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Review

Algae-mediated antibiotic wastewater treatment: A critical review

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ABSTRACT

The existence of continually increasing concentrations of antibiotics in the environment is a serious potential hazard due to their toxicity and persistence. Unfortunately, conventional treatment techniques, such as those utilized in wastewater treatment plants, are not efficient for the treatment of wastewater containing antibiotic. Recently, algae-based technologies have been found to be a sustainable and promising technique for antibiotic removal. Therefore, this review aims to provide a critical summary of algae-based technologies and their important role in antibiotic wastewater treatment. Algal removal mechanisms including bioadsorption, bioaccumulation, and biodegradation are discussed in detail, with using algae-bacteria consortia for antibiotic treatment, integration of algae with other microorganisms (fungi and multiple algal species), hybrid algae-based treatment and constructed wetlands, and the factors affecting algal antibiotic degradation comprehensively described and assessed. In addition, the use of algae as a precursor for the production of biochar is highlighted, along with the modification of biochar with other materials to improve its antibiotic removal capacity and hybrid algae-based treatment with advanced oxidation processes. Furthermore, recent novel approaches for enhancing antibiotic removal, such as the use of genetic engineering to enhance the antibiotic degradation capacity of algae and the integration of algal antibiotic removal with bioelectrochemical systems are discussed. Finally, some based on the critical review, key future research perspectives are proposed. Overall, this review systematically presents the current progress in algae-mediated antibiotic removal technologies, providing some novel insights for improved alleviation of antibiotic pollution in aquatic environments.

1. Introduction

Recently, residual pharmaceuticals and personal care products (PPCPs) have been widely identified in aquatic environments [1]. Antibiotics are a typical category of PPCPs which are increasingly being detected in the natural environment and associated with ecotoxic effects [2]. The increased concentration of antibiotics in the aquatic environment (concentrations of antibiotics rarely exceed 1 $\mu g\ L^{-1}$, but are more commonly in the low ng L^{-1} range) presents a major hazard due to their toxicity and long-term persistence [3,4]. In particular, exposure to residues of antibiotics has attracted significant attention because of the increase in antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) [5]. In 2019, in the US alone, antibiotic-resistant bacteria and fungus caused more than 2.8 million infections and 35,000 deaths [6]. Currently, ARB have been extensively detected in most aquatic environments surrounding point-sources, such as pharmaceutical companies, hospitals, livestock farms, and wastewater treatment plants (WWTPs), leading to a high risk of transformation and migration of ARB and ARGs throughout the aquatic environment [3]. Furthermore, it has been widely demonstrated that WWTPs are not effective at removing ARBs, ARGs, or antibiotics from contaminated wastewater. It has been reported that in the absence of policy interventions, the global consumption of antibiotics in 2030 could increase by 200% relative to their use in 2015 [7]. Accordingly, reducing the release and dispersion of antibiotics throughout the

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environment is critical.

Currently, various technologies have been applied for the removal of antibiotics in the aquatic environment, including biological, physical and chemical methods [8]. Advanced treatment technologies such as adsorption, chlorination, activated carbon filtration, membrane processes, advanced oxidation processes (AOPs), photocatalysis, and the use of nanomaterials and ferrate treatment have been introduced [9–11]. For example, Du et al. [9] reviewed that metal–organic frameworks (MOFs) have excellent photodegradation and adsorption efficiency for antibiotics. Li et al. [12] reported that tetracycline removal efficiency was more than 95% via Fe-based MOF. However, these methods have some notable limitations, for example, the antibiotic removal efficiency of adsorption is extremely adsorbent-dependent, with adsorbents usually being at a high cost [13], while AOPs and photocatalysis are typically efficient but require costly chemical reagents or catalysts [14], while potentially generating secondary pollutants [15], such as large quantities of metal sludge.

Compared to physical and chemical methods, biological treatments provide some unique benefits, such as being environmentally-friendly and having low economic costs [16]. Algae are primary produced in aquatic ecosystems. They can be used as ecological indicators and remove contaminants, due to their short growth cycle, high sensitivity to aquatic pollutants, and initiate stress response mechanisms [17]. Algae-based remediation techniques were first conducted in the 1950's to remove nutrients and dissolved carbon from sewage. The main advantage of algae-based technologies is that they do not need additional energy to effectively remove pollutants [18,19], unlike the conventional approaches, such as micro-filtration and sludge activation, which require significant energy inputs [20]. Recently, algae-based techniques have been wildly utilized for the treatment of wastewater containing antibiotics, providing numerous benefits including efficient fixation of CO_2, low-environmental impact, solar energy driven activity, and providing a potential raw material for the generation of biofuel or other high-value by-products [21,22]. For example, triclosan can be completely removed from water by *Nannochloris* after 7 days of cultivation [23] and diphenhydramine, memantine and trihexyphenidyl can be efficiently degraded by algae, with average degradation rates of 88%, 59% and 83%, respectively [24]. Microalgae-mediated technology could removal groundwater pollution by organic microcontaminants such as pesticides and antibiotics (up to 65%) [25].

Antibiotic degradation and removal methods have been reviewed elsewhere, for example, Peiris et al. [26] and Yan et al. [27] summarized the potential utilization of biochar and bioelectrochemical system to degrade antibiotics. However, investigations into the performance and mechanisms of algae-mediated antibiotic wastewater treatment have not been comprehensively reviewed. This review aims to supplement the emerging literature on algae-based technologies and identify the key challenges and knowledge gaps. Firstly, microalgal removal mechanisms (bioadsorption, bioaccumulation, and biodegradation) as well as the factors affecting antibiotic degradation by algae are introduced. After which the use of algae as a biotemplate for the production of photocatalysts is discussed. Furthermore, the use of materials to improve algal photosynthetic activity is reviewed and the recent novel approaches for enhancing antibiotic removal are summarized and compared, including the potential use of genetic engineering to improve antibiotic degradation and the use of microalgal-bacterial consortia for antibiotic treatment. In addition, microalgal antibiotic degradation can be integrated with other advanced techniques, such as AOPs, ultraviolet (UV) irradiation, integration with other microbial species, hybrid algae-based treatment-constructed wetland systems, and bioelectrochemical systems. Finally, the main research challenges and future prospects are discussed.

2. Perspective #1: biological treatment for antibiotic based on algae

2.1. Algal removal mechanisms: bioadsorption, bioaccumulation, and biodegradation

When exposed to antibiotics, algae initiate stress response mechanisms that degrade toxic antibiotics and assist algal survival [15]. Table 1 summarizes the removal efficiency of different antibiotics by algae-based techniques and the involved mechanisms. Currently, bioadsorption, biodegradation, bioaccumulation, photodegradation, volatilization, and hydrolysis are the main pathways of antibiotic removal that have been identified in algae [28]. As reported by Hena et al. [29], the specific physicochemical properties of PPCPs are the major factor affecting which mechanism algae use to degrade antibiotics in wastewater. Theoretically, algae remove organic contaminants primarily by absorption, due to the physio-chemical characteristics of organic contaminants [30]. According to Nguyen et al. [31] and Xiong et al. [21], photodegradation, volatilization and hydrolysis pathways not being universal and only occur under particular conditions, generally providing a minor contribution to removal. Therefore, this review mainly focuses on bioadsorption, bioaccumulation and biodegradation (Fig. 1).

Bioadsorption is a physico-chemical procedure in which antibiotics are directly removed from wastewater using the adsorptive capacity of biological materials [31]. Biomaterials are structurally complex and diverse due to the wide range of components present in biomass and the variation in functional groups, such as phosphate, thiol, carboxyl, hydroxyl, and amino groups, which are affected by physico-chemical processes to varying degrees [32]. Bioadsorption by algal cells occurs when antibiotics are either adsorbed to components of their cell wall, or onto organic substances (e.g., extracellular polymetric substances (EPS)) that are discharged by algal cells into the surrounding aquatic environment [33]. EPS are a combination of various biopolymers formed by microorganisms which may consist of up to 90% organic matter, such as protein, polysaccharides, enzymes, lipids, and substituents. EPS have various structural and functional roles, such as the enhancement of cell adsorption capacity, surface characteristics, enzyme retention, mass transfer stability, structural stability, and digestive functions [34,35]. Interactions between negatively charged microalgal cell walls or secretions (both collectively termed the cell surface) and antibiotics, occurs via a passive and non-metabolic process [21]. For example, metronidazole (initial concentration was 5 μM) can be removed by *Chlorella vulgaris* via bioadsorption and the removal efficiency can reach 100% [36]. Furthermore, the biomass of nonliving microalgae has also been demonstrated as a promising biosorbent material for the removal of antibiotics. For instance, the residual biomass of the lipid-extracted *Chlorella* sp. has been shown to have a high capability for cephalexin adsorption and removal [37]. Furthermore, after lipid extraction, Daneshvar et al. [38] assessed tetracycline removal from wastewater using microalgal biomass, showing that the maximum adsorption capabilities of *Tetraselmis suecica* and *Scenedesmus quadricauda* were 56.25 and 295.34 mg g^{-1}, respectively. Bioaccumulation is an active metabolic process for antibiotic uptake, noting that it is a key function of live microbial cells, relying on different chemical, physical, and biological mechanisms that include both intra- and extracellular processes. In bioaccumulation, passive diffusion is a poorly defined process with a limited contribution to antibiotic removal, while energy-dependent uptake

Table 1
Algae reported as biological techniques for the removal of different antibiotics and the mechanisms involved.

Antibiotic	Algae	Mechanisms	Removal	Ref.
Tetracycline	*Chlamydomonas* sp. Tai-03	Biodegradation, photolysis, and hydrolysis	100%	[43]
Tetracycline	*Spyrogira* sp.	Photodegradation	89%	[47]
Tetracycline	[a]	Biosorption	295.34 mg g^{-1}	[38]
Tetracycline	[b]	Biosorption	56.25 mg g^{-1}	[38]
Sulfamethoxazole	*Chlamydomonas* sp. Tai-03	Biodegradation	~20%	[43]
Sulfadiazine	*Chlamydomonas* sp. Tai-03	Biodegradation, biosorption, photolysis, and hydrolysis	54.53%	[28]
Sulfathiazole	*Spyrogira* sp.	Biodegradation and indirect photodegradation	36%	[47]
Ciprofloxacin	*Chlamydomonas* sp. Tai-03	Biodegradation, biosorption, photolysis, and hydrolysis	100%	[28]
Ciprofloxacin	*Scenedesmus dimorphus*	Bioadsorption and biotransformation	93%	[48]
Erythromycin	*Scenedesmus obliquus*	Biodegradation, hydrolysis, and photolysis	97%[c]	[45]
Norfloxacin	*Chlorella vulgaris*	Photodegradation	36.9%	[49]
Levofloxacin	*Chlorella vulgaris*	Biodegradation and bioaccumulation	82.35%	[44]
Azithromycin	*Chlorella vulgaris*	Biodegradation	92.77%	[44]
Azithromycin	*Haematococcus pluvialis*	Biodegradation	78%	[44]
Amoxicillin	*Microcystis aeruginosa*	Biodegradation	30.5–33.6%	[50]
Amoxicillin	*Microcystis aeruginosa*	Biodegradation	18.5–30.5%	[51]
Spiramycin	*Microcystis aeruginosa*	Biodegradation	12.5–32.9%	[50]
Cefradine	*Chlorella pyrenoidosa*	Biodegradation	41.47 ± 0.62%	[52]
Cephalexin	[d]	Biosorption	63.29 mg g^{-1}	[37]
Ceftazidime	*Chlorella pyrenoidosa*	Bioadsorption and biodegradation	93%	[21]
Ceftazidime	*Chlorella pyrenoidosa*	Bioadsorption and biodegradation	92.70%	[46]
7-amino cephalosporanic acid	*Chlorella pyrenoidosa*	Bioadsorption and biodegradation	96.07%	[46]
7-amino cephalosporanic acid	[e]	Bioadsorption, hydrolysis and photolysis.	100%	[53]

[a] Dry biomass of lipid-extracted *Scenedesmus quadricauda*
[b] Dry biomass of lipid-extracted *Tetraselmis suecica*
[c] Calculated based on the literature.
[d] Dry biomass of lipid-extracted *Chlorella* sp.
[e] *Chlorella* sp. Cha-01, *Chlamydomonas* sp. Tai-03, *Mychonastes* sp. YL-02.

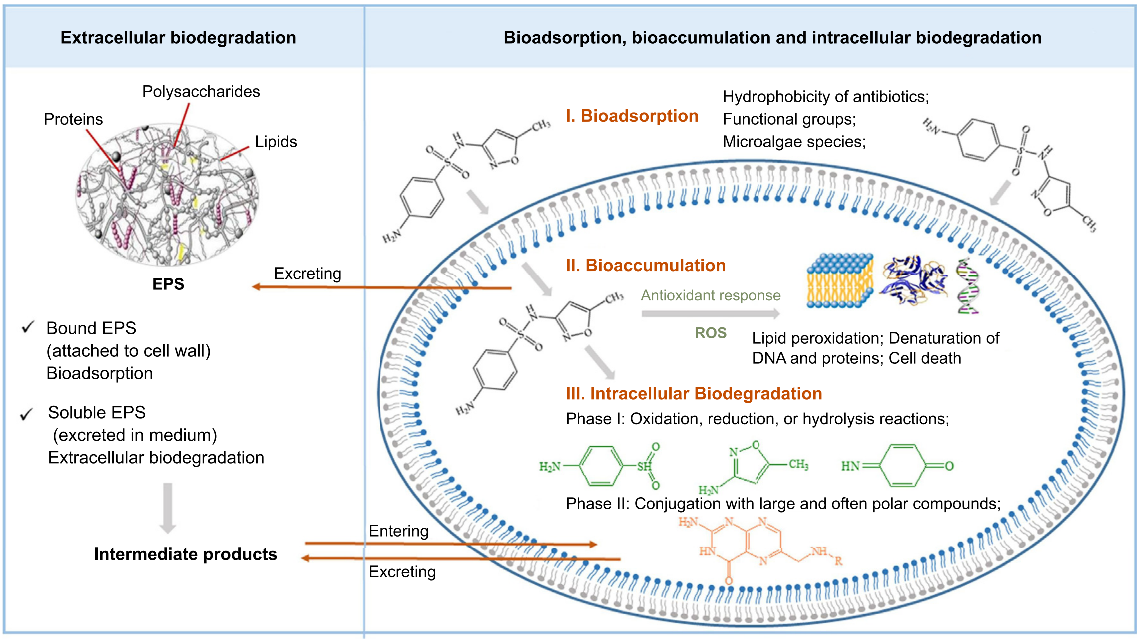

Fig. 1. Mechanisms involved in the removal of antibiotics by algae. Reprinted with permission from Xiong et al. [21], copyright (2021) Elsevier.

is an active transport step driven by energy [32]. For example, bioaccumulation has been observed during the removal of trimethoprim, sulfamethoxazole, florfenicol [39], and carbamazepine [40], in which antibiotics enter algal cells through passive diffusion.

Biodegradation includes the transformation of complicated compounds into simpler small-molecules through catalytic metabolic degradation [41]. According to Tiwari et al. [42], microalgal biodegradation can occur via two major mechanisms: firstly, by metabolic degradation where antibiotics provide a carbon source and serve as electron donors or acceptors for microalgae; and secondly, by co-metabolism where the antibiotics are reduced by enzymes, causing the formation of non-toxic product compounds. Biodegradation has been demonstrated to be the most efficient mechanism for the removal of antibiotics by algae-mediated technologies [43]. For example, Kiki et al. [44] investigated the degradation potential of different algae including *C. vulgaris*, *Selenastrum capricornutum*, *Haematococcus pluvialis*, and *S. Quadricauda* for ten antibiotics, finding that biodegradation was the main mechanism for antibiotics removal, while bioaccumulation, bioadsorption and abiotic factors accounted for relatively smaller contributions. Similarly, Wang et al. [45] inoculated *S. obliquus* into wastewater to determine the erythromycin degradation pathways. Results showed that after 5 days of cultivation, biodegradation (including bioadsorption), hydrolysis and photolysis were the main mechanisms of erythromycin degradation in the microalgae-mediated system, with biodegradation (including bioadsorption) responsible for the highest proportion of removal (57.87%), followed by hydrolysis (<34.13%) and photolysis (<5%). It has previously been reported that ciprofloxacin can be completely removed by *Chlamydomonas* sp. Tai-03, with biodegradation accounting for up to 65.05% of removal [28]. In summary, the degradation of antibiotic by algae-based technologies, can be divided into 3 distinct processes: 1) fast adsorption due to physicochemical interactions between the antibiotics and algal cell walls, 2) the relatively slow transference of molecules through algal cell walls, and 3) biodegradation, bioaccumulation, or both [46].

2.2. Using algae-bacteria consortia for antibiotic treatment

As discussed, algae have the capacity to directly remove antibiotics using extracellular enzymes or heterotrophic metabolism. In addition, the biodegradation capabilities of algae may also be improved indirectly by symbiotic communication with bacteria [54], photosynthetically altering pH conditions, or due to large amounts of oxygen formation [55]. As depicted in Fig. 2a, algae-bacteria consortium techniques can degrade antibiotics in wastewater via mechanisms such as biodegradation, volatilization, photodegradation, and sorption. In algae-bacteria consortia, both bacteria and algae can serve as biosorbents, with EPS formed by bacteria and algae also supply crucial sites for the biosorption of antibiotics [56]. Ismail et al. [57] found that combining *Chlorella* sp. and isolated bacterial strains achieved highly efficient ketoprofen biodegradation and tolerance. Guo et al. [21] developed a novel algae-activated sludge mixed system for the degradation of cephalosporins, which achieved a higher total removal efficiency of 97.91% with green algae shown to have an outstanding cefradine removal rate.

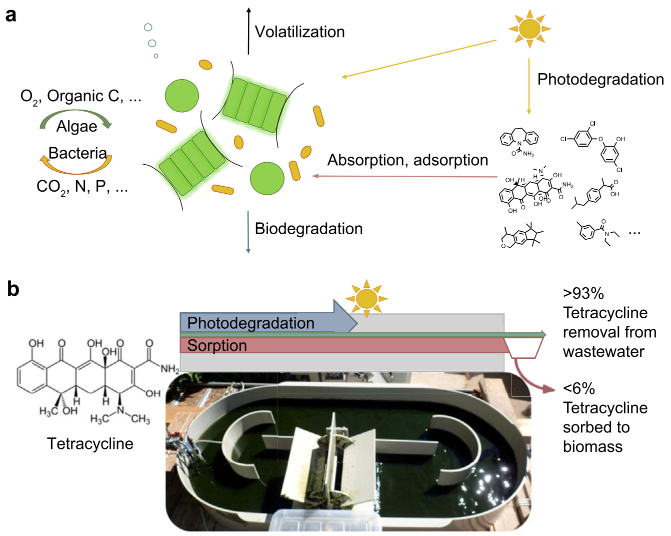

Fig. 2. (a) Processes included in the removal of PPCPs using algae-based techniques, modified from Ref. [56]. (b) Sorption and photodegradation based tetracycline removal during wastewater treatment using algal ponds [60].

High rate algal ponds (HRAPs) have been applied as bioreactors for wastewater treatment since the 1950's [58], effectively utilizing mixed algae-bacteria cultures to achieve energy efficient antibiotic degradation [59]. For example, >93% removal of 100 μg L^{-1} tetracycline from wastewater was achieved mainly via indirect photodegradation, when the HRAP was conducted at a hydraulic retention time (HRT) of 4 days [60] (Fig. 2b). Moreover, de Godos et al. [61] reported effective tetracycline removal in lab-scale HRAPs, demonstrating that sorption and photodegradation were the major removal mechanisms. However, few studies have investigated the fate of antibiotics in HRAP treatment systems. In summary, the results presented above suggest that the feasibility of using algae-bacteria consortia to treat antibiotic containing wastewater is promising in terms of the effectiveness and operational costs, providing a safe and sustainable approach for antibiotic wastewater treatment.

2.3. Integration of algae with other microorganisms (fungi and multiple algal species)

Apart from the aforementioned bacteria, fungi are another important type of microbe that is able to facilitate antibiotic removal by algae. Using fungi to treat high-strength wastewater has shown promising results, providing numerous benefits compared with bacteria in biological wastewater purification. According to Sankaran et al. [62], fungal wastewater remediation not only transforms organic matter into high-value fungal proteins and useful biochemicals (such as amylase, chitin and lactic acids), but it also forms large dewaterable fungal biomass, which can be utilized as an animal feed source and may even be consumed in human diets. Additionally, fungi have high resistance to inhibitory substances and an abundance of extracellular enzymes that promote the bioremediation of recalcitrant compounds. According to Silva et al. [63], pollutant biodegradation by fungi includes either an extracellular enzymatic system (i.e., manganese peroxidase, versatile peroxidase, laccase, and lignin peroxidase) or an intracellular enzymatic system (mainly the cytochrome P450 system). Some filamentous fungi can pelletize, further involving microalgal cells. Contaminant removal mechanisms of algae-fungi consortia are shown in Fig. 3. Algae-fungi consortia have been shown to be capable of treating antibiotic wastewater. For instance, biopellets composed of *C. vulgaris* and *Aspergillus niger* have exhibited significant removal capability for ranitidine [64]. Furthermore, fungi-assisted harvesting of algae has been found to enhance the removal range pharmaceuticals from wastewater [65]. In addition to the potentially high removal rate, biopellets allow harvesting by sedimentation or sieve filtration, leading to significantly reduced treatment costs. Co-pelletization through the addition of fungi and algae does not need extra chemical or energy inputs and may be a promising treatment solution.

Algae are non-target microorganisms for antibiotics and therefore, the disturbance of algae by antibiotics is limited, with no resistant species or resistant genes known to be formed during the procedure of antibiotic treatment [66]. Thus, a combination of multiple algae has been found to be beneficial to the removal of antibiotics. In this regard, the cefradine removal efficiency of *C. pyrenoidosa* cultured in filtered *M. aeruginosa* culture fluid was 75.48 ± 0.29%, which was significantly higher than using *C. pyrenoidosa* alone [52]. These findings suggest that the combination of multiple algal strains is a promising strategy for the treatment of antibiotics in wastewater.

2.4. Hybrid algae-based treatment and constructed wetlands

Constructed wetlands (CWs) have the advantages of being

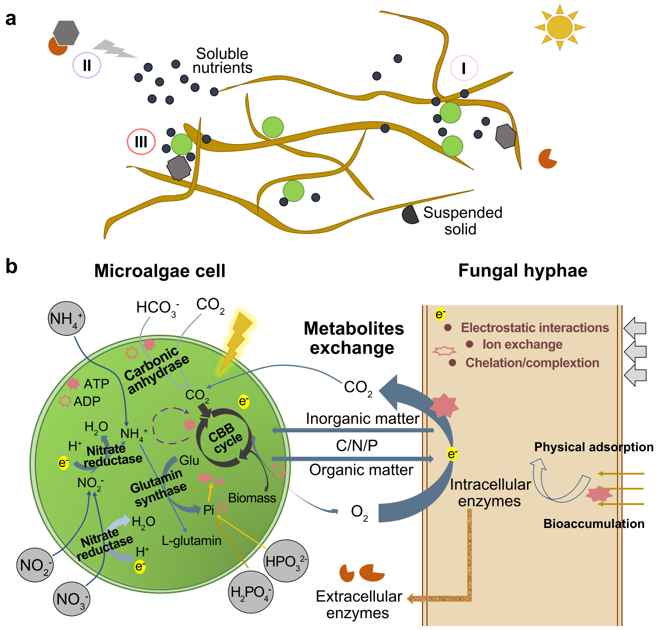

Fig. 3. Contaminant removal mechanisms using the algae-fungi consortia. (a) Algae and fungi work together to degrade wastewater: I. Capture or adsorption of suspended solids; II. Reduction by extracellular enzymes secreted by fungi; III. Assimilation of soluble nutrients by algae and fungi. (b) Detailed description of the assimilation of soluble nutrients via microalgae and fungi. Note: Glu indicates glucose; CBB cycle indicates the Calvin-Benson-Bassham cycle. Modified from Ref. [67].

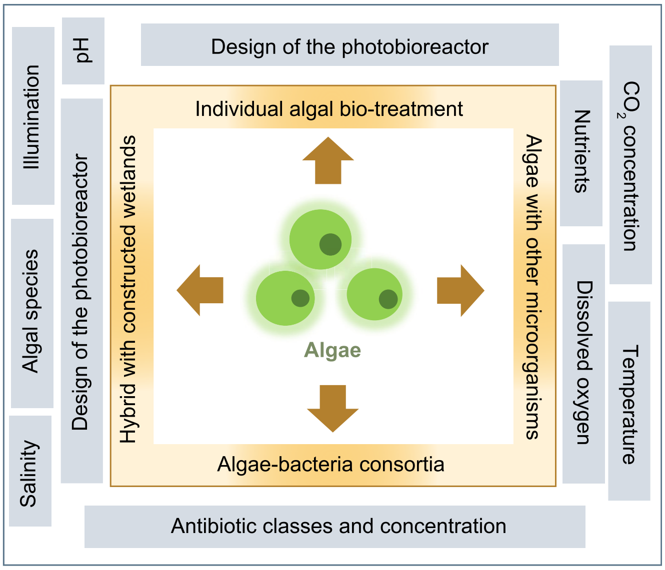

Fig. 4. Factors affecting antibiotic degradation by algae.

sustainable, with easy, low-cost and energy-efficient operation, while also having high efficiency for antibiotics removal due to substrate absorption, biodegradation and plant uptake [68]. Huang et al. [69] reported that more than 84% of oxytetracycline and difloxacin could be removed by vertical flow CWs. CWs can be easily influenced by many factors, such as CW type, plant species, HRT, hydraulic loading rate, pH, substrate type, temperature, and dissolved oxygen content, resulting in a complex range of differences in the mechanisms and removal rates of antibiotics [70]. Algae associated with CW systems have been considered as an outstanding substitute for wastewater treatment and have been developed to a reasonably mature level [71]. For instance, HRAPs were combined with CWs to treat wastewater, with algal biomass enhanced in the HRAP, while the concentration of dissolved oxygen was improved by photosynthesis, resulting in hybrid system removal capacity being significantly higher than CW-only systems [72]. Similarly, Silveira et al. [73] used an integrated system with algae and CWs to degrade wastewater, with results suggesting that the combined system had excellent chemical oxygen demand (COD) and 5-day biochemical oxygen demand (BOD_5) reductions as well as removal efficiency of nearly 98% for N—NH_3. Rabello et al. [74] provided a review of the relationship between the presence of the most broadly explored PPCPs in wastewater and the configuration of CWs and algae tanks designed to reduce them, proposing that integration of CW systems and algae tanks could be a promising method for PPCPs removal from domestic wastewater.

2.5. Factors affecting antibiotic degradation by algae

2.5.1. Effects of algal culture conditions and antibiotic concentrations

Antibiotic degradation by algae is influenced by many factors, such as the pH, temperature, level of CO_2 enrichment, light intensity, HRT and antibiotic concentrations (Fig. 4). The solution pH is a key factor affecting the interactions between the adsorbent and adsorbate [75], not only influencing the charge of algal cell surfaces but also the degree of speciation and ionization of antibiotics. It has previously been reported by de Godos et al. [61] that a cultivation pH ranging from 7.5 to 8, supports tetracycline zwitterion formation, which facilitates adsorption onto the algal surfaces. Temperature affects the metabolic processes of algal cells and enzyme activity with the optimum temperature for microalgae demonstrated to be 25—30 °C [76]. Temperature extremes could disturb kinetic rates and although higher temperatures generally improve kinetics, they can negatively affect other aspects such as enzyme function and cell activity. For example, Rico et al. [77] found that the PSII and growth rates for *S. obliquus* were similar at 30 °C and 20 °C, but the growth rate based on the determined concentration of chlorophyll-a was found to be lower at 30 °C than at 20 °C. In addition, many studies have reported that CO_2 concentrations in the range of 6—15% support the accumulation of *S. obliquus*, *Chlorella* sp., *N. oculata*, and *Spirulina* sp [78,79]. Furthermore, the accessibility and availability of light is an important factor due to the reliance of microalgae on light energy. Light wavelength, intensity, and duration (light/dark cycle) are crucial factors that should be considered to ensure efficient degradation of antibiotics by algae. Furthermore, in order to optimize the operational parameters of photobioreactors for maximum antibiotic removal efficiency by algae, the HRT is a crucial element that requires careful control. Generally, the HRT utilized for wastewater treatment in photobioreactors varies in the range of 2—10 days. Shorter HRT conditions improve nutrient loading and could enhance algae growth, while longer HRT conditions could increase the nutrient removal performance [80].

2.5.2. Effects of algae species and antibiotic structures

The removal of antibiotics using algae as biological methods has been achieved with varying removal efficiencies (%) depending on the species of algae and the structure of antibiotics (e.g., the hydrophobicity of the antibiotic and the functional groups accessible for adsorption) [44]. All algal species are not universally able to remove all types of antibiotics from wastewater and therefore, the choice of species is a crucial step. As reported by Sutherland and Ralph [81], algae from the genera *Scenedesmus*, *Chlorella*, and *Chlamydomonas* are the species most thoroughly investigated and commonly utilized for antibiotic bioremediation because of their high potential and widespread availability. Moreover, hydrophilic substrates are anionic (negatively charged) and have low bioadsorption affinities with microalgal cells, due to cells also being negatively charged. Comparatively, lipophilic cationic compounds have high bioadsorption affinities with microalgae [81]. For example, 68% and 100% of the hydrophilic antibiotics sulfamethoxazole and trimethoprim remained in algal culture after 14 days of cultivation, respectively, exhibiting a low level of removal from the culture [23]. However, the lipophilic antimicrobial triclosan was obviously removed from the culture with 100% triclosan degradation observed after 7 days of cultivation [23].

3. Perspective #2: physico-chemical treatment for antibiotic based on algae

3.1. Modification of algal biochar

Biochar has been considered as a sorbent with high potential for the elimination of antibiotics from aqueous solution [82]. Recently, algal biochar, especially macroalgal biochar, has been utilized for the fabrication and design of various nanocomposite materials for the bioremediation of antibiotics from wastewater [83]. For example, mesoporous biochar derived from *Chlorella* has a large specific surface area of 126.4 m^2 g^{-1}, a mean pore diameter of 11.62 nm and an overall pore volume of 0.55 cm^3 g^{-1}, effectively separating tetracycline from aqueous solution [84]. In general, the biochar production processes and its formation parameters (e.g., heating rate, stay time, pressure, and final temperature) have a major impact on the quality, yield and properties (e.g., crystalline, porous, or amorphous) of biochar (chemical composition, shape, and size) [85]. The heterogeneous chemical composition can be effective to determine the surface properties [86]. While, shape and size of biochar could affect the large specific surface area, which can make it more easily for antibiotic molecules to spread to the interact of the biochar and surface [87]. Furthermore, the composition and proportion of nutrients in algae-derived biochar is probably affected by many abiotic and biotic elements, such as the algal species and culture environment (e.g., saline, brackish, or fresh) [85]. Algal biochar has a smaller carbon component, while it contains more ash, minerals and nitrogen, compared to biochar derived from lignocellulosic biomass [88]. Interestingly, algae rich in a large abundance of proteins and can be directly converted into natural nitrogen-doped biochar *in situ* without the requirement for any additional modification [89]. According to Wan et al. [90], a high content of nitrogen could facilitate the production of π-π bonds and further reinforce the ion adsorption of antibiotics, improving the antibiotic removal efficiency. In general, algal biochar seems to be a promising procedure for antibiotic removal due to the renewable nature of feedstock, high contents of nitrogen, high thermal stability, and highly porous structure [91]. How to use algal biochar more efficiently remains a challenge.

Improving pyrolysis temperatures increase the hydrophobicity, aromaticity, and specific surface area of biochar [91]. Choi et al. [92] evaluated the formation of *Spirulina* sp.–derived biochar at various pyrolysis temperatures for tetracycline removal, with results showing that biochar achieved maximum tetracycline adsorption efficiency (132.8 mg tetracycline per g biochar) due to π-π and hydrophobic interactions, metal complexation and functional groups bindings when the pyrolysis temperature was 750 °C. It has also been reported that the I_D/I_G (intensity ratio of D band and G band) values of biochar decreased from 0.91 to 0.79 with an increase in pyrolysis temperature from 400 to 900 °C, indicating that effective thermal pyrolysis conditions could lead to the formation of an abundance of well-ordered graphitic carbons [82]. The biochar obtained at 900 °C served various roles in organic accumulation and peroxydisulfate activation, as well as the electron shuttle process from sulfamethoxazole to peroxydisulfate (Fig. 5a). However, the antibiotics present in wastewater cannot usually associate with algal biochar, significantly limiting its functional applications. To overcome this limitation and acquire the desired properties, biochar has been combined with trimetallic nanoparticles. For instance, algal biochar reinforced trimetallic nanocomposite (algal biochar@La/Cu/Zr) was successfully fabricated for wastewater remediation due to its promising adsorption/photocatalytic potential [93]. In addition, research has also shown that acid-mediated biochar exhibits an increased adsorption efficiency for sulfadiazine due to the increased specific surface area of biochar [94]. Furthermore, to enhance the sorption efficiency of antibiotics, the properties and structure of *Enteromorpha prolifera* biochar were enhanced by modification with potassium hydroxide under high temperature conditions, achieving the formation of a highly effective sorbent for the removal of antibiotics from wastewater [95] (Fig. 5b).

3.2. The addition of materials to improve the antibiotic removal capacity of algae

3.2.1. Iron (Fe) salts

Iron is considered to be the most abundant transition metal in the natural aquatic environment. Iron (III) salts dissolved in liquid undergoes hydrolysis, forming diverse oligomeric species and hydroxo substances. Therefore, the photochemical properties of Fe (III) salts could be used to form hydroxyl radicals in aquatic environments. It has been well established that hydroxyl radicals are one of the most effective oxidizing agents. Algae can release acidic dissolved organic compounds, such as fulvic acid and humic acid and matter containing carboxyl structures could react with ferric ion to enhance the photolysis rate of antibiotics [96]. For example,

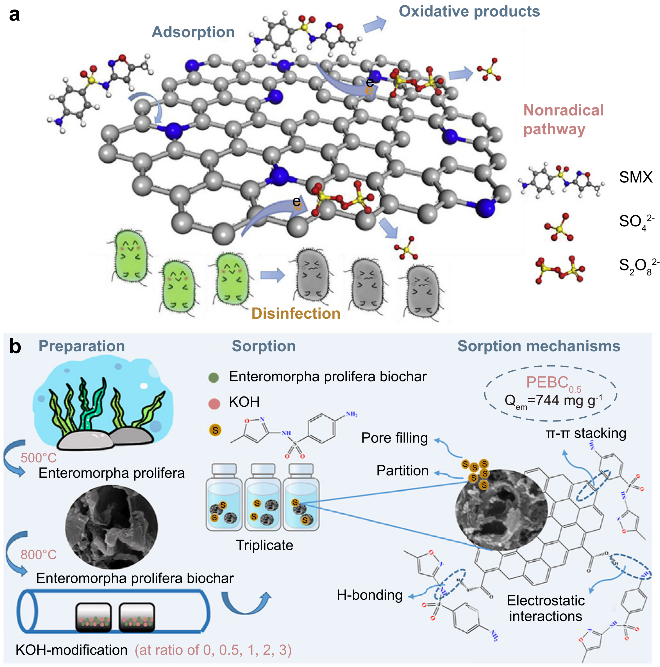

Fig. 5. (a) N-doped graphite biochar generated from C-phycocyanin extracted from *Spirulina* residues for catalytic persulfate activation, organic oxidation and nonradical disinfection, reprinted with permission from Ref. [82], copyright (2019) Elsevier. (b) Potassium hydroxide-modified algae-based biochar for sulfamethoxazole removal, reprinted with permission from Ref. [95], copyright (2021) Elsevier.

Ge and Deng [97] reported that the simultaneous photocatalytic reduction of two fluoroquinolone antibiotics using an Fe (III)-algae system. However, results showed that the efficiency of photodegradation of the two fluoroquinolone antibiotics was better at lower antibiotic concentrations in the Fe (III)-algae system [97]. In another study, Zhang et al. [49] also reported that the cooperative action of algae and Fe (III) could be beneficial to the photodegradation of Norfloxacin. The mechanism of photocatalytic degradation of Norfloxacin shown in Fig. 6a.

3.2.2. Nutrients

The addition of suitable nutrients can effectively improve the removal rate of antibiotics. It has been reported that the addition of glucose and sodium acetate significantly enhanced the antibiotic removal capacity of algae [98]. In addition, HCO_3^- and CO_2 are inorganic carbon species that are used by microalgae for autotrophic growth [99]. Notable improvement was observed in the cefradine removal efficiency when CO_2 was added into the process [100]. Moreover, Zhang et al. [101] revealed that $NaHCO_3$ did not promote self-decomposition of the target antibiotic, instead increasing the removal efficiency and total removal capacity of algal cells. Specifically, results showed that the target antibiotic removal rate could be improved from 10.21% to 92.89% when $NaHCO_3$ was added. Furthermore, the addition of sodium acetate as a source of organic carbon for algae, has been demonstrated to significantly increase the ciprofloxacin removal rate (56%) as compared to the system without sodium acetate (13%) [102]. In another study, the bioremediation of levofloxacin by *C. vulgaris* increased following the addition of NaCl, with the improvement in levofloxacin removal linked to salinity enhanced bioaccumulation and intracellular biodegradation [103].

3.2.3. Photocatalysts

Both algae and photocatalysts depend on the availability of light resources and therefore, the inclusion of photocatalytic reduction in antibiotic removal may produce interesting results and deserves further research [104,105]. Recently, the photosynergistic performance of bismuth vanadate ($BiVO_4$) combined with the microalgae

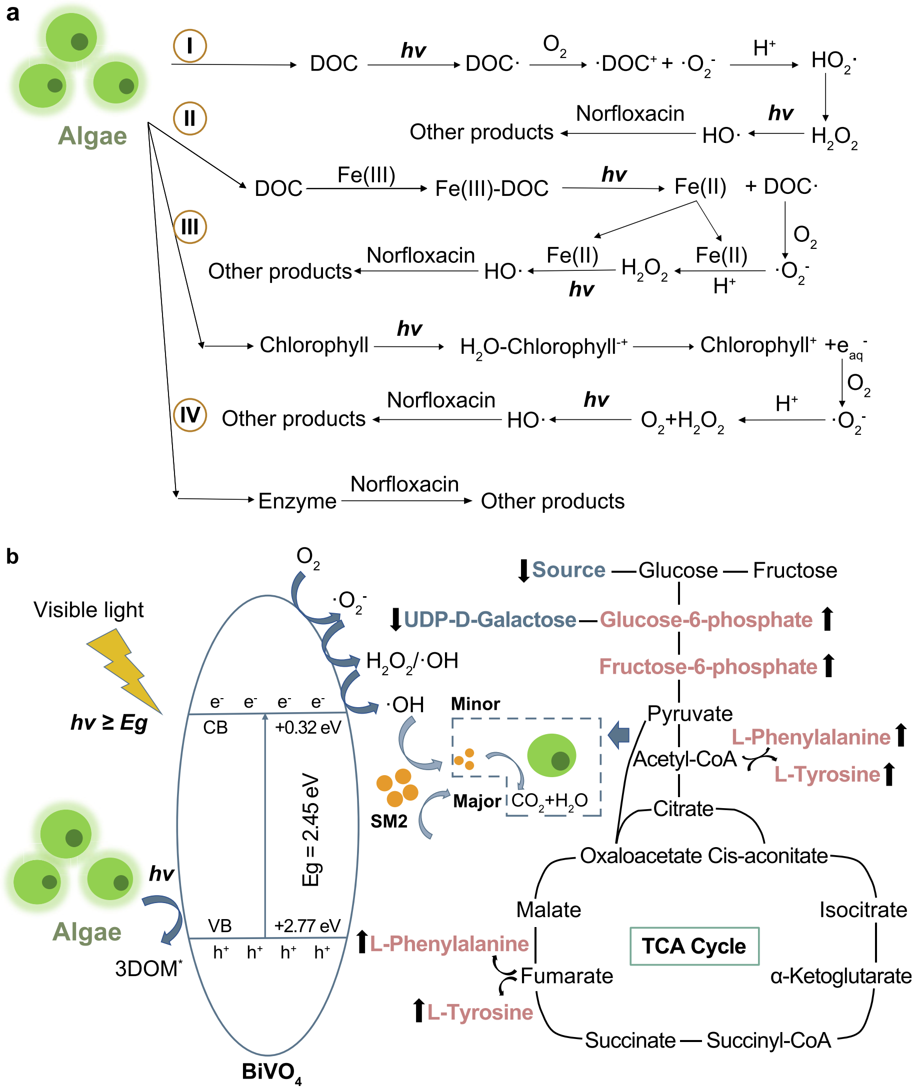

Fig. 6. (a) Photodegradation pathway of norfloxacin in water containing both algae and Fe(III), modified from Ref. [49]. (b) Mechanism of catalytic degradation of sulfamethazine by microalgae and monoclinic $BiVO_4$ under visible-light irradiation, modified from Ref. [106].

Dictyosphaerium sp., was tested under visible-light irradiation for the first time (Fig. 6b) [106], achieving 83% antibiotic removal efficiency via the $BiVO_4$-microalgae system, which was higher than the microalgae only group (41% removal efficiency) [106]. In order to identify the photocatalytic degradation mechanism, metabolomic analysis was performed [106], with results showing that the tricarboxylic acid cycle was activated and glycometabolic pathways were improved when $BiVO_4$ was present. Overall, it is feasible that the addition of specific materials can improve the removal capacity of algae for antibiotics. However, based on the current research status, apart from photocatalytic reactions may inhibit the growth of algae [107], the higher costs will prevent it from being using in a real-world large-scale application.

3.3. Hybrid algae-based treatment with advanced oxidation processes (AOPs)

To reduce the bio-recalcitrance of pollutants in wastewater, a combination of chemical-biological technologies is an emerging method, allowing the removal of inhibitory organics or strongly recalcitrant pollutants. AOPs are efficient and powerful techniques for wastewater treatment [108], such as ozonation, ultrasonication, photocatalysis, UV radiation, and Fenton/photo-Fenton treatment. However, AOP technologies are high-cost and require large energy inputs, especially when complete mineralization of target organic pollutants is required [3]. In general, AOP technologies consist of the production of hydroxyl radicals which serve as a powerful oxidant with these oxidative radicals acting on the target molecules [109]. Recently, AOPs have been increasingly used as a pretreatment process to increase the algal-degradability of antibiotic wastewater, particularly when the resulting intermediates are easily removed by subsequent biological treatment processes (Fig. 7a) [110]. For instance, when the antibiotic concentration was 1000 mg L^{-1}, 97.63% of amoxicillin and 91.08% cefradine were removed, after 12 h of *C. pyrenoidosa* treatment combined with the Fenton process (Fig. 7b and c) [111]. Moreover, a reduction in the amount of Fenton reagents (H_2O_2 and Fe(II)) has been shown not to affect the Fenton treatment contribution, while the overall removal efficiency of the target antibiotic was enhanced [111]. Nevertheless, to date, the number of studies assessing combinations of AOPs and microalgal treatment remain very limited.

In addition, UV light sources can be utilized in combination with bio-treatment [112]. Peng et al. [113] found that algae can improve the photodegradation of pollutants under UV light exposed conditions, speculating that rapid degradation may occur due to hydroxyl radical formation by algae. In order to explore the role of algae in the degradation of cefradine using a combined UV-algae method, Du et al. [114] assessed the removal efficiency of individual *C. pyrenoidosa*, UV activated, and combined UV-*C. pyrenoidosa* treatments, with results showing that reduced effluent toxicity and a relatively high removal rate (22.01% residue) were acquired after the UV-algae process, with the algae treatment being vital for the reduction of effluent toxicity. Subsequently, green algae have been shown to play an important role in the removal of antibiotics, with UV irradiation serving as a trigger for algal treatment. This mechanism was demonstrated by Yang et al. [115], with an excellent removal efficiency (99.84%) obtained when UV-irradiation was applied at 365 nm in combination with *S. obliquus* [115]. Comparatively, Liu et al. [112] established that exposure to UV at shorter wavelengths was crucial for ceftazidime degradation, with wavelengths of 185 nm achieving a removal efficiency of up to 97.26%, while wavelengths >280 nm had a removal efficiency of up to 97.15% and >365 nm achieved a low removal efficiency of only 8.52% (Fig. 7d). However, although UV irradiation has generally been combined with biological approaches for wastewater treatment [116], the research on the capability of algae combined with UV irradiation to remove antibiotics was not sufficient.

4. Perspective #3: novel approaches for enhancing antibiotic removal

4.1. Potential use of genetic engineering to increase algal degradation of antibiotics

Genetic engineering can be used to add a desired characteristic into the target organism. This approach previously used to generate engineered algae, with the aim of developing algae with specific functionality and enhanced metabolic activities [21]. For example,

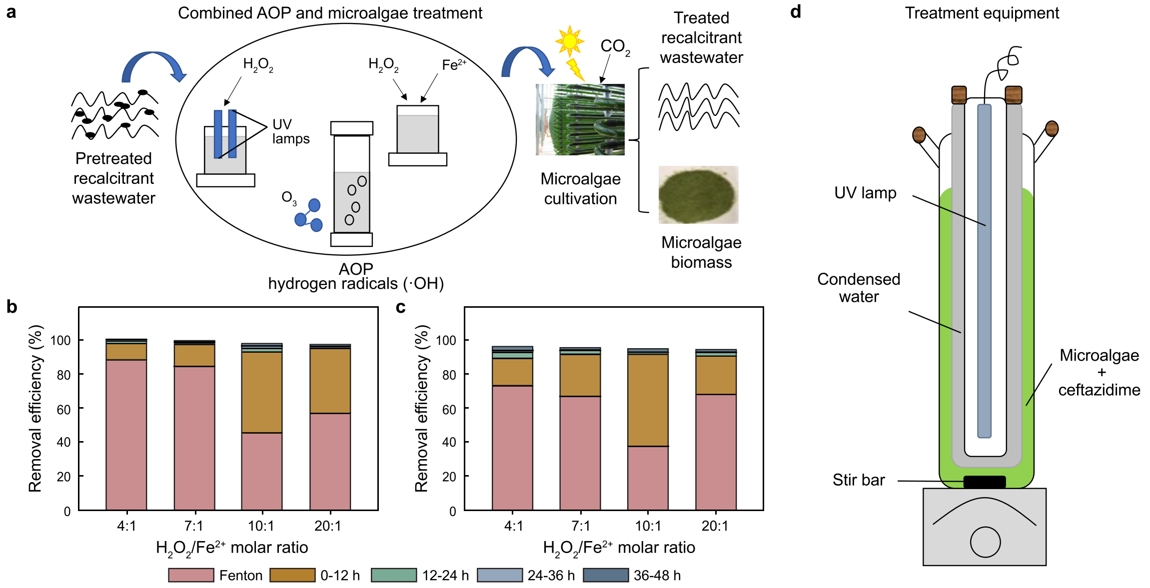

Fig. 7. (a) Advanced oxidation processes combined with microalgae for the treatment of recalcitrant wastewater, modified from Ref. [110]. The removal efficiency of (b) amoxicillin and (c) cefradine, via a combined Fenton-algal treatment process, modified from Ref. [111]. (d) UV/algal treatments, modified from Ref. [112].

algal clones have been engineered with functional enzyme genes, such as for laccase enzymes, which have been demonstrated to increase the stability of oxidoreductases, ensuring efficient biodegradation of pollutants [117]. According to Xiong et al. [21], laccases are extracellular oxidoreductases that are abundant in plants and bacteria. Novel contamination removal genes from bacteria have been effectively transferred into mixotrophic algae for efficient expression, rather than photoautotrophs [117]. For example, the *linA* gene of *P. paucimobilis* UT26 was transferred into *Anabaena* sp. PCC7120, resulting in an increase in the lindane removal efficiency of this cyanobacterium, even in the absence of nitrate [118].

Microalgal genetic engineering still faces some key challenges, including 1) the structure and composition of cell walls, 2) selection agent sensitivity 3) and genomic ploidy [119]. More specifically, the resilient and complex nature of cell walls presents a main challenge. Algae cell walls are a complicated heteropolymer incorporating carbohydrates, lipids, hydrocarbons, carotenoids, tannins, proteins, and lignin, making them highly resistant to infiltration [120]. The selection of a suitable agent plays a crucial role in the effectiveness and efficiency of algal conversion and is dictated by the species present and the antibiotics native resistance [119]. While, in terms of ploidy, which is the number of homologous sets of chromosomes in a biological cell and could play an important role in the efficiency and success of transformation. The main characteristics of different transformation approaches utilized in algae genetic engineering studies are summarized in Table 2.

4.2. Integration of algal antibiotic removal with bioelectrochemical systems

Electricity production from microbes (bioelectricity) has recently attracted increasing attention as a source of sustainable energy as a fourth-generation fuel [122]. Bioelectrochemical systems (BESs) have progressed significantly in the past two decades [123]. In general, microbial electrolysis cells (MECs) and microbial fuel cells (MFCs) are the major applications of BESs, and when coupled with electrochemical redox reactions and microbial metabolisms, provide an excellent option for antibiotics removal [124]. Recently, photosynthetic microbial fuel cells (PMFCs) have been developed as novel BESs, in which photosynthetic organisms act as electron donors in the anode chamber or metabolize and grow to form electron donors in the cathode chamber (Fig. 8a, b, c) [125]. Sharma et al. [125] used *C. reinhardtii* in the cathode chamber of a PMFC, with the system achieving a Coulombic efficiency of 9.068% along with 73.30% COD removal. Aiyer used a co-culture of *Escherichia coli* and *Pseudomonas aeruginosa* as anode microbes and inoculated *C. vulgaris* in the cathode chamber, resulting in an increase in mean power density by 41.7% (Fig. 8d) [126]. Moreover, a new algae-bacteria powered biofuel cell was devised for antibiotic wastewater remediation and energy production, in which algae-bacteria (*C. vulgaris* and anaerobic sludge) cooperation was combined with a cathodic bioelectrochemical procedure for high nitrogen removal, while simultaneously prompting anodic bioelectrochemical reduction of the antibiotic florfenicol, with instantaneous electron absorption from the co-substrate [127]. In another study, pharmaceutical wastewater was pre-treated in a photobioreactor utilizing *S. abundans*, with the effluent further treated in a PMFC for the generation of electricity and biomass, resulting in 93.71% of phosphate and 97.12% of nitrate being removed [128]. Although PMFCs allow sustainable energy formation from wastewater with high removal rates, system scale-up remains a major challenge hindering the utilization of PMFCs in industrial applications. Yang et al. [129] proposed the use of a PMFC stack, including multiple anodic chambers installed in an algal raceway pond, as shown in Fig. 8e, achieving a removal of nearly 98% of the ammonium in the aqueous media of the anode chamber. In summary, these studies suggested that algae-MFC systems seems to be a novel and sustainable alternative for antibiotic wastewater treatment [21].

5. Future perspectives and research potential

Considering the benefits of algae-based techniques in terms of environmental impact, sustainability, and economic viability, they provide alternative biological methods for the degradation of antibiotics from aquatic environments. However, these technologies still face some challenges that require further comprehensive research.

(1) The diversity of physicochemical characteristics of antibiotics are a major factor for algae, requiring the selection of specific combined mechanisms to degrade wastewater contaminants (Table 1). Moreover, apart from bioadsorption, bioaccumulation, biodegradation, photodegradation, volatilization, and hydrolysis, there may be other mechanisms active during the removal process and future research should focus on these aspects.
(2) The potential of algae to degrade antibiotics is high. However, each algal species can degrade different types of pollutants and thus, the development and design of new algal species with increased capacity, affinity, and selectivity for

Table 2
Main characteristics of different transformation methods utilized in algae genetic engineering [121].

Transformation methods	Characteristics
Electroporation	Easy mode of operation. Commonly utilized for distinct genera but restricted on brown algae.
Induced transformation and natural transformation	Mostly in cyanobacteria and is currently rarely utilized.
Silicon carbon whiskers method	Overcomes the cell wall interference to exogenous DNA compared to the glass bead method and has low operational costs. However, strict protection against the hazard of inhalation is required.
Recombinant eukaryotic algal viruses	Promising application potential in brown algae. However, still requires comprehensive and extensive research.
Trans-conjugation	It is mostly in cyanobacteria and is currently rarely used.
Glass beads	The procedures are simple and do not require expensive transgenic equipment. However, it is limited in macroalgae because of immature protoplast regeneration technology.
Agrobacterium tumefaciens-mediated genetic transformation	Performance is reliant on diverse factors, with this approach being technically challenging.
Microinjection	A highly effective and expensive approach with delicate and complicated processes.
Artificial transposon method	Exogenous genes can be directionally integrated into the receptor genome.
Biolistic transformation	Exogenous DNA could be imported into numerous tissues and cells. Diversified vectors could be used to overcome the insufficient genetic background of substances. The manipulation is mature and controllable, but expensive and specialized equipment is required.

the bioremediation of antibiotics is a promising research focus. Genetic engineering approaches also seem to have high potential for enhancing antibiotic degradation.

(3) Biochar has been regarded as a promising biosorbent for organic pollutant removal from aquatic environments [130]. Algae contains an abundance of proteins and can be directly converted into classic nitrogen-doped biochar *in situ* without any additional modification. However, although algal biochar has been applied to treat wastewater, the potential for treatment of antibiotics has not been clearly established to date.

(4) Due to the low operational cost and high performance of microalgal systems, they have been utilized to degrade numerous antibiotics in wastewater. Unfortunately, antibiotics can be bioaccumulated in living organisms (e.g., ciprofloxacin in green algae) leading to the potential feminization of male fish and decreased reproduction processes [109]. For this reason, algae need be associated with other treatment methods when bioadsorption or bioaccumulation is the primary mechanism of antibiotic removal.

(5) Some fungi can form extracellular enzymes with low specificity and are highly convenient for the reduction of some antibiotics, even those exhibiting low water solubility [31]. However, as mentioned above, few studies have investigated the potential for their association in algae-fungi consortia. Therefore, further research is required as multiple algal strain methods have shown promising results for antibiotics treatment.

(6) Some studies using algae for bioremediation have achieved successful operation at an industrial scale [131,132]. Nevertheless, most current research has been at the laboratory-scale, with systems operated using synthetic media despite the well-known complexity of wastewater matrices that vary depending on the WWTPs and geographical regions [63]. Therefore, future work should focus on real polluted wastewater and industrial scale cultivation.

(7) Previous research has primarily focused on enhancing removal efficiency of algae-based technologies for antibiotics, while insight on the fate of ARB and ARGs in aqueous media remains limited [21]. The formation and spread of ARGs in the environment is increasing worldwide, posing a major problem that can severely affect environmental and human health [15]. Thus, the treatment of biomass accumulated downstream is particularly important and this should be considered carefully in future studies. For example, the biomass could be reused as functional biochar for antibiotic removal.

6. Conclusions

This review highlights the application of algae-based technologies for effective antibiotic removal. For the removal of antibiotics, bioadsorption, bioaccumulation, biodegradation, photodegradation, volatilization, and hydrolysis are the main mechanisms that occur during the removal process by algae-based technology. Bioadsorption by algal cells takes place when antibiotics are either adsorbed to cell wall components, or onto organic matter that is secreted by the cell. The bioaccumulation capacity of

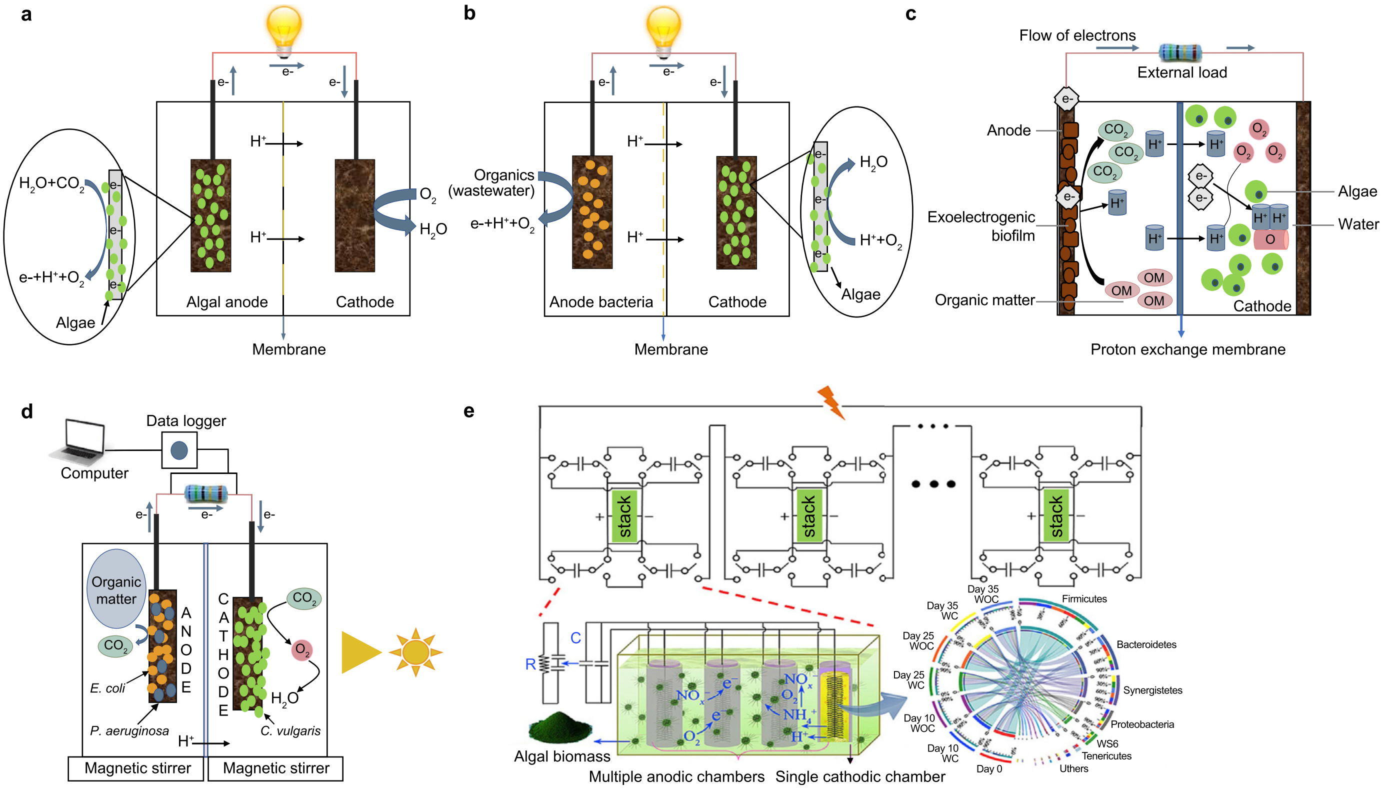

Fig. 8. (a) Algae utilization as a source of carbon in the MFC anode for electricity production. (b) Live algae utilized as an electron donor to the MFC anode for electricity production. (c) A dual-chamber photosynthetic microbial fuel cell configuration, modified from Ref. [125]. (d) Schematic representation of the microbial fuel cell [126]. (e) Multiple anodic chambers combined an algal raceway pond to form a photosynthetic microbial fuel cell stack, reprinted with permission from Ref. [129], copyright (2019) Elsevier.

living organisms is based on various chemical, physical and biological mechanisms, containing both intra- and extracellular processes, whereas passive uptake plays only a minor and poorly defined role. Biodegradation includes the conversion of complicated substrates into simpler molecules via catalytic metabolic reduction. Moreover, the biodegradation capabilities of algae may be improved indirectly by symbiotic communication with bacteria. Fungi are another important type of microbe that is able to facilitate antibiotic removal by algae. In addition, a combination of multiple algae could be beneficial to the removal of antibiotics. Antibiotic degradation by algae is influenced by many factors, such as pH, temperature, CO_2 enrichment levels, light intensity, HRT, algal species, substrate concentration, and the structure of target antibiotics. Algal biochar, especially macroalgal biochar, has been utilized for the fabrication and design of various nanocomposites as a remediation agent for antibiotics removal from the wastewater. Moreover, the nitrogen content of biochar could enhance the production of π-π bonds and strengthen ion adsorption of antibiotics, improving the efficiency of antibiotics removal. The addition of other materials (e.g., Fe (III) salts, appropriate nutrients and photocatalysts) can also improve the photosynthetic activity of algae, further enhancing the antibiotics degradation effect. AOPs could be used as a pretreatment process to increase the algal-degradability of antibiotic wastewater, particularly when the resulting intermediates are easily removed by subsequent biological treatment processes. Furthermore, novel approaches for enhancing antibiotic removal based on algal treatment are also summarized in this review, including genetic engineering and PMFCs, which are demonstrated to be potentially valuable technologies for large-scale antibiotic removal applications.

Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. The work has not been published previously, and not under consideration for publication elsewhere.

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