2 Municipal Wastewater Treatment by Microalgae with Simultaneous Resource Recovery: A Biorefinery Approach

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Abstract

An increase in urbanization and industrialization has led to the increased discharge of wastewater, especially municipal wastewater, causing eutrophication as a large amount of wastewater is discharged into the water bodies without proper treatment. Current municipal wastewater treatment is carried out using the conventional activated sludge process (CAS), where indigenous microbial consortia with external aeration reduce organic matter. But critical issues are associated with the CAS process, including high energy requirements, generation of sludge, and emission of a large amount of carbon dioxide. Therefore, there is a need for alternative strategies in order to deal with these issues. Microalgae-based wastewater treatment process has emerged as a promising alternative technology for treating municipal wastewater. Microalgae offer certain advantages such as sequestration of atmospheric carbon dioxide, effective treatment of wastewater, and resource recovery in the form of microalgal biomass. The current chapter deals with the advancement made during these years for municipal wastewater treatment, including membrane technology, biofilm technology, and photo-sequencing batch reactors. There are also certain disadvantages associated with microalgae-based wastewater, such as scale-up, contamination in raceway ponds, and high energy requirements during the harvesting and dewatering process. In order to recover these costs, a biorefinery approach has been proposed where the microalgal biomass generated during the treatment process is transformed into various products such as biofuel, biochemical, and bioelectricity.

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Keywords

Microalgae · Circular bioeconomy · Wastewater treatment · Bioremediation · Biofuel

Abbreviations

ASP	Activated sludge process
CAS	Conventional activated sludge
CO_2	Carbon dioxide
COD	Chemical oxygen demand
DIC	Dissolved inorganic carbon
IEA	International Energy Agency
LI	Light intensity
MPBR	Membrane photobioreactor
MR	Mixing rate
N	Nitrogen
NH_4^+-N	Ammonium
O_2	Oxygen
P	Phosphorus
PBR	Photobioreactor
PO_4^{3-}P	Phosphate
RAB	Revolving algal biofilm
TAN	Total ammonia nitrogen
Temp.	Temperature
TKN	Total kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus

2.1 Introduction

Rapid industrialization and urbanization have led to the increased exploitation of natural resources by releasing a large amount of wastewater and greenhouse gases (GHGs). The report of International Energy Agency (IEA) fuel combustion 2019 highlights that 2.2, 4.8, and 9.8 Metric gigatons of CO_2 were emitted by India, the United States, and China alone. The high emission of GHGs triggers climate change and global warming (Arun et al. 2020b). The next disadvantage of industrialization and urbanization is the release of different types of wastewater generated from textile and pharmaceutical industries, agricultural lands, domestic, and municipal wastewater (Zhang et al. 2017; Kadir et al. 2018; Rai et al., 2020; Lellis et al. 2019). The wastewater is rich in various types of nutrients, including both inorganic (macronutrients and micronutrients) and organic nutrients (carbon compounds).

When they are discharged into the freshwater sources without the proper treatment, causing the problem of eutrophication poses a threat to the natural ecosystem of the freshwater bodies (Bhatia et al. 2020). It was estimated that eutrophication causes a loss of two billion dollars per year as it severely affects fishing and real estate activities (Lavrinovičs and Juhna 2018).

A large portion of wastewater released every year is constituted by municipal wastewater generated from the urban colonies, institutional setup and small-scale industries (Daverey et al. 2019). The conventional treatment of municipal wastewater is carried out by the activated sludge process (ASP) mediated via the biological approach. In the ASP process, organic matter in the wastewater is degraded via indigenous consortia of microbes and O_2 is supplied to them via an external aeration system. The microbial population in the reactor is maintained via a recycling system that recycles back a portion of sludge into the reactor (Daverey et al. 2019). The main disadvantage of the ASP process is the requirement of a high amount of energy (0.3–0.6 kWh/m^3), constituting about 26% of the net cost of the treatment process (McCarty et al. 2011; Li et al. 2017). The aeration process alone consumes 47–70% of the total energy required by the treatment process. There have been some advancements in the aeration process. Still, the consumption of a large amount of energy by the ASP process remains a major issue (Gikas 2017). Another critical issue of the ASP process is the disposal of a large amount of activated sludge generated during the process. Removal of per kg of chemical oxygen demand (COD) generates about 0.3–0.5 kg of dry biomass of activated sludge (Liu et al. 2018). The sludge can be utilized in the energy recovery process, but its handling process, which includes thickening, dewatering, and digestion process, consumes about 30% of the total plant energy (Zhou et al. 2013). The third and last critical issue of the ASP process is releasing a large amount of CO_2 during the oxidation process of organic matter by microbes (Singh et al. 2016).

To resolve the issues explained above, microalgae-based treatment of municipal wastewater proved to be a promising technology for the advanced treatment of wastewater with simultaneous recovery of nutrients (Li et al. 2019; Singh and Mishra 2021, 2022). Microalgae are the rapidly growing photoautotrophs that utilize sunlight as energy and CO_2 as a carbon source with the release of O_2 and generate a large amount of biomass (Singh and Mishra 2019). Their CO_2 fixation efficiency is 10 to 50 times higher than terrestrial plants (Langley et al. 2012). In recent years they have been applied to treat municipal wastewater by growing them in open raceway ponds or closed photobioreactors (Daverey et al. 2019). The ample amount of inorganic nutrients such as nitrogen and phosphorus and low toxic elements in municipal wastewater makes it a highly suitable medium for microalgae cultivation (Craggs et al. 2013). Some of the advantages offered by microalgae-based wastewater treatment are given as (1) Overall wastewater treatment is reduced as microalgae can assimilate almost every pollutant with resource recovery; thus, there is no need for additional treatment; (2) the pollutant level in the treated water by microalgae has a deficient level of pollutants satisfying the discharge limit criteria (Whitton et al. 2015); (3) microalgae can efficiently grow in the municipal wastewater with or without the need of external nutrient supplementation (Clarens et al. 2010);

(4) when microalgae are grown in symbiosis with bacteria during the treatment process, they provide O_2 required for oxidation of organic matters by bacteria, thus eliminating the need of external aeration device (Jia and Yuan 2018); (5) microalgal biomass generated the end of the process can be further transformed into biofuels, biogas, fertilizers and feedstock for animals (Raheem et al. 2015; Singh and Mishra 2019). However, various challenges are also associated with microalgae-based wastewater treatment, which include contamination in open raceway ponds, scale-up of closed photobioreactors, the significant cost involved in the harvesting and dewatering process, which incurs about 3–15% of the total cost of the treatment process (Razzak et al. 2017; Fasaei et al. 2018). This cost can be overcome by biorefinery or bio-circular economy approach in which a microalgae-based wastewater treatment process is integrated with the production of energy and other valuable products, as explained in detail in Sect. 2.3 (Bhatia et al. 2020).

Therefore, the current chapter's objective is to provide insights into the recent advancements in the treatment of municipal wastewater by microalgae. It further covers the prospective details of the biorefinery approach for decreasing the treatment process cost.

2.2 Recent Advancements in the Treatment of Municipal Wastewater by Microalgae

Various advancements have been made to treat municipal wastewater by microalgae, including the microalgae-bacterial process, photo-sequencing batch reactor, membrane and biofilm technology, and synchronization of microalgae with yeast and macrophytes explained in the upcoming sections. Table 2.1 represents various microalgal species utilized to treat municipal wastewater with the removal efficiencies of various pollutants and biomass concentrations.

Figure 2.1 represents a schematic diagram for integrating conventional activated sludge process with microalgae technology for the treatment of municipal wastewater and simultaneous production of biomass and transforming it into biofuel, representing a biorefinery concept.

2.2.1 Microalgal-Bacterial Process

The microalgal-bacterial process is becoming an alternative method of choice for the treatment of municipal wastewater other than the conventional activated sludge process (CAS), as it demands low energy, low cost, easy operation, and the potential of resource recovery in the form of biomass feedstock (Mata et al. 2010; Quijano et al. 2017; Zhang et al. 2020a). They are a self-sustainable system with mutual synchronization between the microalgae photosynthesis and bacterial respiration processes. Microalgae feed upon the inorganic nutrients such as nitrogen and phosphorus present in the wastewater and assimilate the carbon dioxide generated during bacterial respiration, releasing oxygen. Bacteria then utilize the generated

Table 2.1 Treatment of municipal wastewater by various microalgal species and removal efficiencies

Microalgae species	Photobioreactor configuration	Experimental condition	Pollutant removal efficiency/removal rate	Biomass concentration/ productivity	Biofuel type and concentration	Reference
Nannochloropsis gaditana DEE03	250 mL Erlenmeyer flasks (Batch) and 1 L flat PBR (bioethanol production)	0%–100% wastewater in filtered f/2 medium Temp: 24 ± 2 °C pH: 7.8 Air flow rate: 0.5 L/min LI: 80 μmol/m^2/s	–	2.33 ± 0.12 g/L	89.0 ± 4.0 mg/g (bioethanol)	Onay (2018)
Pantanalinema rosaneae	Glass reactor	pH: 7.0 ± 0.2 LI: 200 ± 10 μmol/m^2/s	Influent organics: 92.69%, ammonia: 96.84%, phosphorus: 87.16%	–	–	Ji et al. (2020)
Chlorella sorokiniana	1 L Erlenmeyer flasks	Room temp. Mixing speed: 120 RPM LI: 120 μmol/m^2/s	Ammonium: 94.29% Phosphate: 83.30%	77.14 mg/L/d	24.91 mg/L/d (lipid)	Ramsundar et al. (2017)
Nannochloropsis oculata and Tetraselmis suecica	250 mL Erlenmeyer flasks	Temp.: 24 ± 2 °C Mixing rate: 150 RPM pH: 8.5 LI: 1300 lm	–	1.285 g/L (*N. oculate*) 1.055 g/L (*T. suecica*)	–	Reyimu and Özçimen (2017)
Hindakia tetrachotoma ME03	1 L Flat PBR	Air flow rate: 0.5 L/min LI: 150 μmol/m^2/s	–	0.78 ± 0.01 g/L	11.2 ± 0.3 g/L (bioethanol)	Onay (2019)

(continued)

Table 2.1 (continued)

Microalgae species	Photobioreactor configuration	Experimental condition	Pollutant removal efficiency/removal rate	Biomass concentration/ productivity	Biofuel type and concentration	Reference
		pH: 7.4 Temp.: 24 ± 1° C				
Chlorella sp.	14 L stirred PBR	Room temperature LI: 100 μmol/ m^2/s Mixing rate: 100 RPM	COD: 37.5–45.7%	1.12 g/L	–	Nguyen et al. (2020)
Chlorella sp.	Open raceway pond	pH: 8 Temp.: 24 °C	–	3.6 ± 0.12 g/L	0.925 ± 0.1 g/L	Ashokkumar et al. (2019)
Chlorella zofingiensis	10 L glass column reactor and 240 L outdoor plate bioreactor	8% pig bio-gas slurry in MW Temp.: 25 ± 1 ° C LI: 150 μmol/ m^2/s 5% CO_2 mixed air ventilation	TN: 93% TP: 90%	2.5 g/L	–	Zhou et al. (2018)
Chlorella sp.	1 L PBR	pH: 7.33 ± 0.06 LI: 80 μmol/m^2/s Temp.: 23–27 °C MR: 150 RPM	NH_4^+-N: 23.25 ± 1.59 mg/L/d PO_4^{-3}-P: 8.05 ± 0.83 mg/ L/d	117.1 ± 2.7 mg/L/d	17.2 ± 0.2 mg/L/ d)	Cho et al. (2017)
Chlorella pyrenoidosa	1 L cylindrical reactor	Aeration rate: 50 mL/min Temp.: 25 ± 2.0 °C LI: 10,000 lux	NH_4^+-N: 91.7%	70 mg/L/d	27.3 mg/L/d (lipid)	Zhou et al. (2020)

Chlorella, diatoms and filamentous cyanobacteria	2 L photo-sequencing batch reactors (PSBR)	Flow rate: 0.7 L/cycle MR: 200 RPM Temp.: 22.2 °C	COD: 87 ± 5% TKN: 98 ± 2% NH4 $^{+}$-N: 99 ± 3%	–	–	Foladori et al. (2018)
Chlorella pyrenoidosa	1 L PBR	Temp.: 22–28 °C LI: 8000–80,000 lx	P accumulation: 9.82 mg/L	0.749 g/L	0.197 g/L	Wang et al. (2019)
Chlorella vulgaris	1.5 L photo-sequencing bioreactors	MR: 50 RPM Temp: 24 ± 2 °C LI: 45 μmol/m^2/s	COD: 89 ± 4% NH_4^+-N: 99 ± 1%	1.1 ± 0.3 g/L	–	Petrini et al. (2020a)
Nostoc ellipsosporum	1 L closed transparent reactors	LI: 2500–6500 lx Aeration rate: 0.05–0.2 vvm Room temp.	N: 87.59% P: 88.31%	2.9 g/L	24.62 wt% (bio-oil yield)	Devi and Parthiban (2020)
Scenedesmus sp.	80 L high rated algal pond	Temp.: 20 °C Paddle speed: 10 RPM Liquid velocity: 0.2 m/s	TN: 60 ± 5% COD: 89 ± 3% P-PO_4^{3-}: 28 ± 7%	12.7 g/m^2/d	–	Arcila and Buitrón (2017)
Chlorella sorokiniana	50 L Flat panel PBR	LI: 196 μmol/m^2/s Aeration rate: 0.6 vvm Temp.: 0.6 vvm	Organic matter removal: >90% DIC: 46–56% PO_4^{3-}-P: 40–60% NH_4^+-N: 100%	1 g/L	–	Leite et al. (2019)
Chlorella sorokiniana	2 L integrated sequencing batch reactor system	Temp.: 24 ± 2 °C	COD: 99% TKN: 88% PO_4^{3-}-P: 91% NH_4^+-N: 90%	45 mg/d	–	Kotoula et al. (2020)

(continued)

Table 2.1 (continued)

Microalgae species	Photobioreactor configuration	Experimental condition	Pollutant removal efficiency/removal rate	Biomass concentration/ productivity	Biofuel type and concentration	Reference
Chlorella vulgaris	Cylindrical glass reactors	Temp.: 25 °C LI: 2000 lx MR: 300 RPM	COD: 93% PO_4^{3-}-P: 91% NH_4^+-N: 90%	1.96 g/L	–	Amini et al. (2020)
Scenedesmus obliquus	1 L Erlenmeyer flasks	Temp: 25 ± 2 °C LI: 100 μmol/ m^2/s	TP: 95.72% TN: 80.30% NH_4^+-N: 87.25% COD: 85.43%	0.891 ± 0.012 g/L	0.477 ± 0.073 g/L	Qu et al. (2020a)
Scenedesmus sp.	5 L batch polyethylene terephthalate (PET) bioreactors	Air flow rate: 0.5 VVM pH: 7	Nitrate: 96% TAN: 100% PO_4^{3-}-P: 3%	0.98 ± 0.10 g/ L	–	Walls et al. (2019)
Tetraselmis sp. NKG2400013	Flat-shaped glass flasks	Temp.: 25 °C LI: 130 μmol/ m^2/s Air flow rate: 0.8 L/L/min	N: 98 ± 0% P: 82 ± 2%	157 ± 5 mg/ L/d	5.5 ± 1.8 mg/ L/d	Aketo et al. (2020)
P. kessleri NKG021201			N: 98 ± 0% P: 20 ± 3%	101 ± 1 mg/ L/d	39 ± 1 mg/L/ d	
C. Saccharophilum NKH13			N: 99 ± 0% P: 39%	127 ± 9 mg/ L/d	35 ± 10 mg/ L/d	
Chlorella sorokiniana	Conical flasks	Temp.: 25 °C LI: 4000 lux	N: 100% P: 39.3%	25.0 ± 0.1 mg/L/d	–	Chen et al. (2020)
Chlorella vulgaris	1 L MPBR	LI: 101.5 to 112.3 μmol/m^2/s Air flow rate: 0.5 L/min pH: 6.8–7.6 Temp.: 25–28 °C	–	1.84 g/L	25.76 mg/L/d	Gao et al. (2019)
Scenedesmus obliquus				1.72 g/L	29.57 mg/L/d	
Chlorella pyrenoidosa	1 L Erlenmeyer flask	Temp.: 20.65 ° C, pH: 7.72 LI: 2500 lux	NH_4^+-N: 98.72% PO_4^{3-}-P: 76.29%	5.36 g/L	–	Singh and Mishra (2020)

Chlorella pyrenoidosa	PBR	LI: 86.5 μmol/ m^2/s pH: 7.56 Temp.: 30 °C	N: 96.7% P: 98%	1.001	–	(Gao et al. 2021)
Microalgae consortia	Raceway Pond	LI: 428.52 μmol/ m^2/s pH: 8 Temp.: 16.5 °C	N: 99% P: 90.16%	0.601	–	Lage et al. (2021)

Temp. temperature, *LI* light intensity, *MR* mixing rate, NH_4^+-*N* ammonium, PO_4^{3-}-*P* phosphate; *N* nitrogen, *P* phosphorus, *COD* chemical oxygen demand, *TAN* Total Ammonia Nitrogen, *TN* Total Nitrogen, *TP* Total Phosphorus, *DIC* dissolved inorganic carbon, *TKN* Total Kjeldahl Nitrogen, *PBR* photobioreactor

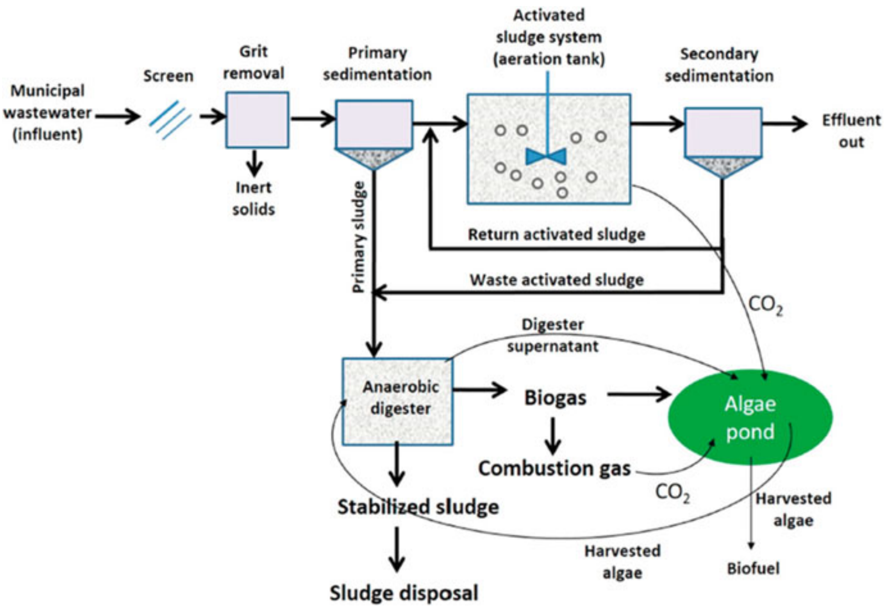

Fig. 2.1 Integration of microalgae-based treatment process with the conventional activated sludge process for municipal wastewater treatment. (Adapted from Daverey et al. (2019))

oxygen to oxidize and degrade organic compounds generating carbon dioxide (Ramanan et al. 2016). Thus, microalgae act as an aeration device, cutting off the need for external oxygen supply and replacing the aeration system (Jia and Yuan 2018). Eliminating the need for external oxygen supply decreases the energy demand by nearly 40–60% (Gikas 2017; Luo et al. 2019). In nature, several micro-ecosystems have been formed by microalgae and bacteria where aggregation of algal cells is facilitated by specific bacterial cells (Subashchandrabose et al. 2011; Powell and Hill 2014). It has also been widely reported that microalgae can recover resources in the form of biomass which can further be processed for the production of biofuels, fertilizers, feedstock, and pigments (Quijano et al. 2017; Singh and Mishra 2019). Various wastewater treatment processes utilizing the microalgae-bacterial process have been reported in Table 2.1. Nguyen et al. (2020) investigated the effect of different inoculation ratios of the microalgae and bacteria for wastewater treatment in the PBR. Inoculation ratios of 1:0 and 3:1 offered the highest biomass concentration, which was 1.06 and 1.12 g/L, respectively, and inoculation ratios of 3:1 and 1:1 showed the highest COD removal, which was in the range of 37.5–47.5% (Nguyen et al. 2020).

But, the commercialization of the microalgal-bacterial process is still not achievable due to the long requirements of time for the reaction (Arcila and Buitrón 2017), poor settleability of biomass (Hu et al. 2017; Quijano et al. 2017), the requirement of external aeration during high pollution load (Abouhend et al. 2018), and low removal efficiency (RE) of the nutrients (Huang et al. 2015; Zhao et al. 2019). A sludge process was developed to eliminate these limitations that utilized engineered microalgal-bacterial granules. The process successfully achieved high REs of 96.84%, 92.69%, and 87.16% for ammonia, organic components, and phosphorous, respectively, within 6 h of operation. No external aeration was supplied to the process (Ji et al. 2020). They also concluded that a mutually symbiotic relationship occurred between the microalgae and bacteria, which was essential in obtaining the above results and self-sustaining the system for a longer time (Ji et al. 2020).

Another limitation in applying the microalgal-bacterial process was the design process of PBR, as the kinetics and parameters used for the ASP may not be applicable for the PBR (Brindley et al. 2010; Qu et al. 2020b). The reason for this can be the difference in the PBR's growth and decay rate of the microalgal-bacterial process (Decostere et al. 2016). Therefore, a method based on the respirometry approach was used by Petrini et al. (2020b) to determine the kinetics of the microalgal-bacterial consortium treating municipal wastewater (Petrini et al. 2020b). Respirometry is a cheap and fast method in which the process's DO (dissolved oxygen) concentration is continuously measured via an automated system. After that, the DO curve is plotted from which the net Oxygen Uptake Rate (OUR, considered negative) of the consortium and net Oxygen Production Rate (OPR, considered positive) of the microalgae are calculated by the slope of the curve. At last, the gOPR (gross oxygen production rate) is calculated by the difference between OPR and OUR (Tang et al. 2014; Ippoliti et al. 2016). Based upon the calculation of Petrnin et al. (2020), gOPR was found to be 9.8 ± 0.2 mg O_2 g TSS^{-1} $h^{-1,}$ and this O_2 was applied for the degradation of COD at the maximum rate of 19.3 TSS^{-1} h^{-1} (Petrini et al. 2020b).

2.2.2 PSBR (Photo-Sequencing Batch Reactor)

The application of the microalgal-bacterial consortium for wastewater treatment has been further extended in photo-sequencing batch reactors (PSBR). An ASP comprising of sequencing batch reactor (SBR) has been applied for the treatment of municipal and agro-industrial wastewater at low and medium scales (Sirianuntapiboon et al. 2005; Wang et al. 2011). SBR offers advantages such as high RE, flexible operation, and an effective control system (Dionisi et al. 2001). Microalgae have been introduced in the SBR process to form a synergistic microalgal-bacterial system to improve its potential for resource recovery. Such an SBR system is called PSBR (Liu et al. 2017). Foladori et al. (2018) cultivated a microalgal-bacteria consortium in PBR to treat municipal wastewater and also evaluated DO, pH, and ORP profiles. No external aeration was supplied to the reactor, and RE of 87 $\pm$ 5% for COD and 98 $\pm$ 2% for total kjeldahl nitrogen (TKN) was obtained (Foladori et al. 2018). However, it should also be noted that an appropriate amount of microalgae inoculum should be supplied to the reactor to maintain the system's excellent performance, as the introduction of microalgae impacts the original microbial flora (Ye et al. 2018). When the microalgae concentration is above 4.60 mg Chl/L, it will inhibit the growth of certain bacteria phylum, including Bacteriodetes and Actinobacteria, and hamper the stable operation of PSBR (Ye et al. 2018).

2.2.3 Supplementation of External Nutrient Source

It has been reported that low-nutrient concentration in municipal wastewater limits its application for microalgae cultivation (Chu et al. 1996). Leite et al. (2019) also reported that municipal wastewater they received from the centralized Brazilian system was highly diluted and not fit for microalgae cultivation both technically and economically (Leite et al. 2019). One of the methods applied to increase the nutrient concentration was the supplementation of artificial nutrient media, which will increase the overall production cost (Lv et al. 2010; Phukan et al. 2011; Itoiz et al. 2012; Lam and Lee 2013; Miriam et al. 2017). Biogas slurry can prove to be an alternative nutrient supplementation source instead of artificial nutrient media. It contains a high amount of nutrients, thus reducing nutrient limitation in municipal wastewater (Wang and Lan 2011). Zhou et al. (2018) cultivated *Chlorella zofingiensis* in the municipal wastewater where pig biogas slurry was supplied as the sole supplementation source of nutrients (Zhou et al. 2018). Their study reported that keeping the concentration of pig biogas slurry up to 8% in municipal wastewater produced significant results. REs of up to 93% for total nitrogen (TN) and 90% for TP were obtained with a 2.5 g/L concentration of biomass and increased lipid productivity by 8% compared to the BG11 medium (Zhou et al. 2018). The problem of nutrient limitation can also be solved by mixing municipal wastewater with another source of wastewater that may have a high-nutrient concentration, such as livestock effluent (Leite et al. 2019). Leite et al. (2019) carried out the pilot-scale cultivation of *Chlorella sorokiniana* in the flat panel PBR by mixing municipal wastewater with piggery wastewater. Biomass concentration reached up to 1 g/L with 46–56% REs for DIC, 40–60% for orthophosphate, and 100% for ammonia (Leite et al. 2019).

Utilization of the tail gas of the power plant to meet the demand for inorganic carbon sources during the cultivation of microalgae in wastewater has gained much importance during these years (Packer 2009; Ho et al. 2010; Sydney et al. 2010; Yoo et al. 2010; Lam et al. 2012). The use of tail gas increases biomass and lipid productivity and is also helpful in successfully sequestering CO_2 from the environment (Tu et al. 2019). During the cultivation of *C. pyrenoidosa* in the wastewater, tail gas was supplied from the power plant, which increased dry biomass weight and lipid productivity by 84.92% and 74.44%, respectively. Their study also suggests that pretreatment of tail gas by desulfurization and denitrification is also needed in order toxic material (Tu et al. 2019).

2.2.4 Membrane Photobioreactor

In the membrane photobioreactor (MPBR), a membrane made up of microfilters is equipped in the PBRs (Gao et al. 2014). Membrane act as a solid-liquid barrier during the cultivation of microalgae in semi-continuous or continuous mode. The filtration module eliminates the problem of a washout as microalgal cells can be retained for a longer duration of time with the continuous and ample supply of wastewater (Honda et al. 2012; Singh and Thomas 2012; Gao et al. 2014; Sun et al. 2018). As hydraulic retention time (HRT) is increased in the MPBR, wastewater containing low-nutrient concentration can also be used to cultivate microalgae (Gao et al. 2016, 2018; Sheng et al. 2017). They also offer other advantages, such as high sludge concentration, high RE, and small footprint (Sun et al. 2018). Several studies have reported that the biomass productivity of microalgae in MPBR is higher than in conventional PBR (Honda et al. 2012; Gao et al. 2014, 2018). Gao et al. (2019) cultivated two green microalgae strains, *Chlorella vulgaris* and *Scenedesmus obliquus,* in MPBR using municipal wastewater having a low-nutrient concentration in the continuous mode (Gao et al. 2019). The result indicated that even though the low-nutrient medium was used for cultivation, the lipid content was increased by 29.8% and 36.9% in *C. vulgaris* and *S. obliquus,* respectively, thus proving MPBR a valuable tool for cultivating microalgae in a low-nutrient medium (Gao et al. 2019). The application of MPBR was further extended to treat wastewater by microalgae-bacteria consortia (Amini et al. 2020). *Chlorella vulgaris* and bacterial inoculum from activated sludge were cultivated in MPBR in semi-continuous mode. RE of 93%, 88 ± 1%, and 84 ± 1% for COD, N-NH_4^+, and P-PO_4^{3-}, respectively, were obtained. Also, the biomass concentration reached up to 1.96 g/L. Thus, the above results indicated that MPBR is useful in both semi-continuous and continuous modes (Amini et al. 2020).

2.2.5 Biofilm Technology

One of the significant problems that hinder the scale-up of the microalgae cultivation system is a less efficient harvesting system, as microalgal cells have low separability in the suspended cultures (Zhu et al. 2017a, b). To tackle this, biofilm technology has been developed in which the microalgal cells are grown on the carrier surface and can be easily separated from the effluent (Wang et al. 2017, 2018a, b). After that, cells are mechanically separated from the carrier surface (Wang et al. 2018a, b). Biofilm technology performs the wastewater treatment process more efficiently and economically as they possess a high mass transfer rate and high penetration efficiency of light (Mantzorou and Ververidis 2019). Carriers supporting microalgal cell growth play an essential role in biofilm technology. Various biofilm technology that has been applied both at lab and pilot scale includes rotating biofilm reactors (Christenson and Sims 2012), algal turf scrubber (Wang et al. 2018a, b), and vertical biofilm reactors (Podola et al. 2017). Zhang et al. (2018) modified the traditional raceway pond by introducing vertical algal biofilm and accessed its efficiency for wastewater treatment and biomass production (Zhang et al. 2018). Their results showed that this modified raceway pond could efficiently remove COD, TN, and TP at 7.52, 6.76, and 0.11 g/m^2/day removal rates. Moreover, lipid productivity reached 7.47–10.10 tonnes/hectare/year (Zhang et al. 2018). In another study, revolving algal biofilm (RAB) reactors were used to treat wastewater generated after sludge sedimentation at pilot scale mode. RE of 80% and 87% were obtained for TP and TKN, respectively, while 100% RE was obtained for NH_4^+-N and PO_4^{3-}-P (Zhao et al. 2018).

But the reported carriers used for the biofilm technology are expensive in nature. Therefore, the study has shifted towards cheap carriers such as natural materials that include loofah sponge (Zhang et al. 2019), filter papers (Aljerf 2018), jute (Cao et al. 2013), linen (Kesaano and Sims 2014), etc. One of the added advantages of these materials is that they have micropores and various functional groups on their surface that function as adsorbent surfaces and are involved in the nutrient removal process with the microalgal cells (Riahi et al. 2017). Zhang et al. (2020b) designed a PBR in which pine sawdust was used as a biofilm carrier and accessed its efficiency for treating both synthetic and real wastewater (Zhang et al. 2020b). Their results showed that RE of 95.54% for TN and 96.10% for NH_4-N^+ was obtained in real wastewater and biomass productivity reached up to 8.10 g/m^2/day. Pine dust acted as a carrier for algal cells and performed the role of adsorbent as it removed 23.60% of COD, 37.30% of TN, 41.08% of NH_4^+-N, and 17.07% of total phosphorus (TP) (Zhang et al. 2020b).

2.2.6 Synchronization of Microalgae with Other Species

Earlier in Sect. 2.1, the application of the microalgal-bacterial process has been discussed in detail as several researchers have focused on its application for wastewater treatment. Microalgae have also been used in synchronization with other species for wastewater treatment. Some of them have been explained in the upcoming sections.

2.2.6.1 Microalgae-Yeast Process

Yeast species are widely used in the baking, brewing, and pharmaceutical industries. But its application for wastewater treatment has not been thoroughly evaluated due to the assumption that it will not grow to its full potential in the non-sterile environment of wastewater (Walls et al. 2019). But the P and N content in the yeast cells are 3–5% and 10%, respectively, higher than the content in microalgal cells (0.87%: P; 6%: N) (Walker 1998; Dalrymple et al. 2013). Thus, yeast can remove the nutrients from the wastewater at a higher RE. Yeast also has good settling properties that can decrease the cost of the harvesting system (Walls et al. 2019). Therefore, the application of microalgae-yeast cells for wastewater emerged as a hot research topic during these years. The synergetic relationship between microalgae and yeast occurs in the same way as the microalgae-bacterial process (i.e., O2 generated during the photosynthetic process of microalgae used by yeast for respiration in turn generates CO_2). Yeast cells can also trap the microalgal cells during harvesting, thus decreasing the cost of harvesting and dewatering. Walls et al. (2019) cultivated the *Scenedesmus* sp. and wild yeast in co-culture mode in a heterotrophic bioreactor, and they showed that this co-culture was efficient in 100% total ammonia nitrogen (TAN), 96% nitrate, and 93% orthophosphate. The biomass concentration of *Scenedesmus* sp. and yeast reached up to 0.98 ± 0.10 g/L and 4.2 ± 0.1 g/L, respectively (Walls et al. 2019). Yeast also offers the added advantage that it can be applied for aerobic fermentation for bioethanol production.

2.2.6.2 Microalgae-Macrophytes Process

Lemna minor belongs to the family of Lemnaceace, characterized as floating microphyte and smallest angiosperms having a rapid multiplication rate (Ekperusi et al. 2019). It is usually applied at the tertiary stage of the wastewater treatment process to treat effluent generated from the secondary treatment plant, mainly to remove toxic micropollutants and biomass production (Gatidou et al. 2017). It has also been applied for nitrogen removal, showing a high nitrogen uptake rate (Toyama et al. 2018). Recently, the co-culture of microalgae and macrophytes gained much importance for treating municipal wastewater by combining their synergistic effects. Kotoula et al. (2020) cultivated *Chlorella sorokiniana* UTEX 1230, Lemna minor in a SBR, and RE was 99% for COD and 88% for TKN, respectively 90% for NH_4^+-N, and 91% for PO_4^{3-}-P. *C. sorokiniana* was able to completely remove the COD while partially removing N and P. On the other hand, *Lemna minor* mainly contributed to the removal of nitrogen (Kotoula et al. 2020).

2.3 Microalgal Biorefinery Perception

As discussed earlier, high energy and cost are required during the microalgae-based wastewater treatment process, especially during the harvesting and dewatering process. The microalgae biorefinery approach (Fig. 2.2) has been proposed to compensate for the cost, where the microalgal biomass is transformed into various liquid and gaseous fuels, as explained below.

2.3.1 Liquid Biofuels

The demand for sustainable energy sources is increasing daily due to the increment of fuel load for the community, global warming effects, and decreasing petroleum reserves. In this context, liquid biofuels play a crucial role because they can put back fossil fuels and diminish carbon dioxide emissions (Williams and Laurens 2010). Some examples of liquid biofuels are bioethanol and biobutanol, which are fermentative biofuel that is derived from carbohydrates present in microalgal biomass.

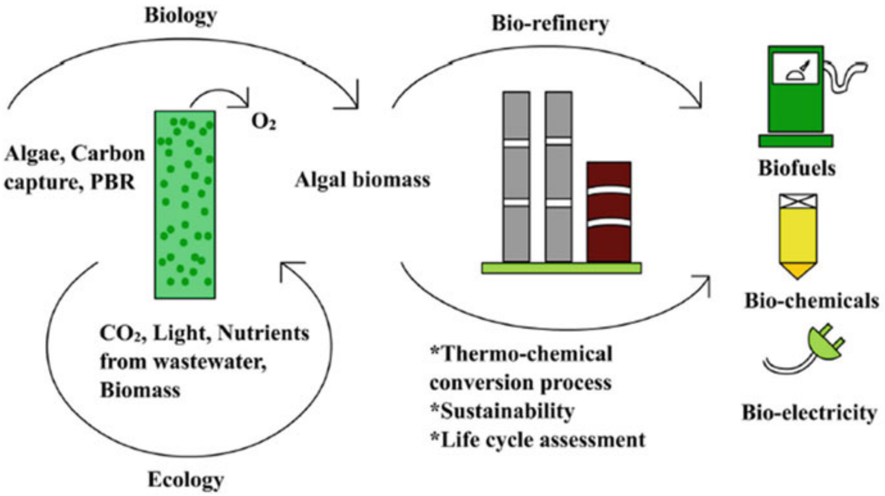

Fig. 2.2 Integration of microalgae-based wastewater treatment process with biorefinery concept (Arun et al. 2020b)

2.3.1.1 Bio-Oil

Bio-oil is obtained by pyrolysis and hydrothermal liquefaction (HTL) of biomass which refers to thermochemical conversion that leads to the polymerization of organic matter in an anaerobic environment (Sun et al. 2020). Initial steps of biomass degradation include degrading it into smaller compounds either individually or in combination with dehydrogenation, dehydration, decarboxylation, and deoxygenation. The obtained molecules are unstable and highly reactive, leading to cyclization, condensation, and polymerization, resulting in oily compounds and a great variety of molecular weight distribution (Arun et al. 2020b). Yang et al. (2007) noted that the quality of Bio-oil depends on the constituents of plant biomass like cellulose, hemicellulose, and lignin. It was found that cellulose, hemicellulose, and lignin degradation occurred at a temperature range of 220–315 °C, 314–400 °C, and 160–900 °C, respectively, and generated high solid residue (40%) (Yadav et al. 2020; Yang et al. 2007).

2.3.1.2 Biodiesel

In 1900, Rudolf Diesel initiated the production of methyl esters (commonly known as diesel) involving crops (Ramadhas et al. 2005). He considered it biodegradable, sustainable, and non-lethal (Demirbas and Fatih Demirbas 2011). Biodiesel consists of an extended chain of methyl ester and is renewable, non-hazardous, and eco-friendly fuel produced by oxidation and disintegration of biomass. Microalgae have been accepted as a good source of biodiesel production because of their high lipid content (50–70%) and multiplication rate (Satputaley et al. 2017). Biodiesel is highly viscous, due to which it accumulates on the fuel injector of the engines.

Processes like pyrolysis, dilution, and emulsification decrease viscosity (Marchetti et al. 2007).

Transesterification is a process through which triglycerides are converted into biodegradable, low atomic weight fatty acid methyl esters (FAMS) compounds suitable for engines. In the presence of methanol or ethanol, the rate of reaction is increased. Biodiesel production depends on the temperature, reaction time, catalyst load, and alcohol concentration (DuPont 2013). It was reported that transesterification, in combination with ultrasonication, reduces the reaction time that results in decreased working costs (DuPont 2013).

2.3.1.3 Bioethanol

It is the preferable liquid biofuel processed from the saccharification of carbohydrates and then alcohol fermentation (Ho et al. 2012). In alcohol fermentation, the components like starch, sugar, and cellulose present in biomass are converted into the fermentative fuel through the metabolic process of fungi, bacteria, or yeast in anaerobic conditions (Costa and de Morais 2011; Yadav et al., 2020). The United States Environmental Protection Agency reported that biofuels are receiving more attention all over the globe, in which bio-ethanol was the preferable biofuel in the last 10 years (Madakka et al. 2020). For the industrial fermentation process, *Saccharomyces cerevisiae* is the preferable strain (Suali and Sarbatly 2012). Through the glycolytic pathway, sugar converts into pyruvate followed by acetaldehyde synthesis, and carbon dioxide is liberated as a by-product. The produced acetaldehyde is then reduced to synthesize ethanol (Costa et al. 2015). In a study, it was mentioned that glucose resulted in ethanol (0.51 kg) and CO_2 (0.49 kg) per kg of substrate used (Hamed 2015). Another study reported that microalgae like *Chlorella vulgaris* yield around 65% ethanol converted from 37% starch content per dry cell weight (Brennan and Owende 2010). The anaerobic fermentation process for bioethanol production for algal biomass is a simple and easy process compared to other fermentative techniques.

2.3.1.4 Biobutanol

In Liquid biofuels, biobutanol provides a high energy profile and may also bring back bioethanol in the future (Vivek et al. 2019). Yeast like *Clostridium acetobutylicum* can digest biomass feedstock (cellulose and starch) and produce biobutanol. Along with biobutanol, they also produce some valuable by-products like ethanol, acetone, and organic acids. Under favourable fermentation conditions, the maximum yield of biobutanol was 0.41 g/g of glucose; unexpectedly, it is less than bioethanol yield (0.5 g/g of glucose) (Chen et al. 2013). Biobutanol production is increased by adding butyrate into acetone-butanol-ethanol (ABE) fermentation because it enhances the metabolic route from acidogenesis to the solvent genesis acetoacetyl-CoA is transformed to butyl Co-A instead of acetoacetate (Kao et al. 2013).

2.3.2 Gaseous Biofuels

2.3.2.1 Biohydrogen

Biohydrogen production is achieved by conventional and anaerobic operations like reverse water gas shift reaction, gasification, water electrolysis, and steam methane reforming (Xue et al. 2013). In the ABE fermentation process, biohydrogen synthesis occurs synchronously with bioethanol and biobutanol. Photosynthetic microorganisms like *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* utilize organic matter present in microalgal biomass resulting in hydrogen and CO_2 generation (Lam and Lee 2011). In recent times hydrothermal gasification is the preferable technique for hydrogen production. Ma et al. (2017) reported that in the presence of a catalyst like alkaline biochar, gasification of biomass results in hydrogen yield of 89.13% (Ma et al. 2017). The gasification route was difficult to clear, but it was reported that it goes through several reactions like water gas shift, methanation, pyrolysis, steam reforming, and hydrolysis (Vo et al. 2020).

$$\textbf{Water-gas shift reaction}: CO + H_2O \leftrightarrow CO_2 + H_2 \ (\Delta H = -41\,\mathrm{KJ/mol}) \tag{2.1}$$

$$\textbf{Methanation reaction}: CO + 3H_2 \leftrightarrow CH_4 + H_2O \tag{2.2}$$

$$\textbf{Steam reforming reaction}: CH_XO_y + (1-y)H_2O \rightarrow CO + \left(1 - y + \frac{x}{2}\right)H_2 \tag{2.3}$$

2.3.2.2 Biomethane

Biomethane is produced by the digestion of biomass anaerobically. In anaerobic digestion, organic matter is converted into biogas, CO_2, methane (CH_4), and trace gases. The three steps involved in anaerobic digestion activity are hydrolysis, fermentation, and methanogenesis (Pragya et al. 2013).

$$\begin{array}{c}\text{Complex organic matter}\\ \text{(Fatty acids, Protein)}\end{array} \rightarrow \begin{array}{c}\text{Soluble organic matter} \quad \text{(Hydrolysis)}\\ \text{(Alcohol, volatile acids)}\end{array} \tag{2.4}$$

$$\text{Alcohols, volatile acids} \rightarrow \text{Hydrogen gas, Acetic acid (Acetogenesis)} \tag{2.5}$$

$$\text{Acetic acid} \rightarrow CH_4, CO_2 \qquad \text{(Methanogenesis)} \tag{2.6}$$

2.3.3 Bioelectricity

In recent years, microbial fuel cells (MFCs) from algal biomass have been a novel technology and attracting attention for bioelectricity generation (Chandrasekhar and

Venkata Mohan 2014). In MFCs, microorganisms are actively involved in bioelectricity generation; hence, they are referred to as a bioelectrochemical system (Deval et al. 2017). In microalgal MFCs, CO_2 is consumed by the photosynthesis process that results in organic biomass synthesis with simultaneous O_2 liberation. This liberated O_2 acts as an electron acceptor throughout the metabolism and ends up in the current synthesis. In MFCs, photosynthesis was also reported to be directly related to the light source intensity and cell density (Lee et al. 2015; Jadhav et al. 2019).

2.4 Environmental Effect of Bio-Refinery Products

2.4.1 Carbon Footprinting

In the past century, the electrical energy and transportation zone restructured society by providing motorized movement to non-professional. It was reported that transportation (14%) and the electricity sector (25%) is responsible for GHG emission globally. Biofuels are eco-friendly as they have reduced the release of GHGs and CO_2 emissions. The car's lifespan determines the ecological impact of an automobile from manufacture to the level of its use. Well-to-Wheel (WTW) practice was developed to check the efficiency of vehicles. Basically, this WTW technique was separated into two steps, one is Well to Tank (WTT), and another is Tank to Wheel (TTW) (Strecker et al. 2014). The equal WTW technique calculates the carbon footprint estimation for electric vehicles. It was also reported that the lifetime of vehicles and carbon footprinting is affected by riding behaviour, use of gadgets (like air-conditioning, heating gadgets, defroster, etc.), and climate condition (Badin et al. 2013).

2.4.2 Negative Emission

The title "carbon negative" refers to the removal of carbon dioxide out of the common (natural) carbon cycle that includes carbon capture and segregation (CCS) through deposited biochar in soil and direct release of carbon dioxide in the wastewater for biomass farming. Here the released carbon dioxide will either be combined with the environment or treated as unfavourable depending on carbonaceous raw materials and the final target of carbon dioxide. Using 1 kg of microalgae biomass, approximately 2 kg (1.83 kg) of CO_2 gas can be isolated from the ecosystem (Rosenberg et al. 2011). This isolated carbon dioxide was transformed into gaseous and liquid fuels through thermochemical and biological processes. Recently, it was reported that through the gasification process, 33.5% of carbon dioxide is obtained from 15 g of *S. obliquus* biomass used (Arun et al. 2020a). Another study also reported that from 15 g of *A. fragilissima*, 34.1% of carbon dioxide and 29.5% of carbon dioxide were obtained by the HTL process and pyrolysis process, respectively. For microalgal biomass, the flow of carbon dioxide was referred to as "carbon negative" because of its removal from the environment (Arun et al. 2020c).

2.5 Conclusion

The current chapter concludes that microalgae present a promising approach for treating municipal wastewater, achieving high REs of up to 90%. Various advancements have been made in the microalgae-based wastewater treatment process, such as synchronizing microalgae with bacteria, yeast, and other species, PSBR, biofilm, and membrane technology. Out of all, the microalgae-bacterial process in the PSBR offers a cost-effective solution with high RE. Biofilm and membrane technology are also effective solutions, but the cost involved in these technologies is high, and, in the future, they may be a feasible solution after the decrease in cost. Integrating the biorefinery concept with the wastewater treatment process can decrease the cost of the process up to a suitable extent as the microalgal biomass can be transformed into various liquid and gaseous fuels and other by-products. This integration also decreases the net carbon emission in the atmosphere, decreasing the effect of global warming.

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