



# Algae and bacteria consortia for wastewater decontamination and transformation into biodiesel, bioethanol, biohydrogen, biofertilizers and animal feed: a review

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## Abstract

Traditional wastewater treatment has been aimed solely at sanitation by removing contaminants, yet actual issues of climate change and depletion of natural resources are calling for methods that both remove contaminants and convert waste into chemicals and fuels. In particular, biological treatments with synergic coupling of microalgae and bacteria appear promising to remove organic, inorganic, and pathogen contaminants and to generate biofuels. Here, we review the use of algae and bacteria in the treatment and valorization of wastewater with focus on cell-to-cell adhesion, wastewater properties, and techniques for algae harvesting and production of biodiesel, bioethanol, biohydrogen, exopolysaccharides, biofertilizers, and animal feeds.

**Keywords** Microalgae · Wastewater treatment and bioremediation · Nutrient removal · Biodiesel and bioethanol production · Biofertilizer production · Emerging contaminants removal

## Introduction

Water is considered the most essential component for all living organisms. Almost 70% of the Earth's surface comprises water, of which nearly 3% accounts for freshwater resources. As freshwater is scarce, sustainable use of water is a pressing need. Recent studies have shown the outbreak of several water-borne diseases among people due to the

consumption of water contaminated with industrial waste and wastewater treatment plants (Hasan et al. 2019; Lin et al. 2022; Ntajal et al. 2022). Various sources of water including municipal, industrial, and agricultural are adding nutrients, toxic metals, colorants, pharmaceutical products, antibiotic residues, pesticides, and inorganic compounds to the water bodies leading to their eutrophication and contamination (Kunhikrishan et al. 2012). The addition of excess nitrogen and phosphorus to wastewater and subsequent eutrophication is considered extremely harmful to aquatic flora and

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fauna (Preisner et al. 2021). Thus, it has become necessary to develop cost-effective, environment-friendly, and efficient methods for the treatment of wastewater.

Wastewater treatments are largely done in the primary, secondary, and tertiary stages following physical, chemical, and biological procedures (Aboagye et al. 2021; Rout et al. 2021; Xu et al. 2021). To dispose of wastewater safely, different methods are followed, which include processes like coagulation, flocculation, filtration, flotation, adsorption, photocatalysis, and electrocatalysis (Tang et al. 2019; Shahedi et al. 2020; Ahmed et al. 2022; Vidu et al. 2020; Saleh et al. 2022). However, the main drawbacks of these processes are their intensive energy requirement, high cost, and less environment-friendly nature. Moreover, these processes tend to waste resources and generate hazardous by-products and sludge, which result in secondary pollution (Edo et al. 2020; Qu et al. 2019; Wang et al. 2022).

The biological treatment of wastewater is considered a less energy-consuming and more sustainable approach for the treatment of wastewater after initial pre-treatment. Moreover, consortia of autotrophic and heterotrophic bacteria indigenous to wastewater remove most of the heavy metals and nutrients such as phosphate and nitrate from the wastewater and help to stabilize the downstream treatment process (Vajda et al. 2011; Xia et al. 2019). They also help to improve the wastewater by reducing the odor and colors and increasing the efficacy of any treatment plants (Del Nery et al. 2016). Biological treatment processes can also be carried out using microalgae, as they show high efficiency in the removal of toxic metals,

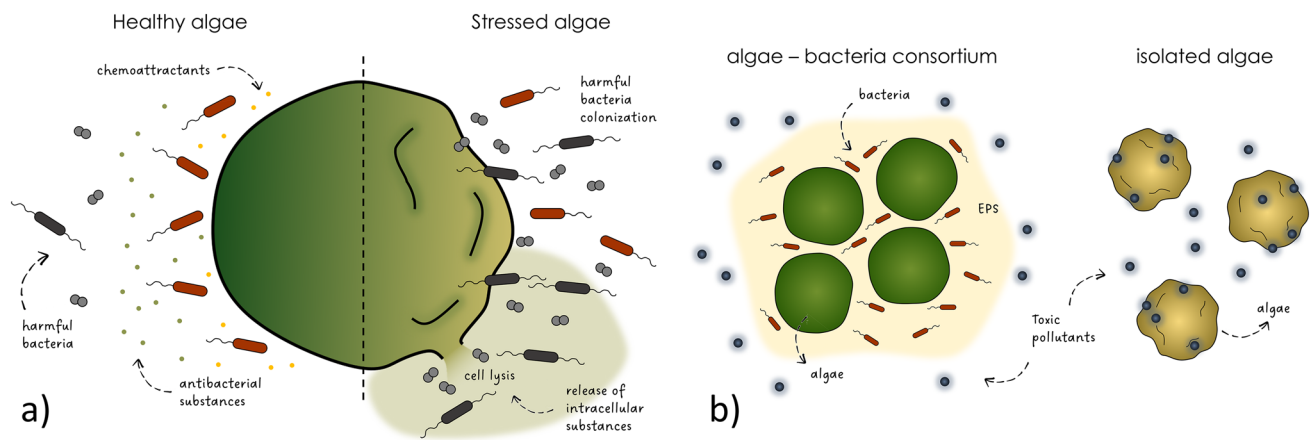
nutrients, and pharmaceuticals (Bolan et al. 2009; Wolmann et al. 2019; Chai et al. 2021; Bhatt et al. 2022). Microalgae also have a great capacity for the uptake of nutrients such as nitrogen and phosphorus, which are required for their growth. Microalgal growth in nutrient-rich wastewater along with a consortium of different bacteria can synergistically recycle the nutrients present in the wastewater and help in the reduction in biological oxygen demand and chemical oxygen demand. They also aid in the removal of nitrates and phosphates from the wastewater. Algal and bacterial-mediated co-bioremediation systems can facilitate the conversion of CO<sub>2</sub> to biobased chemical products, including biofuels and bioalcohol, and reduce greenhouse gasses (Perez-Garcia and Bashan 2015). Figure 1 shows a suitable example of this concept, reporting a picture of the experiments performed in a recent study that used algae and bacteria consortium to promote the removal of organic pollutants by bacteria and the reduction in nutrients by algae in wastewater (Qi et al. 2021). In this work, five different communities were obtained by selecting different biomass proportions of algae and bacteria to find the best choice.

The symbiotic relationship between algae and bacteria is considered the structural pillar of the ecosystem, and their consortia can be effectively used for the treatment of wastewater. However, as the composition of wastewater varies greatly, it can also impact the growth of microalgae depending on the pH, temperature, and light intensity. There have been several reports that state the biological treatment of different wastewaters using algal species like *Chlorella*



**Fig. 1** An algal–bacterial consortium, at a concentration of 500 mg L<sup>-1</sup>, showing the bioreactors made on transparent organic glass with a volume of 6 L. The air inlet is located at the bottom of the bottles, and the air outlet is at the bottom. The culture was mixed with a natural algae community and activated sludge in different ratios, as reported by Qi et al. (2021). The selected proportions of sludge and algae were 1:10, 1:5, 1:1, 5:1, and 10:1. All the samples were cultured in the same aerobic wastewater in the photobioreactors for 48 h.

The efficiency in the removal of nutrients and the productivity in the biomass was evaluated for all the consortia shown in the picture, each having a different ratio between activated sludge and algae. The sample with a proportion of 1:5 achieved the highest nitrogen and phosphorus removal efficiency and better biomass production. This picture from Qi et al. (2021) is under a Creative Commons Attribution 4.0 International License



**Fig. 2** **a** Algae and bacteria release substances that promote the interaction and the consortium's establishment. Stressed algae are colonized by harmful bacteria and cell damage and lysis promote colonization, disturbing the consortium; **b** Cell-to-cell adhesion in the

*zofingiensis* and *Scenedesmus spp.* could successfully reduce the chemical oxygen demand, total nitrogen, and total phosphorus (Wang et al. 2010; Zhu et al. 2013).

The algal biomass generated in wastewater treatment plants can be recovered by flocculation and electrochemical precipitation methods. As this, mass is a very rich source of carbohydrates, lipids, and proteins from which different commercial products, such as carotenoids and polyhydroxyalkanoates, and can be used as a feedstock for microbial fermentation (Bhatia et al. 2017; Goswami et al. 2020; Bolan et al. 2009). Although some reviews on the potential application of microalgae in the treatment of wastewater streams such as farm effluents and municipal effluents (Bolan et al. 2009) have been published, only limited works on the value of microalgae and bacterial consortium in the treatment of wastewater streams are available. This review mainly deals with the use of microalgal as well as bacterial systems in the treatment of wastewater streams. The study design of the paper is reported in the Supporting Information (S1). A detailed discussion on the use of microalgae in the treatment of different types of inorganic contaminants in wastewater is also included. The review also deals with the present state-of-the-art review to highlight economic considerations and the different nutrient recovery processes.

## Cell-to-cell adhesion

Cell-to-cell adhesion between algae and bacteria allows the establishment of a consortium and the co-evolution of both algae and bacteria. Toxic pollutants interfere with the physical contact between cells, thus disturbing the balance, even if

algae-bacteria consortium may reduce the toxicity of pollutants, such as heavy metals and nanoparticles, compared to isolated algae. EPS: extracellular polymeric substances

the mechanism by which pollutants interfere with cell-to-cell adhesion is not fully understood.

Algae extracellular exudates are fundamental in supporting bacterial colonization, promoting the host of the co-existing bacteria. Algae release extracellular products, for example, antibacterial substances, nutrients, and chemoattractants, which have the main role in the regulation of their association with bacteria. Toxic pollutants may interfere with the algae and bacteria release of extracellular polymeric substances, thus interfering with the relationship between organisms (You et al. 2021a) (Fig. 2).

Under stressful environmental conditions, algae undergo stronger bacterial colonization. This is mainly caused by the alteration in extracellular substance release and cell lysis, with the subsequent release of intracellular components used as nutrients by bacteria. Moreover, healthy algae secrete antibacterial substances against the colonization of harmful bacteria, promoting the establishment of fruitful consortiums. This defense strategy may be impaired by toxic contaminants. A reduction in cell mobility and chemotaxis is also observed under stressful conditions, in particular under exposure to heavy metals, such as chromium, cadmium, mercury, and nanoparticles (Yung et al. 2014; Cheng et al. 2019).

Cell-to-cell adhesion in an algae-bacteria consortium may reduce the toxicity of pollutants, such as heavy metals and nanoparticles, compared to isolated algae or bacteria. Bacteria were discovered to reduce toxic uptake by algae as well as to protect cell structure and cell lysis. Environmental factors, such as pH and nutrient availability, may positively or negatively influence these protective mechanisms. For example, Levy et al. (2009) observed the toxicity of copper in *Chlorella spp.* growth was reduced at acid pH, while Wang et al. (2016) observed that high concentrations

of  $\text{PO}_4^{3-}$  enhance the toxicity of arsenic on the consortium between the bacterium *Ateromonas macleodii* and algae *Dunaliella salina*. These effects may be driven by changes in bacterial motility and chemotaxis or algae growth, unbalancing the consortium equilibrium.

Thus, it can be stated that proper cell-to-cell adhesion under suitable environmental conditions is crucial for building an algal bacterial consortium. Moreover, this cell-to-cell adhesion under stressful conditions may also protect the consortium from harmful bacteria and increase the shelf life of such consortium.

## Wastewater characteristics

Wastewater is a complex matrix containing solids, nutrients, dissolved and particulate matter, microorganisms, heavy metals, and micropollutants (Kunhikrishnan et al. 2012; Müller et al. 2007; O'Connor et al. 2022a). The concentration of these components is highly variable depending on the wastewater origin. It can be generated from industrial, domestic, commercial, and agricultural sectors and may contain a wide range of organic substances, such as human excreta, washing waste, nutrient biodegradable waste, and pesticides. The wastewater largely comprises 0.1% suspended and dissolved solids, which consist of

non-biodegradable inorganic waste (Samer 2015). The volatile solids in sewage comprise proteins, carbohydrates, and fats from food industry waste (O'Connor et al. 2022a).

The sewage wastewater consists of a diverse group of microorganisms ranging from viruses, protozoa, antibiotic-resistant bacteria, and helminths which are mostly infectious and considered a menace to human health and the environment (Jia and Zhang 2019). Other microorganisms like algae, *Pseudomonas*, and *Zoogloea* are eco-friendly and can be used to treat wastewater. For suitable growth of algae, the wastewater streams must be rich in nutrients and  $\text{CO}_2$ , which enhances the recovery process of nutrients and help in the production of lipids (Ji et al. 2013; Bolan et al. 2009). The agricultural wastewater, generated from a variety of farm activities, consists of a high amount of ammonia, high nutrient load, suspended solids, and chroma, thus making it unsuitable for algal growth (Zhu et al. 2013). Similarly, dairy wastewater and starch processing wastewater contain chemical oxygen demand values ranging from 1000 to about 70,000 mg/L and 10,000 to about 350,000 mg/L, respectively. The detailed content of different wastewater typologies is enlisted in Table 1.

In a nutshell, it can be said in most of the reports that the wastewater from different sources has been reported to contain high biochemical oxygen demand and chemical oxygen demand associated with high ammonia and total phosphorus

**Table 1** Comparison of different characteristics of wastewater from different sources

Wastewater source	pH	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	TN (mg/L)	TP (mg/L)	References
Dairy	4.53 ± 0.67	170 ± 121.24	1007.3 ± 224.19	299.67 ± 89.97	$\text{NH}_4^+$ : 5.23 ± 5.26 $\text{NO}_2^-$ : 0.35 ± 0.13 $\text{NO}_3^-$ : 25.67 ± 13.68 TKN: 16.31 ± 0.58	$\text{PO}_4^{3-}$ : 46.97 ± 33.59	Noukeu et al. (2016)
Cheese	3.82–5.98	15,500–18,000	44,774–66,739		320.5–436.5	$\text{PO}_4\text{-P}$ : 291–350	Ozturk et al. (2019)
Dairy	6.58 ± 0.1	11,000 ± 50	13,054 ± 5	9622 ± 2.51 Oil and grease: 4203.8 ± 2.25	$\text{NO}_3^-$ -N: 6.62 ± 0.43 TKN: 69.32 ± 1.01	$\text{PO}_4^{3-}$ -P: 13.12 ± 0.7	Amini et al. (2013)
Sugar refinery	4.77–4.94	1164.33–14,491	11,333–357,725	300.667 ± 69.41–2533 ± 540.03	$\text{NH}_4^+$ : 104.98 ± 127.22–177.3 ± 184.16 $\text{NO}_2^-$ : 0.45 ± 0.17–2.79 ± 1.536 $\text{NO}_3^-$ : 127.43 ± 111.28–1477.5 ± 1232.65 TKN: 0.662 ± 0.40–27.07 ± 0.17	$\text{PO}_4^{3-}$ : 46.97 ± 33.59–1426.03 ± 83.01	Noukeu et al. (2016)
Cassava starch processing	4.5–4.92	6300	10,496	827	542.5	94	Sun et al. (2012)
Cassava biogas effluent	7.5 ± 1.0		205 ± 12.3		47.67 ± 2.36	$\text{PO}_4^{3-}$ : 23.53 ± 1.70	Padri et al. (2022a); Padri et al. (2022b)
Fish processing			1128 ± 16.0		$\text{NH}_3\text{-N}$ : 2.0 $\text{NH}_4^+\text{-N}$ : ≈ 320 $\text{NO}_2^-\text{-N}$ : ≈ 0.56 $\text{NO}_3^-\text{-N}$ : ≈ 22.6		Anh et al. (2021)
Tuna wash processing	7.38		139.15 ± 8 (g L <sup>-1</sup> )	23.48 ± 0.7	TKN: 18.2 ± 0.2	$\text{PO}_4^{3-}$ : 1.62 ± 0.1	Hamimed et al. (2022)
Dyeing industry		52.4	111				Choi et al. (2017)
Pharmaceutical industry		120	490	370			Rana et al. (2017)

BOD biochemical oxygen demand, COD chemical oxygen demand, TSS totalsuspended solids, TN total nitrogen. TP total phosphorus, TKN total Kjeldahl Nitrogen, P phosphorus

content, which can serve as a good nutrient source for the growing algal and bacterial biomass.

## Algae for wastewater treatment

Algae are a large and diverse polyphyletic group consisting of predominantly aquatic and photoautotrophic organisms with thallus structures ranging from unicellular to multicellular forms like giant kelp and seaweeds. They include both prokaryotic and eukaryotic organisms and comprise different groups such as *Cyanophyta*, which is also recognized as *Cyanobacteria* due to the huge similarity of these algae with bacteria, *Chlorophyta*, *Rhodophyta*, *Phaeophyta*, *Bacillariophyta*, and *Chrysophyta* (El Gamal (2010; Mutanda et al. (2013; Saber et al. (2022). Despite all the differences in the basic cellular organization, they can use solar energy to assimilate inorganic nutrients into organic substances, thus producing biomass.

Moreover, they play a crucial role in the aquatic food chain and can be regarded as a promising source of renewable energy. They can be grown in nutrient-rich wastewater and help in the recovery of nitrogen, phosphorus, and carbon. Also, they can accumulate a high amount of lipids and carbohydrates during their growth, which makes them suitable for biofuel production (Chen et al. (2018; Sajjadi et al. (2018; Dębowski et al. (2020). Apart from nutrient recovery, they can also remove and/or bio-transform toxic heavy metals and xenobiotic substances from wastewater (Zhao et al. (2018).

The rationale for using algae cells is diverse, as the algal cells are characterized by these main following properties: (i) rapid growth rate, (ii) ease of handling, (iii) requirement of only light, CO<sub>2</sub> and minerals for growth, (iv) ability to grow under extreme environmental conditions, (v) valuable biochemical composition (richness in proteins, lipids, and carbohydrates), (vi) non-requirement of any land for cultivation, (vii) role in CO<sub>2</sub> sequestration, (viii) higher carbon fixation rate than land plants, (ix) ability to evolve oxygen as a by-product, (x) ability to grow in both fresh and saline wastewater, and (xi) ability to carry out nitrogen fixation by selected algal species (Pacheco et al. (2020; Iglina et al. (2022).

In addition, algae exhibit different kinds of metabolism, such as autotrophic, mixotrophic, and heterotrophic, and they can be used to treat various types of waste streams while simultaneously fabricating valuable biomass. Besides the above characteristics, algal biomass requires minimal mechanical aeration thanks to the release of oxygen by photosynthesis, which can be utilized by both the algae and aerobic bacteria to promote the growth of these complex consortia and help in the decomposition of organic matter present in the wastewater (Matamoros et al. (2015; Solimeno

and García, (2017; Udaiyappan et al. (2017; Maryjoseph et al. (2020; Mohsenpour et al. (2021). The reduction in aeration results in an economic benefit for industrial plant. Algae also secrete secondary metabolites that inhibit the further growth of pathogenic organisms (Lee et al. (2022). In addition, they influence the wastewater treatment process by acting as flocculants, thereby increasing the rate of sedimentation (Pieterse and Cloot (1997; Chatsungnoen and Chisti (2016). Another economic advantage of the use of algae in contaminated water treatments is the use of nutrients present in wastewater for the development of microalgae (Pavithra et al. (2020).

These algal biomasses generated can be used to synthesize a wide range of pigments, proteins, polyunsaturated fatty acids, biofuels, biofertilizers, biochar, and production of animal feed which are used in different types of industries such as food, feed, cosmetics, pharmaceutical, and nutraceutical (Santos and Pires (2018).

Nowadays, two different techniques are mostly utilized for the algal bioremediation of wastewater. They are either grown in open systems or ponds such as high-rate algal ponds, which have a low carbon footprint and reduced greenhouse gas emissions. The other system is the closed system, which includes the tubular, the flat panel, and the plastic bag photobioreactors where the environmental factors can be maintained for suitable algal growth (Kaloudas et al. (2021).

William Oswald first used algae to treat wastewater. He was also among the pioneer researchers to observe the collaborative interaction of bacteria and algae in treating wastewater. Early studies on algal wastewater bioremediation were carried out by Oswald and his fellow researchers to evaluate the ability of algae to aerate and bioremediate wastewater (Oswald (1953, (1957; Levin (1965). They proposed an inexpensive, green technique to treat wastewater that is chiefly fueled by sunlight. Subsequently, several researchers have treated various industrial and urban wastewaters with different algal strains which have been discussed in this review in detail. Several other studies were also conducted by different groups in which the production as well as the processes followed during the production of various value-added products from the algal biomass were discussed.

## Nutrients bioremediation by algae

Municipal and agricultural wastewater contains a large amount of nutrients like nitrogen, phosphorus, and other minerals. However, the presence of excessive nutrients can result in the eutrophication of natural water bodies, which occurs through the production of dangerous algal blooms and the depletion of dissolved oxygen (hypoxia) due to the decomposition of algal biomass. It ultimately disturbs the whole aquatic ecosystem and imparts a severe hazard to the aquatic life forms, for example, the death of fish, which in



turn may harm humankind as well. Moreover, a high amount of ammonia and phosphate in water may cause severe health problems in humans, such as methemoglobinemia, which is caused by an excessive quantity of nitrates present in drinking water (Fewtrell (2004).

Nutrients released from wastewater are getting considerable attention and have been strictly controlled throughout the world. The studies on phycoremediation of carbon, nitrogen, and phosphorus from a variety of wastewater effluents, such as agricultural, municipal, refinery, brewery, and industrial effluents, have been performed by several researchers using various algal strains. Algae produce biomass by consuming the nutrients present in wastewater. Algal biomass is harvested and used for different applications. Therefore, nutrients are effectively removed from the contaminated water body by the removal of the biomass. Some of the recent studies on algal nutrient removal and the production of valuable products are shown in Table 2.

The main source of nitrogen in wastewater is primarily fertilizers and human wastes, and most of the phosphorus comes from synthetic detergents used in households and

different industrial activities (Azam et al. (2019); Haddaway et al. (2019); Harder et al. (2019)). The predominant forms in which they occur in wastewater are ammonium ions, nitrite, nitrate, and orthophosphate. Phosphate enters the algal cell actively through a symporter with  $H^+$  or  $Na^+$  ions providing the driving force. Algae also hydrolyze organic phosphorus compounds with membrane-bound as well as free phosphatases, releasing bioavailable phosphorus that is subsequently taken up by the algal cells (Bolan et al. (2004).

Among inorganic nitrogen sources, algae preferentially take up ammonium because of its more energetically favorable assimilation and direct protein incorporation process (Bolan et al. (2004)). Algae uptake ammonium by a group of membrane transporter proteins belonging to the ammonium transporter family. On the other hand, nitrate and nitrite are reduced to ammonium, by nitrate reductase and nitrite reductase, respectively, for intracellular uptake, which is energy intensive. Moreover, the entry of nitrate inside the algal cell involves ATP hydrolysis. In addition to inorganic nitrogen, algae can also assimilate nitrogen from a broad array of organic sources such as amino acids, nucleosides,

**Table 2** Nutrient removal and production of value-added chemicals using algal strains

Algal strain	Wastewater type	Nitrogen removal (%)	Phosphorus removal (%)	Product/Co-product	References
<i>Dunaliella</i>	Anaerobically digested poultry litter wastewater	63.8 TN	87.2 TP	7.26 mg L <sup>-1</sup> $\beta$ -carotene	Han et al. (2019)
<i>Tetraselmis indica</i>	Pharmaceutical wastewater	67.17 (nitrate)	70.03 (PO <sub>4</sub> <sup>3-</sup> -P)	Lipid Productivity: 15.69–17.15 mg/L/d	Nayak and Ghosh (2020)
<i>Chlorella sorokiniana</i>	Palm oil mill effluent	98.6 TN	96 TP	Lipid content: 14.43% (NPBR)	Cheah (2020)
<i>Scenedesmus obliquus</i>	Municipal wastewater	96 TN	80 TP	Lipid content: 56%	Qu et al. (2020)
<i>Desmodesmus sp</i>	Piggery wastewater	79.2 TN	65.3 TP	Total fatty acid/dry weight (%): 29.4 ± 0.17 28.3 ± 0.21 SFA 39.9 ± 0.93 MUFA 31.3 ± 1.74 PUFA	Chen et al. (2020)
<i>Isochrysis sp.</i>	Sewage discharge	5.57 TN	84–94	63.0, 16.92% MUFA, 20.00% PUFA	Singh (2021)
<i>Chlorella vulgaris</i> , <i>Chlorococcum vitiosum</i> , <i>Chroococcus turgidus</i> , <i>Desmococcus olivaceus</i> , <i>Scenedesmus acutus</i> , <i>Scenedesmus dimorphus</i> and <i>Oocystis solitaria</i>	Coke plant wastewater	42.7 (NH <sub>4</sub> <sup>+</sup> N)	NA	NA	Nagi et al. (2021)
<i>Scenedesmus sp.</i>	Domestic wastewater	80 (NH <sub>4</sub> <sup>+</sup> N), 99 (NO <sub>2</sub> <sup>-</sup> N), 86 (NO <sub>3</sub> <sup>-</sup> N)	66 (PO <sub>4</sub> <sup>3-</sup> -P)	43.3% SFA, 44.4% MUFA, 12.3% PUFA	Baldev et al. (2021)

TN total nitrogen, TP total phosphorus, SFA saturated fatty acids, UFA unsaturated fatty acids, PUFA polyunsaturated fatty acids, MUFA mono-unsaturated fatty acids

purines, and urea. The incorporation of organic nitrogen inside the algal cell may occur in both autotrophic and heterotrophic conditions (Feng et al. (2016)). Thus, an algal system can be very efficiently used for the removal of nutrients from wastewater sources in which ammonium ions, nitrate, nitrite, and orthophosphate can be successfully removed.

### Removal of contaminants by algae

The presence of potentially toxic elements in wastewater pollutes natural water bodies like lakes, rivers, and seas and can lead to several health issues, such as kidney damage, reduced lung function, bone mineral loss, nerve problems, and cancer (Kunhikrishnan et al. 2012). Despite the presence of several conventional methods, the use of algae in the removal of potentially toxic elements offers an innovative technology that is more proficient, ecologically secure, and inexpensive (Pavithra et al. (2020)). *Scenedesmus* and *Chlorella*, in particular, are considered hyper-adsorbents and hyper-accumulators due to their remarkable ability to remove these substances (Travieso et al. (1999; Terry and Stone (2002)).

Algae can withstand the stress of potentially toxic elements and require heavy metals like zinc, molybdenum, manganese, iron, cobalt, copper, and boron as trace elements for their growth and metabolism; however, other potentially toxic elements like cadmium, chromium, lead, arsenic, and mercury are harmful to them. Furthermore, a trace amount of toxic heavy metals is required to stimulate algal growth, a process known as hormesis. Algae can also recover precious metals, such as silver and gold, and can also remove toxic radioactive elements from water. Algae tolerate potentially toxic elements through various mechanisms such as gene regulation, heavy metal immobilization, chelation, exclusion, and the production of different enzymes that decrease the toxicity of these substances (Monteiro et al. (2012; Tripathi and Poluri (2021; Manikandan et al. (2022)). Algae control heavy metal concentrations in the cytoplasm by forming organometallic complexes and further separating them inside the vacuoles. Potentially toxic elements induce the production of phytochelators, several antioxidant enzymes, like catalase, peroxidase, superoxide dismutase, glutathione reductase, and ascorbate peroxidase, and also various non-enzymatic antioxidants, like glutathione, ascorbic acid, proline, carotenoids, and cysteine, that ultimately reduce the stress of potentially toxic elements.

Algae remove these contaminants from wastewater largely through biosorption and bioaccumulation. Biosorption is a rapid, reversible, metabolism-independent, passive physicochemical process that involves the binding of metal ions to the dead or inactive algal cell wall through adsorption, electrostatic interaction, ion exchange, chelation, and micro-precipitation. In contrast, bioaccumulation by living

algal cells takes place in two phases. The initial phase is like the passive biosorption process, in which the metal ions bind to several binding groups such as hydroxyl, phosphoryl, carboxyl, amine, imidazole, and sulfate present on the algal cell surface. During the second phase, the potentially toxic elements can be actively transported inside the algal cells at the cost of cellular energy. This phase is known as intracellular uptake, which is dependent on cellular metabolism and plays a huge role in these pollutants' biosorption and detoxification (Bolan et al. (2013)).

There are several reports of potentially toxic elements being removed from wastewater using algae, some of which are shown in Table 3. It shows that non-living algal biomass has been predominantly used to treat wastewater, as live algae show restricted sorption due to the poisoning of the living cells. Furthermore, the absorption process by live algal biomass is more complex as the live cells accumulate metal ions intracellularly, and the intracellular uptake is in turn affected by several factors, like the growth phase of the algae used. In contrast, non-living or inactive algal biomass acts as an assemblage of polymers, like cellulose, glycoproteins, pectins, and sugars, and adsorbs metal ions only at the extracellular level (Shakoor et al. (2016)).

Nevertheless, the use of dead cells makes the whole process cost-effective and simple. The use of extremophilic algae growing under harsh environmental conditions also appears to be an encouraging choice. Table 3 also shows that the potentially toxic elements removal efficiency varies with different algal strains and usually reaches a satisfactory level within 120 min. It is also evident that low pH favors metal ion uptake. Sheng (2004) suggested that the functional groups present on the cell walls of algal biomass influence the effect of pH on metal uptake. Besides pH, phytoremediation is also affected by several factors, such as concentrations of algal biomass and metal ions, temperature, and the presence of competing ions (Danouche et al. (2021)).

Electrostatic interactions between algal cells have a considerable effect on metal uptake. High biomass concentrations exert a 'shell effect' on the outer structure of the biomass, which prevents the binding of metal ions to the cell surface functional groups, leading to reduced uptake per gram of biomass. Moreover, higher metal ion concentrations lower the metal removal efficiency of live algal biomass as well. It could be because an excess of potentially toxic elements can destroy algal cells by denaturing protein structure or causing oxidative damage (Pavithra et al. (2020)). Temperature variations show diverse biosorption behavior in different algal species with different metal ions. Moreover, wastewater polluted with numerous potentially toxic elements shows competition between them for binding to the algal cell wall, which in turn is affected by some chemical characteristics, like electronegativity, ionic radius, and the metal ions, that are present. Light intensity, the amount of

**Table 3** Biosorption efficiency of toxic elements using macro- and microalgal strains under optimal conditions

Metal	Algal strain	Initial metal concentration (mg/L)	Biomass (g/L)	Temp (°C)	Optimal pH	Time	Max. sorption (mg g <sup>-1</sup> )	References
As(III)	<i>Ulothrix cylindricum</i>	10	NA	20	6	60 min	67.2	Tuzen et al. (2009)
	The mixture of green and blue-green algae	50	10	20	4	180 min	3.5	Sulaymon et al. (2013)
	<i>Scenedesmus almeriensis</i>	12	1	NA	10	180 min	5	Saavedra et al. (2018)
Al (III)	<i>Laminaria japonica</i> #	NA	1	NA	5	30 h	75.27	Lee et al. (2004)
Au (III)	<i>Fucus vesiculosus</i> #	100	1	23	7	8 h	74.05	Mata et al. (2009)
Cd(II)	<i>Chlorella minutissima</i>	NA	4	28	6	20 min	303	Yang et al. (2015)
	<i>Scenedesmus sp.</i>	200	1.5	NA	6	NA	48.4	Jena et al. (2015)
	Lipid-extracted <i>Chlamydomonas sp.</i>	NA	1	30	8	60 min	23.3	Zheng et al. (2016)
	Lipid-extracted <i>Chlorella sp.</i>	NA	1	30	8	60 min	25.5	Zheng et al. (2016)
Cr(III)	<i>Parachlorella sp.</i>	100	1	35	7	NA	96.2	Dirbaz and Roosta (2018)
	<i>Chlorella miniate</i>	100	NA	25	5	24 h	41.12	Han et al. (2006)
	<i>Spirogyra sp.</i>	50	NA	25	5	3 h	30.21	Bishnoi et al. (2007)
Cr(VI)	<i>Chlorella sorokiniana</i>	NA	1	25	4	NA	58.8	Akhtar et al. (2008)
	<i>Rhizoclonium hookeri</i> #	1000	1	NA	2	45 min	67.3	Kayalvizhi et al. (2015)
	<i>Chlorella vulgaris</i>	147	1	25	2	240 min	63.2	Sibi (2016)
	<i>Spirulina platensis</i>	500	NA	60	1	90 min	59.6	Nithya et al. (2019)
Cu(II)	Lipid-extracted <i>Spirulina platensis</i>	500	NA	60	1	90 min	45.5	Nithya et al. (2019)
	<i>Sargassum sp.</i> #	NA	1	22	6	180 min	72.5	Karthikeyan et al. (2007)
	<i>Fucus vesiculosus</i> #	NA	NA	23	5	120 min	105.48	Mata et al. (2008)
Pb(II)	<i>Cladophora sp.</i> #	100	NA	25	5	60 min	13.7	Lee et al. (2011)
	<i>Phormidium sp.</i>	10	4	25	5	40 min	2.305	Das et al. (2016)
Se(IV)	<i>Rhizoclonium hookeri</i> #	NA	NA	40	5	NA	81.7	Suganya et al. (2017)
	<i>Cladophora hutchinsiae</i> #	NA	8	20	5	60 min	74.9	Tuzen and San (2010)
U (VI)	<i>Chlorella vulgaris</i>	23.8	0.8	NA	4	96 h	27	Vogel et al. (2010)
Ni (II)	<i>Sphaeroplea sp.</i>	NA	1	33	6	60 min	199.55	Srinivasa Rao et al. (2005)
	<i>Codium vermilara</i> #	50	0.5	NA	6	120 min	13.2	Romera et al. (2007)
Zn (II)	<i>Scenedesmus obliquus</i>	75	0	25	6 to 7	24 h	836.5	Monteiro et al. (2011)
Hg (II)	Transgenic <i>Chlorella sp.</i>	8	0.3	30	NA	120 min	7.33	Huang et al. (2006)
	<i>Chlorella vulgaris</i>	48	2	20	5	120 min	17.49	Solisio et al. (2019)

#Seaweed/macroalgae. NA: Not applicable

dissolved nitrates, and growth rate also play a key role in the phycoremediation of potentially toxic elements. Furthermore, several chemicals and physical pre-treatments of algal biomass enhance the uptake capacity of these pollutants.

Owing to the different cell wall compositions in different groups of algae, the biosorption capability varies among

different strains. For example, seaweed, green macroalgae, and their alginate derivatives can remove many metal ions. In algae belonging to the family *Phaeophyceae*, alginate serves as the chief means for heavy metal binding, and its availability and macromolecular conformation directly influence the biosorption process. Several other factors, such as



the number of functional groups on the algal cell surface, the accessibility of binding groups for metal ions, and the coordination number of the metal ion to be absorbed, play a major role in determining the biosorption efficiency of a particular ion by a specific alga (Escudero et al. (2019)). Proteins and polysaccharides present in algal cell walls are also involved in metal binding. On the other hand, intracellular uptake is mediated by several cytosolic proteins, and inside the cells, metal ions are accumulated in the vacuoles.

The current literature shows that together with the identification of the most suitable algal strain for the wastewater to be treated, molecular genetics can also provide some possibilities to make available new genetical-modified algal strains, able to remove specific heavy metals from the wastewater (Kaloudas et al. (2021)).

### Removal of emerging contaminants using algae

Emerging environmental contaminants present in wastewater are drawing significant awareness as they exhibit several bad qualities such as high polarity, the ability to be bioaccumulated by aquatic organisms, and resistance to biodegradation. They harm the aquatic ecosystem and human health as well. The most common contaminants include not only pharmaceuticals products but also several personal care products,

perfluorinated compounds, gasoline additives, surfactants, organometallic compounds, disinfection by-products, brominated and organophosphate flame retardants, endocrine-disrupting compounds, nanoparticles, and plasticizers (Müller et al. (2007; O'Connor et al. (2022a; Morin-Crini et al. (2022)). Algae-based technologies have demonstrated greater efficiency in removing emerging contaminants (Morin-Crini et al. (2022) at both laboratory scales and in real wastewater, some of which are shown in Table 4, respectively.

Bioremediation of emerging contaminants by algae takes place in three steps, such as bioadsorption, bioaccumulation, and biodegradation or biotransformation, as shown in Fig. 3. Biodegradation is the breakdown of complex materials into environmentally acceptable, simpler forms, which takes place intra- and/or extracellularly. It occurs via two key mechanisms, for example, metabolic degradation and cometabolism (Maryjoseph, (2020; Bolan et al. (2013)). Algae take up emerging contaminants as their carbon source during metabolic degradation. In co-metabolism, enzymatic breakdown of the contaminants takes place, and a threshold concentration of contaminants is required for enzymatic activity.

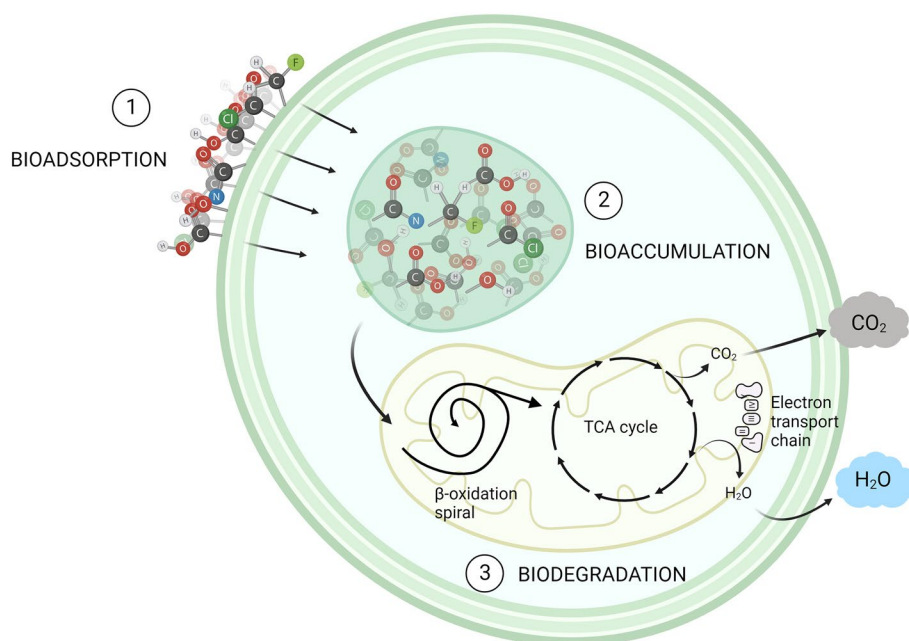
Several factors influence biodegradation in various ways, including algal strain, pollutant feature, enzymatic pathway, and environmental conditions. Moreover, algae, especially microalgae, boost the degradation process by forming a

**Table 4** Removal efficiency of emerging contaminants by algae

Emergent contaminants	Removal (%)	Algal strain	Experimental conditions	References
Pharmaceutical: ciprofloxacin	100	<i>Chlamydomonas</i> sp.	Synthetic wastewater medium, 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 12/12 light/dark cycle, 2%, $\text{CO}_2$	Xie et al. (2020)
Pharmaceutical: sulfadiazine	54.53		25 $\pm$ 1 $^\circ\text{C}$ temperature, 5–6 days time	
Pharmaceutical: Sulfamerazine	84	<i>Haematococcus pluvialis</i>	Pre-sterilized synthetic wastewater medium, 12 h: 12 h dark/light cycle, 25 $\pm$ 1 $^\circ\text{C}$ temperature, 40 days time	Kiki et al. (2020)
Pharmaceutical: Sulfamethoxazole	74			
Pharmaceutical: Sulfamonomethoxine	75			
Pharmaceutical: Acetaminophen	67	<i>Chlorella sorokiniana</i>	Mann and Myers medium, 25 $\pm$ 1 $^\circ\text{C}$ temperature, pH 7.5 $\pm$ 0.5, 370 $\mu\text{E m}^{-2} \text{s}^{-1}$ light intensity 12/12 light/dark cycle, 144 h time	Escapa et al. (2019)
Pharmaceutical: Sulfamethazine	31.4–62.3	<i>Scenedesmus obliquus</i>	Sterilized Bold's Basal medium, 27 $^\circ\text{C}$ temperature, 45–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 16/8 light/dark cycle, 14 days	Xiong (2019)
Pharmaceutical: Sulfamethoxazole	27.7–46.8			
Pharmaceutical: Carbamazepine	<21	<i>Chlorella vulgaris</i>	Synthetic wastewater medium, 22 $^\circ\text{C}$ temperature, 90–160 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 16/8 light/dark cycle, 25 days time	Larsen et al. (2019)
Pharmaceutical: Ibuprofen	60			
Pharmaceutical: Gemfibrozil	<27			
Personal care product: Methylisothiazolinone	100	<i>Scenedesmus</i> sp.	BG11 medium, 25 $\pm$ 1 $^\circ\text{C}$ temperature, 55–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 14:10 h light/dark cycle, 4 days time	Wang et al. (2020)
Industrial Chemicals: Para-xylene (aromatic hydrocarbons)	100	<i>Rhodomonas</i> sp.	F/2 medium, 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, 14 h:10 h light/dark cycle, 20 $^\circ\text{C}$ temperature, 6 days time	Li et al. (2020)

The experiments were carried out at a laboratory scale. The experimental conditions are also reported

**Fig. 3** Emerging contaminants removal mechanisms using microalgal metabolism. Algal-based bioremediation takes place in three steps: (1) bioadsorption, (2) bioaccumulation, and (3) biodegradation. As a result, contaminants are degraded into environmentally acceptable compounds in the metabolic pathways. As an example,  $\beta$ -oxidation and aerobic respiration are illustrated. TCA: tricarboxylic acid. The TCA cycle consists of a series of chemical reactions to generate energy from carbohydrates, fatty acids, and proteins.  $\beta$ -oxidation is a spiral reaction that involves repeated enzymatic steps. Created with [BioRender.com](https://www.biorender.com)



mutual relationship with bacteria (Subashchandrabose et al. (2011)). It is also important to note that not all emerging contaminants are easily biodegradable and, as a result, can be toxic to a variety of algal species, particularly in large-scale treatment plants. Acclimatization of algae to wastewater, on the other hand, may overcome this challenge through genetic adaptation and the production of counteracting enzymes.

Several factors influence algae-based bioremediation of emerging contaminants, including nutrient deficiency in wastewater and competition between contaminants for binding sites. The presence of several contaminants in wastewater increases its toxicity as compared to the occurrence of a single contaminant. Surprisingly, some specific contaminants seem to boost the removal rate of other contaminants as well. For instance, the removal rate of sulfamethazine increased several times in the presence of sulfamethoxazole (Xiong (2019)). Co-metabolism is another mechanism that enhances the elimination efficacy of a variety of emerging contaminants. Xiong (2017) reported an increased removal rate of ciprofloxacin by *Chlamydomonas mexicana* after adding sodium acetate to the medium. Temperature plays an important role in the removal of emerging contaminants, and a higher temperature usually enhances the whole process (Vijayaraghavan and Yun (2007)). Light intensity also has a significant effect on removal efficiency as it affects algae growth. Hom-Diaz (2017b) showed reduced degradation of pharmaceutically active compounds under low irradiance in the high-rate algal community; optimizing all these factors would help us enhance the removal process.

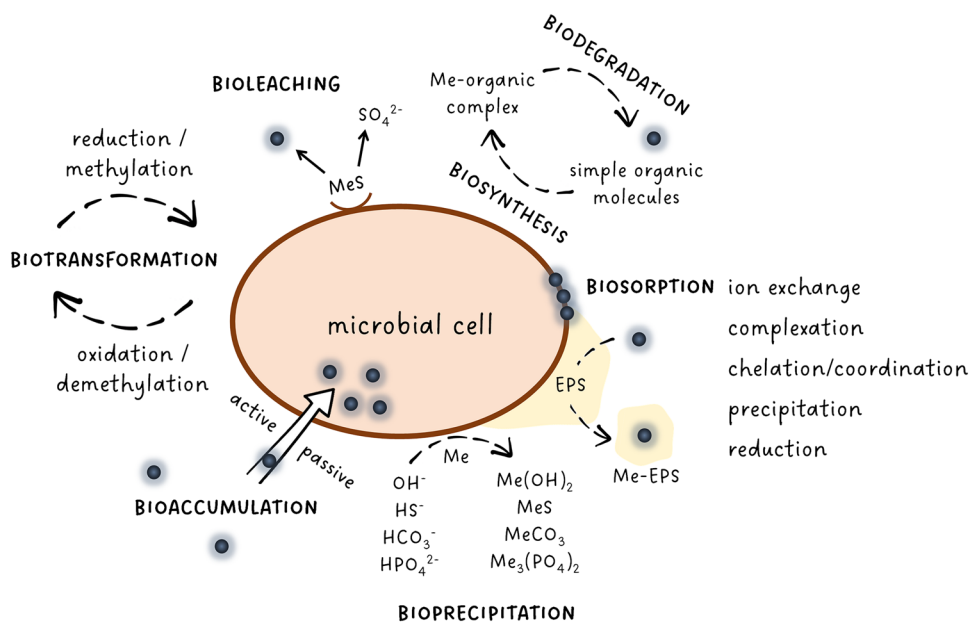
It is also important to check the effluent quality, which has been ignored since most of the studies are restricted to laboratory conditions only. Many studies found that the effluent

contained more contaminants than the influent (Zhou, (2014; Garcia-Galan, (2020; O'Connor et al. (2022a)). Moreover, the formation of by-products may turn out to be more toxic in comparison with the primary compounds. For example, bioremediation of textile wastewater by *Oscillatoria tenuis*, *Chlorella pyrenoidosa*, and *Chlorella vulgaris* showed degradation of azo dyes into simple aromatic amines, which are carcinogenic as well as persistent (Fazal (2018)). However, before the use of algal systems in the removal of emerging contaminants, a proper study of the effects of other environmental factors on the algal system and the factors monitoring their growth must be thoroughly studied for the successful application of the process.

## Bacteria for wastewater treatment

The biological treatment of wastewater is gaining importance and is considered a cost-effective and eco-friendly process for the mitigation of pollutants. Bioremediation of wastewater by bacteria can be done by different processes such as biosorption, biodegradation, biomineralization, bioaugmentation, and bioreduction of pollutants into less toxic and harmless products (Bolan et al. (2013; Ramadass et al. (2015; Bouabidi et al. (2019; Morin-crini et al. (2019) (Fig. 4). Generally, biological wastewater treatment is associated with complex biochemical metabolic processes, which occur mainly through the interaction between bacteria and different inorganic and organic pollutants (Laurenson et al. (2013)). Mostly, a consortium of microbes is used for wastewater treatment, which has high biodegradation efficiency

**Fig. 4** Biointeractions of bacterial cells with metals. The different physicochemical mechanisms of microbial interaction with soluble metal and metalloid species include complexation, coordination, chelation, ion exchange, precipitation of inorganic species, metal accumulation, reduction/oxidation, and alkylation. The result is the immobilization of metals and metalloids. EPS: extracellular polymeric substances



and can use a wide range of different substrates present in wastewater.

Several bacteria have been reported to produce a plethora of enzymes such as chromate reductase and arsenate oxidase which can convert Cr(VI) and toxic arsenic As(III) to less toxic Cr(II) and As(V) (Panneerselvam et al. (2013); Sanyal et al. (2016)). Bacteria can also eliminate radionuclides by changing their oxidation states (Tsezos (2009)). As a result, the element can be dissolved and transported or precipitated, or immobilized. However, the efficiency of bioremediation is largely dependent on pH, temperature, and other environmental factors. Bacteria like *Pseudomonas spp.* and *Lysinibacillus spp.* have been extensively studied for their ability to remove potentially toxic elements and act as carrier matrix of natural non-polymeric electrospun cyclodextrin fibers (Park et al. (2011a, (b); Park and Bolan (2013); Safdari et al. (2018); Orellana et al. (2018)). These cyclodextrin fibers along with microbial consortium chiefly function as a biosorbent of heavy metals and textile dyes (Yadav et al. (2019)). Isolates like *Aeromonas hydrophila* have been used to decolorize triarylmethane dyes; however, the efficiency was largely dependent on temperature, pH, and oxygenation state (Imran et al. (2015)).

Heterotrophic bacteria are known to degrade a wide range of biodegradable organic components by using them as terminal electron donors. Under aerobic, anaerobic, or anoxic conditions, different substrates such as oxygen and different nutrients such as nitrite and sulfates present in the wastewater are used as electron acceptors. Several respiratory products, such as sulfide, nitrogen gas, carbon dioxide, and biomass, are produced depending on the substrate present in wastewater (Gao et al. (2010)). Autotrophic bacteria procure energy by oxidizing ammonia to nitrate or nitrite and also

by using organic substances as a carbon source. Both phototrophs and chemotrophs use solar energy or chemical energy using both organic and inorganic substances and can be phototrophic, chemoorganotrophic, or chemolithotrophic in their nutritional mode. Other essential nutrients for growth included in the wastewater include nitrogen, magnesium, sulfur, phosphorus, iron, potassium, and calcium.

In some cases, some technologies for cell immobilization are applied in polluted water treatments due to some advantages in comparison with biodegradation using free cells, such as providing cell reuse, high resistance to toxic chemicals, and eliminating cell washout problems (Bouabidi et al. (2019)). Cell immobilization is obtained by its entrapment by using organic or inorganic water-insoluble materials. In the next section, the role of heterotrophic as well as autotrophic bacteria in the treatment of different pollutants from wastewater is discussed in detail. Also, the role of different genera in processes like nitrification, denitrification, and other metabolic functions associated with nutrient removal from wastewater was analyzed in detail.

### Role of heterotrophic bacteria

The heterotrophic bacteria such as *Agrobacterium spp.* and *Pseudomonas spp.* are known to degrade readily biodegradable chemical oxygen demand. They do, however, have low efficacy for converting less biodegradable and slowly hydrolyzable chemical oxygen demand to biodegradable chemical oxygen demand (Cyzdik-Kwiatkowska and Zielińska (2016)). The accumulation of nonbiologically degraded chemical oxygen demand results in high biosolids loads in the wastewater treatment plants and requires additional treatment, aeration, and disposal costs. The heterotrophic bacteria use

inorganic and organic macronutrients (Orchard et al. (2010) including inorganic phosphate and dissolved organics, for growth, metabolism and bioremediation (Sisma-Ventura and Rahav (2019).

As wastewater contains a high concentration of phosphate from phosphorus-containing biomass, this helps increase the heterotrophic bacterial population and its productivity. Nitrification and denitrification are processes that aid in the removal of total nitrogen and ammonium nitrogen (Zehr and Ward (2002). Many competitive heterotroph species, such as *Pseudomonas spp.*, are known to inhibit ammonium nitrogen conversion into nitrate, thus preventing several metabolic processes and decreasing efficiencies. However, when a consortium of nitrifying and denitrifying bacteria has been used, an increase in performance has been noted (Yang et al. (2020a, (b). Thus, heterotrophic bacteria can be used extensively for the treatment of wastewater using different metabolic activities depending on the prevailing abiotic conditions of the water.

### Role of autotrophic bacteria

Autotrophic bacteria such as *Nitrosomonas spp.* and *Leptospirillum spp.* play a determining role in the nitrification process. Both physiological and molecular in situ techniques have been used to investigate the role of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in wastewater treatment plants. Their distribution pattern is highly dependent on the different biological and environmental conditions of the wastewater (Cai et al. (2018). Autotrophic bacteria play a crucial role in activated sludge systems; however, a detailed study on the growth pattern, operational strategies, and degradation products formed by the autotrophs is still required to clearly understand the entire process (Ni et al. (2008). However, in an activated sludge system, a high nitrite concentration inhibits the growth of autotrophs (Zhang et al. (2018) and requires a considerable amount of energy during maintenance. The details of different types of microorganisms and their role in the degradation of different pollutants in wastewater are tabulated in Table 5. Table 5 highlights the wide range of pollutants that can be successfully degraded and removed by different genera of aerobic and microaerophilic bacteria and their consortium in an eco-friendly manner.

### Consortia of algae and bacteria for wastewater treatment

Studies demonstrate that algal and bacterial consortiums show better wastewater treatment and efficient nutrient recovery than single algal or bacterial systems (Tang et al. (2018). This can be efficiently done through direct and

indirect ecological interactions between microalgae and wastewater bacteria. However, to establish an effective system, a detailed knowledge of ecological interactions between microalgae and bacteria is required, which may vary from mutualism or commensalism to competition or amensalism (Zhang et al. (2020). Under suitable conditions, algal and bacterial consortium formation occurs over several days. Both algae and bacteria present in the consortium need to be compatible (Qi et al. (2018) and promote mutual growth through complex interaction and substrate exchange (Liu et al. (2017). Algae need CO<sub>2</sub> and nutrients for photosynthesis and release oxygen, which can be used by the bacteria for metabolism by oxidizing organic matter and ammonia, as shown in Fig. 5.

Oxygen is used as an electron acceptor for the bacteria's metabolism when oxidizing organic matter and ammonia. This interdependence promotes robust growth of algae and bacteria, helps stabilize the ecosystem against continuous oscillations of abiotic conditions, and also reduces invasion by other pathogenic bacteria. The mutualism can also be found in the bacteria's supply of B12 vitamins to the algal species. Commensalism is also evident in this situation, where only algae benefit from the interaction, using vitamin B12 produced by bacterial metabolism. Similarly, parasitism can occur, in which many bacteria lyse algal cells and use their nutrients for growth.

A classic example of an algal–bacterial consortium is the interrelationship between microalgae and ammonia-oxidizing bacteria, which can be both favorable and unfavorable for the partner depending on the abiotic conditions. If high pH, temperature, and ammoniacal nitrogen persist, the equilibrium between free ammonia nitrogen and ammonium nitrogen shifts toward free ammonia nitrogen, which prevents the growth of microalgae by inhibiting their metabolism (Rossi et al. (2020). Moreover, in similar conditions, there can be an inhibition of nitrite-oxidizing bacteria, as reported by González-Camejo et al. (2020), which promotes amensalism.

Competition can also exist for ammonium nitrogen between algae and ammonia-oxidizing bacteria under different light intensities, and after a few generations, the better competitor can outlive the other and establish a stable community (González-Camejo et al. (2019). It was also reported that under non-limiting ammonium and suitable light, pH, and temperature conditions, microalgae supply oxygen for nitrification, so only ammonia-oxidizing bacterial communities benefit via commensalism. However, in most cases, this interrelationship between the algal and bacterial partners varies depending on the prevailing abiotic conditions and shows a non-discrete interface. Controlling metabolic interactions and interrelationships among the consortium's algae bacterial partners allow wastewater to be efficiently mitigated in an economically sustainable manner. However,

**Table 5** Microorganism species used in the remediation of contaminants

Contaminants	Microorganisms	References
<i>Hydrocarbon organic compounds</i>		
Aromatic hydrocarbons	<i>Acinetobacter spp.</i> , <i>Microbacterium spp.</i> , <i>Pseudomonas spp.</i> , <i>Ralstonia spp.</i>	Simarro et al. (2013)
Dibenzothiophene (DBT)	<i>Pseudomonas putida</i> KT2440	Martínez et al. (2016)
Haloalkanes	<i>Pseudomonas putida</i> KT2440	Benedetti et al. (2016)
Pyrene, benzo(a) pyrene and phenanthrene	A synthetically microbial consortium	Zafra et al. (2017)
Anthracene, 9-metil anthracene, striatum pyrene, dibenzothiophene lignin peroxidase	<i>Gloeophyllum striatum</i>	Birolli et al. (2018)
<i>Oil and grease contaminants</i>		
Oil	<i>Alcaligenes odorans</i> , <i>Bacillus subtilis</i> , <i>Corynebacterium propinquum</i> , <i>Pseudomonas aeruginosa</i>	Paikhomba Singha et al. (2017)
<i>Azo dye wastewaters</i>		
Oil-based paints	<i>Bacillus subtilis</i> strain NAP1, NAP2, NAP4	Phulpoto et al. (2016)
Textile azo dyes	<i>Micrococcus luteus</i> strain SSN2, <i>Providencia rettgeri</i> strain HSL1 <i>Pseudomonas sp.</i> SUK1, <i>Pseudomonas fluorescens</i> <i>Staphylococcus spp.</i>	Ghosh et al. (2016; Sadeghi et al. (2019)
<i>Heavy metals</i>		
Cadmium, cobalt, copper, chromium, and lead	<i>Bacillus safensis</i> (JX126862), <i>Bacillus safensis</i> (PB-5) <i>Bacillus safensis</i> (RSA-4) <i>Lysinibacillus sphaericus</i> (CBAM5)	Fauziah et al. (2017)
Copper, iron, manganese, zinc, lead, and uranium	<i>Geobacter metallireducens</i> , <i>Geobacter spp.</i> , <i>Pseudomonas syringae</i> , <i>Pseudomonas fluorescens</i> <i>Pseudomonas aeruginosa</i> , <i>Vibrio harveyi</i>	Igiri et al. (2018; Choudhary et al. (2017)
Cadmium and lead	<i>Escherichia coli</i>	Liu et al. (2021a)
<i>Pesticides</i>		
Endosulfan, coragen	<i>Achromobacter sp. M6</i> , <i>Bacillus</i> , <i>Klebsiella pneumonia</i> , <i>Klebsiella spp.</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas spp.</i> , <i>Rhodococcus sp. M2</i>	Alvarez et al. (2017)
Decis 2.5, EC, Fitoraz WP 76, Ridomil MZ 68 MG	<i>Acinetobacter sp.</i> , <i>Arthrobacter spp.</i> , <i>Pseudomonas putida</i> , <i>Rhodococcus rhodochrous</i> , and <i>Sphingomonas spp.</i>	Mónica et al. (2016) Tarla et al. (2020)
Bensulfuron-methyl (BSM)	<i>Methylomonas sp.</i> strain LW13	Liu et al. (2021a)
Organochloride pesticides, organophosphorus pesticides, carbamates, and pyrethroid	<i>Escherichia coli</i> strain BL21	Li et al. (2020)

detailed knowledge of the associated abiotic factors is necessary for the increased efficiency of the system.

The detailed report on bacterial and algal consortiums used for the treatment of wastewater is discussed in Table 6.

## Techniques for harvesting algae

The algal biomass generated in a wastewater treatment plant can be reused to produce different algae-based product formulations (Sarwer et al. (2022)). To obtain different products from algal biomass, the algal mass needs to be procured. This procedure can be done by centrifugation, flocculation,

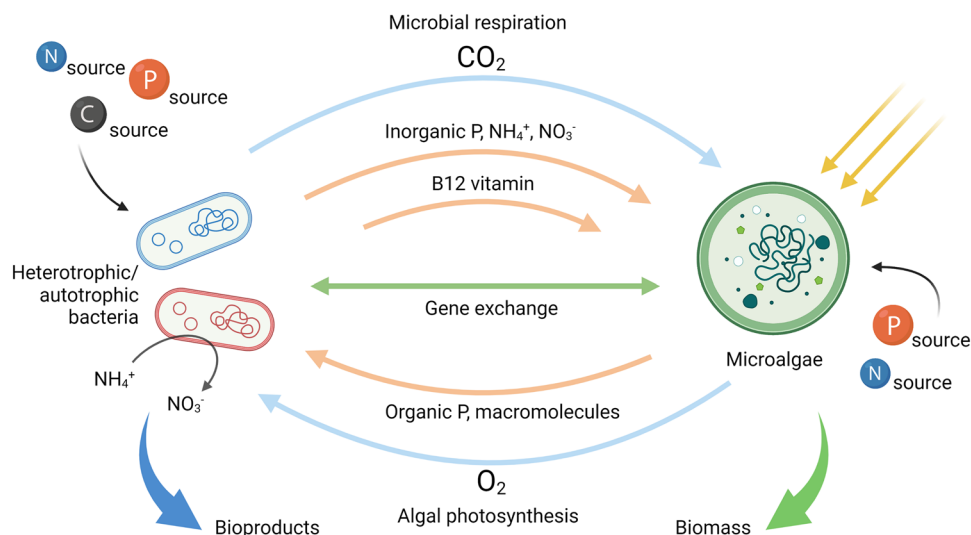
and sedimentation, which face many constraints due to the substantial cost and energy involved.

## Chemical and mechanical processes

Flocculation is largely done by the application of alum or ferric chloride. The factors which affect clump formation include surface properties, net charge, pH, ionic strength, the concentration of coagulant/flocculent, and hydrophobicity (Papazi et al. (2010)). There have been several reports in which biomass of microalgae can be recovered by flocculation which includes *Chlorella vulgaris* and *Chlorella minutissima* by application of nano-aminoclays,  $\text{Fe}_2(\text{SO}_4)_3$  and  $\text{CaOH}_2$  as a coagulant (Farooq et al. (2013;



**Fig. 5** Illustration of the interactions between algae and bacteria in a consortium. Microalgae fix inorganic elements into organic macromolecules via photosynthesis, providing bacteria with oxygen and organic compounds necessary for their metabolisms; bacteria degrade organic matter and produce  $\text{CO}_2$  from aerobic respiration, which enters the algae photosynthetic pathway. Both algae and bacteria produce substances that can promote or inhibit mutualistic growth, exchange genes, or alter gene expression ( $\text{NH}_4^+$ : ammonium ion;  $\text{NO}_3^-$ : nitrate ion). Created with [BioRender.com](https://www.biorender.com)



Papazi et al. (2010; Vandamme et al. (2012). It has been also reported that there exist various ways to induce flocculation to microalgal biomass which include processes like the application of the electrostatic patch, bridging, and sweep flocculation (Vandamme et al. (2013). During the process of using chemicals, care should be taken that there is no biomass contamination associated with high-efficiency biomass settling and minimum impact on the environment. Besides the addition of chemicals, the flocculation process must be also cost-effective and non-toxic when they are applied on a large-scale (Molina Grima et al. (2003). Usually, more electronegative ions lead to faster coagulation without disruption of cellular structure (Papazi et al. (2010).

However, for rapid and reliable recovery of algal biomass mechanical methods like centrifugation are usually used, and on the other hand, a separate filamentous algae filtration method is employed. For smaller suspended algal masses, tangential flow filtration is used but this may lead to disruption of the filter membrane, and replacement of the membrane can be quite expensive (Danquah et al. (2009; Uduman et al. (2010). This mechanical separation process shows several drawbacks including membrane fouling, high operational cost, and slow energy intensiveness (Greenwell et al. (2009; Park et al. (2011a, (b). Normally, with microalgal-rich waste waters, dissolved air flotation is the preferred technique over sedimentation methods (Teixeira and Rosa (2006).

## Biological methods

Bioflocculation is an eco-friendly technique used to harvest microalgae using aggregation of diverse types of bacteria and filamentous fungi and autoflocculating microalgae. A combination of microalgae and bacteria can increase the recovery of algal biomass from wastewater treatment plants

(de Godos et al. (2014). Different polymers obtained from different groups of microbes were also efficient in bioflocculation. Ndikubwimana et al. (2016) and Choi et al. (2020) reported that poly  $\gamma$ -glutamic acid produced by *Bacillus licheniformis* CGMCC 2876 and activated sludge-derived extracellular polymeric substance can efficiently increase the flocculation ability of *Desmodesmus brasiliensis* and *Chlorella vulgaris*, *Chlamydomonas asymmetrica*, and *Scenedesmus spp.*, respectively. Moreover, the addition of magnesium and calcium hydroxide can lead to auto flocculation of microalgae. Microalgal cells can also be co-pelletized using a coculture of filamentous fungal species which can be later harvested using a sieve (Zhang and Hu (2012); similarly, bacterial floc can also be used for microalgal cell harvesting (Nguyen et al. (2019b).

However, in most cases, these technologies are not economically and ecologically viable in a field experiment and there is a need for a cost-effective, and efficient eco-friendly process is necessary. Moreover, a combination of chemical, mechanical, and biological methods for the harvesting of algal biomass can serve as an efficient technique for industrial and large-scale purposes.

## Valorisation of algal and microbial biomass

### Production of biodiesel, bioethanol and biohydrogen

Microalgal biomass can be used for biohydrogen production which can be done via both direct and indirect photolysis of water and fermentation in the dark yielding hydrogen along with various volatile fatty acids (Rajesh Banu et al. (2021). However, factors like carbon–nitrogen ratio, pH, temperature, cultural set-up, pre-treatment methods, and the species

**Table 6** Algal–bacterial consortia for the treatment of wastewaters

Wastewater type	Relationship between the algal and bacterial partner	Microalgae	Bacteria	COD recovery efficiency (%)	Nitrogen recovery efficiency (%)	Phosphorus recovery efficiency (%)	References
Dairy wastewater	Mutualism	Chlorophyceae	Trebouxiophyceae	Chroococcales	100	93	Biswas et al. (2021)
Dairy wastewater	Mutualism/Commensalism	<i>Tetraselmis indica</i>		<i>Pseudomonas aeruginosa</i>	83.8	87.5	Talapatra et al. (2021)
Starch wastewater and piggery wastewater	Mutualism	<i>Chlorella vulgaris</i>		<i>Rhodobacter sphaeroides</i>	97	96	You et al. (2021b)
Synthetic aquaculture wastewater	Mutualism	<i>Coelastrrella</i>		<i>Rhodobacteraceae</i>	84.9	64.8	Fan et al. (2021)
Raw dairy wastewater	Mutualism	<i>Chlorella sp. DBWC7</i> , <i>Chlorella sorokiniana</i> , <i>DBWC2</i>		<i>Acinetobacter calcoaceticus</i> , <i>ORWB3</i> , <i>Klebsiella pneumoniae</i> , <i>ORWB1</i>	84.7	90.5	Makut et al. (2019)
Anaerobically digested starch wastewater, alcohol wastewater	Mutualism/Parasitism	<i>Chlorella pyrenoidosa</i>		Bacterial from digested starch wastewater	87	97	Tan et al. (2018)

COD chemical oxygen demand

of microalgal species affect the hydrogen production rate. Microalgal species such as *Chlorella*, *Scenedesmus*, and *Saccharina* are extensively used for biohydrogen production (Wang and Yin 2018). Microalgae *Scenedesmus obliquus* showed 56.8 mL H<sub>2</sub>/g<sub>VS</sub> under controlled conditions and sulfur deprivation (Batista et al. (2015)). Blue light is known to increase algal biomass production, whereas purple light increases biohydrogen production. Moreover, the entire production process is also dependent on light, enzyme activity, and CO<sub>2</sub> fixation efficiency (Schiano et al. (2019)).

Microalgal biomass can also be used for biodiesel production. Biodiesel consists of esters of methylated fatty acids which are usually formed by the transesterification of oils with alcohols. Microalgae growing in wastewater accumulate lipids which can be used for biodiesel (Otari et al. (2020); Peter et al. (2021); Aravind et al. (2020)). The crucial steps of biodiesel production involve the steps like cultivation, drying, and extraction of oils followed by transesterification to fatty acid methyl esters. To extract lipids from biomass, different methods like mechanical extraction, solvent extraction, ultrasonic, and enzymatic extraction are performed. Transesterification is an important step in which a reaction occurs between triglycerides or fatty acids and alcohol, like methanol, ethanol, butanol, and amyl alcohol. In most biodiesel formation processes, methanol and ethanol are used for their low cost and easy availability. Algal cells can interact with a wide range of nano- and microparticles, and metallic nanoparticles, such as copper ferrite (CuFe<sub>2</sub>O<sub>4</sub>) nanoparticles, are often used as a support for the immobilization of the enzymes (Otari et al. (2019)). Different types of bioreactors, such as membrane microreactors, microchannel reactors, microwave reactors, and microtubular microreactors, have been developed to enhance the efficiency of transesterification (Bhatia et al. (2021)).

Kong et al. (2010) reported that around 505 mg/L per day of *Chlamydomonas reinhardtii*, bio-oil is produced from municipal waste. Moreover, the incorporation of nanoparticles is an emerging technology used for biodiesel production. According to the studies conducted by Pattarkine and Pattarkine (2012) and Safarik et al. (2016), the modification of algal cells with hydrous Fe(III) oxide particles, magnetic particles incorporated with aluminum sulfate, and silver nanoparticles was reported to increase the biomass production of *Anabaena*, *Aphanizomenon*, *Chlamydomonas reinhardtii*, and *Cyanothece* 51,142 microalgae, respectively, leading to greater biodiesel formation.

During the entire production, process glycerol is continuously produced as a by-product which needed to be continuously removed to increase biodiesel yield; also, the entire production process is dependent on the content of free fatty acids, carbon chain length, degree of unsaturation, branching, density, and oxidation stability (Bhatia et al. (2021)).

From an economic point of view, for cost-effective and large-scale production of bioethanol, it is necessary to select suitable microalgal biomass and cultivation it in a suitable substrate, so their content of fermentable carbohydrates is relatively low. In most cases, microalgae contain less amount of lignin when compared to highly fermentable carbohydrates. However, till now, there are several constraints regarding the large-scale production of bioethanol and its industrial implementation and requires more research on improving carbohydrate content and biomass productivity (De Farias and Bertucco (2016)). Furthermore, the accumulation of toxins in biomass as a result of a large-scale wastewater medium, which may be undetectable at the laboratory scale, may limit the use of biomass valorisation products (Peter et al. (2021)). However, the preliminary life cycle assessment studies have already shown that microalgae plants for biofuel production seem to provide a positive contribution to the environment. In particular, the environmental advantages have been associated with a significant reduction in carbon dioxide, nitrogen oxide, and sulfur oxide emissions (Sarwer et al. (2022)).

### Production of polyhydroxyalkanoates

Extensive use of plastic is causing huge pollution and harming aquatic flora and fauna (Sridharan et al. 2021). Plastics can be replaced by a sustainable alternative bioplastic produced from biopolymers obtained from living organisms. These biopolymers are mostly produced from natural substrates under nitrogen limitation conditions and have mechanical and thermal properties like petroleum-based polymers with the added advantage of being biodegradable. Depending on the strain, the bioplastic production can be largely modified by altering the nutrient source and co-substrates. Mostly, polyhydroxyalkanoates such as poly-3-hydroxybutyrate, poly-3-hydroxyvalerate, and their copolymer have structural stability like polypropylene. These polymers are also used in different industries sectors such as pharmaceutical, medicinal use, disposables, and agriculture (López et al. (2018)). Polyhydroxyalkanoates obtained from cyanobacteria and microalgae can serve as a cost-effective alternative which may boost the competitiveness of biological-based biopolymers (Devadas et al. (2021)).

Several studies have been conducted to access the accumulation of intracellular polyhydroxyalkanoates under nutrient deprivation conditions. *Spirulina subsalsa* was able to accumulate 147.75 mg of polyhydroxyalkanoates per g of cell dry weight under nitrogen-deprived conditions (Shrivastav et al. (2010)). Later, Kamravamanesh et al. (2017) reported nearly 13% of cell dry weight of intracellular polyhydroxybutyrate production by *Synechocystis* sp. PCC 6714 under nitrogen and phosphorus limitation. However, in

most of these cases, the algae were harvested in a synthetic medium. To make the process sustainable and cost-effective, the medium can be replaced with wastewater or digestate treatment to achieve a similar biopolymer synthesis. Similar production of polyhydroxyalkanoates was also seen with *Spirulina platensis* under specific growth conditions (Laycock et al. (2013)). However, in most cases, the downstream processes of polyhydroxyalkanoates recovery and purification are difficult and represent the main drawbacks of full-scale implementation.

### Production of exopolysaccharides

The microbial cell produces a wide range of exopolysaccharides which are loosely bound to the cell wall surface (Sooriyakumar et al. (2022)). These exopolysaccharides are easy to extract and can possess anticoagulant, antimutagenic, anti-cancer, antiulcer, immunomodulatory, and anti-inflammatory bioactivities (Bhatia et al. (2021)). Exopolysaccharides are usually negatively charged biopolymers that mainly consist of glucose, fructose, galactose, xylose, arabinose, mannose, and rhamnose. Among algal groups, both cyanobacteria and red algae produce exopolysaccharides to adapt to extreme conditions. Halotolerant microalgae under salt stress produce a complex mixture of polyelectrolytes and polysaccharides to protect the cell from desiccation (Mishra and Jha (2009)). These exopolysaccharides contain uronic acid and sulfates which can immobilize positively charged metal ions (Freire-Nordi et al. (2005)) which can be used for water purification.

Freire-Nordi et al. (2005) reported complexing capacity against  $Zn^{2+}$  and  $Cd^{2+}$  and  $Cu^{2+}$ ,  $Pb^{2+}$ , and  $Hg^{2+}$  by *Chlorella stigmatophora* and *Anabaena spiroides*, respectively. Moreover, exopolysaccharides produced by *Cyanothece spp.* also have biofloculant properties which can be used in the bioremediation of micro- and nano-plastics from wastewater streams (Cunha et al. (2020); Sooriyakumar et al. (2022)). Polysaccharides obtained from *Gyrodinium impudicum* KG03, *Nostoc flagelliforme*, *Porphyridium cruentum*, and *Aphanothece sacrum* exhibit antiviral and antibacterial activity against encephalomyocarditis virus, Vaccinia virus, African swine fever virus, and *Salmonella enteritidis* (Arora et al. (2021)). Similarly, the exopolysaccharides produced from *Rhodella reticulata*, *Chlamydomonas reinhardtii*, and *Arthrospira platensis* show free radical scavenging and antioxidant properties (Bafana (2013)). Several other research reports show that these exopolysaccharides show the biotechnological, pharmaceutical, and food industries.

### Production of biofertilizers and animal feed

The algal biomass used in wastewater treatment can be used as a soil amendment or biofertilizer which can increase the

nitrogen and phosphorus content of the soils (Das et al. (2018)). These biomasses can also increase calcium, potassium, iron, and manganese in the soils; however, there lies a risk of the presence of heavy metals which needs to be avoided. Moreover, the biofertilizers made from microalgae are slow-release biofertilizers, which can be used to enhance the organic content of soils (Das et al. (2019)). Also, they can introduce various plant-stimulating compounds as well as potentially pathogenic and other micropollutants to the soil which poses a concern for their application as biofertilizers. Cyanobacteria have been reported to assimilate more nitrogen compared to inorganic fertilizers and are considered to be more suitable for rice plant cultivation. Similarly, biofertilizers made from immobilized *Chlorella pyrenoidosa* grown in dairy wastewater also reported increased growth in paddy (Yadavalli and Hegggers (2013)). It was also reported that a consortium of cyanobacteria and bacteria increased better growth in *Lupinus termis* when compared to seed treated with indole acetic acid, gibberellic acid, and cytokines (Baskar et al. (2022)).

Thus, various value-added products such as biohydrogen, bioethanol, polyhydroxyalkanoates, exopolysaccharides, and biofertilizers can be obtained from microalgal, and bacterial biomass obtained from wastewater treatment plants. They are not only cost-effective but also serve as environmentally friendly and sustainable options to boost the bioeconomy and biotechnology sectors.

### Conclusion

Algae and bacteria allow to improve wastewater treatments. However, certain considerations are to be taken care of. First, screening of suitable algal and microbial strains with specific attributes, such as high tolerance, ability to produce valuable products, low nutrient requirements, high  $CO_2$  capturing ability, robustness toward the existence of other microorganisms, resistance to predation by grazers, and having the self-flocculation capability, is crucial. Hence, research work based on genetic engineering to raise suitable algal and microbial strains is a prospective area of research. Investigations to understand the mechanisms of algal bioremediation are also very crucial. Moreover, innovation of new harvesting techniques is necessary to make the entire process inexpensive. Surface or chemical modification of algal biomass and integration of other pollutant removal techniques may also enhance the removal efficiency of potentially toxic elements. In addition, heavy metal stress can be exploited to alter the fatty acid composition of algal biomass to facilitate the production of biodiesel with desirable properties. More research on the cultivation and purification process is needed to attain adequate removal of heavy metals and simultaneous synthesis of value-added products.

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## Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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