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Review

Can Microalgae Remove Pharmaceutical Contaminants from Water?

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The increase in worldwide water contamination with numerous pharmaceutical contaminants (PCs) has become an emerging environmental concern due to their considerable ecotoxicities and associated health issues. Microalgae-mediated bioremediation of PCs has recently gained scientific attention, as microalgal bioremediation is a solar-power driven, ecologically comprehensive, and sustainable reclamation strategy. In this review, we comprehensively describe the current research on the possible roles and applications of microalgae for removing PCs from aqueous media. We summarize several novel approaches including constructing microbial consortia, acclimation, and cometabolism for enhanced removal of PCs by microalgae, which would improve practical feasibility of these technologies. Some novel concepts for degrading PCs using integrated processes and genetic modifications to realize algal-based bioremediation technologies are also recommended.

Trends

Water contamination with numerous pharmaceutical contaminants (PCs) has been one of the most important emerging environmental problems facing humanity due to their ecotoxicities and health issues.

Culturing microalgae in wastewater can create a 'zero-waste concept' and stimulate an effective and sustainable practice for the microalgae biofuel industry.

Constructing microbial consortia, acclimating microorganisms, and cometabolic approaches can improve the engineering feasibility of microalgae-based biotechnologies.

Some innovative concepts, such as integrated processes (algae-based technologies with advanced oxidation processes, constructed wetlands, and microbial fuel cells) and genetic modifications, can help to realize algae-based bioremediation technologies.

Pharmaceutical Contaminants: An Emerging Concern

The security and scarcity of clean water for the safe livelihood of human beings have drawn concern worldwide due to the contamination of water resources by various micropollutants, including **pharmaceutical contaminants** (**PCs**, see Glossary) [1,2]. These synthetic chemicals can be transported through the atmosphere and water and, in many cases, find their way into sediments and soils (Box 1). Moreover, **multidrug-resistant efflux pumps** are present in all organisms and can exist in large numbers within single microorganisms, such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*. Low concentrations of PCs can be beneficial for bacteria by triggering specific transcriptional changes that are independent of the bacterial stress response pathways; this is especially pertinent for antibiotics, to which bacteria can develop resistance [3]. PCs can accumulate in different **trophic level** organisms including human beings through biomagnification in food chains due to their hydrophobic and persistent nature. Conventional activated-sludge processes in current wastewater treatment plants are not designed for the efficient removal of PCs [4]. Although several advanced treatment technologies are available for removing PCs (Box 2), the low removal efficiencies and/or limitations of these technologies motivated researchers to investigate better treatment strategies for the removal of these trace PCs. **Microalgae**-mediated **bioremediation** of PCs is of growing scientific interest. Its advantages include being driven by solar energy, the relatively small amounts of operational inputs, eco-friendliness, its role in the fixation and turnover of carbon, and its simultaneous production of high-value products such as nutraceuticals and cosmetics, low-value food products for aquaculture, and microalgal biomass for the production of biofuel and animal feed [5–7]. Mixotrophic microalgae can switch their metabolism between autotrophic and heterotrophic depending on the availability of carbon

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 http://dx.doi.org/10.1016/j.tibtech.2017.09.003
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Box 1. Ecotoxicological Concerns of Pharmaceutical Contaminants in the Environment

Pharmaceutical compounds (PCs) are a large and diverse group of organic compounds that are widely consumed throughout the world. PCs residues are ubiquitous in aquatic environments, which can be excreted from agricultural activities, hospital effluents, industrial wastes and domestic wastes. Although most of the recognized concentrations of PCs in the environment are at ng/l to μg/l levels, there is mounting evidence to show that they can induce adverse ecological effects on target- and non-target organisms, such as altering microbial communities, inhibiting the growth of microbes, reducing microbial activity of soil, and affecting the denitrification rate of bacteria. PCs are also normally persistent to biodegradation and have a long half-life time in the environment. Moreover, steroid PCs (estrone, 17β-estradiol, and 17α-ethynylestradiol) can act as endocrine disruptors, causing the widespread feminization of fish [71]. Amine-based PCs (e.g., ranitidine, nizatidine, doxylamine, and carbinoxamine) are the precursors to produce N,N-nitrosodimethylamine (NDMA), which forms due to reactions with disinfecting chemicals used in disinfection of drinking water. NDMA poses great health risks to human beings due to its carcinogenic properties, and California's Office of Environmental Health Hazard Assessment has a health goal of a maximum of 3 ng/l of this species in wastewater [71]. Antibiotics (macrolides, sulfonamides, tetracyclines, quinolones) can induce genetic resistance in bacteria. For example, the bacterium *Acinetobacter baumannii* is resistant to all of the current antibiotics as a result of its chronic adaptation in hospital settings [72]. Once the resistant genes are transferred into human beings through biomagnification in food webs, they compromise public health by preventing treatment of infections caused by these bacteria [73].

sources and nutrients in the surrounding environment, which provides them with great flexibility to survive and thrive in extreme environments. This capability of microalgae can overcome some of the major limitations associated with bacteria and fungi that require carbon and other nutrients in stoichiometric balance for growth and degradation of pollutants. The adaptability of mixotrophic microalgae makes them promising candidates for removal of contaminants in wastewater [5–9]. The capacity of mixotrophic microalgae to remove and uptake PCs from wastewater and/or synthetic wastewater has been demonstrated (Table 1) using different microalgae species (Box 3). Culturing microalgae in wastewater substantially reduces the need for chemical fertilizers/nutrients and their related burden on the life cycle. A 'zero-waste' concept can be implemented through the utilization of wastewater as nutrient source for the cultivation of mixotrophic microalgae (wastewater treatment through microalgal remediation) followed by the subsequent utilization of produced biomass as a feasible feedstock for sustainable biofuel production to stimulate a more sustainable practice for the microalgae biomass based biofuel industry. In this review, we comprehensively describe the current status of research activities and perspectives on the mechanistic aspects and applications of microalgae for removing PCs from wastewater.

Removal and Metabolic Mechanism of Pharmaceutical Contaminants by Microalgae

The mechanisms of PCs removal by microalgae include **bioadsorption**, **bioaccumulation**, and intracellular and extracellular **biodegradation** (Figure 1).

Bioadsorption of PCs by Microalgae

The bioadsorption of PCs by microalgae cells has been well reported. The amount of studied PCs (including diclofenac, ibuprofen, paracetamol, metoprolol, trimethoprim, carbamazepine, estrone, β-estradiol, and ethinylestradiol) adsorbed by microalgae has varied from 0 to 16.7% [10]. Additionally, the dead cell biomass of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* was found to adsorb approximately 10% of the available progesterone and norgestrel [11]. Algae cell walls possess assemblages of polymers with notable similarities to cellulose, pectins, hemicelluloses, arabinogalactan proteins, extensin, and lignin; the cell wall of microalgae is negatively charged as a result of the dominant functional groups, such as carboxyl, phosphoryl and amine. Because adsorption is extracellular, the sorption process varies significantly according to the hydrophobicity, structure, and functional groups of different PCs and microalgal species. Pollutants with cationic groups are actively attracted toward the microalgal surface through electrostatic interaction, resulting in effective biosorption.

Glossary

Acclimation: the process by which organisms improve their tolerance to stresses in order to adapt to a new environment.
Advanced oxidation processes (AOPs): a set of chemical treatment procedures designed to remove organic matter in water and wastewater by oxidation using highly reactive oxygen species such as hydroxyl radicals.
Antioxidant system: a defense system used by plants and microalgae that consists of enzymatic and nonenzymatic antioxidants to scavenge reactive oxygen species.
Bioaccumulation: an active metabolic process in a living organism to take up compounds, driven by energy.
Bioadsorption: the process of collecting soluble substances that are in solution on a suitable interface of a microorganism.
Biodegradation: disruption of compounds caused by enzymatic processes due to cellular metabolism.
Biofilm matrix: a mixture of extracellular polymeric substances (EPS), such as proteins, lipids and even nucleic acids in some cases, which provides skeletal support for member microbes. The matrix functions as an active part of a biofilm, where it acts as an electroconductive medium, as well as serving as a reservoir for nutrients, exoenzymes, siderophores, and signaling molecules.
Bioremediation: a biological treatment technique that uses organisms such as bacteria, fungi, and microalgae to remove contaminants from polluted sites. It is an environmentally friendly, ecologically comprehensive and sustainable reclamation strategy.
Cometabolism: any oxidation of substances without using the energy derived from oxidation to support microbial growth. It describes the situation in which an organism can biotransform a substrate but is unable to grow on it. A second meaning of cometabolism is to describe the degradation of a given compound by the combined effects of several organisms pooling their biochemical resources for mutual efforts.

Bioaccumulation of PCs in Microalgae

Bioaccumulation is an active metabolic process to uptake substrates, which is driven by energy. Microalgae can take up organic pollutants along with growth nutrients through bioaccumulation, as described in several reports. For example, a green alga, *Desmodesmus subspicatus*, accumulated approximately 23% of the radiolabeled 17α-ethinylestradiol (^{14}C-EE2) in 24 h [12]. The bioaccumulation of micropollutants by algae was determined to be an essential route in the removal of triclosan, trimethoprim, and sulfamethoxazole [13]. Carbamazepine has been reported to accumulate in several microalgal species such as *Chlamydomonas mexicana* and *Scenedesmus obliquus* [14]. The accumulated PCs in microalgae cells can induce the generation of **reactive oxygen species (ROS)**. ROS play dual roles in cells: (i) at normal levels, ROS act as essential signaling molecules to control cellular metabolism, including pathogen defense, programmed cell death, and stomatal behavior; and (ii) in excess, the strong oxidative properties of ROS cause severe damage to cellular components, such as lipids, proteins, and DNA, and an increased rate of mutagenesis ultimately leads to programmed cell death [15]. PCs do not exhibit lethal inhibition of microalgal activities at their environmental relative concentrations, demonstrated by the negligible growth inhibition of microalgae in the presence of PCs [14,16]. Therefore, microalgal species can become more resistant to PCs at low environmental concentrations through ROS, which can monitor and improve the production of related genes and expression of signals [15,17]. Expression of photosynthesis-related genes (*psbA, psaB, rbcL*, and *mcyB*) in *Microcystis aeruginosa* was significantly increased after exposure to antibiotics (spiramycin and amoxicillin) [18]. The pre-exposed microalga could remove cefradine more effectively than its wild species. The adaptation of this microalgal species to the antibiotic cefradine might have been responsible for the enhanced removal [19].

Intracellular Biodegradation of PCs by Microalgae

Biodegradation is the most effective way by which microalgae eliminate organic contaminants from an aqueous phase. Microalgae form simpler molecules by catalytically degrading complex parent compounds. In one example of biodegradation of progesterone by two freshwater microalgal species, *S. obliquus* and *C. pyrenoidosa* showed 95% biodegradation of the available progesterone in an aqueous medium [11]. In other studies, approximately 30–80% of pharmaceutically active compounds such as ibuprofen, caffeine, carbamazepine, and tris(2-chloroethyl) phosphate were biodegraded by microalgae in urban or synthetic wastewater [20–22].

Microalgae have a complex enzyme system that consists of phase I and phase II enzyme families. The initial attacks begin with the phase I enzyme (cytochrome 450), which makes a compound more hydrophilic by adding or unmasking a hydroxyl group and usually involves hydrolysis, oxidation, or reduction reactions (Figure 1B). Different types of enzymatic reactions of the PCs by microalgae, such as hydroxylation, carboxylation, oxidation, hydrogenation, glycosylation, demethylation, ring cleavage, decarboxylation, dehydroxylation, and bromination, have been reported during microalgal degradation [11,12,14,20,22–24]. Phase II enzymes, such as glutathione-S-transferases, catalyze the conjugation reaction between electrophilic compounds and glutathione. Phase II enzyme conjugation results in the opening of the epoxide ring to protect against oxidative damage. For example, glycosylation of ibuprofen by a freshwater diatom, *Navicula* sp., has been demonstrated [22]. An active role of various intracellular enzymes of microalgae, such as mono(di)oxygenase, soluble inorganic pyrophosphatase, glutamyl-tRNA reductase, malate/pyruvate dehydrogenase, uroporphyrinogen III carboxylase/decarboxylase, dehydratase, alkaline and acid phosphatase, transferase, catalase, laccases, violaxanthin de-epoxidase, and hydrolases, in biotransformation and detoxification of endogenous organic compounds has been reported [25–29].

Constructed wetlands (CWs): treatment systems that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages for treating municipal or industrial wastewater, greywater or storm water runoff.

Laccase: multicopper oxidoreductase enzymes that have broad substrate specificity and relative autonomy. They are considered to be the ultimate 'green catalysts' in environmental biotechnology because they use molecular oxygen from the air as an electron acceptor and only produce water as a byproduct.

Microalgae: eukaryotic organisms (of micrometer size) that are different from plant species. Microalgae do not possess roots, stems, and leaves, but can use solar energy and fix carbon dioxide through photosynthetic activities. They are often considered to prefer aquatic environments, but they also grow under numerous non-aqueous terrestrial habitats including soils, rocks, caves, modern buildings, and even living animals and plants. The ubiquity of algae in these habitats, besides freshwater, estuaries, and oceanic environments, and evidence of their presence in numerous stromatolites of the Archaean and the Mesozoic era give credence to the theory that they were one of the first organisms to appear on the Earth.

Microbial fuel cell (MFC): a device that converts chemical energy to electrical energy by microbial activity. These electrochemical cells are constructed using either a bioanode and/or a biocathode. Most of the MFCs contain a membrane to separate the compartments of the anode (where oxidation takes place) and the cathode (where reduction takes place).

Multidrug-resistant efflux pumps: proteinaceous transporters localized in the cytoplasmic membrane of cells. The genetic elements encoding efflux pumps may be encoded on chromosomes and/or plasmids, thus contributing to both intrinsic (natural) and acquired resistance, respectively. As an intrinsic mechanism of resistance, efflux pump genes can survive a hostile environment (for example in the presence of antibiotics), which allows

The biotransformation of these persistent organic compounds in microalgae is highly complex, and the exact role of these enzymes in biodegradation has not been fully investigated. However, omics analysis has started to lead to breakthroughs in discovering the enzymatic mechanism of PCs degradation. Metagenomics analyses are well suited to identify wild adapted microbial communities and to discover pollutant-degrading enzymes, which are naturally exposed to the contaminants in their own habitat and constitute a huge reservoir of enzymes [30]. Metatranscriptomics analyses have been used to accurately reveal the metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems of microalgae. For example, transcriptome analysis of *Picochlorum* SENEW3 showed that one-half of the coding regions were differentially expressed in response to a contaminant [25,32]. Proteomics and metabolomics also have been used to identify the enzymes responsible for biocatalysis and to reveal microalgal responses to pollutant-induced stresses [29,31,32].

Extracellular Degradation of PCs

Microalgae can also excrete various extracellular polymeric substances (EPS), including polysaccharides, protein, enzymes, substituents (polysaccharide-link methyl and acetyl groups), and lipids to their surrounding environment. The EPS can form a hydrated **biofilm matrix** that acts as an external digestive system by keeping extracellular enzymes close to the cells and enabling them to metabolize organic compounds, which are either in dissolved, colloidal, or solid form. The charged polysaccharides and proteins allow for the accumulation of nutrients from the environment and the sorption of xenobiotics, contributing to environmental detoxification [33]. The EPS can act as a surfactant and emulsifier to increase the bioavailability of the persistent PCs [34]. These interactions of different EPS and the interactions between EPS and microalgal cells can induce extracellular degradation of the organic compounds, such as PCs, and can also influence intracellular degradation through the byproducts formed [33,34].

Novel Approaches for Enhanced Removal of PCs by Microalgae

Bioremediation by Microbial Consortia

Microalgae are an effective platform for promoting electron and energy flow in microalgae–bacteria symbiosis (Figure 2A–C). Consortia of microalgae with cyanobacteria and bacteria are highly efficient for detoxifying organic and inorganic pollutants, and removing nutrients from wastewater, more than that of individual microorganisms [5,8,35]. A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photosequencing batch reactor has been developed [36]. This artificial algae and bacteria consortium demonstrated an elevated efficiency in degrading both the aliphatic and aromatic hydrocarbons of crude oil, whereas the contaminants were persistent to the individual strains [37]. Natural substrate colonization and artificial inoculation are the two main methods to obtain microalgae–bacteria consortium [38]. The oxygen and carbon dioxide cycles in the algae and bacteria consortium are beneficial. The metabolites from bacterial degradation of the contaminants can also act as promoters for the growth of algae. Here, algal exudates are the main carbon sources (carbohydrate, protein, and lipid) for bacteria. In addition, the cell surfaces of microalgae can provide a stable habitat for the bacteria [39,40]. Bacteria decompose organic matter into mineral form and secrete extracellular metabolites such as auxins and vitamin B12, which are essential for microalgal growth [40]. Thus, using algae–algae and algae–bacteria consortia to remediate emerging organic contaminants can be advantageous because synergistic interactions between the cocultivated microorganisms can be established, enhancing the overall uptake efficiency [36,37].

Competitive interaction between algae and bacteria also exists. For example, the growth of algae can significantly increase the pH of a solution to 9–10; some bacteria can secrete toxic active substances such as algaecides, which may affect photosynthesis-related gene transcription and block electron transport of microalgae [38]. However, multicultural systems tend to be more resistant to oscillations in environmental conditions due to the combination of

for the selection of mutants that overexpress these genes.

Pharmaceutical contaminants (PCs): antibiotics, analgesics, steroids, antidepressants, antipyretics, stimulants, antimicrobials, disinfectants, and many other chemicals that are widely manufactured, used, and emitted daily, mainly for protecting human beings from infection and diseases and as growth promoters in veterinary clinics.

Reactive oxygen species (ROS): both free radical (O_2^-, superoxide radicals; OH^-, hydroxyl radical; HO_2^-, perhydroxy radical and RO^-, alkoxy radicals) and nonradical forms (H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen), caused by various environmental stresses, which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates, and DNA. This damage ultimately results in oxidative stress.

Trophic level: the position of an organism occupies in a food chain.

Box 2. Current Treatment Technologies for the Removal of PCs

Different advanced technologies have been developed to remove PCs (Figure I). Advanced oxidation processes (AOPs) are effective technologies to remove these PCs; however, their utilization in large-scale applications has been restricted due to high operational and maintenance costs. Moreover, the incomplete mineralization of PCs during AOPs can generate byproducts that exhibit toxicity comparable to or greater than that of the parent compound [74]. Adsorption using powdered activated carbon or granular activated carbon as the sorbent to remove PCs was also investigated. Background organic matter can significantly affect the removal efficiencies, and such techniques only remove the contaminants from aqueous phase-by-phase separation instead of by mineralization [75]. Bioremediation using bacteria and fungi has primarily been investigated. In bacteria, the initial degradation efficiency of these PCs is low. To overcome this drawback, isolated bacteria with high PCs-biodegradability were used to remove these PCs. The utilization and acclimation of bacteria to remove antibiotics is a significant threat to humanity due to the fact that this can develop the antibiotic resistance in bacteria. Fungal morphology and slow catabolism kinetics prevent their utilization in large-scale applications. Moreover, additional carbon and nutrient sources are required to sustain the normal growth of bacteria and fungi for such treatments [67]. There is also a disadvantage to mineralize organic pollutants by bacteria and fungi as the fixed carbon is ultimately released to the atmospheric CO_2 pool [8]. A purified oxidative enzyme, laccase, was utilized to remediate these PCs. The high cost, low activities, selectivity, and poor stabilities under the process conditions and the low rate of reaction and limitation of the reaction environment of the enzymatic remediation restrict their large-scale utilization to remove PCs [76].

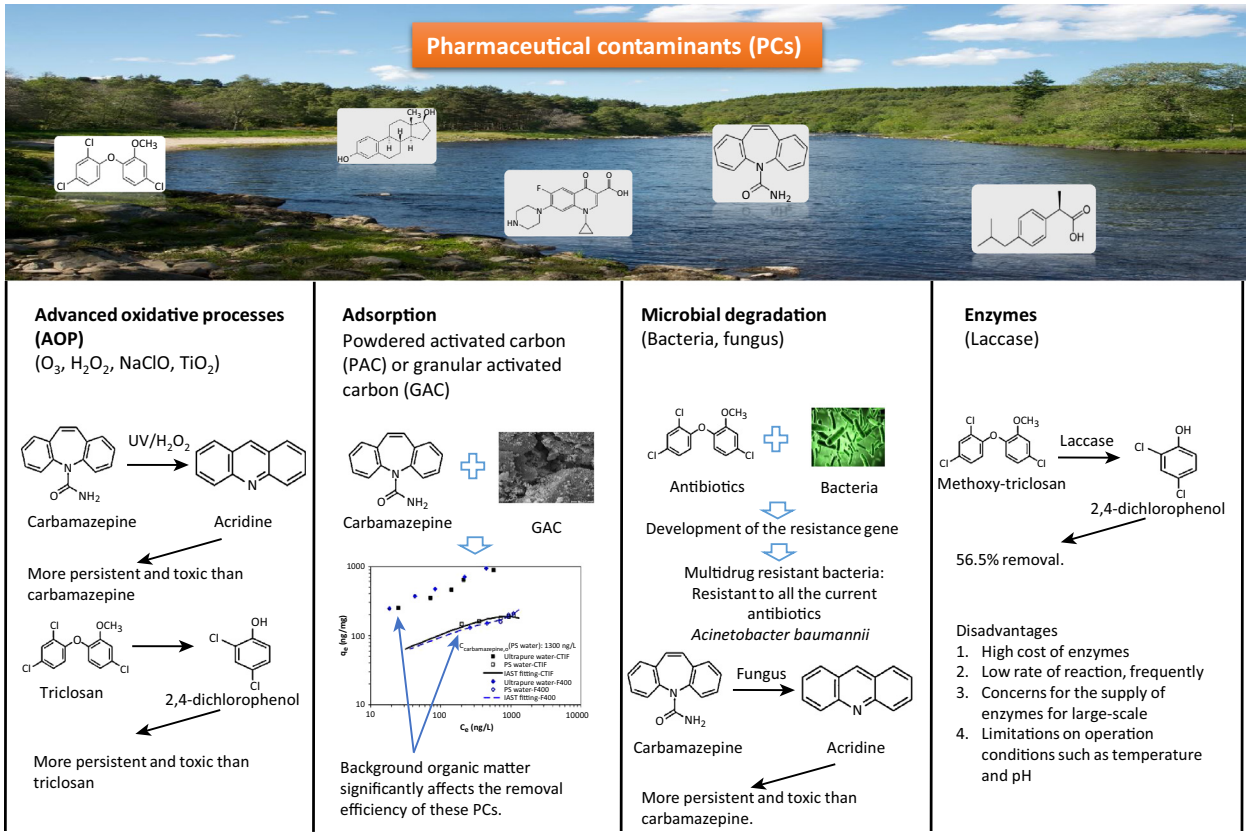

Figure I. Current Treatment Technologies for the Removal of PCs from the Aqueous Phase. Technologies include advanced oxidation processes, adsorption by PAC and GAC, microbial degradation using bacteria and fungi, and degradation by laccases.

microorganisms with different metabolic activities and resistance that are adapted to different environmental variations [5].

Acclimation

Extreme environments often support microalgae communities that live beyond their normal limits and tolerance. It is possible to improve the microalgal tolerance and biodegradable capacity toward hazardous compounds after an **acclimation** process by changing their living

Box 3. Tolerant Microalgae Species Used in Bioremediation of Different Wastewaters

Different microalgae species have been investigated for the bioremediation of different types of wastewater. The most desired characteristics of algae for use in wastewater treatment include high growth rate, high biochemical characteristic content and productivity, higher tolerance to possible pollutants (e.g., metal ions and toxic compounds) present in wastewater, high NH_4^+ tolerance, high O_2 generation rates, high CO_2 sinking capacity, and robust growth properties with improved tolerance for varied environmental conditions. Those tolerant microalgae species have been used to treat municipal wastewater, anaerobic digestion effluent, textile dye wastewater, agro-industrial wastewater, metal-containing wastewater, industrial effluent, sugar mill effluent, and various pharmaceutical contaminants in various algal bioreactors and high rate algal ponds (Figure I).

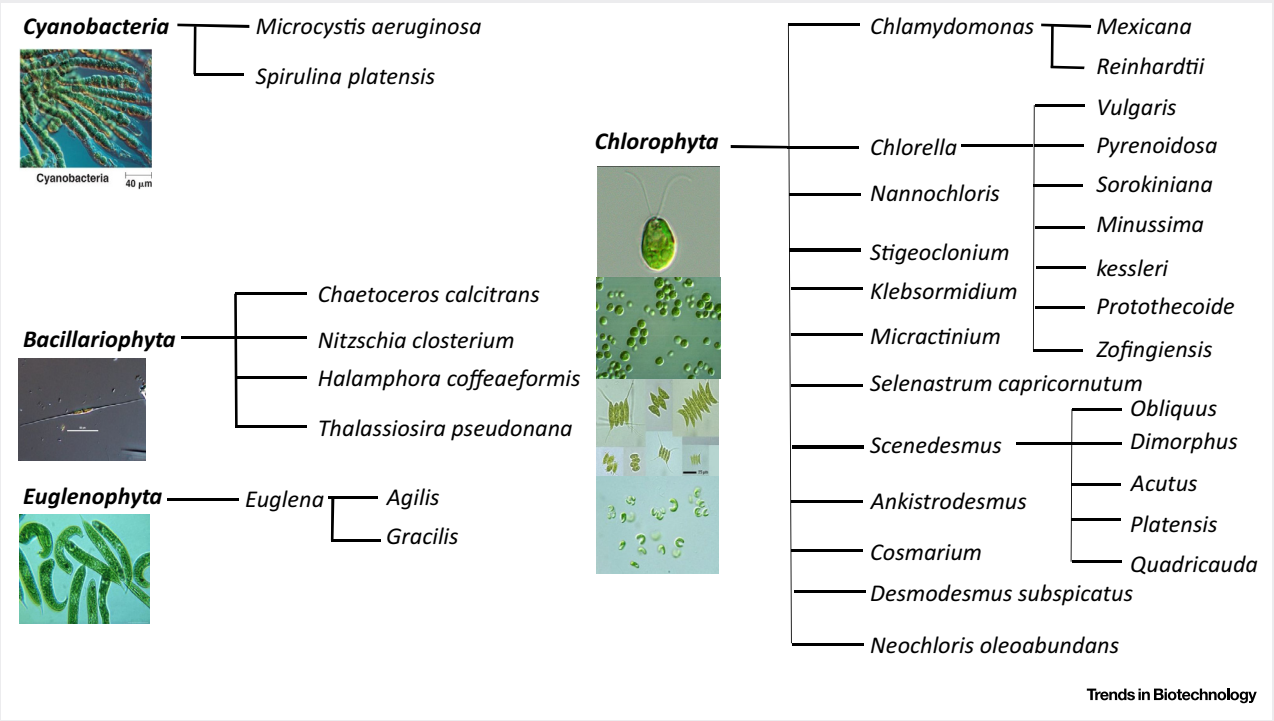

Figure I. Microalgal Species (Cyanobacteria, Bacillariophyta, Euglenophyta, and Chlorophyta) Commonly Used for Bioremediation of Wastewater and PCs.

environments [41–44]. It is most likely that the acclimated microalgae gain higher tolerance and biodegradation capacity to achieve better removal of contaminants at more realistic concentrations than the wild-type microalgal species. Previous studies reported that the acclimation of *Chlorella pyrenoidosa* to 60 mg/l cefradine resulted in a better removal efficiency [19]. The acclimation of six different microalgae species to wastewater for 8 weeks resulted in a significantly higher growth rate and biomass productivity than the normal one [42]. The acclimation of *Dunaliella salina* to high doses of phenol induced a higher growth rate and accumulation of valuable biochemical characteristics than the wild type microalgal species, which was significantly inhibited at the same concentrations [43]. The acclimation of *Chlorella vulgaris* into 200 mg/l of levofloxacin for 11 days resulted in a better removal efficiency (28%) than the wild species (16%) [44]. The adaptation mechanism of microalgae to extreme conditions, such as high doses of organic compounds, salt water, and strong light, has been explained by genetic changes caused by spontaneous mutation or physiological adaptation [42,43]. The improved capability of the acclimated microalgae may be explained by enhanced photosynthesis, carotenoid biosynthesis, **antioxidant system** activities, metabolism processing, genetic information processing, environmental information processing, or cellular processes [42–45]. However, in-depth investigation at the molecular level, especially omics

Table 1. Removal of Pharmaceutical Contaminants from Aqueous Phase by Microalgae

Algal species	Contaminants and removal efficiency (%)	Wastewater category	Refs
Chlorella sorokiniana	Diclofenac (40–60), ibuprofen (100), paracetamol (100), metoprolol (100), carbamazepine (30), trimethoprim (40)	Urine, anaerobically treated black water and synthetic urine	[10]
Scenedesmus obliquus, Chlorella pyrenoidosa	Progesterone (95), norgestrel (60–100)	BG11 medium	[11]
Desmodesmus subspicatus	17α-Ethynylestradiol (68)	M4 medium	[12]
Nannochloris sp.	Ibuprofen (40), trimethoprim (10), ciprofloxacin (100), carbamazepine (20), triclosan (100)	Lake Mead water	[13]
Chlamydomonas mexicana, Scenedesmus obliquus	Carbamazepine (30–37)	Bold's Basal medium	[14]
Microcystis aeruginosa	Amoxicillin (18–31)	BG11 medium	[18]
Chlorella pyrenoidosa	Cefradine (76)	BG11 medium	[19]
Microalgae consortia in high-rate algal ponds dominated by *Chlorella sp. and Scenedesmus sp.*	Caffeine (99), ibuprofen (99), carbamazepine (20%),	Urban or synthetic wastewater	[20]
Microalgae consortia in microalgal photobioreactor	Ketoprofen (36–85), naproxen (10–70), ibuprofen (98), acetaminophen (99), salicylic acid (33), paroxetine (94), lorazepam (30–60), alprazolam (87), atenolol (85–98), hydrochlorothiazide (44–84), erythromycin (85), azithromycin (89), ofloxacin (66), ciprofloxacin (47), diltiazem (72–77)	Lake water and pharmaceutical wastewater	[21]
Navicula sp.	Ibuprofen (60)	D1 medium	[22]
Chlorella vulgaris	Levofloxacin (10–92)	Bold's basal medium	[44]
Chlamydomonas mexicana	Ciprofloxacin (10–56)	Bold's basal medium	[49]
Microalgae consortia in high-rate algal ponds	Caffeine (98), acetaminophen (99), ibuprofen (99), naproxen (89), carbamazepine (62), diclofenac (92), triclosan (95)	Urban wastewater	[77]
Selenastrum capricornutum	Estradiol (88–100), 17α-ethynylestradiol (60–95)	Wastewater digestate and growth medium	[78]
Chlamydomonas reinhardtii	Estradiol (100), 17α-ethynylestradiol (100)		
Nannochloris sp.	Trimethoprim (0), sulfamethoxazole (32), triclosan (100)	F/2 algal culture medium	[79]
Floating aquatic macrophyte system, *Iris pseudacorus*, *Scirpus* sp. and *Carex* sp., *Lemna* and floating algae	Fluconazole (0–19), carbamazepine (0–15), diclofenac (65–71), venlafaxine (72-76), 2-hydroxy-CBZ (35–41), 3-hydroxy-CBZ (34–50), tramadol (54–62), oxazepam (27–37), sulfamethoxazole (49–53), trimethoprim (95–97), erythromycin (66–80), clarithromycin (51–70), metoprolol (73–75), atenolol (93–96), bezafibrate (73–80), acyclovir (92–97), codeine (92–95), diatrizoate (23–43), iomeprol (44–46)	Wastewater influent and effluents	[80]
Chlorella pyrenoidosa	Amoxicillin (77), cefradine (23)	BG11 medium	[81]
C. reinhardtii, S. obliquus, C. pyrenoidosa, C. vulgaris	17α-Boldenone (82–83), 17β-boldenone (75–86), carbamazepine (4–15), carbendazim (14–30), ciprofloxacin (74–79), clarithromycin (100), climbazole (30–70), clofibric acid (0–30), diclofenac (0), enrofloxacin (75–77), erythromycin–H_2O (63–86), estrone (85–88), fluconazole (25–28), gemfibrozil (0), ibuprofen (0), lincomycin (80–81), norfloxacin (41–53),	Wastewater influent	[82]

Table 1. (continued)

Algal species	Contaminants and removal efficiency (%)	Wastewater category	Refs
	ofloxacin (43–52), paracetamol (88–94), progesterone (83–87), roxithromycin (87–94), salicylic acid (97–99), salinomycin (71–79), sulfadiazine (52–75), sulfadimethoxine (56–78), sulfameter (81–88), sulfamethazine (18–48), sulfamethoxazole (0–18), sulfamonomethoxine (0), sulfapyridine (98–100), testosterone (100), triclocarbon (81–99), triclosan (31–58), trimethoprim (0–37), tylosin (75–77)		

analysis, is needed to explore the role of the stress conditions in improved removal of organic contaminants.

Cometabolism

Emerging organic contaminants showed susceptibility to microbial decomposition in a **cometabolic** system. Although only 19.1% of tetrabromobisphenol A was removed by the bacterium *Pseudomonas* sp. as a sole carbon source, more than 96% was removed with the addition of formate through a cometabolic mechanism [46]. Addition of acetate significantly improved the removal efficiency of clofibric acid, diclofenac, carbamazepine, and propyphenazone by conventional activated sludge [47]. Combining phenol and glucose with 4-chlorophenol significantly increased the removal efficiency of 4-chlorophenol, while only minor amounts of 4-chlorophenol were removed as a sole source for *Comamonas testosteroni* [48]. The remediation efficiency of ciprofloxacin was increased by more than a factor of three with the addition of a cometabolic substrate, acetate, in microalgae [49]. The additional organic substrates not only serve to sustain biomass production, but also act as an electron donor for the **cometabolism** of the non-growth substrate. The addition of growth substrate in culture can promote the activities of specific catabolic enzymes that are responsible for the degradation of the emerging organic contaminants. A previous study demonstrated that the presence of a cometabolic organic source, such as acetate, can induce specific enzymes involved in the degradation of toxic compounds like monooxygenase and N-deethylase [50]. The biotransformations of ibuprofen, ketoprofen, carbamazepine, dexamethasone, and iopromide were significantly improved by a stable nitrifying enrichment culture due to elevated ammonia monooxygenase [51]. The activities of dehydrogenase, catalase, and phenol oxidase were identified in the rhizosphere sediment of a plant, which improved the degradation of isoproturon, a polycyclic aromatic hydrocarbon, by adding the organic acid as a cometabolic substrate [52]. By contrast, the presence of some organic substrates can also decrease the removal efficiency of the contaminants [49,53]. This negative influence might be due to catabolite repression. Therefore, it is necessary to investigate the effects of various organic substrates and their mixtures on the removal efficiency of the contaminants when considering the cometabolic mechanism as an enhancement tool.

Integrated Processes for Removal of Pharmaceutical Contaminants

Integration of Algae-Based Biotechnologies and Advanced Oxidation Processes

Advanced oxidation processes (AOPs) can provide an advantage as pretreatment methods, which can transform the resistant parent compounds into biodegradable intermediates. Then, the following biological process can completely mineralize these byproducts into nontoxic entities, which can reduce the cost and ecological risks. For example, a mixed bacterial inoculum can fully mineralize the byproducts after oxidation of carbamazepine by AOPs [54],

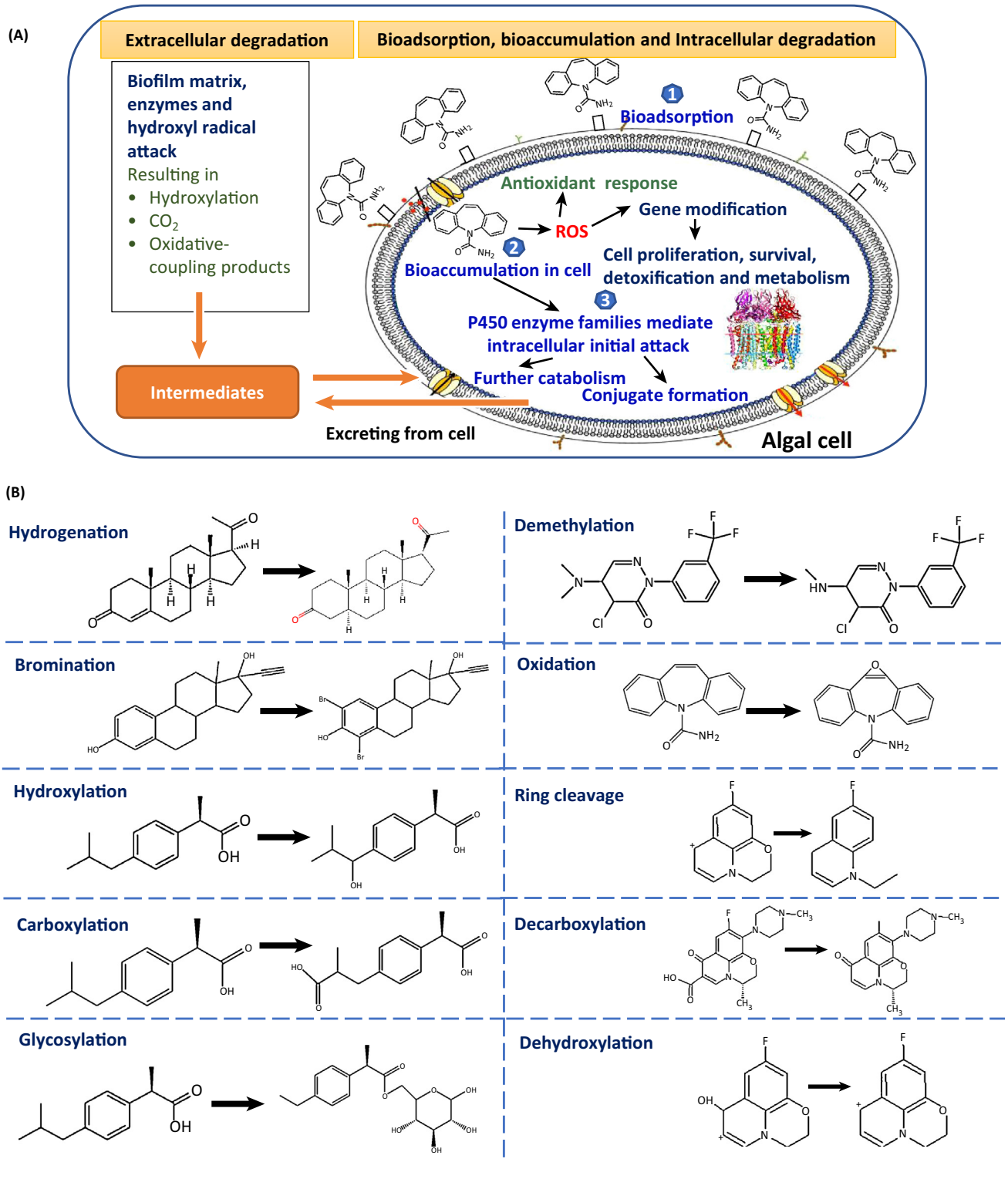

Figure 1. Proposed Mechanisms of Removal of Pharmaceutical Contaminants and Microalgal Metabolism. (A) Removal mechanisms of PCs by microalgae include bioadsorption, bioaccumulation, and intracellular and extracellular degradation. The metabolic mechanism of PCs in microalgae is induced by

(Figure legend continued on the bottom of the next page.)

and the biodegradation of quinoline was enhanced by 19–50% with UV photolysis [55]. Some concerns can arise while constructing combined systems, such as the biodegradability of intermediates produced from AOPs, overoxidation, achieving optimum oxidation conditions and life cycle assessments, treatment flexibility, and the relationship between the photoreactor effluent composition and bioreactor performance [56]. More efforts and investigations should be devoted to overcome these concerns. Integrated algae-based biotechnologies with AOPs have rarely been reported, although the microbial consortium (algae and bacteria) in the biological process shows greater efficiency and flexibility than the individual system (Figure 3, Key Figure). The use of microbial consortium-mediated bioremediation as a biological process for treatment of AOP influent is highly recommended.

Integration of Algae-Based Biotechnologies and Constructed Wetlands

Constructed wetlands (CWs) have been demonstrated to be an effective technology for wastewater treatment and have gained attention due to their low cost of construction, operation, and maintenance. Nitrification and denitrification play key roles in CWs. However, the low oxygen availability in CWs makes nitrification the rate-limiting step, as nitrification depends on sufficient oxygen supply and carbon sources. Artificial aeration can increase the oxygen but it is expensive and inhibits denitrification in the absence of anaerobic condition. Microalgae can produce oxygen through photosynthesis, and the algal debris can be used as the organic substrate (Figure 3). Thus, including an algal pretreatment unit within a CW water treatment system has been recognized as an attractive option for wastewater treatment. The removal efficiency of selenium was significantly increased in the integrated process (algal remediation with CW) and reduced the risk of the buildup of ecotoxic forms of selenium [57]. The nitrogen removal performance was enhanced in the high rate algal ponds/constructed wetlands hybrid systems and was significantly higher than that in a single CW [58]. There is a limited amount of data on the utilization of integrated CW systems for the removal of PCs in wastewater. Therefore, more study is needed to fully understand the behaviors of these integrated systems for wastewater containing PCs. The configuration design, hydraulic mode, temperature and seasonality, pH, oxygen, and redox potential are the key factors that should be carefully evaluated during the construction of integrated systems, since these parameters play essential roles in the removal capacities of the combined system.

Integration of Algae-Based Biotechnologies and Microbial Fuel Cells

Microbial fuel cells (MFCs) have been suggested as an effective approach to treat wastewater, replace energy intensive wastewater treatment processes, and produce clean electrical energy and valuable products [59–61]. Anodic conversion of organic carbon into electricity is a relatively easy process in MFCs. However, removal of nutrients and PCs in MFCs has not been well studied so far [59]. This technology is also relatively more expensive; the employed membranes are costly, and a considerable amount of energy is consumed for nitrification in terms of mechanical aeration. Moreover, MFCs have little capacity to remove phosphorus [60]. Fortunately, the emergence of microalgae technology can be a good complement to MFCs (Figure 3). The establishment of a symbiotic system, based on the cooperation between microalgae and electrochemically active bacteria, for simultaneous removal of organic matter, nitrogen, and phosphorus along with electricity production without energy input is a highly attractive and beneficial approach [59]. The removal efficiencies of organic carbon, nitrogen, and phosphorus in a photomicrobial fuel cell containing an algae–bacteria consortium were

enzymes such as cytochrome P450 and conjugation Phase II enzymes. (B) Proposed enzymatic reactions in microalgae for bioremediation of PCs according to the reported degradation pathways, including hydroxylation [20], carboxylation [20], oxidation [14], hydrogenation [11], glycosylation [22], demethylation [23], ring cleavage [24], decarboxylation [24], dehydroxylation [24], and bromination [12].

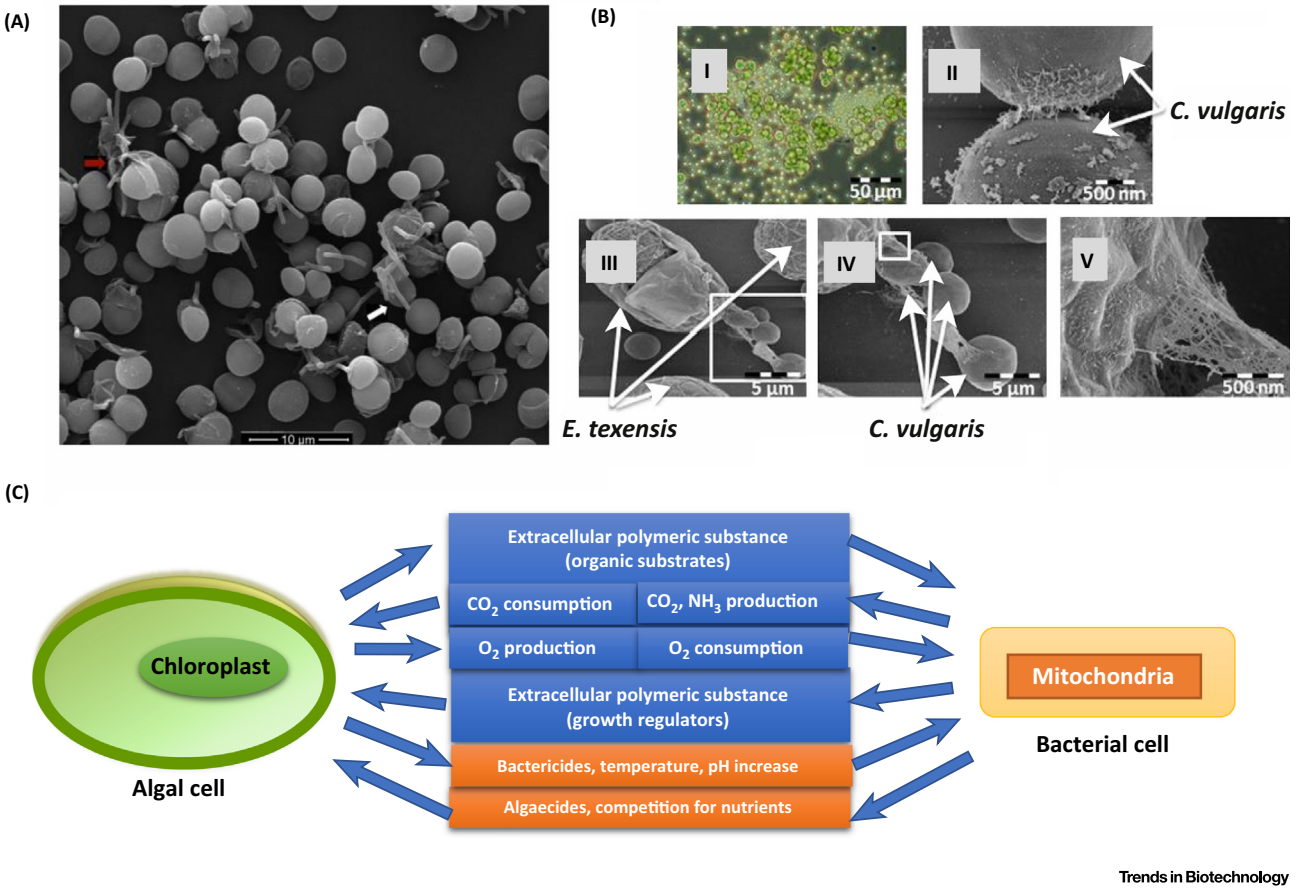

Figure 2. Microalgae Provide a Habitat for Bacteria. For example, (A) *Chlorella vulgaris* houses bacteria on the cell wall (white arrow) and beneath the cell sheath (red arrow) [39]. (B) Microscopic picture of *Ettlia texensis* and *Chlorella vulgaris* suspension (i). SEM picture of two *C. vulgaris* cells with extracellular polymeric substances (EPS) released from *E. texensis* in the suspension and attached to the surface of *C. vulgaris* cells (ii). *E. texensis* and *C. vulgaris* suspension (iii), several *C. vulgaris* cells attached to EPS from *E. texensis* (iv) and EPS structure attached between *E. texensis* and *C. vulgaris* cells (v). White box in (iii) is magnified in (iv) and white box in (iv) is magnified in (v). (C) Nutrient and energy flow in a microalgae–bacteria consortium. Reproduced, with permission, from [39,40].

observed to be 99.6%, 87.6%, and 69.8%, respectively [60]. Under illumination, MFCs embedded with microalgae consortia continuously produced electricity without the external input of exogenous organics or nutrients [61]. All of these results suggest that the MFC–algae integrated process would be a breakthrough idea for the remediation of a variety of wastewaters.

Although these integrated processes showed benefits and more flexibility, most of the current experiments are still laboratory-scale investigations using individual methods, and only a few scientists are working on real field application of algae-based technologies for removal of PCs. Therefore, more studies should be conducted to investigate the removal efficiencies of PCs using integrated technologies for commercialization. Meanwhile, omics technologies including metagenomics, metatrascriptomics, metaproteomics, and metabolomics may be useful tools in the integrated processes as these analyses can give a full understanding of the microbial communities, genes, proteins, and metabolites changes during the construction of these integrated processes. Moreover, better economic models must be developed to estimate how the cost of this combined process varies with specific industrial wastewater characteristics, overall decontamination efficiency, and the relative cost of the different treatment processes versus algae-based biotechnologies.

Key Figure

Novel Approaches (Algae–Bacteria Consortia, Acclimation of Microalgae, and Cometabolic Mechanism) for the Enhancement of Microalgal Removal of Pharmaceutical Contaminants

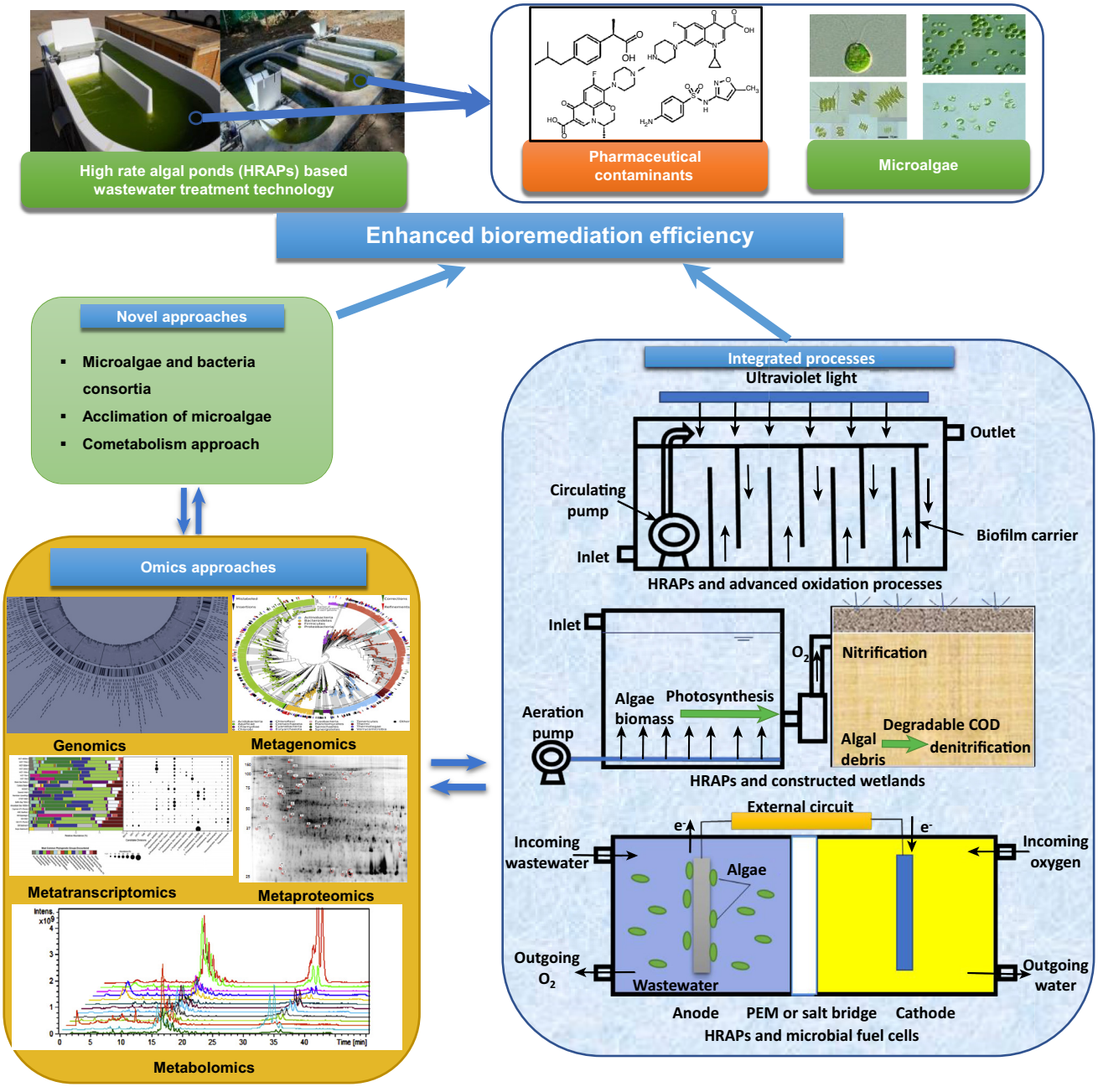

Figure 3. The proposed integrated processes (algae-based biotechnologies with advanced oxidation processes, constructed wetlands, and microbial fuel cells) for the enhancement of removal of pharmaceutical contaminants. Abbreviations: COD, chemical oxygen demand; PEM, proton exchange membrane.

Proposed Genetic Modifications of Microalgae for Enhancement of PC Remediation

The major bottleneck in microalgal biomass production is its low productivity, which is usually <1 g/l. Genetic engineering of algae is a crucial systemic technology to overcome the microalgal biomass problem in industrial applications, to modify the metabolic pathway for high-product yield, and to design and construct artificial photoautotrophs in the rising and promising field of synthetic biology [62]. However, genetically engineered microalgae for bioremediation have rarely been reported. We suggest that attention should be focused on engineered microalgae cloned with the laccase enzyme gene for effective bioremediation. Laccases are multicopper oxidoreductases and have a broad substrate specificity and relative autonomy. They are considered to be the ultimate 'green catalysts' in environmental biotechnology, since they use molecular oxygen from the air as an electron acceptor and only produce water as a by-product [63]. A laccase has been engineered in *Streptomyces coelicolor* by structure-based design and site-directed mutagenesis to improve activity on commercially relevant substrates. The variants generated showed up to a 40-fold increase in efficiency on 2,6-dimethoxyphenol and the ability to use mediators with considerably higher redox potentials [64]. Laccase activity has been successfully observed in the green microalgae *Tetracystis aeria* and *Chlamydomonas moewusii* [28,65]. An extracellular laccase monomeric enzyme produced from a cyanobacterium, *Spirulina platensis,* was purified recently [66]. Algal enzymes are most active at circumneutral or even alkaline pH with phenolic substrates, which is a most useful feature in order to exploit laccases in bioremediation of emerging contaminants. Microalgal laccases require appropriate redox mediators to transform phenolic micropollutants, including endocrine-disrupting chemicals [67]. By contrast, bacterial/fungal laccases can efficiently catalyze the organic compounds without involving the redox mediators. Thus, cloning of bacterial/fungal laccase and expressing it in algae would provide an opportunity to improve the algal bioremediation.

Concluding Remarks

The overall evaluation of microalgal bioremediation suggests a positive outlook for microalgae as a potential candidate for the removal of PCs (see Outstanding Questions). Coupling microalgae with microbial consortia, acclimation, and abiotic redox processes provide a new approach for the effective treatment of PCs in aqueous phase. Integrated treatment processes (algae-based technologies combined with advanced oxidation processes, constructed wetlands, and microbial fuel cells), and genetic modifications would be feasible for the enhanced remediation of emerging organic contaminants such as PCs. In addition, the microalgal treatment technologies generate commercially valuable biomass that is useful as feedstock to produce biofuels and other value added chemicals. Microalgae-based biotechnological strategy for the bioremediation of PCs is a relatively new topic and involves challenges, such as long hydraulic retention time, environmental factors (pH, temperature, nutrients, light), toxicities, and the interactions and toxicities of co-contaminants [66–70]. Under the current operational conditions, this microalgae-based treatment can be a part of the advanced wastewater treatment system to achieve effective removal of nutrients (e.g., nitrogen or phosphorus) with simultaneous degradation of low concentrations of PCs [39,66]. Nevertheless, more scientific investigations on scale-up studies and technical aspects of microalgal bioremedial system are needed. Further in-depth studies using molecular approaches, such as metatranscriptomics, metagenomics, metaproteomics, and metabolomics, to reveal the community and species-specific interactions should be comprehensively studied. Analytical approaches, such as LC-MS/MS and GC-MS, to illustrate the mechanisms of biodegradation in the microalgae-based biotechnologies should be considered in depth.

Outstanding Questions

What are the ecotoxicological effects of PCs in the environment?

What are the best technologies currently used to remove PCs from the environment?

What are the advantages and drawbacks of using microalgae-based biotechnologies over other treatment methods?

What are the removal mechanisms of PCs by microalgae?

How can we enhance the removal efficiency of PCs from the aqueous phase by microalgae?

How can we improve the engineering feasibility of microalgae-based biotechnologies to remove PCs?

How can we use omics technologies to achieve real applications of microalgae-based biotechnologies?

Acknowledgments

This study was supported by National Research Foundation of Korea (NRF) grants funded by the Ministry of Education, Science, and Technology (MEST) of the South Korean government (No. NRF-2013R1A2A2A07069183 and No. 2017R1A2B2004143).

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