CHAPTER 20

Microalgae-based Remediation of Wastewaters

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INTRODUCTION

Microalgae are increasingly considered a green expedient for wastewater treatment. Key reasons [1,2] include (a) cost-effective treatment with (b) low energy requirements, that can be achieved through system optimization. Other prospects are (c) their low land requirements, (d) the residual organic and nutrient concentration is also low, and (e) they do not require further addition of chemicals. Furthermore, (f) sludge formation is lower than in conventional aerobic treatment, and (g) algal biomass can be used for biofuel or added-value product formation. Microalgae are efficient in removing nutrients, such as nitrogen (N) and phosphorus (P) as well as removing minerals and reducing the organic load. The latter includes the removal of phenolic and aromatic compounds. Nevertheless, intensive cultivation of microalgae might require large amounts of various nutrients that cannot be retrieved from the environment, as the utilization of nutrients is interwoven with their bioavailability. Microalgae are also capable of efficiently removing heavy metals (HMs), as well as emerging contaminants (ECs), even when they are contained in trace concentrations. A great advantage is that the use of microalgae for potentially human health-threatening compounds removal does not afflict the valorization of microalgal biomass for biofuel production (as would happen for applications such as fertilizers and feed products). The objective of this chapter is to highlight the challenges and opportunities of wastewater treatment using microalgae, as well as the potential valorization routes of the produced microalgal biomass (Fig. 20.1).

Availability of Nutrients in Wastewater for Microalgal Growth

The use of wastewater for microalgal growth is considered beneficial for minimizing the use of freshwater and reducing the cost of nutrient addition because nitrogen (N) and phosphorus (P) are removed from wastewater as bioresources for the production of biofuel or other added-value products [3]. Microalgae have been successfully cultivated in a variety of domestic and agroindustrial wastewaters (see Section 3). In case of lack of nutrients within a wastewater treatment plant a potentially significant source could be either raw or anaerobically digested agricultural wastes added in the influent of the system [4,5]. Although microalgae may have numerous advantages in wastewater treatment, their application is still challenged by the stringent discharge limits of nutrients [6]. Specifically, to achieve effective growth, microalgae require a basic nitrate (NO_3^-) concentration, which varies between 200 and 400 mg/L, in addition to other macronutrients such as phosphorous (P) and carbon (C) as well as micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), sulfur (S), copper (Co), selenium (Se), potassium (K), and magnesium (Mg) [7–9].

Carbon

Microalgae assimilate inorganic carbon via photosynthesis. Through this process, solar energy is converted into chemical energy producing oxygen (O_2) as a by-product, while in the second step chemical energy is used to assimilate carbon dioxide (CO_2) into sugars [9]. Microalgae are known to exist only in aqueous environments where the inorganic carbon required for their growth occurs in the form of CO_2, bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). Microalgae mainly metabolize inorganic carbon in the form of CO_2, while the enzyme carbonic anhydrase is required to convert HCO_3^- into CO_2 [10]. The most commonly used CO_2 source for microalgal growth is atmospheric air (CO_2 as 0.04% v/v, where v/v denotes volume of CO_2 per volume of air). However, about 2%–5% (v/v) of CO_2

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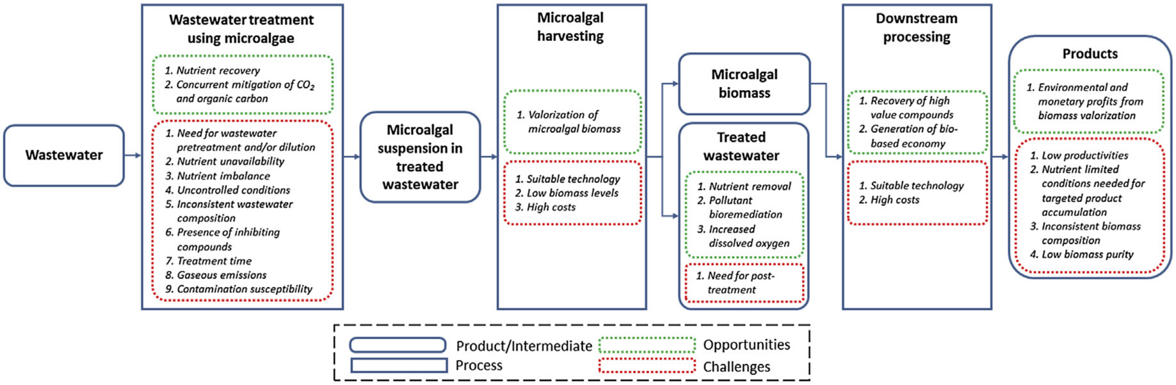

FIG. 20.1 Challenges and opportunities of wastewater treatment using microalgae.

in the supplied gas stream is usually required for sufficient microalgal biomass production [9]. As a matter of fact, it has been reported that 1 kg of microalgal biomass can use 1.83 kg of CO_2 but the theoretical yield can be predicted only when nitrogen source is defined [9]. The amount of CO_2 dissolved in wastewater might vary according to the pH. Specifically, at higher pH values ($pH > 9$) most of the inorganic carbon is in the form of carbonate (CO_3^{2-}), which cannot be assimilated by microalgae and as a result great care should be taken regarding the pH of wastewater [11]. In such a case, atmospheric CO_2 may be provided to the wastewater via forced aeration (air enriched by 1%–5% v/v CO_2) although this method has been proved economically unfeasible [9,12]. In case CO_2 becomes limited some microalgae can metabolize organic compounds such as sugars, organic acids, acetate, or glycerol as carbon source [9,13–16]. Scientific studies have reported that about 25%–50% of the algal carbon can be derived from heterotrophic utilization of organic carbon sources [14]. In fact, based on their metabolism, microalgae can be photoautotrophic, photoheterotrophic, mixotrophic, and heterotrophic while in several species the mode of carbon utilization can be shifted from autotrophy to heterotrophy, or even mixotrophy according to the variety and availability of the carbon source [14,16,17]. Many recent studies have targeted on the addition of various carbon sources along with wastewater for enhanced microalgal biomass production [16,18]. In addition, the use of food and agricultural waste along with wastewater for production of microalgae could be a beneficial approach from an economic point of view. Likewise, according to Chew et al. [13], the use of 25% of organic food waste along with inorganic residues can provide an enhanced *Chlorella vulgaris* FSP-E biomass up to 11% compared with microalgal cultivation in a fully inorganic medium. Another approach is the use of mixed cultures of photosynthetic bacteria and microalgae, which could simultaneously remove all nutrients contained within synthetic wastewater [8]. Finally, temperature, illumination, pH, salinity, aeration, and substantial nutrients are the most important operating parameters to optimize for maximized CO_2 sequestration targeting sufficient microalgal biomass production.

Nitrogen

Nitrogen is the second most important macronutrient essential for microalgal growth and as such could directly influence their production yield. Nitrogen can be found in many forms within wastewater and is usually assimilated by microalgae as an inorganic substrate in the preference of ammonium (NH_4^+) > nitrate (NO_3^-) > nitrite (NO_2^-) > organic–N [19]. Many different wastewater streams contain high ammonium concentrations (100–9000 mg/L NH_4^+–N) and can be successfully used for production of microalgae [5]. Wastewater treatment plants present one of the greatest opportunities for ammonium recovery due to wastewater containing high concentrations of ammonium ions [5]. Ammonium shows higher bioavailability compared with nitrate or organic–N, and as a result, when it occurs in wastewater, alternative nitrogen sources are not assimilated [20]. High temperatures or high pH values could lead to an equilibrium of free ammonia (NH_3) and ammonium (NH_4^+) shifting toward ammonia production, and therefore inhibiting microalgal growth [20]. In some cases, this equilibrium can be used for the removal of excess nitrogen from wastewater as ammonia stripping and loss to the atmosphere can range between 17% and about 80% depending on wastewater pH [21]. Specifically, nitrogen is found in the form of ammonia at pH 10.5−11.5 and can be transferred from the liquid to the air through aeration. However, this process might result in high costs and can cause air pollution, and ammonia emissions may distort nitrogen recovery through microalgal treatment [22]. Some strains, on the other hand, have been reported to grow in high ammonia concentrations in a range up to 130 mg/L [8,23]. In addition to the inorganic nitrogen forms, urine ($CO(NH_2)_2$) derived mainly from urban wastewaters can also be used as a nitrogen source, but only nitrification and stabilization could lead to high microalgae production yields [24]. When other nitrogen sources have become limited, amino acids can be assimilated by some species of cyanobacteria, but this method has been proved to be most cost-effective for microalgae growth [25]. As reported previously, ammonium recovery in wastewater seems preferable than other nitrogen forms. To this end, valorization of wastewater containing less desirable nitrogen entities seems feasible only by a combination of various wastewater sources (domestic wastewater, piggery wastewater, landfill leachate, and urine) that could lead to enhanced microalgal growth [5].

Phosphorus

Phosphorus (P) is a macronutrient essential for microalgal growth especially for the formation of DNA, RNA, adenosine triphosphate (ATP), and phospholipids in algal biomass [19]. Phosphorus is a nonrenewable resource, and thus its recycling is considered vital for future use. It can be retrieved from wastewater and can be assimilated by microalgae as an inorganic substrate preferably as orthophosphate (PO_4^{3-}). This substantial macronutrient should be present in wastewater at c.a. 40 mg/L PO_4−P to support efficient microalgal biomass production [23,26]. Nevertheless, nutrient uptake for microalgal growth depends on the associated concentration of nutrients inside the microalgal biomass. Because the molar N:P ratio in freshwater microalgal biomass ranges between 8:1 to 45:1 [19], phosphorus removal from wastewater is consistently lower than nitrogen due to the greater intracellular nitrogen content. There are also cases, for example, in Norway, in which phosphorous concentrations in the reject water from dewatering of anaerobically digested sludge are very low because all plants use metal salts for phosphorus precipitation and removal. As a result, this wastewater cannot be used as nutrient source for microalgal biomass production [23]. When inorganic phosphate is limited, many microalgal species can utilize organic phosphate sources converting it to orthophosphate using phosphatases located at the cell surface [27]. On the other hand, when inorganic phosphate appears in excess in wastewater, microalgae have the ability to store it within the cells in the form of acid-insoluble polyphosphate (volutin) granules [19]. Microalgae cultivated in wastewater may hence contain higher amounts of phosphorus than actually needed. These processes, which may lead to an extra uptake of phosphorus by microalgae, can be sufficient for prolonged growth in case phosphate concentration becomes limiting. As a result, the growth rate of microalgae may be immediately influenced by external concentration of phosphorus, in opposite to the responses to temperature, pH, or light [19,27].

Micronutrients

Micronutrients play important physiological roles in plant growth, and therefore they should be present in wastewater for efficient microalgal growth. Different species of microalgae require a range of micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), sulfur (S), copper (Cu), selenium (Se), potassium (K), and magnesium (Mg), which might be considered essential. Micronutrient requirements usually differ among taxa, whereas in some cases their presence may have a toxic effect if accumulated in excess of cells' requirements [28]. As a result, the presence of different micronutrients in wastewater could be characterized as essential, required, replaceable, or toxic for microalgal growth [28].

Iron, zinc, and manganese are considered essential for microalgal growth as they take part in the oxygenic photosynthesis. Iron has also been reported as essential for the assimilation of nitrate and nitrite [29]. Generally, iron can be found in wastewater in two oxidation states; soluble (Fe^{2+}) and in a sparingly soluble oxidized form (Fe^{3+}). On the other hand, in oceanic water, the majority of dissolved iron (>99%) is found bound with organic complexes [30]. Nevertheless, microalgae have the ability to utilize both free and bound iron. A problem might occur when micronutrients such as iron are bound with other essential nutrients, inhibiting microalgal growth. For instance, it has been reported that when wastewater contains a lot of sediments, phosphate can be chemically bound to iron or aluminum resulting in phosphates' lower bioavailability [31]. Generally, microalgae can efficiently assimilate these micronutrients when found in adequate amounts according to each species. For example, according to Saavedra et al. [32] removal efficiency of manganese from contaminated river water (2.1 mg/L) was reported up to 99.4% by *C. vulgaris* at pH 7 within 3 h. Likewise, it has been reported that the monometallic effluents of zinc (150 mg/L) used for *Scenedesmus obliquus* cultivation supported an uptake of 22.3 mg/g [33]. However, zinc or other micronutrients could be inhibitory for the growth of microalgae if present at elevated concentrations, or as a result of antagonistic behaviors in the presence of other micronutrients [32]. For example, the copper uptake efficiency of *C. vulgaris* is decreased by 10% in the presence of zinc [34]. In multimetallic systems, biosorption might be another process taking place in parallel explaining the removal of metallic components from wastewater. The biosorption mechanisms in this case are very complex and are not yet fully understood.

Speciation in Contaminant Removal

As stated previously, microalgae use organic or inorganic carbon sources, nitrogen (N), phosphorus (P), and trace metals as nutrients for their growth, depending on the cultivation method [35]. Phycoremediation extends beyond the aforementioned chemicals, which are just a limited example of the substances that microalgal species can remove from the environment. Nowadays, the scientific concern is oriented toward the so-called EC such as pharmaceutical organics, endocrine disrupting compounds (EDCs), polycyclic aromatic hydrocarbons (PAHs), surfactants, personal care products (PCPs), pesticides, perfluorinated compounds, substituted phenolics, flame retardants, industrial additives, and nanomaterials, among others [36–39]. The biochemical routes that microalgae follow to remove these contaminants are still under investigation, but it is believed that cell adsorption, biodegradation, bioaccumulation, stripping (volatilization), and/or photodegradation are the possible mechanisms [36].

Numerous studies have been conducted regarding specific microalgal species and their removal capacity on certain contaminants. *Navicula* sp., which is a typical freshwater diatom, was used to degrade the antimicrobial agent Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol, TCS) in a synthetic medium. The presence of potassium permanganate ($KMnO_4$) as well as the pH value of the culture medium seem to influence the outcome, regarding the toxicity of TCS in this diatom. Researchers also identified the transformation products of the contaminant and suggested a possible biochemical pathway of TCS degradation in *Navicula* sp. [40]. The same research group investigated the biouptake, toxicity, and biotransformation of TCS in the freshwater algae *Cymbella* sp. and how humic acid (HA), as a typical constituent of dissolved organic matter found in aquatic environments, affects these mechanisms [41].

Natural organic matter and its role on the removal of bisphenol A by *Monoraphidium braunii* was the research subject of Gattullo et al. [42]. Organic matter affects algal growth rate and may hinder or promote xenobiotic removal under certain circumstances. *M. braunii* reached 48% removal of bisphenol A [42]. *Cymbella* sp. and *Scenedesmus quadricauda* were evaluated also in terms of their bioremediation efficiency regarding naproxen [40]. Wang et al. [43] studied the removal and metabolism of TCS by using *Chlorella pyrenoidosa*, *Desmodesmus* sp., and *S. obliquus*. They stated 99.7% removal by the three microalgae and detected that cellular uptake was the metabolic mechanism for the reduction of TCS by *C. pyrenoidosa*, whereas *Desmodesmus* sp. and *S. obliquus* cells use biotransformation to eliminate the contaminant. Previously published studies, referred to a 77.2% removal percentage by *C. pyrenoidosa* after its cultivation with 800 ng mL^{-1} TCS for 96 h [44].

C. vulgaris is a species thoroughly examined by many scientists for its capacity to remove various pollutants. Among six tested species, Xiong et al. [45] reported that *C. vulgaris* reached a 12% removal of the antibiotic levofloxacin (LEV) at 1 mg/L, which was the highest. Furthermore, acclimation of the microalgae and addition of 1% w/v sodium chloride seem to boost its removal efficiency [45]. The decrease of metal concentrations such as Zn, Cd, Cu, Zn, Fe, Al, Ni, and Mn ions [46–48], organic matter, nutrients [48], diclofenac [49], bisphenol A [50], diazinon [51], and enrofloxacin (ENR) [52] was also evaluated for *C. vulgaris* commercially available species, mutants [53], and/or local isolates [54].

Desmodesmus sp., *Chlorella sorokiniana*, *S. obliquus*, *Chlamydomonas mexicana*, *Chlamydomonas pitschmannii*, *Ourococcus multisporus*, *Micractinium resseri* are some other species that were tested for their removal capacity in comparison with *C. vulgaris* or in a consortium, regarding the abovementioned chemicals.

Liu et al. [55] reported the biodegradation and reduction of 17β-estradiol (E2) and diethylstilbestrol (DES) by *Raphidocelis subcapitata*. E2 and DES are two estrogens that can be found both in waste and natural water, and it is believed that their activity is linked to endocrine disruption in humans and may affect future generations due to their accumulation in the food chain. *R. subcapitata* was chosen because of its reported ability to remove and degrade also benzo(a)pyrene (BaP) through its dioxygenase enzyme system, parent PAHs by a cytochrome P-450 system, and via monooxygenase and dioxygenase enzymatic pathways remove phenanthrene, fluoranthene, and pyrene. In the mentioned study, *R. subcapitata* presented an efficient removal capacity for E2 and DES, achieving up to 88.5% removal through accelerated bioaccumulation and biodegradation. Parlade et al. [56] approached the same contaminant problem from a different angle. They investigated the removal of 17β-estradiol by an algal–bacterial consortium in indoor and outdoor conditions as well as the outcome of bioaugmentation with *Scenedesmus*. It was concluded that algal-based systems removed E2 totally, and the high diversity of algal community acts beneficially on the contaminant removal [56].

The importance of this field is reflected in various published reviews of the scientific community, which deals with the different species that have been tested and all the outstanding questions or research gaps that are still need to be unraveled [36,37,39,57].

The elucidation of metabolic pathways that microalgal species use to remove contaminants as well as the identification of transformation products is a valuable tool for risk assessment of the chemicals that can be found in natural waters. The screening of the capabilities of each species, regarding micropollutant elimination is crucial for an effective design of water treatment installations and protocols.

Treating Municipal, Agroindustrial and Industrial wastewater

This section focuses on the treatment of municipal wastewater effluent (MWE), palm oil mill effluent (POME), olive mill wastewater (OMW), textile wastewater (TW), HM-containing wastewater, petroleum wastewater (PW), and ECs. A typical composition of POME, OMW, TW, and PW is presented in Table 20.1, whereas the HM- and EC-containing wastewaters are not included because of their high variability depending on the origin.

Municipal wastewater

Existing literature focuses on treatment of different effluents of municipal wastewater treatment, for example, primary settling, denitrification tank, and secondary settling effluent; therefore, direct comparison is not possible.

This subsection focuses on the microalgal treatment of secondary effluent, further referred to as MWE. The physicochemical characteristics of this wastewater are substantially deviating from those of any (agro)industrial stream, due to the distinctively low concentrations (Table 20.1) [58]. MWE treatment with microalgae results in efficient removal of N and P, while it also offers the advantages of removing HMs, increasing the dissolved oxygen in the effluent and limiting the growth of bacteria [2]. Nevertheless, the latter is mostly observed in attached growth systems rather than suspended. Gómez-Serrano et al. [63] cultivated *Muriellopsis* sp., *C. vulgaris*, *Chlorella fusca*, *Chlorella* sp., *Scenedesmus subspicatus*, and *Pseudokirchneriella subcapitata* in semicontinuous mode. The final biomass concentrations ranged between 0.7 and 1.7 g/L, while the N and P were totally eliminated from the cultivation medium (initial concentrations of 17.1 and 10 mg/L for TKN and TP, respectively). Shi et al. [59] used a twin-layer photobioreactor to immobilize *Halochlorella rubescens* for the treatment of MWE. Removal of 0.45 and 6.26 mg/L day was achieved for PO_4^-–P and NO_3^-–N, respectively (achieving 73.2% and 83.2% removal, respectively). The authors noted that this treatment achieved effluent values that meet the requirements of the European Water Framework Directive. Furthermore, Lv et al. [58] observed that the immobilization of microalgae as well as the establishment of a specific consortium enhances the nutrient/pollutant removal. Continuous cultivation of *Chlorella* and *Scenedesmus* sp. in MWE resulted in biomass concentrations varying between 0.1 and 1.3 g/L (at hydraulic retention time [HRT] 0.04–10 day). This resulted in TN removal of 36.0%–95.3% and TP removal of 40%–100%. It was noted that construction of microalgal consortia, apart from resulting in higher nutrient removals, also results in a more robust system that can face environmental fluctuations and hinders the invasion and prevalence of other species. Similarly, Wu et al. [64] found that the use of a coculture of *Scenedesmus* sp. LX1 and *Haematococcus pluvialis* resulted in better performance than the individual microalgae. It was concluded that the main mechanism responsible for this higher nutrient removal (7.2–7.5 mg/L residual concentration for monocultures compared with 3.3–5.0 mg/L for the mixed culture starting from 27.4 mg/L) was the interspecific nutrient competition between the two microalgae. Phosphorus was eliminated except from the case where *H. pluvialis* was the sole microalga grown. Finally, Wang et al. [2] summarized the mechanisms of N removal in MWE as: assimilation by microalgae (NH_4^+–N, NO_3^-–N, and NO_2^-–N and minor amounts of organic–N); loss as NH_3 due to increase in pH and temperature as well as mixing; and loss as N_2 due to bacterial nitrification–denitrification. On the other hand, P is removed through assimilation (for their anabolic and catabolic products); it is up-–taken to form polyphosphate granules (luxurious P uptake); or precipitated as $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$ (in the presence of Ca^{2+} and Mg^{2+}, using the photosynthesis derived O_2, when pH > 8.5).

TABLE 20.1
Typical Characteristics of Raw Municipal Wastewater Effluent (MWE), Palm Oil Mill Effluent (POME), Olive Mill Wastewater (OMW), Textile Wastewater (TW), and Petroleum Wastewater (PW).

Parameters	Units	Municipal Wastewater Effluent (MWE)	Palm Oil Mill effluent (POME)	Olive Mill wastewater (OMW)	Textile Wastewater (TW)	Petroleum Wastewater (PW)
pH	–	8.40 ± 0.41	4.30 ± 0.21	5.13 ± 0.03	7.63 ± 5.34	7.82 ± 1.07
Alkalinity	$g\ CaCO_3\ L^{-1}$	–	–	0.75 ± 0.10	–	–
Total solids (TS)	$g\ L^{-1}$	–	–	112 ± 1	5.86 ± 8.24	–
Volatile solids (VS)	$g\ L^{-1}$	–	–	82.3 ± 6.8	0.29 ± 0.34	0.22 ± 0.23
Total suspended solids (TSS)	$g\ L^{-1}$	–	19.6 ± 5.59	40.6 ± 0.6	0.09 ± 0.09	–
Volatile suspended solids (VSS)	$g\ L^{-1}$	–	28.7 ± 2.0	39.9 ± 1.1	–	–
Biochemical oxygen demand (BOD)	$g\ O_2\ L^{-1}$	0.01 ± 0.01	0.35 ± 0.07	–	–	0.18 ± 0.20
Total chemical oxygen demand (COD)	$g\ O_2\ L^{-1}$	0.05 ± 0.03	1.74 ± 0.54	169 ± 9	0.61 ± 0.54	0.39 ± 0.30
Soluble chemical oxygen demand (COD)	$g\ O_2\ L^{-1}$	–	22.5 ± 2.8	89.2 ± 3.9	–	–
Total carbohydrates[a]	$g\ L^{-1}$	–	–	37.5 ± 1.7	–	–
Soluble carbohydrates[a]	$g\ L^{-1}$	–	–	26.0 ± 0.3	–	–
Phenolic compounds[b]	$g\ L^{-1}$	–	–	8.61 ± 0.98	–	–
Oil and grease	$g\ L^{-1}$	–	–	22.0 ± 4.0	–	0.69 ± 1.31
Total Kjeldahl nitrogen (TKN)	$mg\ N\ L^{-1}$	14.8 ± 5.6	–	735 ± 10	–	–
Total ammonium nitrogen (TAN)	$mg\ N\ L^{-1}$	9.17 ± 8.99	650 ± 212	86.1 ± 5.3	25.7 ± 35.6	19.6 ± 22.2
Total phosphorus	$mg\ P\ L^{-1}$	2.32 ± 3.19	–	513 ± 1	–	–
Soluble phosphorus	$mg\ P\ L^{-1}$	–	400 ± 283	309 ± 4	2.04 ± 2.79	–
Zinc (Zn)	mg/L^{-1}	–	–	–	1.52 ± 1.99	–
Manganese (Mn)	mg/L^{-1}	–	–	–	0.03 ± 0.02	–
Cadmium (Cd)	mg/L^{-1}	–	–	–	0.03 ± 0.03	–
Copper (Cu)	mg/L^{-1}	–	–	–	0.05 ± 0.07	–
Lead (Pb)	mg/L^{-1}	–	–	–	0.09 ± 0.01	–
Nickel (Ni)	mg/L^{-1}	–	–	–	0.03 ± 0.01	–
Arsenic (As)	mg/L^{-1}	–	–	–	<0.001	–
Iron (Fe)	mg/L^{-1}	–	–	–	0.14 ± 0.04	–
Reference	–	[58,59]	[60]	[60a]	[61]	[62]

"–" indicates unavailable values.
[a] in glucose equivalents.
[b] in syringic acid equivalents.

Palm oil mill effluent

POME is a high-strength wastewater, characterized by high concentrations of organic load (BOD and COD), organic N and P and other nutrients (Table 20.1). It consists of 95%–96% of water, 4%–5% of solids of which 50%–100% are suspended, and 0.6%–0.7% of oil [65]. Kamyab et al. [65] used *Chlamydomonas incerta* to treat POME in COD concentrations of 250 mg/L, 500 mg/L, and 1000 mg/L. The highest COD removal (67.4%) was observed at 250 mg/L in 28 days, gradually decreasing for the two higher concentrations (43.2% and 34.1%). The corresponding organic carbon removal was 56.2%, 54.7%, and 49.8%. POME concentrations higher than 500 mg/L resulted in slow growth of *C. incerta* due to substrate inhibition as indicated by the lower growth rates. Therefore, this wastewater requires pretreatment before microalgal remediation. Vairappan et al. [66] bioremediated 5% aerobically digested POME using *Isochrysis* sp. Biomass productivities of 69.0 and 91.7 mg/m^2 day were achieved with an indoor photobioreactor and an outdoor system, respectively. The outdoor system produced higher lipid content biomass (52.8% compared with 44.5% for the photobioreactor), a fact ascribed to stress induced by variations in irradiance and temperature during outdoor cultivation. The nutrient removals were comparable in both systems, achieving 42.2%, 84.4%, and 21.9% reductions of nitrate, phosphate, and BOD_3, respectively (corresponding to 3.55, 0.03, and 3.02 mg/L, respectively). Hadiyanto et al. [67] applied a two-stage treatment of anaerobically treated POME. The first stage (3–8 days) consisted of treatment with aquatic plants, hyacinth (*Eichhornia crassipes*), or water lily (*Nymphaea* sp.), whereas the second treatment stage (15 days) was performed using *Arthrospira platensis*. *A. platensis* achieved maximum growth rate of 0.412 day^{-1} while reducing the COD, N and P content by 50.8%, 96.5%, and 85.9% (reaching values between 400 and 790, 8.29–145, and 3.14–10.0 mg/L, respectively).

Olive mill wastewater

The biological treatment of OMW, the side-stream of olive oil production, is challenging due to its phytotoxic and antibacterial properties derived from the high (poly)phenolic content as well as the high organic load and nutrient content. OMW can be effectively treated through membrane filtration, resulting in a wastewater with a limited amount of polyphenols [68]. Nevertheless, a sustainable membrane-based treatment method requires the valorization of the concentrates, accounting to about 20% of the initial volume. Cicci et al. [68] selected *Scenedesmus dimorphus* and *A. platensis* to treat OMW as well as ultra- and nanofiltration concentrates (all in 1:1 v/v). Both strains displayed higher polyphenol and phenol removals when treating ultrafiltration (UF) retentate (60.2%–72.7% and 72.7%–100%, respectively), while *A. platensis* eliminated phenolics during this treatment. OMW and UF retentate reduced microalgal growth due to the high polyphenol content, whereas nanofiltration concentrate promoted growth, as indicated by the lower specific growth rates (−59% to −30%, −124% to −26% and 53%–142% difference compared with the control for OMW, UF concentrate, and NF concentrate, respectively). The increase in growth in the latter case is ascribed to the reduced concentration of toxic compounds coupled with the presence of suitable soluble organic carbon. Hodaifa et al. [69] remediated physicochemically pretreated OMW using *C. pyrenoidosa*. Microalgal treatment contributed to 1.4% COD decrease, 8.0% phenolic compound (corresponding to 0.57 mg/L) as well as N removal of 19.2% (or 11.3 mg/L). The maximum specific growth rate and biomass productivity increased with the increase in concentration until 50% OMW (v/v), where the values of 0.07 h and 1.25 mg/L h were presented. Higher OMW concentrations resulted in drop of these parameters either due to inhibition or due to increased toxic effect. The low biomass productivities hinder the use of the biomass as a high-value product and therefore decrease the potential of economic viability. To this end, centrifuged OMW was supplied to *Scenedesmus* sp. through different strategies: (A) batch, providing 9% v/v; (B) fed-batch, with daily addition of 1% OMW; and (C) two-stage supply, consisting of photoautotrophic cultivation, followed by 9% OMW supplementation for heterotrophic growth [70]. The highest biomass concentration was achieved by the two-stage strategy (1.4 g/L compared with 0.9 g/L and 0.5 g/L during fed-batch and batch supply, respectively). Sugars were removed at the levels of 74.0%, 62.9%, and 61.0% during batch, fed-batch, and two-stage strategy, respectively. Phenol removal was reversely proportional to the concentration of phenolics (at concentrations >100 mg/L), reaching values of 66.0% in day 1 and gradually decreasing to 12.0% until day 7 of fed-batch cultivation. A slight phenol increase was observed during batch cultivation, as well as during the first days of the two-phase strategy, whereas 55% decrease was noted on day 12 of the heterotrophic phase. Therefore, three hypotheses were formed: (A) phenol degradation occurs after the depletion of highly biodegradable compounds, (B) phenol-tolerant microorganisms (bacteria or fungi) degraded the phenols, and (C) polyphenols were degraded from the beginning of the phase but this was not detected from the method used. On the other hand, Pinto et al. [71] did not observe high-molecular-weight phenol (e.g., lignins, tannins) degradation when treating OMW (1:10 v/v) with *Ankistrodesmus braunii* and *Scenedesmus quadricauda.* In contrast, low-molecular-weight phenols (<300 Da) were biotransformed during this short treatment, with hydroxytyrosol, catechol, and ferulic and sinapic acid completely removed in all cases. Experiments performed in the dark resulted in higher phenol removal efficiency (75%–100% compared with 35%–100% under light conditions), while light was found to result in high toxicity mainly due to autooxidation. The overall phenolic compound reduction after 5 days amounted to 12%. Finally, physicochemical analysis revealed that phenols were biotransformed into other aromatic compounds.

Textile wastewater

Textile industry generates various organic and inorganic streams that fluctuate from alkaline to acidic, the color of which can be orange, red, purple, blue, and black, presenting highly variable composition [72]. This wastewater, a typical composition of which is shown in Table 20.1, constitutes significant environmental threat, as apart from the nutrients and COD, also contains HMs. Biological treatment with microalgae seems promising, as these microbes degrade dyes to utilize them as N source [73]. Ghazal et al. [72] tested *Anabaena flos-aquae*, *Nostoc elepsosporum*, *Nostoc linckia*, *Anabaena variabilis*, and *C. vulgaris* for the treatment of TW. The highest color removal was noted by *N. elepsosporum*, followed by *C. vulgaris*, *A. variabilis*, *N. linckia*, and *A. flos-aquae* (50.8%–100% removal). The treatment increased the pH (from 9.5 up to 9.95) and DO (from 0.6 to 2.01–2.75 mg/L) due to photosynthesis. Furthermore, a drop was noted for EC (from 2.90 to 0.92–2.03 dS/m), COD (60.0%–98.9% removal at initial concentration of 430 mg/L) and BOD (80.0%–97.6% removal starting from 96 mg/L). The cell dry weight remained at low levels, with the best results presented by *N. elpsosporum* (11.5 mg/L), while the growth was 12.1%–19.4% reduced compared with the control. The best overall performance was noted by *N. elepsosporum*, achieving removals of 98.9% for COD, 97.6% for BOD, and 95.0%–99.0% for HMs, while color was totally removed. Moreover, *C. vulgaris* was used for TW bioremediation in high-rate algae ponds (HRAPs) [61]. NH_4–N was reduced by 44.4%–45.1%, PO_4–P by 33.1%–33.3% (achieving values of 3.43–3.57 mg N L and 4.76–64.23 mg P L) and COD by 38.3%–62.3% (reaching values of 102–170 mg/L), while the color was 41.8%–50.0% removed through biosorption. Color removal decreased with the increase in initial color content. TW reduced the specific growth rate of *C. vulgaris* to 0.05 day^{-1} compared with 0.40 day^{-1} for the control pond (containing nutrient medium), while nutrient addition almost restored the specific growth rate to the control level (0.39 day^{-1}). Nevertheless, biomass concentrations remained low in all TW treatments (107–203 mg/L compared with 613 mg/L for the control). Nutrient deficiency was identified as the main limiting factor for *C. vulgaris* growth in TW. Finally, additional treatment with immobilized in alginate *Chlorella* cells would be suitable to further remove the color of TW because this approach creates a cell-dense system that can enhance dye adsorption.

Heavy metal–containing wastewater

The anthropogenic HM disposal into the environment causes detrimental effects, due to the inability of biodegradation that causes bioaccumulation. This provoked the penetration of HMs, with Cd, Cr, Cu, Hg, Pb, and Zn most commonly occurring into the food chain, causing environmental, public health, and economic impacts [74]. The main HM removal mechanisms in microalgae are cell incorporation and biosorption (passive adsorption process), while the great superiority of the microalgal HM remediation over conventional processes is that it has a great efficiency in removing HMs in trace levels [74,75]. Kumar et al. [74] categorized the factors affecting the HM remediation to biotic, that is, algal species used, tolerance, capacity, biomass concentration, size and volume of microalgal cell; and abiotic factors, that is, pH, ionic stress, salinity, temperature, metal speciation, and effect of combined metals. It was noted that the species of choice for HM removal are *Chlorella* and *Scenedesmus*, whereas the highest HM removals are generally presented at pH 5 [74]. This study summarized that *Chlamydomonas reinhardtii*, *S. platensis*, as well as several strains of *Scenedesmus* spp., *Tetraselmis* spp., and *Chlorella* spp. are efficient in removing Cd, taking it up in levels of 0.64–292 mg/g dry biomass. Co is more efficiently removed by *Spirogyra* spp. (12.8 mg/g) and *Oscillatoria angustissima* (15.3 mg/g). The most notable Cr^{6+} as well as Cu^{2+} uptake was performed by *Arthrospira* spp. reaching levels of 333 and 389 mg/g, respectively [76]. Immobilized in alginate *C. reinhardtii* presented the most prominent Hg^{2+} removal capacity of 107 mg/g as well as Pb^{2+} removal (381 mg/g) [74], while the highest Zn removal potential was presented by *Desmodesmus pleiomorphus* (360 mg/g) [77]. Finally, *A. flos-aquae*, *N. elepsosporum*, *N. linckia*, *A. variabilis*, and *C. vulgaris* were able to remove Cr (60.4%−99.2%), Pb (73.0%−98.8%), Fe (73.1%−98.9%), Cu (60.4%−99.0%), Mo (60.8%−96.9%), and As (46.7%−95.0%) present in initial concentrations 2.60−95.2 mg/L [72].

Petroleum wastewater

Petroleum by-product contamination is a considerable polluting factor of surface- and groundwater occurring due to leakage of storage tanks, spills as well as incorrect disposal of PW. Compounds commonly found in petroleum by-products include benzene, toluene, ethylbenzene, and xylenes (BTEX). Takáčová et al. [78] investigated BTEX biodegradation using *Parachlorella kessleri*. The number of *P. kessleri* cells after 48 h was 13% lower compared with the control, whereas BTEX degradations of 39%−50% and 56%−64% were observed after 24 and 48h, respectively (initial BTEX concentration of 100 μg/L). Ansari et al. [79] used *Isochrysis* sp. for the treatment of crude oil and heavy duty marine diesel (HDMD), to assess the potential of degrading the water-soluble fractions of oceanic oil spill. Crude oil affected the growth due to oil toxicity. Specifically, concentrations of 30%−50% resulted in initial drop in cell density during early cultivation. HDMD had more toxic effect, notable even at 5%−10% concentration, while higher concentrations (20%) resulted in culture death. The higher toxicity of HDMD compared with crude oil is noted by the 7.4% decrease in maximum growth rate (0.24 day^{-1}) compared with the 58% decrease (0.55 day^{-1}) for crude oil, when both were supplemented at 10%. The lower toxicity of crude oil's water-soluble fractions is attributed to the lower concentration of polyaromatic hydrocarbons compared with HDMD. On the other hand, *Prototheca zopfii* degraded 10.7% motor oil and 41.4% crude oil [80]. More specifically, 10%−23% of the saturated aliphatic hydrocarbons as well as 10%−26% of the aromatic compounds contained in motor oil were removed. Regarding crude oil, the respective percentages were 38%−60% and 12%−41%. The higher removal concerning crude oil is ascribed to the higher content of alkanes (17% compared to 9% for motor oil) and the lower percentage of aromatics and cycloalkanes (35% and 45% for motor oil and 28% and 39% for crude oil). The degradation of cycloalkanes was inversely proportional to the number of rings, and the six-ring cycloalkanes did not get utilized whatsoever. Hodges et al. [81] compared two different systems for the treatment of petroleum refining wastewater. Specifically, they used rotating algae biofilm reactors (RABRs) as well as suspended-growth open pond lagoons, containing mainly *Pseudanabaena*, *Oscillatoria*, and *Chroococcus*. N was reduced by 70.8%−72.4% (corresponding to 17.7−18.1 mg/L reduction), P was reduced by 50.0%−55.6% (0.90−1.00 mg/L) while the TSS concentration was 53.6%−61.3% lower (20.9−23.9 mg/L) through the RABR treatment. The open pond growth lagoon operated in 13.9% (3.47 mg/L) and 18.9% (0.34 mg/L) lower N and P content, while the TSS content increased by 46.9% (18.3 mg/L) due to the suspended growth of microalgae. The biomass productivity of the open pond amounted to 0.4 g m^2 day, whereas the RABR resulted in average biomass productivity of 4.11 g m^2 day while presenting the advantage of not requiring intensive biomass harvesting.

Emerging contaminants

ECs have recently penetrated natural environments. They are mostly found in municipal and pharmaceutical plant wastewater and landfills and derive from daily household products, cosmetics, and medicine [39]. Even though their concentration in aquatic environments is low (ranging from ng/L to μg/L), they can have deleterious ecological effects. As mentioned in section 17.2, pharmaceuticals, PCPs, EDCs, and pesticides are among the prime examples of ECs [39]. Microalgae have shown the potential to remove many types of ECs at levels of 9−24 μg/L, however, presenting difficulties in removing pesticides [39]. Ahmed et al. [39] identified the trend of removal: pharmaceuticals > PCPs > EDCs > pesticides. Furthermore, Wang et al. [44] found that *C. pyrenoidosa* can remove 50% of triclosan (antimicrobial agent) during the first 1 h of exposure when the initial concentration is 100−800 μg/L. The microalga removed 77.2% of triclosan after 96 h (initial concentration of 800 μg/L). Nevertheless, this compound negatively affected the microalga, as indicated by the disruption of the chloroplast. Matamoros et al. [82] tested the removal of 26 different ECs from urban wastewater using pilot scale HRAPs fed with 7−29 g of COD m^{-2} day^{-1}. The targeted compounds included pharmaceuticals and PCPs, fire retardants, surfactants, anticorrosive agents, pesticides, and plasticizers. The system was able to remove up to 90% of the most commonly occurring compounds (caffeine, acetaminophen, and ibuprofen), whereas the concentration of others was not reduced. Specifically, the authors of this study identified four different groups according to their removal efficiency: (A) > 90% biodegradation: caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate, and hydrocinnamic acid; (B) 60%−90% biodegradation: oxybenzone, ketoprofen, 5-methyl/benzotriazole, naproxen, galaxolide, tonalide, tributyl phosphate, triclosan, bisphenol A, and octylphenol; (C) 40%–60% biodegradation: diclofenac, benzotriazole, OH–benzothiazole, triphenyl phosphate, cashmeran, diazinon, celestolide, and atrazine; (D) < 30% biodegradation: carbamazepine, benzothiazole, methyl paraben, tris(2-chloroethyl) phosphate, and 2,4-D. Moreover, it was noted that the main mechanisms were biodegradation and photodegradation. Xiong et al. [83] evaluated the biodegradation of carbamazepine using *C. mexicana* and *S. obliquus*. The growth of *C. mexicana* was 30% inhibited by the supplementation of 200 mg/L carbamazepine, whereas *S. obliquus* presented 97% inhibition. The maximum biodegradations achieved were 28% and 35% by *S. obliquus* and *C. mexicana* when 1 mg/L of the compound was supplemented, while 13% and 32% carbamazepine was biodegraded after 10 days of incubation (initial concentration of 25 mg/L). Batch reactors with *Chlorella* sp. and *Scenedesmus* sp. as prevalent species were used for the biodegradation of caffeine, ibuprofen, galaxolide, tributyl phosphate, 4-octylphenol, tris(2-chloroethyl) phosphate, and carbamazepine [84]. 4-octylphenol, galaxolide, and tributyl phosphate were removed up to 99% after 10 days due to air stripping. However, caffeine and ibuprofen were biodegraded by 99% and 95%, respectively. The authors observed that the initial lag phase of 3 days for ibuprofen removal was eliminated to nondetectable time. Finally, Ahmed et al. [39] concluded that the combination of physicochemical treatment (e.g., ozonation, ultrasound treatment, UF) with biological treatments, such as microalgal treatment, will result in more effective EC removal. Summarizing, Table 20.2 illustrates the maximum EC removals by various microalgal species reported in recent literature.

Challenges in Wastewater Bioremediation

The challenges concerning the microalgal wastewater bioremediation need to be identified and elucidated, to advance the field of microalgal biotreatment. To this end, the following limitations were identified by Wang et al. [2]: (A) relatively long treatment time is required; (B) the separation of algae from the wastewater is cumbersome; and (C) reduced performance is observed when bacteria and zooplankton dominate. Additionally, (D) it is common to observe increase in organic compound and solid content due to the often

TABLE 20.2
Maximum Emerging Contaminant Removals by Various Microalgal Species.

Microalgal Species	Contaminant	Max. Removal (%)	References
Cymbella sp.	Triclosan (5–chloro–2–(2, 4–dichlorophenoxy)–phenol)–phenol	69	[41]
	Naproxen	97.1	[40]
Monoraphidium braunii	Bisphenol A	48	[42]
Scenedesmus quadricauda	Naproxen	58.8	[40]
Scenedesmus obliquus	Triclosan (5–chloro–2–(2, 4–dichlorophenoxy)–phenol)–phenol	99.7	[43]
	Diclofenac	79	[49]
Chlorella pyrenoidosa	Triclosan (5–chloro–2–(2, 4–dichlorophenoxy)–phenol)–phenol	69.3, 77.2	[43,44].
Chlorella vulgaris	Levofloxacin	12	[45]
	Nitrogen, phosphorus	87.7, 76.7	[48]
	Metal ions (Zn, Cd, Cu, Zn, Fe, Al, Ni, and Mn)	64.7–100	[46–48]
	Diclofenac	21.58	[49]
	Bisphenol A	23	[50]
	Diazinon	94	[51]
	Enrofloxacin	26	[52]
Desmodesmus sp.	Triclosan (5–chloro–2–(2, 4–dichlorophenoxy)–phenol)–phenol	92.9	[43]
Raphidocelis subcapitata	17β-Estradiol (E2)	88.5	[55]
	Diethylstilbestrol (DES)	71.8	[55]

not efficient harvesting of cells, or due to the intracellular products release from the lysis of the cells [2]. Even though membranes can shorten the HRT as well as retain the microalgal biomass, the costs and risk of fouling limit their full-scale implementations. Another approach is the stimulation of attached growth by introducing substratum for biofilm creation. In this case, the most common method of harvesting, mechanical scraping, is difficult to perform in full-scale installations. Furthermore, the reasons for the unpredictable biomass detachment need to be elucidated.

The characteristically low concentration of nutrients and organics in some wastewaters, for instance MWE, render its treatment challenging. Lv et al. [58] reported that the lack of organic carbon is one of the main challenges in the cultivation of microalgae in this effluent. The biggest fraction of organics contained in MWE are inert and therefore not degradable by microalgae [2]. Another challenge for treating MWE is the imbalanced concentration of nutrients and particularly the N:P ratio [2]. The nutrient-limiting conditions prevailing under the cultivation in MWE result in low biomass productivity, while also increasing the lipid and carbohydrate accumulation in the cell [63]. On the other hand, (agro)industrial wastewaters often need high dilution to be eligible for microalgal remediation. *Scenedesmus* sp. was used for the treatment of OMW, in concentration of 9% v/v during batch growth, and 1% v/v during fed-batch growth [70]. The lower growth during batch cultivation was ascribed to the exposure at high concentrations of growth-inhibiting compounds, as well as the dark color of the wastewater that does not permit light penetration. Therefore, special attention should be paid with wastewaters containing high amounts of inhibiting compounds, as for instance phenolics [69]. Di Caprio et al. [70] stated that a low OMW supply rate is suitable for efficient phenol removal from microalgae, for a low phenol as well as light variation. Moreover, wastewaters such as PW provoke oxidative stress, and therefore high dilution is required for microalgal treatment [85]. Cicci et al. [68] highlighted that apart from the compositional characteristics, the color of the wastewater hinders its treatment since a reduced fraction of light penetrates dark-colored wastewaters (e.g., OMW). This could be solved by cultivation in thin-layer bioreactors, nevertheless, the biomass productivity in such systems is yet to be determined. Substrate inhibition is a common reason for the lower growth of microalgae, even during heterotrophic conditions [86], where nutrient and organic load levels can generally be higher. For example, POME supplied in concentrations more than 500 mg/L inhibited the growth of *C. incerta* [65]. Furthermore, wastewaters are often nutrient poor and therefore do not favor the growth of microalgae [61,66]. To satisfy all the microalgal needs, Vairappan et al. [66] treated 5% POME, diluted in seawater and supplemented with 0.075% NPK fertilizer. The low percentage was selected due to (A) the high organic and nutrient load of POME, (B) the dark color that hinders the phototrophic cultivation, and (C) it is a freshwater-type wastewater that cannot be used for the cultivation of a marine microalga (*Isochrysis* sp.).

Furthermore, agroindustrial streams in many cases need extensive pretreatment before they become suitable for bioremediation using microalgae, mainly targeting suspended solids and COD reduction [68,69]. For instance, Hadiyanto et al. [67] used anaerobically digested POME, that was subsequently treated with aquatic plants prior to the treatment with *A. platensis*. For the latter, POME was filtered and used at 80% v/v, supplemented with N, P, and vitamin sources. Hodaifa et al. [69] proposed a complete treatment process composed of (A) flocculation−sedimentation to remove the biggest part of solids from the liquid, (B) photolysis to reduce the organic load and photodegrade the phenolic compounds, (C) microfiltration (0.2 μm) to remove the suspended particles and sterilize the wastewater, and finally (D) biotreatment of OMW using *C. pyrenoidosa*. The authors stated that this pretreatment was essential for ensuring microalgal growth in OMW. Similarly, Cicci et al. [68] used OMW that was filtered, flocculated through acidification, subsequently centrifuged and photocatalyzed. *S. platensis* appeared to be more sensitive to media composition than *Scenedesmus dimorphus*, as indicated by the high variation in specific growth rate under different media. The latter indicates that some strains are more sensitive than others, indicating that several experiments need to be performed before an optimal treatment is established.

Treatment efficiency is also affected by environmental conditions. For instance, when temperature deviates from the optimal range growth rates decline [87], having as a result the reduced pollutant removal in a given period of time. Similarly, increased temperature or pH can result in ammonia volatilization, resulting in the escape of the main nitrogen source [20]. Additionally, reduced nutrient bioavailability due to wastewater composition reduces the potential of wastewater bioremediation through microalgal treatment [87]. More specifically, nutrient speciation has an important role in the overall performance, as microalgae show preference in specific nutrient forms (for instance ammonium instead of organic−N) [19]. All these factors can lead to insufficient microalgal growth and therefore inefficient wastewater treatment. Special attention should be paid during wastewater treatment aiming at HM or EC removal. Kumar et al. [74] stated that there are several compounds that form complexes with HMs (e.g., amino acids, HAs, fulvic acid, EDTA) rendering them unavailable for microalgal treatment. Furthermore, there is a selectivity in EC removal, as for instance difficulties are presented in the elimination of EDCs and pesticides [39]. Moreover, EC removal efficiencies are season dependent as they get reduced with lower temperatures [39]. These compounds can present growth inhibition up to 97% [83] and therefore, extensive study is required to establish the suitable strains and cultivation conditions for EC biotreatment. Finally, it is also important to note that there are several phenomena occurring during the microalgal wastewater remediation, that remain yet to be identified. For instance, the biotreatment of OMW under illumination results in phenol autooxidation increasing therefore the toxicity of the wastewater [71].

End-use of Microalgae Cultivated in Wastewater

In modern societies, renewable sources of energy, dietary ingredients, and valuable chemicals have been in the limelight of scientific research, due to challenges concerning the increasing population, climate change, water availability, and energy supply. In this regard, microalgae represent a promising feedstock for biofuels production, as well as unlimited number of added-value compounds, including lipids, proteins, carbohydrates, pigments, and numerous secondary metabolites used for cosmetics, pharmaceuticals, and other purposes, as shown in Fig. 20.2 [88,89].

From an environmental point of view, microalgal cultivation makes no use of arable land and enables consumption of sea water, wastewaters, and effluents, as well as flue gases contributing to recycling of nutrients, CO_2 mitigation, and substantial improvement in terms of cost of biomass production [90,91]. However, wastewater valorization in microalgal cultivation can be a demanding task that needs further investigation. Especially in the case where the produced biomass ends up in the food chain, wastewater pretreatment is needed to diminish concentration of several contaminants, including pathogens, HMs, and xenobiotic substances present in the biomass produced by specific types of effluents [6].

Biofuels

Microalgal biomass can be converted, through thermal, chemical, and biological processes to third-generation biofuels, including biodiesel, biogas, biohydrogen, bioethanol, and biocrude oil. In contrast to first- and

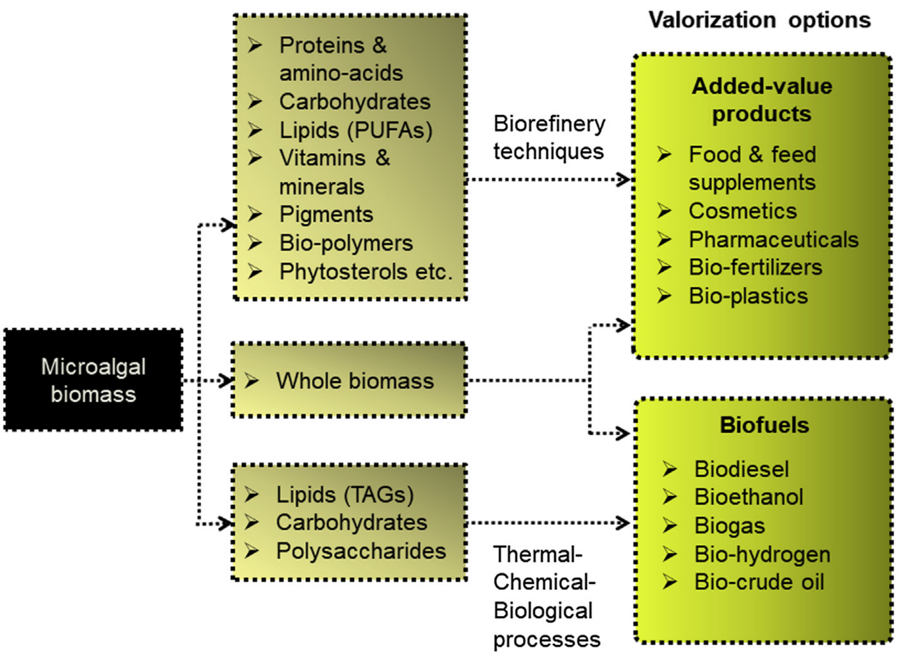

FIG. 20.2 Valorization options for microalgal biomass.

second-generation biofuels, produced from food crops and lignocellulosic biomass, agricultural and forestry residues, respectively, microalgal biofuels represent a sustainable alternative to fossil-based fuels, which can dominate the energy sector the following decades, provided that mass cultivation and downstream processing make substantial progress [92,93].

One of the most well-investigated valorization options for microalgae produced in wastewaters is biodiesel, associated with the lipid fraction of microalgal biomass. Typically, microalgae are characterized by 20%–50% intracellular lipids at dry weight basis, mainly consisting of C16–C18 fatty acids, which can be transesterified to high-quality biodiesel. Lipid content can reach up to 80% usually under nutrient-depleted conditions resulting in high lipid productivity. Concurrently, high biomass productivity can compensate for moderate lipid content inducing high biodiesel production [94]. Lipid productivity in wastewaters can be optimized through two-stage cultivation, where nutrients and COD are initially depleted maximizing biomass production, while lipid content is subsequently increased in the second stage characterized by nutrient starvation [95]. Low nitrogen concentration resulted in high lipid content (32.7%) of *C. vulgaris* cultivated in municipal wastewater [96]. Cultivation of *Nephroselmis* sp. with diluted industrial wastewater resulted in high specific growth rate (0.87 day^{-1}), higher lipid productivity (14.2 mg/L day) than that of the synthetic medium BBM and high percentage of palmitic and oleic acid for biodiesel production [97]. In addition, lipid content of *Chlorococcum* sp. RAP13 cultivated in dairy wastewater increased from 31% under mixotrophic conditions, to 42% under heterotrophic growth after the addition of 6% waste glycerol from biodiesel industry [98]. Microalgae produced from wastewater treatment can also be used as feedstock for biocrude oil production through hydrothermal liquefaction, a sustainable process that can be effectively applied in wet biomass, mixed cultures, and low-lipid microalgae, resulting in a potential jet fuel [92,99].

Carbohydrates represent another major component of microalgal biomass that can be further valorized for bioethanol production. In contrast to ethanol feedstock of former generations, microalgae are characterized by high concentration of the easily fermentable cellulose and starch, along with low concentration of hemicelluloses and absence of lignin [100]. With view to decreasing the cost of biomass production and materializing algal biofuels, carbohydrate-rich biomass can be cultivated in wastewater. *Nannochloropsis oculata* and *Tetraselmis suecica* were effectively grown in municipal wastewater, and the produced biomass was fermented by *Saccharomyces cerevisiae*, after alkaline pretreatment, resulting in almost 4% bioethanol yield, despite the relatively low carbohydrate content [101].

In contrast to biodiesel and bioethanol production which make use of a single fraction of microalgal biomass, anaerobic digestion exploits the whole biomass produced toward biogas production [93] and can be applied without prior drying. Anaerobic digestion is highly determined by the rigidity of microalgal cell wall and biomass composition and can be performed after biomass pretreatment for cell permeability and lipid extraction or codigestion with other substrates for enhanced nutrient balance [102,103]. Biomass produced by a consortium of *C. vulgaris*, *S. obliquus*, and *C. reinhardtii* cultivated in swine wastewater under batch and semicontinuous mode of operation, resulted in methane yields up to 146 and 171 mL/g COD, respectively [104]. Methane yield of 346 and 415 mL/g VS was also recorded in case of *Chlorella kessleri* and *C. vulgaris*, which were effectively grown in municipal wastewater [105]. Another type of wastewater used for cultivation of microalgae intended for biogas production among others, is the effluent from anaerobic digesters, called digestate, in the framework of an integrated approach for zero-waste production of energy [106,107]. Lastly, clean energy, in the form of biohydrogen, is a highly promising option for microalgal technology and can be produced through direct biophotolysis or dark fermentation [92]. Carbohydrate-rich biomass produced during municipal wastewater treatment was fermented by *Enterobacter aerogenes*, resulting in hydrogen yield of 56.8 mL/g VS in case of *S. obliquus* [108]. In addition, an output of 11.7 mL/L hydrogen was recorded during cultivation of *Chlorella* sp. with crude glycerol, a by-product from biodiesel industry, under anaerobic conditions [109].

Added-value products

Despite the high potential of microalgal biomass for biofuels production, formation of added-value products should accompany energy applications, in the framework of a biorefinery approach, which will make scale-up of microalgae-based systems economically feasible [110]. To this end, utilization of wastewater and effluents to produce valuable compounds, including proteins, pigments, vitamins, and numerous bioactive substances represents an emerging and highly promising field of microalgal technology. Since ancient times, people have been using microalgae as a food and feed ingredient, while currently microalgae have been considered as a novel source of protein. This type of protein is often called single-cell protein or microbial protein. In case effluents are used as cultivation media for microalgal production, several concerns arise in terms of safety and hygiene of the produced protein source [6,111]. Especially in the case of fecally contaminated wastewater, multiple pretreatment steps should be implemented before their use for microalgal biomass destined for feed and/or food applications, to enable public acceptance [112]. Wastewater characteristics and operational mode significantly affect biomass composition, with high levels of nitrogen, semicontinuous operation, and low hydraulic retention time inducing protein accumulation [104]. Biomass of a *Chlorella* strain (PY-ZU1) cultivated in swine digestate was considered suitable for feed applications, owing to its 46% protein content and low accumulation of HMs [113]. However, microalgae used for wastewater treatment usually coexist with different types of microorganisms, including bacteria, zooplankton, and debris, affecting total biomass quality. It has been found that microalgae grown on agroindustrial wastewaters demonstrate efficient nutrient removal capacity, although poorer biomass characteristics and lower than 30% algae abundance, when compared with purple phototrophic bacteria [114].

Microalgal biomass also constitutes a valuable source of pigments, mainly chlorophylls and carotenoids, which have been correlated with numerous health benefits, including prevention of cancer and cardiovascular diseases, antioxidant, antiinflammatory, and antidiabetic activity, as well as several applications as natural colorants, cosmetics, food, and feed supplements [115,116]. With view to alleviating environmental concerns and decreasing the upstream cost, POME was used to cultivate *Phaeodactylum tricornutum* for production of bioactive compounds, resulting in fucoxanthin productivity of 25.4 μg/L day, under 30% (v/v) effluent supplemented with urea [117]. In addition, carotenoid production of *Phormidium autumnale* at industrial scale can reach up to 108 ton year, under heterotrophic cultivation with slaughterhouse waste [118]. Besides pigments, several benefits and subsequent applications can stem from valuable biomass ingredients, including vitamins, minerals, polyunsaturated fatty acids, phenols, and phytosterols. Additionally, agriculture represents another area of application for microalgal biomass, due to the similarities and even enhanced characteristics compared to conventional fertilizers. Several microalgal species, including *Chlorella* and *Arthrospira* with high nutrient removal capacity, can return valuable nutrients and micronutrients, as well as soil organic carbon, substantially improving soil quality and crop growth and coping with macro- and micronutrient deficiencies [119]. Lastly, high ash content of microalgae can be an advantageous characteristic, such in case of biomass of *Scenedesmus* and *Desmodesmus* sp. grown in effluent from a stabilization lagoon, which ash concentration close to 20% and low concentration of HMs can result in a high-quality digestate used as biofertilizer [120].

Concluding Remarks

In conclusion, microalgae-based wastewater treatment and biomass valorization represent a wide area of research and development with great potential. Even though having several bottlenecks, mainly in terms of economics, downstream processing and scale-up have to be handled, many strategies anticipated to meet these challenges exist. To this end, exploitation of wastewater and effluents for microalgal cultivation and bioproduct formation offer significant advantages both for the environment and modern societies' needs, thus it is expected to culminate in the near future.

REFERENCES

[1] J.P. Maity, J. Bundschuh, C.Y. Chen, P. Bhattacharya, Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: present and future perspectives − a mini review, Energy 78 (2014) 104−113.

[2] J.-H. Wang, T.-Y. Zhang, G.-H. Dao, X.-Q. Xu, X.-X. Wang, H.-Y. Hu, Microalgae-based advanced municipal wastewater treatment for reuse in water bodies, Appl. Microbiol. Biotechnol. 101 (2017) 2659−2675.

[3] S. Raghuvanshi, V. Bhakar, R. Chava, K.S. Sangwan, Comparative study using life cycle approach for the biodiesel production from microalgae grown in wastewater and fresh water, in: Procedia CIRP, Elsevier, 2018, pp. 568−572.

[4] E. Koutra, G. Grammatikopoulos, M. Kornaros, Microalgal post-treatment of anaerobically digested agroindustrial wastes for nutrient removal and lipids production, Bioresour. Technol. 224 (2017) 473−480.

[5] Y. Ye, H.H. Ngo, W. Guo, Y. Liu, S.W. Chang, D.D. Nguyen, H. Liang, J. Wang, A critical review on ammonium recovery from wastewater for sustainable wastewater management, Bioresour. Technol. 268 (2018) 749−758.

[6] G. Markou, L. Wang, J. Ye, A. Unc, Using agro-industrial wastes for the cultivation of microalgae and duckweeds: contamination risks and biomass safety concerns, Biotechnol. Adv 36 (2018) 1238−1254.

[7] H. Kamyab, F. Friedler, S. Chelliapan, S. Rezania, J. Sultan Yahya Petra, K. Lumpur, Bioenergy production and nutrients removal by green microalgae with

cultivation from agro-wastewater palm oil mill effluent (POME)-A review, Chem. Eng. Trans. 70 (2018).

[8] E.J. Olguín, Phycoremediation: key issues for cost-effective nutrient removal processes, in: Biotechnol. Adv., Elsevier, 2003, pp. 81−91.

[9] R. Verma, A. Srivastava, Carbon dioxide sequestration and its enhanced utilization by photoautotroph microalgae, Environ. Dev. 27 (2018) 95−106.

[10] M.A. Borowitzka, Chapter 3 − biology of microalgae, in: I.A. Levine, J. Fleurence (Eds.), Microalgae Heal. Dis. Prev., Academic Press, 2018, pp. 23−72.

[11] V.C. Eze, S.B. Velasquez-Orta, A. Hernández-García, I. Monje-Ramírez, M.T. Orta-Ledesma, Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration, Algal Res 32 (2018) 131−141.

[12] E. Becker, Microalgae — Biotechnology and Microbiology, Cambridge University Press, 1994.

[13] K.W. Chew, S.R. Chia, P.L. Show, T.C. Ling, S.S. Arya, J.S. Chang, Food waste compost as an organic nutrient source for the cultivation of *Chlorella vulgaris*, Bioresour. Technol. 267 (2018) 356−362.

[14] J. Hu, D. Nagarajan, Q. Zhang, J.-S. Chang, D.-J. Lee, Heterotrophic cultivation of microalgae for pigment production: a review, Biotechnol. Adv. 36 (2018) 54−67.

[15] W.B. Kong, H. Yang, Y.T. Cao, H. Song, S.F. Hua, C.G. Xia, Effect of glycerol and glucose on the enhancement of biomass, lipid and soluble carbohydrate production by *Chlorella vulgaris* in mixotrophic culture, Food Technol. Biotechnol. 51 (2013) 62−69.

[16] N. Poddar, R. Sen, G.J.O. Martin, Glycerol and nitrate utilisation by marine microalgae *Nannochloropsis salina* and *Chlorella* sp. and associated bacteria during mixotrophic and heterotrophic growth, Algal Res 33 (2018) 298−309.

[17] L. Lu, J. Wang, G. Yang, B. Zhu, K. Pan, Biomass and nutrient productivities of *Tetraselmis chuii* under mixotrophic culture conditions with various C:N ratios, Chin. J. Oceanol. Limnol. 35 (2017) 303−312.

[18] A. Otondo, B. Kokabian, S. Stuart-Dahl, V.G. Gude, Energetic evaluation of wastewater treatment using microalgae, *Chlorella vulgaris*, J. Environ. Chem. Eng. 6 (2018) 3213−3222.

[19] R. Whitton, F. Ometto, M. Pidou, P. Jarvis, R. Villa, B. Jefferson, Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, Environ. Technol. Rev. 4 (2015) 133−148.

[20] L. Leng, J. Li, Z. Wen, W. Zhou, Use of microalgae to recycle nutrients in aqueous phase derived from hydrothermal liquefaction process, Bioresour. Technol. 256 (2018) 529−542.

[21] G. Markou, D. Vandamme, K. Muylaert, Ammonia inhibition on *Arthrospira platensis* in relation to the initial biomass density and pH, Bioresour. Technol. 166 (2014) 259−265.

[22] F.M. Santos, J.C.M. Pires, Nutrient recovery from wastewaters by microalgae and its potential application as bio-char, Bioresour. Technol. 267 (2018) 725−731.

[23] A.K. Sahu, J. Siljudalen, T. Trydal, B. Rusten, Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion, J. Environ. Manag. 122 (2013) 113−120.

[24] J. Coppens, R. Lindeboom, M. Muys, W. Coessens, A. Alloul, K. Meerbergen, B. Lievens, P. Clauwaert, N. Boon, S.E. Vlaeminck, Nitrification and microalgae cultivation for two-stage biological nutrient valorization from source separated urine, Bioresour. Technol. 211 (2016) 41−50.

[25] H. Kageyama, R. Waditee-Sirisattha, Mycosporine-like amino acids as multifunctional secondary metabolites in cyanobacteria: from biochemical to application aspects, in: Stud. Nat. Prod. Chem., Elsevier, 2019, pp. 153−194.

[26] A. Solovchenko, A.M. Verschoor, N.D. Jablonowski, L. Nedbal, Phosphorus from wastewater to crops: an alternative path involving microalgae, Biotechnol. Adv. 34 (2016) 550−564.

[27] S.T. Dyhrman, Nutrients and their acquisition: phosphorus physiology in microalgae, in: Physiol. Microalgae, Springer International Publishing, Cham, 2016, pp. 155−183.

[28] A. Quigg, Micronutrients, in: R.J. Borowitzka MA, J. Beardall (Eds.), Physiol. Microalgae, Springer International Publishing, Cham, 2016, pp. 211−231.

[29] A. Anbalagan, S. Schwede, C.F. Lindberg, E. Nehrenheim, Influence of iron precipitated condition and light intensity on microalgae activated sludge based wastewater remediation, Chemosphere 168 (2017) 1523−1530.

[30] D. Lannuzel, M. Grotti, M.L. Abelmoschi, P. van der Merwe, Organic ligands control the concentrations of dissolved iron in Antarctic sea ice, Mar. Chem. 174 (2015) 120−130.

[31] T.R. Devlin, A. Di Biase, V. Wei, M. Elektorowicz, J.A. Oleszkiewicz, Removal of soluble phosphorus from surface water using iron (Fe-Fe) and aluminum (Al-Al) electrodes, Environ. Sci. Technol. 51 (2017) 13825−13833.

[32] R. Saavedra, R. Muñoz, M.E. Taboada, M. Vega, S. Bolado, Comparative uptake study of arsenic, boron, copper, manganese and zinc from water by different green microalgae, Bioresour. Technol. 263 (2018) 49−57.

[33] C.M. Monteiro, S.C. Fonseca, P.M.L. Castro, F.X. Malcata, Toxicity of cadmium and zinc on two microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*, from Northern Portugal, J. Appl. Phycol. 23 (2011) 97−103.

[34] M.S. Rodrigues, L.S. Ferreira, J.C.M. de Carvalho, A. Lodi, E. Finocchio, A. Converti, Metal biosorption onto dry biomass of *Arthrospira (Spirulina) platensis* and *Chlorella vulgaris*: multi-metal systems, J. Hazard Mater. 217−218 (2012) 246−255.

[35] D. Hoh, S. Watson, E. Kan, Algal biofilm reactors for integrated wastewater treatment and biofuel production: a review, Chem. Eng. J. 287 (2016) 466−473.

[36] S.P. Cuellar-Bermudez, G.S. Aleman-Nava, R. Chandra, J.S. Garcia-Perez, J.R. Contreras-Angulo, G. Markou, K. Muylaert, B.E. Rittmann, R. Parra-Saldivar, Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater, Algal Res 24 (2017) 438−449.
[37] Z.N. Norvill, A. Shilton, B. Guieysse, Emerging contaminant degradation and removal in algal wastewater treatment ponds: identifying the research gaps, J. Hazard Mater. 313 (2016) 291−309.
[38] Y. Wang, S.-H. Ho, C.-L. Cheng, W.-Q. Guo, D. Nagarajan, N.-Q. Ren, D.-J. Lee, J.-S. Chang, Perspectives on the feasibility of using microalgae for industrial wastewater treatment, Bioresour. Technol. 222 (2016) 485−497.
[39] M.B. Ahmed, J.L. Zhou, H.H. Ngo, W. Guo, N.S. Thomaidis, J. Xu, Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review, J. Hazard Mater. 323 (2017) 274−298.
[40] T. Ding, K. Lin, B. Yang, M. Yang, J. Li, W. Li, J. Gan, Biodegradation of naproxen by freshwater algae *Cymbella* sp. and *Scenedesmus quadricauda* and the comparative toxicity, Bioresour. Technol. 238 (2017) 164−173.
[41] T. Ding, K. Lin, L. Bao, M. Yang, J. Li, B. Yang, J. Gan, Biouptake, Toxicity and biotransformation of triclosan in diatom *Cymbella* sp. and the influence of humic acid, Environ. Pollut. 234 (2018) 231−242.
[42] C.E. Gattullo, H. Bährs, C.E.W. Steinberg, E. Loffredo, Removal of bisphenol A by the freshwater green alga *Monoraphidium braunii* and the role of natural organic matter, Sci. Total Environ. 416 (2012) 501−506.
[43] S. Wang, K. Poon, Z. Cai, Removal and metabolism of triclosan by three different microalgal species in aquatic environment, J. Hazard Mater. 342 (2018) 643−650.
[44] S. Wang, X. Wang, K. Poon, Y. Wang, S. Li, H. Liu, S. Lin, Z. Cai, Removal and reductive dechlorination of triclosan by *Chlorella pyrenoidosa*, Chemosphere 92 (2013) 1498−1505.
[45] J.Q. Xiong, M.B. Kurade, B.H. Jeon, Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris*, Chem. Eng. J. 313 (2017) 1251−1257.
[46] M.A. Alam, C. Wan, X.Q. Zhao, L.J. Chen, J.S. Chang, F.W. Bai, Enhanced removal of Zn^{2+} or Cd^{2+} by the flocculating *Chlorella vulgaris JSC-7*, J. Hazard Mater. 289 (2015) 38−45.
[47] L. Rugnini, G. Costa, R. Congestri, L. Bruno, Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media, Sci. Total Environ. 601−602 (2017) 959−967.
[48] F. Gao, C. Li, Z.H. Yang, G.M. Zeng, J. Mu, M. Liu, W. Cui, Removal of nutrients, organic matter, and metal from domestic secondary effluent through microalgae cultivation in a membrane photobioreactor, J. Chem. Technol. Biotechnol. 91 (2016) 2713−2719.
[49] C. Escapa, R.N. Coimbra, S. Paniagua, A.I. García, M. Otero, Comparative assessment of diclofenac removal from water by different microalgae strains, Algal Res 18 (2016) 127−134.
[50] M.K. Ji, A.N. Kabra, J. Choi, J.H. Hwang, J.R. Kim, R.A.I. Abou-Shanab, Y.K. Oh, B.H. Jeon, Biodegradation of bisphenol A by the freshwater microalgae *Chlamydomonas mexicana* and *Chlorella vulgaris*, Ecol. Eng. 73 (2014) 260−269.
[51] M.B. Kurade, J.R. Kim, S.P. Govindwar, B.H. Jeon, Insights into microalgae mediated biodegradation of diazinon by *Chlorella vulgaris*: microalgal tolerance to xenobiotic pollutants and metabolism, Algal Res 20 (2016) 126−134.
[52] J.Q. Xiong, M.B. Kurade, B.H. Jeon, Ecotoxicological effects of enrofloxacin and its removal by monoculture of microalgal species and their consortium, Environ. Pollut. 226 (2017) 486−493.
[53] J. Cheng, Q. Ye, K. Li, J. Liu, J. Zhou, Removing ethinylestradiol from wastewater by microalgae mutant *Chlorella PY-ZU1* with CO_2 fixation, Bioresour. Technol. 249 (2018) 284−289.
[54] Q.T. Gao, Y.S. Wong, N.F.Y. Tam, Removal and biodegradation of nonylphenol by different *Chlorella* species, Mar. Pollut. Bull. 63 (2011) 445−451.
[55] W. Liu, Q. Chen, N. He, K. Sun, D. Sun, X. Wu, S. Duan, Removal and biodegradation of 17β-estradiol and diethylstilbestrol by the freshwater microalgae *Raphidocelis subcapitata*, Int. J. Environ. Res. Public Health 15 (2018) 452.
[56] E. Parladé, A. Hom-Diaz, P. Blánquez, M. Martínez-Alonso, T. Vicent, N. Gaju, Effect of cultivation conditions on β-estradiol removal in laboratory and pilot-plant photobioreactors by an algal-bacterial consortium treating urban wastewater, Water Res. 137 (2018) 86−96.
[57] J.Q. Xiong, M.B. Kurade, B.H. Jeon, Can microalgae remove pharmaceutical contaminants from water? Trends Biotechnol. 36 (2018) 30−44.
[58] J. Lv, J. Feng, Q. Liu, S. Xie, J. Lv, J. Feng, Q. Liu, S. Xie, Microalgal cultivation in secondary effluent: recent developments and future work, Int. J. Mol. Sci. 18 (2017) 79.
[59] J. Shi, B. Podola, M. Melkonian, Application of a prototype-scale twin-layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae, Bioresour. Technol. 154 (2014) 260−266.
[60] H. Kamyab, M.F. Md Din, C.T. Lee, A. Keyvanfar, A. Shafaghat, M.Z.A. Majid, M. Ponraj, T.X. Yun, Lipid production by microalgae *Chlorella pyrenoidosa* cultivated in palm oil mill effluent (POME) using hybrid photo bioreactor (HPBR), Desalin. Water Treat. 55 (2015) 3737−3749.
[60a] K. Tsigkou, M. Sakarika, M. Kornaros, et al., Inoculum origin and waste solid content influence the biochemical methane potential of olive mill wastewater under mesophilic and thermophilic conditions, Biochem. Eng. J. 151 (2019) 107301.

[61] S.L. Lim, W.L. Chu, S.M. Phang, Use of *Chlorella vulgaris* *f*or bioremediation of textile wastewater, Bioresour. Technol. 101 (2010) 7314–7322.

[62] B.H. Diya'uddeen, W.M.A.W. Daud, A.R. Abdul Aziz, Treatment technologies for petroleum refinery effluents: a review, Process Saf. Environ. Prot. 89 (2011) 95–105.

[63] C. Gómez-Serrano, M.M. Morales-Amaral, F.G. Acién, R. Escudero, J.M. Fernández-Sevilla, E. Molina-Grima, Utilization of secondary-treated wastewater for the production of freshwater microalgae, Appl. Microbiol. Biotechnol. 99 (2015) 6931–6944.

[64] Y.H. Wu, S.F. Zhu, Y. Yu, X.J. Shi, G.X. Wu, H.Y. Hu, Mixed cultivation as an effective approach to enhance microalgal biomass and triacylglycerol production in domestic secondary effluent, Chem. Eng. J. 328 (2017) 665–672.

[65] H. Kamyab, M.F.M. Din, A. Keyvanfar, M.Z.A. Majid, A. Talaiekhozani, A. Shafaghat, C.T. Lee, L.J. Shiun, H.H. Ismail, Efficiency of microalgae *Chlamydomonas* on the removal of pollutants from palm oil mill effluent (POME), Energy Procedia 75 (2015) 2400–2408.

[66] C.S. Vairappan, A.M. Yen, Palm oil mill effluent (POME) cultured marine microalgae as supplementary diet for rotifer culture, J. Appl. Phycol. 20 (2008) 603–608.

[67] M.C.,D.S. Hadiyanto, Phytoremediation of palm oil mill effluent (POME) by using aquatic plants and microalge for biomass production, J. Environ. Sci. Technol. 6 (2013) 79–90.

[68] A. Cicci, M. Stoller, M. Bravi, Microalgal biomass production by using ultra- and nanofiltration membrane fractions of olive mill wastewater, Water Res. 47 (2013) 4710–4718.

[69] G. Hodaifa, A. Malvis Romero, M. Halioui, S. Sánchez, Combined process for olive oil mill wastewater treatment based in flocculation, photolysis, microfiltration and microalgae culture, in: N. Khelifi, A. Kallel, M. Ksibi, H. Ben Dhia (Eds.), Recent Adv. Environ. Sci. From Euro-Mediterranean Surround. Reg. EMCEI 2017. Adv. Sci. Technol. Innov. (IEREK Interdiscip. Ser. Sustain. Dev., Springer, Cham, 2018, pp. 1127–1129.

[70] F. Di Caprio, P. Altimari, F. Pagnanelli, Integrated microalgae biomass production and olive mill wastewater biodegradation: optimization of the wastewater supply strategy, Chem. Eng. J. 349 (2018) 539–546.

[71] G. Pinto, A. Pollio, L. Previtera, M. Stanzione, F. Temussi, Removal of low molecular weight phenols from olive oil mill wastewater using microalgae, Biotechnol. Lett. 25 (2003) 1657–1659.

[72] F.M. Ghazal, E.-S.M. Mahdy, M.S.A. EL-Fattah, A. Elg, Y. EL-Sadany, N.M.E. Doha, The use of microalgae in bioremediation of the textile wastewater effluent, Nat. Sci. 3 (2018) 98–104.

[73] H.Y. El-Kassas, L.A. Mohamed, Bioremediation of the textile waste effluent by *Chlorella vulgaris*, Egypt, J. Aquat. Res. 40 (2014) 301–308.

[74] K. Suresh Kumar, H.-U. Dahms, E.-J. Won, J.-S. Lee, K.-H. Shin, Microalgae – a promising tool for heavy metal remediation, Ecotoxicol. Environ. Saf. 113 (2015) 329–352.

[75] H.V. Perales-Vela, J.M. Peña-Castro, R.O. Cañizares-Villanueva, Heavy metal detoxification in eukaryotic microalgae, Chemosphere 64 (2006) 1–10.

[76] H. Doshi, A. Ray, I.L. Kothari, Bioremediation potential of live and dead *Spirulina*: spectroscopic, kinetics and SEM studies, Biotechnol. Bioeng. 96 (2007) 1051–1063.

[77] C.M. Monteiro, A.P.G.C. Marques, P.M.L. Castro, F.X. Malcata, Characterization of *Desmodesmus pleiomorphus* isolated from a heavy metal-contaminated site: biosorption of zinc, Biodegradation 20 (2009) 629–641.

[78] A. Takáčová, M. Smolinská, M. Semerád, P. Matúš, Degradation of BTEX by microalgae *Parachlorella kessleri*, Pet. Coal. 57 (2015) 101–107.

[79] Z.A. Ansari, M.C. Saldanha, R. Rajkumar, Effects of petroleum hydrocarbons on the growth of a microalga, *Isochrysis sp. (*Chrysophyta), Indian J. Mar. Sci. 26 (1997) 372–376.

[80] J.D. Walker, R.R. Colwell, L. Petrakis, Degradation of petroleum by an alga, Prototheca zopfii., Appl. Microbiol. 30 (1975) 79–81.

[81] A. Hodges, Z. Fica, J. Wanlass, J. VanDarlin, R. Sims, Nutrient and suspended solids removal from petrochemical wastewater via microalgal biofilm cultivation, Chemosphere 174 (2017) 46–48.

[82] V. Matamoros, R. Gutiérrez, I. Ferrer, J. García, J.M. Bayona, Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study, J. Hazard Mater. 288 (2015) 34–42.

[83] J.-Q.Q. Xiong, M.B. Kurade, R.A.I.I. Abou-Shanab, M.-K.K. Ji, J. Choi, J.O. Kim, B.-H.H. Jeon, Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana a*nd S*cenedesmus obliquus and* the determination of its metabolic fate, Bioresour. Technol. 205 (2016) 183–190.

[84] V. Matamoros, E. Uggetti, J. García, J.M. Bayona, Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study, J. Hazard Mater. 301 (2016) 197–205.

[85] K. Ramadass, M. Megharaj, K. Venkateswarlu, R. Naidu, Toxicity of diesel water accommodated fraction toward microalgae, *Pseudokirchneriella subcapitata a*nd Ch*lorella sp*, *MM3*, Ecotoxicol. Environ. Saf. 142 (2017) 538–543.

[86] M. Sakarika, M. Kornaros, Kinetics of growth and lipids accumulation in *Chlorella vulgaris* during batch heterotrophic cultivation: effect of different nutrient limitation strategies, Bioresour. Technol. 243 (2017) 356–365.

[87] A. Juneja, R.M. Ceballos, G.S. Murthy, Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review, Energies 6 (2013) 4607–4638.

[88] M.A. Borowitzka, High-value products from microalgae-their development and commercialisation, J. Appl. Phycol. 25 (2013) 743–756.
[89] M. Koller, A. Muhr, G. Braunegg, Microalgae as versatile cellular factories for valued products, Algal Res 6 (2014) 52–63.
[90] T.-Y. Zhang, H.-Y. Hu, Y.-H. Wu, L.-L. Zhuang, X.-Q. Xu, X.-X. Wang, G.-H. Dao, Promising solutions to solve the bottlenecks in the large-scale cultivation of microalgae for biomass/bioenergy production, Renew. Sustain. Energy Rev. 60 (2016) 1602–1614.
[91] E.S. Salama, M.B. Kurade, R.A.I. Abou-Shanab, M.M. El-Dalatony, I.S. Yang, B. Min, B.H. Jeon, Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation, Renew. Sustain. Energy Rev. 79 (2017) 1189–1211.
[92] B. Behera, A. Acharya, I.A. Gargey, N. Aly, P. Balasubramanian, Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production, Bioresour. Technol. Reports. 5 (2019) 297–316.
[93] P. Vo Hoang Nhat, H.H. Ngo, W.S. Guo, S.W. Chang, D.D. Nguyen, P.D. Nguyen, X.T. Bui, X.B. Zhang, J.B. Guo, Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation? Bioresour. Technol. 256 (2018) 491–501.
[94] S.-Y. Chiu, C.-Y. Kao, T.-Y. Chen, Y.-B. Chang, C.-M. Kuo, C.-S. Lin, Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource, Bioresour. Technol. 184 (2015) 179–189.
[95] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: status and prospects, Renew. Sustain. Energy Rev. 19 (2013) 360–369.
[96] M.K. Lam, M.I. Yusoff, Y. Uemura, J.W. Lim, C.G. Khoo, K.T. Lee, H.C. Ong, Cultivation of *Chlorella vulgaris* using nutrients source from domestic wastewater for biodiesel production: growth condition and kinetic studies, Renew. Energy 103 (2017) 197–207.
[97] M.K. Ji, H.S. Yun, B.S. Hwang, A.N. Kabra, B.H. Jeon, J. Choi, Mixotrophic cultivation of *Nephroselmis sp.* using industrial wastewater for enhanced microalgal biomass production, Ecol. Eng. 95 (2016) 527–533.
[98] S.B. Ummalyma, R.K. Sukumaran, Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load, Bioresour. Technol. 165 (2014) 295–301.
[99] W.T. Chen, Y. Zhang, J. Zhang, G. Yu, L.C. Schideman, P. Zhang, M. Minarick, Hydrothermal liquefaction of mixed-culture algal biomass from wastewater treatment system into bio-crude oil, Bioresour. Technol. 152 (2014) 130–139.
[100] C.Y. Chen, X.Q. Zhao, H.W. Yen, S.H. Ho, C.L. Cheng, D.J. Lee, F.W. Bai, J.S. Chang, Microalgae-based carbohydrates for biofuel production, Biochem. Eng. J. 78 (2013) 1–10.
[101] Z. Reyimu, D. Özçimen, Batch cultivation of marine microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal wastewater toward bioethanol production, J. Clean. Prod. 150 (2017) 40–46.
[102] A.L. Marques, F.P. Pinto, O.Q.F. Araújo, M.C. Cammarota, Assessment of methods to pretreat microalgal biomass for enhanced biogas production, J. Sustain. Dev. Energy, Water Environ. Syst. 6 (2018) 394–404.
[103] J.C. Meneses-Reyes, G. Hernández-Eugenio, D.H. Huber, N. Balagurusamy, T. Espinosa-Solares, Oil-extracted *Chlorella vulgaris* biomass and glycerol bioconversion to methane via continuous anaerobic co-digestion with chicken litter, Renew. Energy 128 (2018) 223–229.
[104] B. Molinuevo-Salces, A. Mahdy, M. Ballesteros, C. González-Fernández, From piggery wastewater nutrients to biogas: microalgae biomass revalorization through anaerobic digestion, Renew. Energy 96 (2016) 1103–1110.
[105] M.P. Caporgno, A. Taleb, M. Olkiewicz, J. Font, J. Pruvost, J. Legrand, C. Bengoa, Microalgae cultivation in urban wastewater: nutrient removal and biomass production for biodiesel and methane, Algal Res 10 (2015) 232–239.
[106] L. Yang, X. Tan, B. Si, F. Zhao, H. Chu, X. Zhou, Y. Zhang, Nutrients recycling and energy evaluation in a closed microalgal biofuel production system, Algal Res 33 (2018) 399–405.
[107] E. Koutra, C.N. Economou, P. Tsafrakidou, M. Kornaros, Bio-based products from microalgae cultivated in digestates, Trends Biotechnol. 36 (2018) 819–833.
[108] A.P. Batista, L. Ambrosano, S. Graça, C. Sousa, P.A.S.S. Marques, B. Ribeiro, E.P. Botrel, P. Castro Neto, L. Gouveia, Combining urban wastewater treatment with biohydrogen production - an integrated microalgae-based approach, Bioresour. Technol. 184 (2015) 230–235.
[109] D. Sengmee, B. Cheirsilp, T.T. Suksaroge, P. Prasertsan, Biophotolysis-based hydrogen and lipid production by oleaginous microalgae using crude glycerol as exogenous carbon source, Int. J. Hydrogen Energy 42 (2017) 1970–1976.
[110] C.V. García Prieto, F.D. Ramos, V. Estrada, M.A. Villar, M.S. Diaz, Optimization of an integrated algae-based biorefinery for the production of biodiesel, astaxanthin and PHB, Energy 139 (2017) 1159–1172.
[111] M. van der Spiegel, M.Y. Noordam, H.J. van der Fels-Klerx, Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production, Compr. Rev. Food Sci. Food Saf. 12 (2013) 662–678.
[112] W. Verstraete, P. Clauwaert, S.E. Vlaeminck, Used water and nutrients: recovery perspectives in a 'panta rhei' context, Bioresour. Technol. 215 (2016) 199–208.
[113] J. Cheng, J. Xu, Y. Huang, Y. Li, J. Zhou, K. Cen, Growth optimisation of microalga mutant at high CO2 concentration to purify undiluted anaerobic digestion effluent

of swine manure, Bioresour. Technol. 177 (2015) 240−246.
[114] T. Hülsen, K. Hsieh, Y. Lu, S. Tait, D.J. Batstone, Simultaneous treatment and single cell protein production from agri-industrial wastewaters using purple phototrophic bacteria or microalgae − a comparison, Bioresour. Technol. 254 (2018) 214−223.
[115] G. Markou, E. Nerantzis, Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions, Biotechnol. Adv. 31 (2013) 1532−1542.
[116] A. Gille, U. Neumann, S. Louis, S.C. Bischoff, K. Briviba, Microalgae as a potential source of carotenoids: comparative results of an in vitro digestion method and a feeding experiment with C57BL/6J mice, J. Funct. Foods. 49 (2018) 285−294.
[117] M.M.A. Nur, W. Muizelaar, P. Boelen, A.G.J. Buma, Environmental and nutrient conditions influence fucoxanthin productivity of the marine diatom *Phaeodactylum tricornutum* grown on palm oil mill effluent, J. Appl. Phycol. (2018) 1−12.
[118] D.B. Rodrigues, É.M.M. Flores, J.S. Barin, A.Z. Mercadante, E. Jacob-Lopes, L.Q. Zepka, Production of carotenoids from microalgae cultivated using agroindustrial wastes, Food Res. Int. 65 (2014) 144−148.
[119] E. Alobwede, J.R. Leake, J. Pandhal, Circular economy fertilization: testing micro and macro algal species as soil improvers and nutrient sources for crop production in greenhouse and field conditions, Geoderma 334 (2019) 113−123.
[120] G.S. Diniz, A.F. Silva, O.Q.F. Araújo, R.M. Chaloub, The potential of microalgal biomass production for biotechnological purposes using wastewater resources, J. Appl. Phycol. 29 (2017) 821−832.