Current Progress on the Microalgae-mediated Treatment of Pharmaceutical Active Compounds

S. Ahmed,[a,b] G. Mujtaba,[b] M. Rizwan,[c,*] S. Ahmed,[a] M. R. Brohi,[b] and S. W. Ali[b]

[a]Institute of Environmental Engineering & Management, Mehran University of Engineering & Technology, Jamshoro, Sindh 76062, Pakistan

[b]Water and Wastewater Laboratory, Department of Energy & Environment Engineering, Dawood University of Engineering & Technology, Karachi, Sindh 74800, Pakistan

[c]U.S. Pakistan Center for Advanced Studies in Water (USPCAS-W), Mehran University of Engineering & Technology, Jamshoro, Sindh 76062, Pakistan

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Review
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Pharmaceutical active compounds (PACs) are under consideration due to their potential risk to human health and the ecosystem. Researchers are exploring economical and effective wastewater treatment methods to address these issues. Conventional wastewater treatment facilities cannot successfully remove PACs. The co-existence of several PACs and various microalgal species provides an opportunity for microalgae-based bioremediation of PACs. This review paper focuses primarily on antibacterial resistance genes (ARGS), removal of PACs through microalgal systems, essential factors influencing PACs removal, ecotoxicity of microalgae, bioprospecting strategy for microalgae, and challenges and future aspects for microalgae in PACs removal.

Keywords

microalgae, pharmaceutical active compounds, toxicology, bioremediation, wastewater treatment

Introduction

Pharmaceutical active compounds (PACs) in wastewater effluent have posed significant challenges to the environment and human health[1,2]. The demand for pharmaceutical products is increasing day by day, with more than a thousand tons of various classes of compounds being consumed daily in both veterinary and human medicine. It is projected that the use of pharmaceuticals will reach 4.5 trillion daily doses in adults worldwide by 2020[3]. PACs are considered emerging pollutants[4,5], encompassing several different chemicals, their metabolites, and transformation products, with diverse medical and physicochemical characteristics, primarily organic[6]. PACs enter water through two main pathways: (i) production in pharmaceutical industries, and (ii) usage of pharmaceuticals. Consequently, these contaminants can be found in urban and municipal wastewater[5,7].

Conventional wastewater treatment plants are not effectively removing PACs[5,7,10]. The discharge of PACs into water bodies leads to the emergence of antibiotic-resistant bacteria and antibiotic-resistance genes[3,11,16]. Although various advanced methods, such as photocatalytic degradation, carbon adsorption, advanced oxidation, and membrane filtering, have shown promising results in PACs removal from wastewater, there widespread use is hindered by high costs[9]. Due to the shortcomings associated with existing methods, researchers are seeking cost-effective and efficient wastewater treatment alternatives[17,18]. Biologically based technologies are often more environmentally friendly and cost-effective compared to those found in physical or chemical processes[19,20]. Numerous studies have investigated the biodegradation of these pollutants in various settings, such as constructed wetlands (CW), sequencing batch reactors, and membrane bioreactors[21]. Microalgae-based wastewater treatment is a suitable alternative method for removing PACs from wastewater. After wastewater treatment, the algal biomass can be converted into biofuel and biofertilizer[22,24]. *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Tetradesmus* sp, and *Monoraphidium* sp microalgae were used in removing the ciprofloxacin and norfloxacin antibiotic from wastewater. The microalgae strains could remove ciprofloxacin and norfloxacin antibiotics 52.4 % and 87.5 %[25]. The *Chlamydomonas acidophila* strain was collected from Sammlung von Algenkulturen Göttingen [SAG], Germany, for removing the nutrients and

*Corresponding author: e-mail: drmrizwan.uspcasw@faculty.muet.edu.pk

PACs from wastewater. The species removed erythromycin, clarithromycin, 93–95 % and 64–50 %[2]. *Chlorella* sp. and *Scenedesmus* sp. were used to remove caffeine and ibuprofen. Caffeine and ibuprofen were removed at 99 % and 95 %, respectively. Shanthamareen *et al.* suggested that co-culture can also remove the PACs[13].

Microalgae-based wastewater treatments present a more sustainable alternative for PACs removal compared to conventional wastewater treatment systems[12,26,27].

Antibacterial resistance genes (ARGs)

The lack of public awareness regarding the hazards of antibiotic pollution and improper antibiotic handling contribute to the excessive introduction of antibiotics into the environment. This situation poses a significant risk, as bacteria are exposed to the possibility of developing antibiotic resistance. As public awareness of infectious diseases and the demand for antibiotics rows, it becomes crucial to address the associated risks. One of the most substantial threats to public health is the surge in antibacterial resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) resulting from antibiotic use. The primary contributors to the release of antibiotics and the emergence of antibiotic resistance (AR) in the environment are thought to be aquaculture, agriculture, and wastewater treatment plants (WWTPs)[28,29]. Contrarily, acquired resistance refers to ARGs that microbes do not naturally possess but acquire through transposition, horizontal gene transfer, and transformation. The expression of acquired resistance genes in bacteria arises from mutations in the genes encoding antibiotic-modifying enzymes, drug transporter regulators, drug transporters, and drug targets. Water systems in hospitals and medical facilities serve as endemic breeding grounds for germs that produce genes for antibiotic resistance[30,31]. Municipal wastewater treatment releases bacteria and unmetabolized antibiotics into the aquatic ecosystem, where bacteria from various sources and antibiotics interact, exchange genes, and produce antibiotic-resistant species. In the deep-sea sand of the Pearl River Estuary, Chen *et al.* found antibiotic residues and multidrug-resistant bacteria, including sulfamethazine, norfloxacin, ofloxacin, tetracycline, and erythromycin. Moreover, biosolids (remaining solids from sewage treatment) that are dumped in landfills, indirectly contribute to antibiotic contamination of terrestrial ecosystems. Bacteria with antibiotic-resistance genes have been discovered in soils in non-agricultural areas such as the University of Washington, and in agricultural land[33]. According to Blanco-Pena *et al.* animals may spread antibiotic-resistant genes from aquatic ecosystems to terrestrial ones[34]. Wild animals meet antibiotic-resistant bacteria through their diet. A minimum of one antibiotic-resistant bacteria has been detected in the gut microbiota of wild seabirds, which flourish in complex microbial environments full of wastewater and agricultural waste[33]. Antibiotics and ARGs have long-term consequences on the landfill system and the ecosystem. Antibiotics typically kill bacteria, leading to alterations in the structure and functionality of microbial communities[35].

Removal of PACs through microalgal systems

Conventional wastewater treatment plants face challenges in effectively removing pharmaceutically active compounds (PACs)[5,8,36,37]. The release of PACs into water bodies contributes to ecotoxicological issues and antibiotic resistance among bacteria[3,14,40]. Scientists are actively seeking cost-effective and efficient wastewater treatment solutions to address these concerns, and microalgae-based wastewater treatments emerge as sustainable alternatives compared to conventional wastewater treatment systems [12,26].

The increased attention on PACs stems from their potential threat to human health and the environment. Microalgae have regained popularity as a means of bioremediating PACs from wastewater over the last decade[1,38,39]. This review highlights the challenges facing commercial microalgae bioremediation of PACs. Researchers have noted that the bioabsorption and bio-uptake processes of microalgae have not provided a sustainable solution, often leading to toxicity problems. Additionally, a study into biodegradation-assisted bioremediation is required before using algal biomass[13].

In a study by Xiong *et al.*, the effect of sulfamethazine (SMZ) and sulfamethoxazole (SMX) on *S. obliquus* microalgae was investigated. The results indicated that *S. obliquus* could resist high doses of SMZ and SMX, substantially impacting the biochemical features of *S. obliquus* (total chlorophyll, carotenoid, carbohydrate, and FAMEs). The enhanced biodegradation observed at higher SMZ and SMX concentrations suggests that SMZ and SMX degradation by microalgae might be an effective antibiotic resistance mechanism[15]. Ali *et al.* modified the biomass of an alga (*Scenedesmus obliquus*) with an alkaline solution and employed it for the biosorption of tramadol (TRAM) and other medications. The kinetics and isotherms of adsorption were studied. Langmuir isotherms were better at fitting TRAM adsorption by modified algal biomass (MAB) biosorbents than the other isotherm model, which had a maximum adsorption capacity of 140.25 mg g^{-1} with the most significant

correlation coefficient (0.942). According to the findings, MAB might be used as a potentially reusable sorbent to remove pharmaceuticals from wastewater[11].

Algae are either micro or macro in size. Microalgae include green-blue algae (prokaryotes/cyanobacteria), while macroalgae include kelp and seaweed. With approximately three lack fifty thousand microalgae species discovered[38], microalgae also contain protein (65 %), carbohydrates (60 %), and lipids (70 %)[41]. Microalgae are 40–50 % more efficient than plants in CO_2 mitigation[41] and can consume CO_2, e.g., 1 kg of microalgae can consume 1.83 kg of CO_2 mitigating almost 40 % of global CO_2 emissions. Acting as a bioindicator for detecting climate change in water environments, microalgae can consume 80 % to 100 % of nutrients from wastewater[42].

Several studies have explored the microalgal removal of PACs from wastewater. Table 1 presents the laboratory-scale research using microalgae to bioremediate antibiotics and other pharmaceutical compounds. In this proof-of-concept research, *Scenedesmus* sp, *Chlamydomonas*, and *Chlorella* are the most often reported and thoroughly investigated species.

Singh *et al.* cultivated three types of microalgal strains, *Chlorella* sp., *Chlorococcum* sp., and *Neochloris* sp., in river water contaminated with pharmaceutical effluent. These strains not only removed the organic pollutants from the drug effluent but also produced lipids[43]. The *Chlorella vulgaris* strain, cultivated on domestic wastewater, demonstrated more efficient removal of nutrients and biomass production compared to microalgae grown in distilled water[44]. The *Chlamydomonas acidophila* strain, collected from Sammlung von Algenkulturen Göttingen [SAG], Germany, exhibited effective removal of nutrients and PACs from wastewater. The species removed erythromycin, clarithromycin 93–95 %, and 64–50 %[2]. The removal of cephalexin by *Chlorella* sp., non-living modified by lipid extraction, was explored in this research. At the lowest starting concentration, a notable antibiotic elimination of 71.19 % and 82.77 % was achieved, suggesting that the bio sorbent presents a feasible substitute for removing Cephalexin[45]. In the elimination of tetracycline, Chlorophyte alga, specifically, *Chlorella pyrenoidosa* was employed. More than 98.0 % of the tetracycline was removed by *M. aeruginosa* in just two days, as opposed to 36.7 % to 93.9 % by *C. pyrenoidosa*[46].

C. vulgaris and *S. capricornutum* exhibited greater efficacy in removing fluoroquinolones and macrolides compared to sulfonamides, while *S. quadiricauda* and *H. pluvialis* showed higher removal results for sulfonamides[26]. *Chlorella* sp. and *Scenedesmus* sp. were used to remove caffeine and ibuprofen, achieving removal rates of 99 % and 95 %, respectively. Shanthamareen *et al.* suggested that co-culture also could remove PACs; further, microalgae could be easily harvested after co-culture[13]. Pharmaceutical removal fell within the range from 60 to 100 %, except for antibiotic sulfapyridine and sulfamethoxazole (46 %), which were not removed[3]. Microalgal consortiums were cultivated in periphyton photobioreactors to remove pharmaceutical compounds, personal care products, and nutrients from effluent. The results showed the removal of carbamazepine, ibuprofen, and gemfibrozil. *Scenedesmus obliquus* algal bacteria, used in a photobioreactor, demonstrated moderate elimination of ibuprofen (26.2 % – 48.7 %), with treatment effectiveness improving with longer hydraulic retention time, although the effects of lighting on the removal of nutrients and PACs were inconsistent[19]. Erythromycin (ERY) and Cephalexin (CEP) were removed from wastewater from a neighbouring wastewater treatment plant (WWTP) through the consortium. The incubation findings showed that the higher concentrations of certain antibiotics had not affected the consortium growth. Cephalexin (CEP) and erythromycin (ERY) had nearly complete removal, with total removal of 96.54 % and 92.38 %, respectively. The symbiotic connection between bacteria and microalgae affects the kinetics of CEP and ERY elimination[47]. Diclofenac, ibuprofen, paracetamol, and metoprolol were removed between 60 % and 100 % through photolysis and biodegradation. Trimethoprim and carbamazepine were only partially removed, with removal rates of 30 % and 60 %, respectively. Less than 20 % of the micropollutant elimination was due to sorption to algal biomass[48].

Consistent findings on contaminants of emerging concern (CEC) elimination in HRAP were observed. In July, with an HRT of 4 days, Matamoros *et al.* reported removal efficiencies of NP, IB, MePB, and HM-BP of 83 %, 99 %, 59 %, and 97 %, respectively[46]. In contrast, MePB demonstrated the highest removals, while NP and IB had the lowest. Villar-Navarro *et al.* and Garca-Galán *et al.* both indicate slightly higher removal rates than in this research, with NP and IB being removed 79 % on average in July at an HRT of 6 days and 4.5 days, respectively[49,50]. The only research indicating marginally lower removal was by Vassalle[51].

The research was conducted in Spain and Brazil, both particularly favourable for HRAP due to their sunny weather. For example, Ajdovina, Slovenia, receives roughly 1250 kWh m^{-2} annually, while Barcelona, Spain, and Belo Horizonte, Hungary, receive about around 1750 kWh m^{-2} per year[14].

Table 1 – *Removal of pharmaceutical active compounds using microalgae*

Name of species	PACs	Concentration of PACs/mg L^{-1}	Concentration of algae/mg L^{-1}	Contact time/ days	Removal/%	Toxic metabolites	Types of wastewater	Process	References
Microalgae bacterial consortium	tetracycline, ciprofloxacin, sulfadiazine, and sulfamethoxazole	0.02–1	1000	4	99.9, 78, 52.6 and 5	–	Synthetic	Batch	52,53
Alagl-algal consortium	sulfamethoxazole and ofloxacin	0.01–0.1	–	7–10	77.3 and 55.1	–	Domestic	Batch	53
Chlorella vulgaris, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Tetradesmus* sp and *Monoraphidium* sp.	ciprofloxacin and norfloxacin	0.5–6.5	–	4	52.4 and 87.5	Effect on biochemical product	–	–	25
Chlamydomonas sp.	ciprofloxacin and sulfadiazine	50	80	9	100 and 54.53	–	Synthetic	Batch	54
C. sorokiniana	paracetamol and salicylic	25–250	–	1–2	69 and 98	–	Industrial	Semiconti-nuous	55
Chlorella vulgaris, Chlorella sorokiniana and Scenedesmus obliquus	diclofenac paracetamol and salicylic acid	25	–	10	41, 99 and 79	–	–	Semiconti-nuous	56
Chlamydomonas acidophila	erythromycin and clarithromycin	0.0023–0.008	–	7	93–95 and 64–50	–	Domestic	Batch	2
Scenedesmus obliquus	sulfamethazine and sulfamethoxazole	0.05–6	–	11	17.3 and 29.3	Hydroxy-lation, methylation, nitrosation, deamination, and bond cleavage	–	Batch	57
Chlorella pyrenoidosa	roxithromycin	0.0625–2	0.1–1	21	53.3	Algal growth	Synthetic	–	58
M. aeruginosa and *C. pyrenoidosa*	tetracycline	10–100	–	2	98	Microcystin-LR	Synthetic	–	46
Chlorella vulgaris, Scenedesmus quadricauda Haematococcus pluvialis, and *Selenastrum capricornutum,*	levofloxacin, lomefloxacin, sulfamonomethoxin, sulfamethoxazole, sulfamerazine and clarithromycin, trimethoprim, azithromycin, flumequine, roxithromycin	0.02–0.1	–	40	–	Fish and green algae	Synthetic	Batch	26

Name of species	PACs	Concentration of PACs/mg L^{-1}	Concentration of algae/mg L^{-1}	Contact time/ days	Removal/%	Toxic metabolites	Types of wastewater	Process	References
Chlorella sorokiniana Bacterial consortium	cephalexin and erythromycin	0.05	–	7	96 and 92	–	Industrial	Batch	47
Spirulina platensis	chlortetracycline	0.5–20	0.833	13	98–100	Chl-a and γ-linolenic acid	Livestock	–	59
Chlorella sp.	sulfonamide	–	–	30	24–38	–	Synthetic	Batch	60
Scenedesmus obliquus	sulfamethazine and sulfamethoxazole	0.15	–	12	31.4–62.3 and 27.7–46.7	High effect on aquatic organisms	Treatment effluent	–	15
Nannochloris sp.	trimethoprim, sulfamethoxazole, carbamazepine, ciprofloxacin and triclosan	0.002–0.003	–	14	90, 60, 100, 100 and 100	High effect on aquatic species	Domestic	–	61
Chlorella sp., *Chlamydomonas* sp. and *Mychonastes* sp.	cephalosporin	25–150	–	16	60–75	Microalgae growth	Treatment effluent	Batch	62
Chlorella sp.	amoxicillin	–	–	13	99.3	–	Synthetic	–	63,64
Chlorella vulgaris				4–5	88				64
Chlorella sp.	thiamphenicol	156.8	–	14	46.2–95	Chlorination, chlorine substitution, dehydration and hydroxylation	BG-11	–	65
Chlorella sorokiniana	trimethoprim, diclofenac, ibuprofen, paracetamol and metoprolol	0.1–0.35	–	37	trimethoprim 30–60 and others 60–100	Effect on algal growth	Synthetic	Batch	48
Navicula sp.	ibuprofen, atenolol and naproxen	0.5	–	21	46–53	Hydrophobicity	–	–	66

Essential factors influencing PACs removal

Co-metabolism

While microalgae play a crucial role in removing medicine pollutants through indirect photodegradation, biodegradation, bioaccumulation, and bioadsorption, the clearance efficiencies of some pharmaceutical pollutants can still be relatively low. To enhance removal efficiency, artificial improvements have been made to accomplish co-metabolism, by adding organic substrates (glucose, sodium acetate, or methanol)[37]. In co-metabolism, the pharmaceutical waste products are transformed into their intermediates, which are more readily biodegradable and participate in the central metabolic pathways for further biotransformation (such as acetate or methanol). With the presence of sodium acetate, the removal rate of ciprofloxacin by the microalgae *C. mexicana* increased by more than three times. The addition of glucose produced a comparable outcome, elevating the cefradine removal efficiency from 27.11 % to 85.1 %. However, due to catabolite repression, adding some organic substrates, such as sodium format, reduced the removal efficiency of pharmaceutical contaminants. The production of enzymes involved in the catabolism of carbon sources during the co-metabolism process is hindered by the presence of organic substrates. Factors like class of pharmaceutical contaminants, concentration of organic substrates, microalgae species, and reaction time are additional variables that can affect the co-metabolism process[40].

It is essential to investigate the effect of organic substrates on this process along with other mentioned elements. The metabolic mechanisms involved in the removal of pharmaceutical excess by algae are more frequently investigated in pure culture of microalgae considering cultivation factors such as pharmaceutical compounds, concentration light, temperature, and hydraulic retention time[77].

Co-metabolism mechanisms remain largely unexplored, particularly in diverse aqueous environments like surface water and wastewater. These environments present multiple influencing factors, including microbial species and dissolved organic matter, which may act as potential inhibitors in the removal of pharmaceutical contaminants. In-depth studies are essential for eliminating these impurities from surface waters and wastewater.

Acclimation

Wastewater treatment must enhance the microalgae response, which can be attained through acclimation of microalgae species under limiting or harsh conditions, such as exposure to toxicants and nutrient deficiency. Earlier evidence indicates that acclimated microalgae exhibit higher biodegradation and tolerance capabilities than their wild-type counterparts, ensuring greater removal efficiency of contaminants[15,67]. Acclimation is widely used for the removal of contaminants and nutrients from wastewater. Chen *et al.* highlighted enhanced removal efficiency with 60 mg L^{-1} cefradine by acclimated *Chlorella pyrenoidosa*[86]. Similarly, Xiong *et al.* found *Chlorella vulgaris* effective with 200 mg L^{-1} of levofloxacin exposure for 11 days, and showed higher removal efficiency (28 %) of levofloxacin compared to the wild species (16 %)[68]. Liao *et al.* revealed enhanced biodegradation of sulfanilamide by microbe communities incubated for 28 days at an increased temperature of 45 °C [70]. Cho *et al.* reported that *Dunaliella salina*, acclimated to phenol, exhibited a significantly elevated growth rate and increased fatty acid levels compared to wild species[99]. In general, these studies indicate that acclimation is a favourable perspective for enhancing the tolerance of microalgae in bioremediation and biomass production. The microalgal capability to adapt to harsh environments is ascribed to gene changes caused by physiological adaptation or spontaneous mutation[67]. The enhanced ability of acclimated microalgae species may be attributed to increased photosynthesis, antioxidant system activities, metabolism processes, or carotenoid biosynthesis[15,67,69]. However, the capability of microalgae to acclimatize to harsh environments is species-dependant, necessitating detailed molecular analysis to elucidate the effects of stress conditions on contaminant removal.

Ecotoxicity of microalgae

In the research conducted by Scheurell *et al.,* five transformation products and diclofenac were identified in the waters of the Lyari River and Malir River, Karachi, Pakistan. The presence of transformation products and diclofenac may lead to mixture toxicity[71]. The research focused on Pakistan hospital waste to identify the commonly used pharmaceutical compounds and calculate ecological risk assessments. In the wastewaters of Gujrat, Pakistan, six pharmaceutical compounds were detected: amlodipine, diclofenac, naproxen, rosuvastatin, ibuprofen, and paracetamol[72].

Research was carried out on the wastewaters of two large hospitals in Lahore, Pakistan. The pharmaceuticals found in the hospital wastewater included emifloxacin, sparfloxacin, ciprofloxacin, and ofloxacin. The study conducted an environmental hazard assessment of these pharmaceuticals, employing ecological risk assessment in terms of risk quotient (RQ) computed for ofloxacin, ciprofloxacin, and moxifloxacin against numerous freshwater

species such as *Pseudomonas putida*, green algae, *Vibrio fisheri*, fish, *Daphnia*, and *Pseudokirchneriella subcapitata*. Ofloxacin posed a very high risk to all six species evaluated, while ciprofloxacin posed a risk to species like *Microcystis aeruginosa* and green algae[73].

In the study, 52 pharmaceuticals and personal care products (PPCPs) from various classes were examined in water and sediment samples obtained from Lahore's urban drains and canals, along with their risk evaluation. Non-steroidal anti-inflammatory drugs (NSAID) were the most prevalent PPCPs identified in the surface water and wastewater of the urban drains. Acetaminophen had the greatest concentration, with a median concentration of 13,880 ng L^{-1}, followed by caffeine sediment samples, with ofloxacin (median weight of 1980 µg kg^{-1}) being the highest with a median value of 6200 ng L^{-1}. Antibiotics, such as ciprofloxacin and oxytetracycline, were the most prevalent PPCPs in the study. According to the ecological risk assessment based on RQ, most PPCPs pose a severe threat to the aquatic community. Khan *et al.,* examined the presence of pharmaceuticals in domestic wastewater in Mardan and surface water in the Kalpani stream, River Kabul, and River Indus[74]. NSAIDs, particularly paracetamol and ibuprofen, exhibited the highest concentration in sewage and surface water. The computed RQ for ibuprofen, paracetamol, and diclofenac in the Kabul and Kalpani streams indicated toxicity for daphnia, fish, and algae[75].

Some microalgae species may be hazardous to wastewater media, particularly in large-scale real-world applications. This is the greatest obstacle facing microalgae-based wastewater treatment system (WWTS). The toxicity of wastewater is determined by the source and composition of pollutants[76]. Techniques such as water quality index, direct toxicity evaluation, and in-vitro and in-vivo bioassays are utilised to assess wastewater toxicity. Metals are neurotoxic and carcinogenic, and the organisms' dose, duration of exposure, and immune system determine their toxicity[43]. Toxicological research on microalgae will assist in establishing a firm platform for ecotoxicological assessments in aquatic environments[78]. Significant factors, such as heavy metals (mercury, cadmium), high ammonium concentration, predatory zooplankton, and increased oxygen concentration can induce substantial toxicity in urban wastewater[67]. To address toxicity challenges, acclimatization or adaptation of microalgae to the wastewater system is extensively researched. The concentrations of diclofenac, naproxen, ibuprofen, and carbamazepine range from 0.4 to 20.6 ng L^{-1}, whereas iohexol, iopromide, and gabapentin range from 4,500 to 7,400 ng L^{-1}. In general, nonsteroidal anti-inflammatory drugs pose a threat to aquatic organisms. According to reports, illegal chemicals are toxic to marine life and exist in a usable form. These drugs enhance lipid peroxidation, protein carboxylation, and DNA damage[79]. Benthic creatures in interstitial water, sediments, and overlying water are at risk from PACs pollutants[80]. PACs' primary adverse effects on human health include reproductive system dysfunction, potent carcinogenicity, and endocrine disruption. Endocrine-disrupting compounds found in the surface water of a river contaminated by sewage water can interfere with the reproduction of fish and mammals. These disruptors mimic endogenous hormones, bind to their receptors, and block the regular activity of the endogenous hormone[81]. It was discovered that genetic adaptation enabled microalgae to withstand high concentrations of antibiotics, herbicides, and debris[82,83].

In addition, microalgae have been found to adapt to various sublethal stressors, including intense light, singlet oxygen, salinity, and heavy metals[84,85]. In harsh settings, microalgae release hazardous degradation enzymes[43,85]. Research by Chen *et al.* suggests that pre-exposed microalga can eliminate antibiotics like cefradine more effectively compared to native species. According to another study, the growth rate of acclimated strains in untreated wastewater was substantially higher than that of non-acclimated strains. *Chlorella luteoviridis* and *Parachlorella kessler* were successfully adapted to secondary-treated urban wastewater medium after an 8-week acclimatization period[86].

Moreover, a correlation was identified between the acclimatization to wastewater, tolerance, ascorbate peroxidase activity, and the accumulation of carotenoid pigments[67]. Microalgae *P. kessler,* isolated from wastewater effluent, exposed to high-temperature, oxidative, acidic, and alkaline conditions, exhibited high growth potential in saline environments while collecting radioactive particles. The presence of pharmaceuticals, personal care items, insecticides, etc., in low quantities[36,87] is a central challenge of conventional wastewater treatment for handling PACs. Notably, the EC_{50} (concentration of PACs at which 50 % of algal growth is inhibited) of most microalgae species is much higher than the average concentration of PACs in the entire wastewater system[88].

Bioprospecting strategy for microalgae

Selecting microalgae and bioprocess technologies to produce high-value-added compounds (HVAC) is essential for developing a successful biorefinery process[89,90]. Standard methods for isolating single cells might be more efficient but time-consuming. Creating a fast and sophisticated method for identifying HVAC is required to facilitate the screening of promising microalgal strains

for commercial applications. Casella *et al.* proposed that spectrophotometric selection identifies microalgal strains rapidly producing carotenoids. Numerous wavelengths, including 480, 478, and 488 nm, have excellent astaxanthin absorbance. Although astaxanthin, lutein, and β-carotene exhibited maximum absorbance at different wavelengths during spectrophotometric measurement, astaxanthin had the highest absorbance at 492 nm[90].

Due to the overlap of carotenoid spectra, the maximum absorbance of lutein was approximately 460 nm, and that of β-carotene 466 nm. Carotenoids must be analyzed using HPLC to prevent the overlap of carotenoid spectra. Rasid *et al.* examined astaxanthin accumulation in *Tetraselmis* sp. and *C. sorokiniana* under various stress conditions (nitrate, salt, sugar, pH) using two factorial designs to alter the parameters. The generated astaxanthin was measured at 480 nm using a UV-vis spectrophotometer[91]. In addition, Peraman and Nachamuthu utilized HPLC to select microalgal strains that produce fucoxanthin[97]. Using a high-throughput screening technique based on HPLC-DAD-MS, Asker and Awad, evaluated lutein-producing microalgae strains[92]. Similarly, McGee *et al*. utilized HPLC's C18 monolithic column for rapid chemotaxonomic or chemotaxonomic pigment profiling[93]. Nile Red Fluorescence and fatty acid analysis required accurate methods for lipid identification. BODIPY (borondipyrro-methane) is a quick method for selecting and screening microalgae containing lipids. BODIPY is a lipophilic dye linked to the lipid membranes of microalgal cells[94]. Colorimetric sulfo-phospho-vanillin is a sophisticated alternative method for selecting microalgal strains containing lipids[95].

In addition, fluorescence-activated cell sorting and microfluidic devices facilitate the selection and screening of microalgal species rich in lipids[96]. Katayama *et al.* discovered that the combination of flow cytometry and Nile red labelling is a successful method for isolating, selecting, and screening microalgae strains with high lipid content[98]. Han *et al.* recently utilized a microfluidic device based on Di electrophoresis to select and screen high lipid-producing microalgae strains[94]. Bioprospecting microalgal strains with a high carbohydrate content are better suited for microplate-based techniques. Using microplate techniques, Olia *et al.* isolated a microalgal strain (*Picochlorum* sp. D8) with a high carbohydrate production rate[89,96]. Bioprospecting rich microalgae strains can enhance biomolecule output, reducing algal biorefinery's overall cost[89]. However, additional study is necessary to create innovative, quick, and effective methods for bioprospecting high-yielding microalgal strains for HVAC production.

Challenges and future aspects for microalgae

In numerous WWTPs around the world, PACs still need thorough investigation despite extensive research. Therefore, it is of the utmost importance to investigate treatment efficacy, expanding our understanding of the fate of PACs and potentially discovering new bioremediation strains. In most laboratory-scale studies, only target contaminants were tested under controlled conditions. However, wastewater contains various pollutants, leading to competition for binding sites and altering the stability of interactions between algae and pollutants. Many past works have overlooked the interference among impurities and microalgae cells due to synergistic, antagonistic, and additive effects of toxicity from multiple pollutants. Some microalgae species may be hazardous to wastewater media, particularly in large-scale real-world applications. The most reported and in-depth investigated species in these proof-of-concept investigations are *Chlorella, Scenedesmus* sp., and *Chlamydomonas.* Given the wide variety of microalgae species, it is apparent that only a small number of them have received sufficient research into their bioremediation potential. Screening methods are required to validate and assess various microalgae strains for selectively removing contaminants. A rapid and sophisticated method for identifying high-value-added compounds, HVAC, is needed to facilitate screening promising microalgal strains for commercial applications.

It is worth noting that most of the proof-of-concept studies were conducted in batch reactors under controlled laboratory conditions. Therefore, pilot scale studies are needed to examine the challenges of removing contaminants in continuous-flow reactors under dynamic environmental conditions. More research is required to explore the sustainable pathways for the biodegradation of pollutants while reducing the toxicity of contaminants in the microalgae. Moreover, microalgae biomass after bioremediation needs special consideration for large-scale applications. The effects of multiple pollutants in wastewater in real-world applications are unpredictable. In such cases, a pre-treatment unit before the microalgae-based system may be needed to selectively remove the pollutants in the conventional system, followed by microalgae-based systems. BODIPY (borondipyrro-methane) is a quick method for selecting and screening microalgae containing lipids. BODIPY is a lipophilic dye linked to the lipid membranes of microalgal cells[94]. Bioprospecting microalgal strains with a high carbohydrate content is better suited for microplate-based methods. Olia *et al.* isolated a microalgal strain (*Picochlorum* sp. D8) with a high carbohydrate production rate using microplate techniques[96]. However, additional

study is necessary to create innovative, quick, and effective methods for bioprospecting high-yielding microalgal strains for HVAC production. Bioprospecting rich microalgae strains can enhance biomolecule output, reducing algal biorefinery's overall cost[95]. The quality of wastewater effluent after separating microalgal biomass needs to be better reported in the literature. Future research must focus on characterizing and identifying the byproducts formed during bioremediation. The relative toxicity of such byproducts and their presence might hinder the ability to reuse such wastewater.

Conclusions

The microalgal strains most frequently cited and extensively investigated for removing PACs in literature are *Scenedesmus* sp., *Chlamydomonas*, and *Chlorella*. Microalgae-based wastewater treatment methods are gaining attention for their ability to remove various antibiotics like ciprofloxacin, levofloxacin, norfloxacin, sulfadiazine, sulfamethoxazole, tetracycline, roxithromycin, and other pharmaceutical compounds, such as paracetamol, ibuprofen, diclofenac from wastewater. In most laboratory-scale studies, only target pollutants were assessed under controlled conditions. However, wastewater contains various contaminants that can lead to competition for binding sites and alterations in the stability of interactions between microalgae and pollutants. Future research studies must be focused on identifying and characterizing the byproducts formed during bioremediation.

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