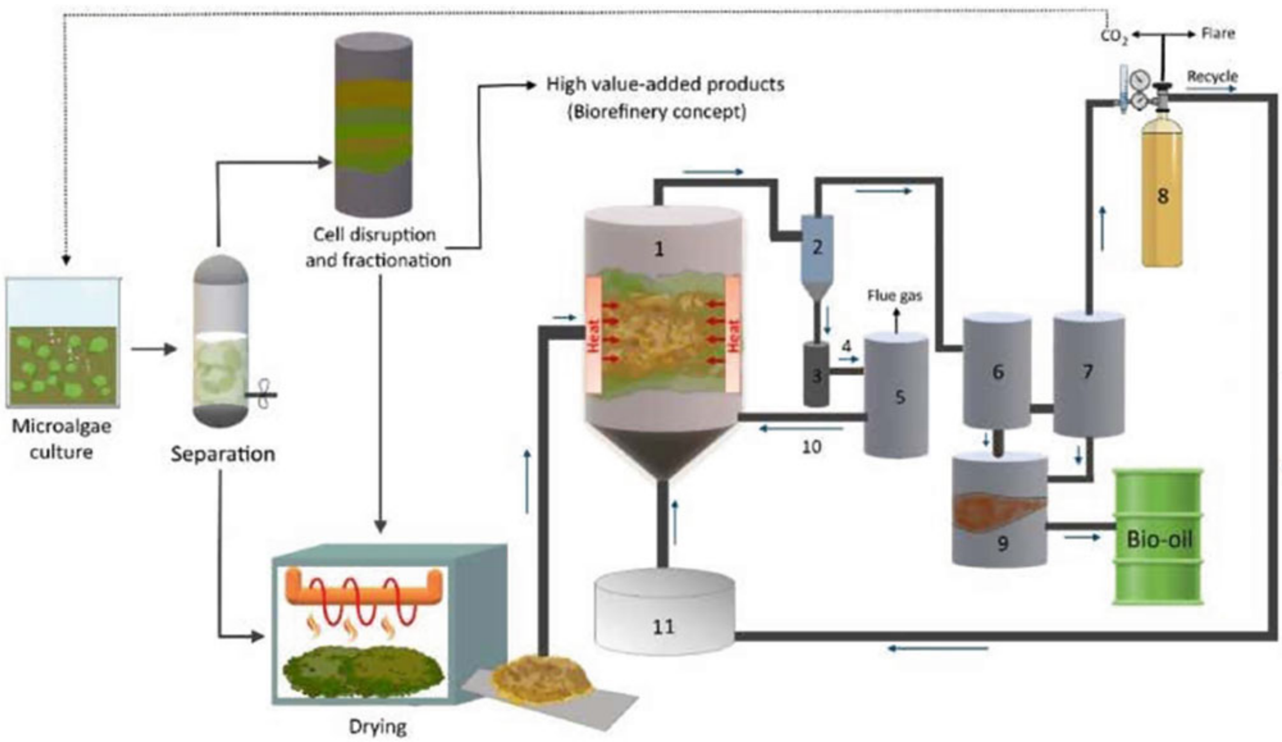

Fig. 10.17 Schematic diagram of HTL process of microalgae biomass. (1) Slurry reservoir (2) high-pressure pump (3) reactor (4) gas cylinder (5) heat exchanger (6) condenser (7) separation vessel. (*Source*: Kazemi Shariat Panahi, H., Tabatabaei, M., Aghbashlo, M., Dehhaghi, M (2019). Recent updates on the production and upgrading of bio-crude oil from microalgae. *Bioresource Technology Reports*, *7*, 100216. https://doi.org/10.1016/j.biteb.2019.100216. Licence 4906430857910 dated 12th September 2020)

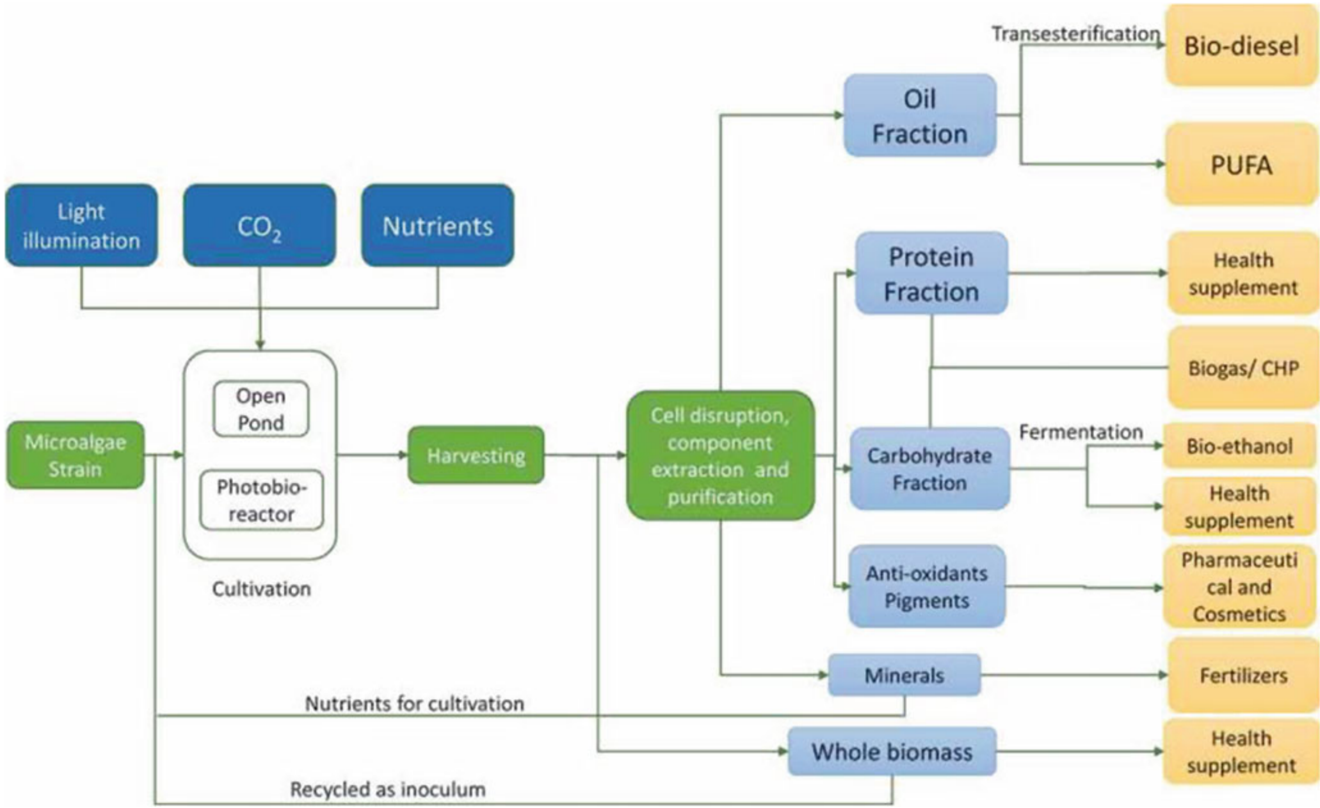

Fig. 10.18 Microalgae biorefinery model. (Koyande AK, Show PL, Guo R, Tang B, Ogino C, Chang JS. Bio-processing of algal bio-refinery: a review on current advances and future perspectives. Bioengineered. 2019 Dec;10(1):574-592. https://doi.org/10.1080/21655979.2019.1679697. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

rates and biomass production; (3) high harvesting index in short rotation period; (4) high content of bioenergy-producing molecules (5) ability to grow on marginal lands and lack of competition with agricultural crops for arable lands; (6) low costs for growth and harvest; and (7) production of high-value co-products (Henry 2010).

However, the mitigation through biological CO_2 fixation is an economically practical and environmentally sustainable technology which has achieved much attention as an alternative method in the long term (Kumar et al. 2010, 2018, 2020; Kumar 2013, 2018).

Microalgae are autotrophic organisms that capture CO_2 present in the air for oxygenic photosynthesis. Because of high tolerant capability to elevated levels of CO_2 in the atmosphere, microalgae are preferred over other carbon sequestration methods (Behera et al. 2019).

The relative supplies of different mineral nutrients and light can have strong phytoplankton and their value as biofuel feedstocks. Large supplies of P relative to N may favour competitive dominance by heterocystous N_2-fixing cyanobacteria (Schindler et al. 2008), which have low cellular lipid contents. Several species of eukaryotic algae accumulate lipid in their cells under conditions of N-starvation (Shurin et al. 2013).

There are three main sources of wastewater, municipal (domestic), agricultural, and industrial wastewater, which contain a variety of ingredients (Chiu et al. 2015). Industrial waste from metal processing industries as well as textile, leather, tannery, and electroplating, have varying amounts of toxic metal ions. Likewise heavy metal ions such as Cd, Cr and Zn, as well as organic chemical toxins such as surfactants, hydrocarbons, and biocides are all present in industrial wastewaters (Ahluwalia and Goyal 2007).

Water from domestic wastewater treatment plants or livestock operations offers a potential source of cheap nutrients that might otherwise be discharged into surface waters (Craggs et al. 2011; Sturm et al. 2012). Recent development of hybrid technologies (biomass production, wastewater treatment, GHG mitigation) for production of prime products as biofuels offer atmospheric pollution control such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Maity et al. 2014). Marine microalgae constitute a useful form of biomass to overcome environmental problems as they can be grown on saline water. However, the availability of innovative approaches for maximizing the treatment efficiency, coupled with biomass productivity, remains the major bottleneck for commercialization of microalgal technology (Abinandan et al. 2018). However in addition to algae culture and growth, it is also essential to develop cost-effective technologies for efficient biomass harvesting, lipid extraction, and biofuels production (Safi et al. 2014). As single-celled molecular factories, microalgae can also be cultivated on marginal land unsuitable for agriculture, using waste streams or saline water supplies (Clarens et al. 2010; Marjakangas et al. 2015). *Nannochloropsis* is a genus of robust, oleaginous microalgae that synthesizes eicosapentaenoic acid (EPA) during balanced growth and is a promising candidate for commercial applications (Davis et al. 2011; Kilian et al. 2011; Radakovits et al. 2012; Sharma and Schenk 2015).

Although some components in the wastewater, such as nitrogen and phosphorus, act as nutrients for microalgae, algal growth rates are lower in different industrial wastewaters, and thus potential for the large-scale treatments of industrial wastewaters containing high levels of heavy metal ions for algal culture are not so promising at the moment. The main challenges are not limited to: industrial-scale capturing of carbon dioxide using microalgal species, the tolerances of specific algal strains grown in a wide variety and concentration of heavy metal ions in industrial wastewaters but optimization of several parameters like simultaneous removal of CO_2 and treatment of wastewaters containing heavy metal ions (Molazadeh et al. 2019).

10.11 Conclusion

Renewable energy plays a critical role in addressing issues of energy security and climate change at global and domestic scales. Biomass offers only means of absorption of global carbon dioxide the major cause of global warming. However, first- and second-generation biofuels compete mostly for land and water with arable lands. Third-generation biofuels derived from algae (or a consortium of microbes) offer triple benefits like algal biomass production, wastewater treatment, and greenhouse gas mitigation. This is receiving increasing attention worldwide as an alternative and renewable source for energy production. Algal biomass can be grown using non-arable areas such as lakes, oceans, or deserts, thus avoiding the current problem of land use competition with the food supply chain. The potential for remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is considered an environmental approach to nutrient polishing. It also has benefits of (a) sequestering of CO_2 from the atmosphere during photosynthesis (b) oxygenating the treated effluent and biofuel production. The chapter presented some of these processes in detail.

Acknowledgements Authors acknowledge with thanks the authors of the papers and figures used in this chapter with permission: Figure 10.1 *Source*: Abdel-Raouf, N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access: Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink. Figure 10.2 Maity, J. P., Bundschuh, J., Chen, C.-Y., & Bhattacharya, P. (2014). Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104–113. https://doi.org/10.1016/j.energy.2014.04.003. Reproduced with licence no 5064180534847 dated 8th May 2021. Figure 10.3 Li H, Watson J, Zhang Y, Lu H, Liu Z. Environment-enhancing process for algal wastewater treatment, heavy metal control and hydrothermal biofuel production: A critical review. Bioresour Technol. 2020 Feb; 298: 122421. doi: https://doi.org/10.1016/j.biortech.2019.122421. Epub 2019 Nov 14. PMID: 31767428. Licence no 5065350239998 dated 10th May. Figure 10.4 Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). Figure 10.5 Kumar A., Singh J.S. (2017)

Cyano Remediation: A Green-Clean Tool for Decontamination of Synthetic Pesticides from Agro- and Aquatic Ecosystems. In: Singh J., Seneviratne G. (eds) Agro-Environmental Sustainability. Springer, Cham. https://doi.org/10.1007/978-3-319-49727-3_4. Reproduced with RightsLink licence number 5071420183103 dated 17th May 2021. Figure 10.6 Integration of microalgal wastewater treatment with resource recovery for maximizing the derivable products. (Source: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licence number 4906420744566 dated 12th September). 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Open access Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink. Figure 10.12 Yustinadiar, N., Manurung, R. & Suantika, G. Enhanced biomass productivity of microalgae Nannochloropsis sp. in an airlift photobioreactor using low-frequency flashing light with blue LED. Bioresour. Bioprocess. 7, 43 (2020). https://doi.org/10.1186/s40643-020-00331-9. This article is licensed under a Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/. Figure 10.13 Miranda, A.F., Ramkumar, N., Andriotis, C. et al. Applications of microalgal biofilms for wastewater treatment and bioenergy production. *Biotechnol Biofuels* **10**, 120 (2017). https://doi.org/10.1186/s13068-017-0798-9 (http://creativecommons.org/licenses/by/4.0/). Figure 10.14 Shi, J., Podola, B. & Melkonian, M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J Appl Phycol* **19**, 417–423 (2007). https://doi.org/10.1007/s10811-006-9148-1. Reproduced with Licence no 4881430070033 dated 3rd August 2020. Figure 10.15 Harun R, Doyle M, Gopiraj R, Davidson M, Forde GM, Danquah MK (2013) Process economics and greenhouse gas audit for microalgal biodiesel production, in: advanced biofuels and bioproducts. Springer, New York, pp 709–744. Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Figure 10.16 Rodionova, M. V, Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., Allakhverdiev, S. I. (2017). Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy*, *42*(12), 8450–8461. https://doi.org/10.1016/j.ijhydene.2016.11.125. License Number 4883421498494 dated Aug 07, 2020. Figure 10.17 Kazemi Shariat Panahi, H., Tabatabaei, M., Aghbashlo, M., Dehhaghi, M (2019). Recent updates on the production and upgrading of bio-crude oil from microalgae. *Bioresource Technology Reports*, *7*, 100216. https://doi.org/10.1016/j.biteb.2019.100216. Licence 4906430857910 dated 12th September 2020. Figure 10.18 Allen et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5.

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