



Microalgae-Based Wastewater Treatment and Recovery with Biomass and Value-Added Products: a Brief Review

Xiao-ya Liu¹ · Yu Hong¹

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Abstract

Purpose of Review With economic development and population increase, environmental pollution and water shortages have become inevitable global problems. Microalgae-based wastewater treatment technology can not only purify wastewater and solve environmental pollution problems but also use the nutrient elements in wastewater to produce algal biomass, which has attracted more and more attention. This work reviews the current status of microalgae bioremediation of wastewater, aiming to provide a reference for further research in this field.

Recent Findings Microalgae have been proven to be used to treat municipal wastewater, agricultural wastewater, and industrial wastewater and can convert nutrients into biomass. In order to further improve the wastewater treatment efficacy and algal biomass productivity, it is necessary to understand the mechanism of microalgae to remove nutrients and pollutants from wastewater. Currently, open ponds and enclosed photobioreactors are used for large-scale cultivation of microalgae, and various harvesting technologies are developed to achieve low-cost capture of microalgae as much as possible. Microalgae are rich in pigments, proteins, lipids, carbohydrates, vitamins, and antioxidants and can produce a variety of value-added products, making this biotechnology more cost-effective.

Summary This review discusses the purification efficiencies of microalgae on wastewater from different sources and introduces the mechanism and influencing factors by which microalgae remove carbon, nitrogen, phosphorus, heavy metals, and antibiotics in details. Moreover, the advantages and disadvantages of different microalgae cultivation systems are analyzed. Finally, the different harvesting methods and the current application of microalgae biomass in various fields are summarized.

Keywords Microalgae · Wastewater treatment · Metabolisms · Photobioreactor · Value-added products

Introduction

With the continuous growth of the global population and economic development, we will inevitably face the two major problems of environmental pollution and resource scarcity [1]. It is estimated that by 2025, more than 3 billion people worldwide will experience water shortages [2, 3]. Annual production of wastewater from municipal, agricultural, and industrial aspects is huge, which contains excessive nutrients, and improper treatment may lead to environmental problems such as eutrophication

of water bodies [4••]. Currently, conventional wastewater treatment technologies are mainly based on physical, chemical, and biological methods, such as activated sludge method to remove organic matter and nutrients and adsorption method to remove heavy metals [5]. However, these methods have the disadvantages of large land area, high energy consumption, and large amount of activated sludge discharge [6]. Besides, the nutrients in wastewater have not been effectively recycled, resulting in waste of resources that could be recycled [7, 8], while microalgae-based wastewater treatment technology is a promising technology that can be used to replace conventional treatment methods [9••].

Microalgae have a wide variety of characteristics, such as high photosynthetic efficiency, fast reproduction speed, and strong environmental adaptability, and can convert nutrients in wastewater into algal biomass [4••, 10, 11]. Therefore, it is considered to be an ideal biological material for comprehensive utilization of wastewater. The research on the application of microalgae in

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✉ Yu Hong
yuhong829908@gmail.com

¹ Beijing Key Lab for Source Control Technology of Water Pollution, College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China

wastewater treatment began in the 1950s. Oswald et al. found that the symbiosis of microalgae and bacteria can improve the removal of pollutants in wastewater [9••, 12]. At present, numerous studies have shown that microalgae has high nutrient removal efficiency for urban [13, 14], agricultural [15, 16], and industrial wastewaters [17, 18•]. At the same time, it is reported that the microalgae biomass harvested from wastewater can provide high value-added products for human production and life, such as being used as raw materials for biofuels, biofertilizers, and animal feeds [19, 20]. Figure 1 depicts a brief process of microalgae-based wastewater treatment and nutrient recovery, which can simultaneously achieve multiple purposes such as wastewater purification, nutrient recycling, and production of high-valued microalgal biomass.

Up to now, researchers have carried out long-term exploration and a large number of studies on wastewater treatment technology based on microalgae. At present, some reviews about microalgae wastewater treatment and resource conversion are more focused and concerned at certain aspects. For example, Gonçalves et al. [21] focused on carbon, nitrogen, and phosphorus in their review of pollutants removal mechanisms. Li et al. [9••] focused on the factors affecting nutrients recovery, and the mechanism of nutrients removal has not been mentioned. This article gives a comprehensive and updated review of the literature regarding the status quo of microalgae-based wastewater treatment technology, including the treatment status of different types of wastewater by microalgae, mechanism and influencing factors of nutrients and pollutants removal, microalgae growth mode, cultivation system, biomass recovery method, and biomass application. In addition, the challenges and future prospects of the technology are also discussed. This review can provide references for the development of microalgae wastewater treatment and biomass recovery application technologies.

Wastewater Resources for the Production of Microalgal Biomass

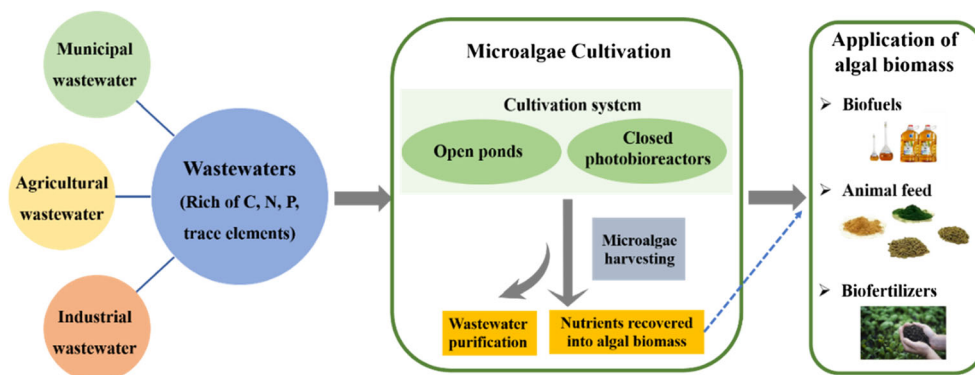
It is estimated that the global population will reach about 9 billion by 2050 [22]. The ever-mounting population has

increased the consumption of freshwater resources, most of which are converted into wastewater due to human daily activities, production, and sustainable development. According to the source of wastewater, it can be classified as municipal, agricultural, and industrial wastewater [23]. These types of wastewater contain nutrients available for microalgae, such as carbon, nitrogen, phosphorus, and other trace elements. A large number of studies have shown that the use of wastewater can not only realize the reuse of wastewater itself but also can transform and obtain a large amount of biomass, especially in the production of microalgae biofuels and other applications, which has great application prospects [24–29]. The treatment of all types of wastewater requires huge capital investment, and the win-win for the production of microalgae biomass would be to reduce treatment costs while purifying wastewater. The following sections will be focused on the growth of microalgae and the efficiency of pollutants removal from municipal, agricultural, industry, and other wastewaters.

Municipal Wastewater

Municipal wastewater is one of the most studied wastewaters used for the research of microalgae cultivation in recent years. Generally, municipal wastewater can be divided into four categories, including raw sewage before primary sedimentation, wastewater after primary sedimentation, wastewater after activated sludge treatment (called secondary effluent), and centrate which was produced by sludge dehydration [30•, 31]. The nutrient distribution of these four types of municipal sewage is quite different, leading to different growth statuses of microalgae. Wang et al. [31] found that *Chlorella* sp. can adapt to the above four types of wastewater, and its growth in centrate was better than the other three types of wastewater. And the removal rate of nitrogen, phosphorus, and chemical oxygen demand (COD) by *Chlorella* was positively correlated with the concentration of nutrients in the wastewater. AlMomani and Örmeci [32] investigated the growth and purification capabilities of *Chlorella vulgaris*, *Neochloris oleoabundans*, and mixed native microalgae in primary sewage, secondary sewage, and centrate. The results showed that

Fig. 1 Microalgae-based wastewater bioremediation and its application



the same kind of microalgae grew at different rates in three different kinds of wastewater. In addition, the growth rates of the three microalgae were different in the same wastewater, and the mixed native microalgae had better wastewater purification ability than the other two microalgae species. Lima et al. [33] used autochthonous microalgae to purify municipal wastewater after primary sedimentation and found that the tested strains had the highest removal rates of TN and TP, reaching 77 and 61%, respectively, but the COD and biochemical oxygen demand concentrations could not be effectively reduced. The nutrient removal of different types of municipal wastewater by microalgae is summarized in Table 1. In order to further improve the removal efficiency of nutrients in municipal wastewater by microalgae, many related studies have been carried out. For example, adding a certain concentration of CO₂ (15%) to municipal wastewater can increase the nutrient removal and simultaneously improve the growth of microalgae and lipid productivity [34]. Another study found that pre-treatment of phosphorus starvation on microalgae can significantly improve phosphorus removal [35].

Agricultural Wastewater

Agricultural wastewater is discharged in the process of crop cultivation, livestock breeding, and agricultural product production, including animal manure wastewaters and agricultural product processing wastewater [36–38]. At present, several studies have shown that the agricultural product processing wastewater can be used for microalgae cultivation, such as potato processing wastewater, palm oil mill effluent, and starch processing wastewater [37, 39–41]. In addition, as one of the main sources of agricultural wastewater, animal manure wastewater has a high

content of nutrients. By comparing with the mineral composition of several commonly used media for microalgae, it was found that animal manure wastewaters seem to be suitable as a medium for microalgae growth, so as to achieve the dual purpose of reducing the cost of microalgae cultivation and resource utilization of wastewater [28, 42–44].

However, the high turbidity and chromaticity of animal manure wastewater is not conducive to light penetration, and high ammonia nitrogen concentration will inhibit the growth of microalgae by affecting the electron transfer of photosystem II, which makes it impossible to directly apply to microalgae cultivation [45]. At present, the method of diluting animal manure wastewaters is commonly used for the microalgae-based wastewater treatment, so as to obtain a high nutrient removal rate [46]. Cañizares-Villanueva et al. [43] reported that the biomass of *Spirulina maxima* and *Phormidium* sp. obtained by culturing in swine wastewater (diluted to 50% by distilled water) can be used as animal feed. Wang et al. [28] found that after 21 d of cultivation, compared with the other dilution times (15, 20, 25) of dairy wastewater, only when *Chlorella* sp. was cultivated in dairy wastewater with dilution times of 10, the highest removal rate of nitrogen, phosphorus, and COD was obtained. Zhu et al. [15] used a tubular bubble-column photobioreactor to cultivate microalgae in piggery wastewater with different dilution ratios for 10 d and found that the removal of COD, TN, and TP were 65.81–79.84, 68.96–82.70, and 85.00–100%, respectively. And the lipid and biodiesel productivities were 48.69–110.56 and 11.85–30.14 mg·L⁻¹·d⁻¹. Chen et al. [47] demonstrated that *Chlorella sorokiniana* AK-1 showed strong tolerance to piggery wastewater, and

Table 1 Removal of nutrients in municipal wastewater by microalgae

Types of municipal wastewater	Microalgae species	Nutrient removal rate (%)				References
		COD	NH ₄ ⁺ -N	TN	TP	
Wastewater treatment plant effluent	<i>Chlorella</i> sp.	Null	—	76.4	61	[33]
Raw sewage before primary sedimentation	<i>Chlorella</i> sp.	50.9	82.4	—	83.2	[31]
Wastewater after primary sedimentation	<i>Chlorella</i> sp.	56.5	74.7	—	90.6	[31]
Primary effluent	Mixed indigenous microalgae	64.9	63.2	63.2	70.0	[32]
Secondary effluent	Mixed indigenous microalgae	70.3	67.5	67.3	30.8	[32]
Secondary effluent	Consortium of filamentous blue-green algae and bacteria	98.2	—	88.3	64.8	[163]
Concentrated municipal wastewater	<i>Auxenochlorella protothecoides</i> UMN280	88.0	—	59.0	81.0	[27]
Centrate	<i>Chlorella</i> sp.	90.8	93.9	89.1	80.9	[13]
Centrate	<i>Chlorella</i> sp.	83.0	78.3	—	85.6	[31]
Centrate	Mixed indigenous microalgae	69.3	71.7	80.8	50.0	[32]
Centrate	<i>Tetraselmis</i> sp. NKG2400013	—	—	99.0	82.0	[164]

Note: “—” means that the parameter has not been measured

when a sponge was used as a carrier of microalgae to purify 50% strength piggery wastewater, the removal efficiencies of COD, TN, and TP of the wastewater were all above 90%.

Considering that a large amount of freshwater is required to dilute animal manure wastewater for cost reduction, on the one hand, it can be tried to use other wastewaters instead of freshwater to dilute the concentration of pollutants, making the manure wastewater more suitable for microalgae growth. On the other hand, how to improve the nutrient removal rate in undiluted animal manure wastewaters is also worthy of further investigation, which will make the microalgae-based wastewater treatment technology more cost-effective. For example, the brewery wastewater was used to dilute piggery wastewater; when the dilution multiples was 4, the removal rates of TN and $\text{PO}_4^{3-}\text{-P}$ by *Chlorella* sp. MM3 were the highest, reaching 89.36 and 56.56%, respectively, and the high biomass yield can be obtained to produce a large amount of biofuel [48]. It is reported that *Chlorella vulgaris* can be adapted to the undiluted cow's farm wastewater and effectively remove pollutants in the wastewater, and after two stages of *C. vulgaris*-based biological treatment, the removal rate can be further improved [49].

Industrial Wastewater

According to the different processing objects, industrial wastewater can be divided into metallurgical wastewater, papermaking wastewater, chemical fertilizer wastewater, textile printing and dyeing wastewater, tanning wastewater, and pesticide wastewater. This category of wastewater contains a variety of pollutants such as grease, heavy metals, antibiotics, and some other chemical toxins, with high organic content and poor biodegradability [18]. Based on the abovementioned, the use of microalgae to treat this category of wastewater faces many bottlenecks. At present, relevant research mainly focuses on the removal and degradation of toxic substances, rather than the subsequent high-valued utilization of algal biomass [50, 51]. Due to the above characteristics of industrial wastewater, it is generally considered unsuitable for microalgae cultivation, but some studies have been confirmed that some specific microalgae strains exhibit excellent performance in treating various industrial wastewaters [52–54]. For example, *Chlorella vulgaris* was reported to be a promising species of microalgae used to treat textile wastewater, which can effectively remove nitrogen, phosphorus, COD, and color in wastewater [53, 55]. A microalgae consortium composed of 15 native algal strains was used to purify carpet wastewater, and the results showed that after 3 d of cultivation, the nutrients removal rate can reach up to 96%, and the highest biomass and lipid yield were obtained [10]. Another study done by Pena et al. [56] used a microalgae consortium mainly composed of *Tetraselmis* sp. to treat tannery wastewater and found that under continuous light conditions, the removal rates of TN, TP, and COD could reached 71.74, 97.64, and

56.7%, respectively. Besides, the microalgae consortium shows excellent biosorption performance for heavy metals in wastewater. Moreno-García et al. [57] found that a native microalgae consortium which isolated from a secondary settler can adsorb 99% of Cr (III) in the tannery wastewater. Table 2 lists the removal of nutrients in different types of agricultural wastewater and industrial wastewater by microalgae.

In short, wastewater containing nutrients and trace elements necessary for the growth of microalgae can usually be used as a potential medium for microalgae cultivation, such as municipal wastewater, agricultural wastewater, and industrial wastewater can all be used as a source of nutrients for the growth of microalgae. At present, most of the research on microalgae-based wastewater treatment technology is still in the laboratory-scale stage. Screening suitable microalgae species that can adapt to different kinds of wastewaters, looking for wastewater resources that can replace freshwater, and developing effective cultivation systems are a series of effective methods to realize large-scale applications of microalgae-based wastewater treatment.

The Mechanism and Influencing Factors of Nutrients and Pollutants Removal During the Microalgae-Based Wastewater Treatment

The Mechanism of Nutrients and Pollutants Removal

The main function of microalgae-based wastewater treatment is to remove nutrients from wastewater and convert them into algal biomass. Due to the abundant high-valued substances such as protein, lipid, and pigments in microalgae, it can be used to produce animal feed, fertilizer, and biofuels [58]. This whole process is inseparable from the uptake and transformation of nutrients such as carbon, nitrogen, and phosphorus in wastewater by microalgae. Additionally, certain toxic pollutants contained in wastewater such as heavy metals would influence the accumulation of microalgae biomass and further resource utilization. Therefore, revealing the mechanism of microalgae metabolizing nutrients in wastewater and elucidating the mechanism of microalgae to remove pollutants in it is of great significance to the development of microalgae-based sewage treatment and biomass production technologies.

Carbon

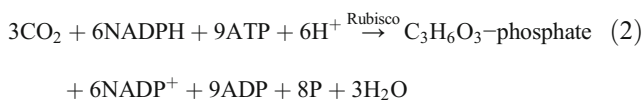
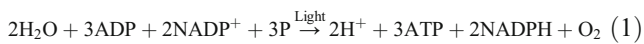
As the basic element of cell composition, carbon accounts for about 50% of the total weight of microalgae [59, 60]. The carbon sources that microalgae can utilize are inorganic carbon (such as CO_2 , HCO_3^-) and organic carbon (sugars, alcohols, and acids). Figure 2 shows the mechanism of microalgae fixing inorganic and organic carbon. The carbon dioxide is

Table 2 Removal of nutrients in agricultural and industrial wastewater by microalgae

Wastewater	Microalgae species	Nutrient removal rate (%)				References
		COD	NH ₄ ⁺ -N	TN	TP	
Piggery wastewater (diluted to five different concentrations)	<i>Chlorella zofingiensis</i>	65.8–79.8	—	69.0–82.7	85.0–100.0	[15]
10% diluted swine wastewater +BG11	<i>Chlorella sorokiniana</i> AK-1	88.8	—	78.3	97.7	[47]
Swine wastewater (diluted 10×)	<i>Chlorella vulgaris</i>	96.0	—	91.0	85.0	[165]
Undiluted raw piggery wastewater	<i>Chlorella sorokiniana</i> , <i>Coelastrella</i> sp., <i>Acutodesmus nygaardii</i>	92.0	90.0	—	100.0	[166]
Piggery wastewater (after anaerobic digestion and activated sludge aeration treatment)	<i>Chlorella vulgaris</i> + <i>Exiguobacterium</i> sp.	86.3	78.3	84.4	87.2	[167]
Digested dairy manure	<i>Chlorella</i> sp.	27.4–38.4	100.0	75.7–82.5	62.5–74.7	[28]
Undiluted cattle farm wastewater	<i>Chlorella vulgaris</i>	62.3	81.2	—	85.3	[49]
Palm oil mill effluent	<i>Chlorella vulgaris</i>	50.5	61.0	—	84.0	[168]
Palm oil mill effluent	<i>Scenedesmus dimorphus</i>	86.0	99.5	—	99.8	[168]
Textile wastewater	<i>Chlorella</i> sp. + <i>Scenedesmus</i> sp.	52.0	—	71.0	98.0	[169]
Tannery wastewater	Microalgae consortium (contains mainly <i>Tetraselmis</i> sp.)	56.7	—	71.7	97.6	[56]

Note: “—” means that the parameter has not been measured

fixed by the microalgae through the Calvin cycle. First, under the light reaction, light energy and water molecules were used to release oxygen and generate ATP and nicotinamide adenine dinucleotide phosphate (NADPH). In the dark reaction stage, CO₂ is assimilated under the action of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The chemical reaction formulas are shown in Eqs. 1 and 2 [61••]. But in the process of high-density cultivation of microalgae, the atmosphere CO₂ cannot meet the demand for carbon, and CO₂ from industrial waste gas has been proven to be a cheap gaseous carbon source [62]. Besides, under the action of carbonic anhydrase, microalgae can convert soluble carbonate into CO₂ according to the carbon demands and convert CO₂ into organic matter through the Calvin cycle [63].



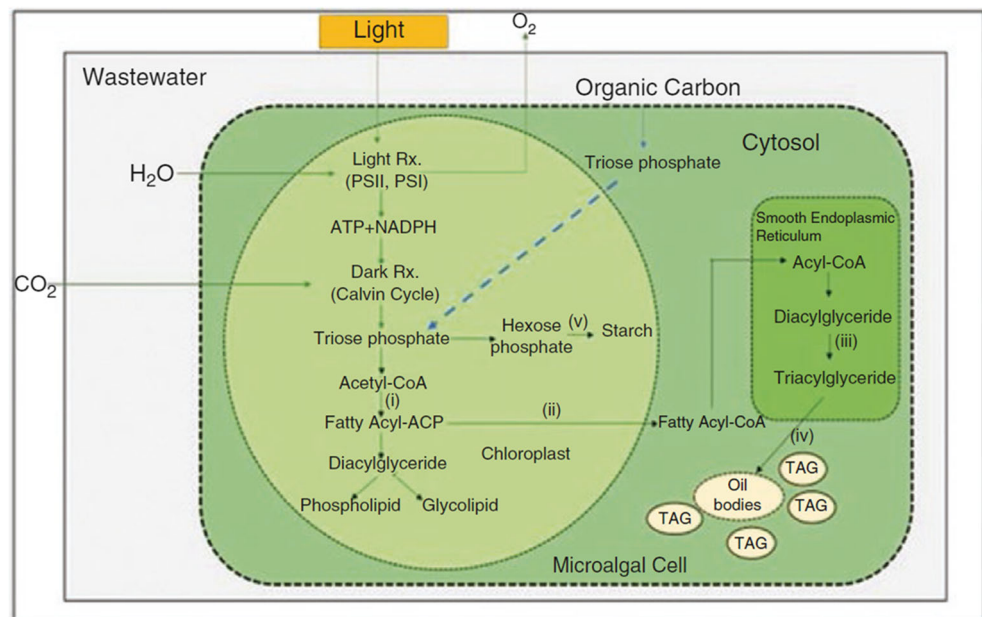
In the heterotrophic mode, microalgae can utilize the organic carbon compounds in wastewater (such as glucose, galactose, glycerol, ethanol, acetate) as a carbon source for facultative or heterotrophic growth [64]. These organic carbon sources enter microalgae cells by passing through the plasma membrane or phagocytosis [65]. Taking glucose as an example, as the organic carbon source of microalgae, it provides energy and carbon for growth and biomass accumulation, and has two metabolic pathways in the cytoplasm, one is the

glycolytic pathway under light conditions, and the other is the hexose monophosphate shunt under dark conditions [64]. The synthesized precursor fatty acids are transported to the endoplasmic reticulum, where triacylglycerides (TAG) are synthesized under the action of TAG biosynthetic enzymes [66].

Nitrogen

Nitrogen is an essential nutrient element for the growth of microalgae, and it participates in the synthesis of peptides, proteins, chlorophyll, enzymes, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), adenosine diphosphate (ADP), adenosine triphosphate (ATP), and other substances in microalgae cells [67, 68]. For most algae species, nitrogen starvation can promote the accumulation of lipids or carbohydrates in microalgae and inhibit protein synthesis. On the contrary, the abundant nitrogen in the culture medium can promote protein synthesis [69]. It is reported that microalgae can assimilate ammonia nitrogen (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻), and simple organic nitrogen such as urea and amino acids in wastewater to synthesize proteins, nucleic acids, and phospholipids [70, 71]. The mechanism between algae growth and nitrogen uptake is very complicated, which is usually closely related to the existence form of nitrogen source [72]. An ideal microalgae cell can absorb and assimilate a series of nitrogen substrates into the cell for growth; a simplified inorganic nitrogen assimilation pathway is summarized in Fig. 3. Nitrate is transported into algae cells passively or actively through the cell membrane and is reduced to NO₂⁻ under the

Fig. 2 The diagram of the mechanism of microalgae fixing inorganic and organic carbon [61••]



catalysis of nitrate reductase. Next, the nitrite is then transferred to the chloroplast, and under the action of nitrite reductase, the reduced form of ferredoxin (Fd) transfers six electrons to reduce it to ammonia nitrogen. Finally, ammonia nitrogen is assimilated to amino acids under the action of glutamine synthetase and glutamine oxoglutarate amidotransferase [4••, 73, 74]. As the energy required to assimilate NH_4^+ is less, the nitrogen source preferentially used by microalgae is NH_4^+ [75].

Phosphorus

In the process of microalgae metabolism, phosphorus is another critical macronutrient, because it is essential for the synthesis of nucleic acids, ATP, phospholipids, and proteins [67]. Phosphorus deficiency will reduce cell division and affect biological processes such as protein synthesis, transcription, and carbon cycle. Inorganic phosphate (such as PO_4^{3-} , HPO_4^{2-} , and H_2PO_4^-) is the most preferentially assimilated phosphorus form by microalgae [76•]. The pathway of phosphorus uptake and transformation by microalgae is shown in Fig. 4. In the case of insufficient inorganic phosphate, microalgae cells can mineralize organic phosphate into orthophosphate through phosphatase present on the cell surface and further assimilate them [59, 77]. For example, some marine diatoms can promote the production of phosphodiesterase under phosphorus-deficient conditions [78]. In the case of excess phosphate, microalgae cells can utilize them and, at the same time, convert them into polyphosphate granules (in the form of acid-insoluble polyphosphate) under the action of polyphosphate kinase, which are stored in the cells to continue

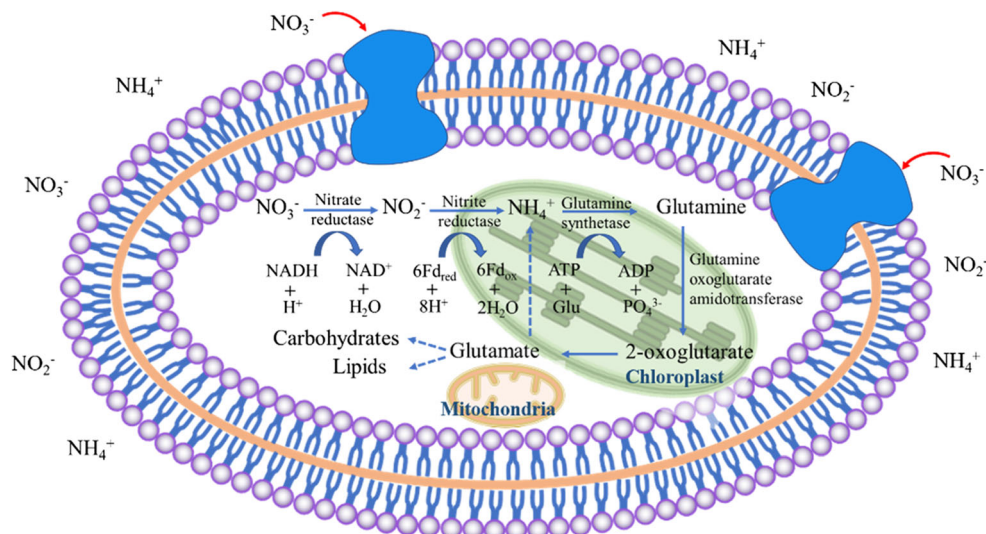
to maintain microalgae viability in the absence of phosphate [79].

Heavy Metals

Heavy metals (HMs) are one of the most common types of pollutants in wastewater, and microalgae have the ability to remediate HMs in wastewater. Some HMs (such as boron, copper, iron, zinc, cobalt, molybdenum) can be used as trace elements for the growth of microalgae to promote the occurrence of enzymatic reactions and cell metabolism in microalgae cells [80]. In addition, microalgae have high affinity for the binding of metal substances or their ionic forms, have abundant binding sites on cell membranes and cell walls, and have a large cell surface area, making them a good material for heavy metal adsorption [81•, 82]. As reported, both live algae cells and microalgae powder can be used for the removal of HMs in wastewater [83, 84]. Figure 5 shows the biosorption and detoxification mechanism of heavy metal ions by microalgae. The biosorption of HMs by microalgae involves the following two stages: (i) metals are quickly negatively charged to the cell surface through electrostatic interaction (bioadsorption), and this process is usually reversible; (ii) a slower metabolic process occurs in the cell for HMs bioaccumulation and biotransformation, which is usually irreversible. The HMs are actively transported across the cell membrane into the cytoplasm, then diffuse, and bind to the internal binding sites of proteins and polypeptides [81•, 85, 86].

In short, microalgae can remove HMs in various wastewaters through biosorption and biotransformation, and biosorption may be the main removal mechanism [75]. Therefore, how to properly dispose of the microalgal biomass

Fig. 3 Simplified diagram of the assimilation of inorganic nitrogen (NH_4^+ , NO_3^- , and NO_2^-) by microalgae (this figure was made using Microsoft Office PowerPoint)



loaded with HMs is a key issue, and solving it is of great significance to prevent the secondary pollution of HMs in the biomass.

Antibiotics

Antibiotics are usually used to treat and prevent bacterial infections and have been widely used in human life and production. Antibiotics in urban sewage generally come from hospitals, domestic sewage, and wastewater from pharmaceutical factories [87, 88]. In addition, antibiotics are used to prevent and treat livestock and poultry diseases, so they are widely present in animal manure wastewaters [89]. Microalgae have been proven to have removal effects on most classes of antibiotics, and the biological treatment of antibiotic-containing wastewater based on microalgae is a promising technical

means [90–93]. The mechanisms of microalgae to remove antibiotics include biosorption, bioaccumulation, biodegradation, photodegradation, and hydrolysis (Fig. 6). Biosorption is a process in which antibiotics are adsorbed on the cell surface through binding sites existing on the cell wall. Antibiotics that enter cells through bioaccumulation can induce the production of reactive oxygen species, which can regulate the normal metabolism of cells, but if excessive, they will cause severe cell damage or eventually death [94, 95•]. Biodegradation refers to the process by which algae decompose antibiotics inside or outside the cell, and some of the decomposed derivatives are further consumed by the algae cells [96]. In addition, the hydrolysis reaction caused by algae metabolites also belongs to biodegradation [95•]. The photodegradation of antibiotics includes the direct photolysis of antibiotics under the condition of no algae and the indirect photolysis of antibiotics

Fig. 4 Schematic illustration of phosphorus absorption and transformation pathway by microalgae (solid lines, under sufficient phosphorus conditions; dotted lines, under phosphorus deficiency conditions). Modified from Su [76•]

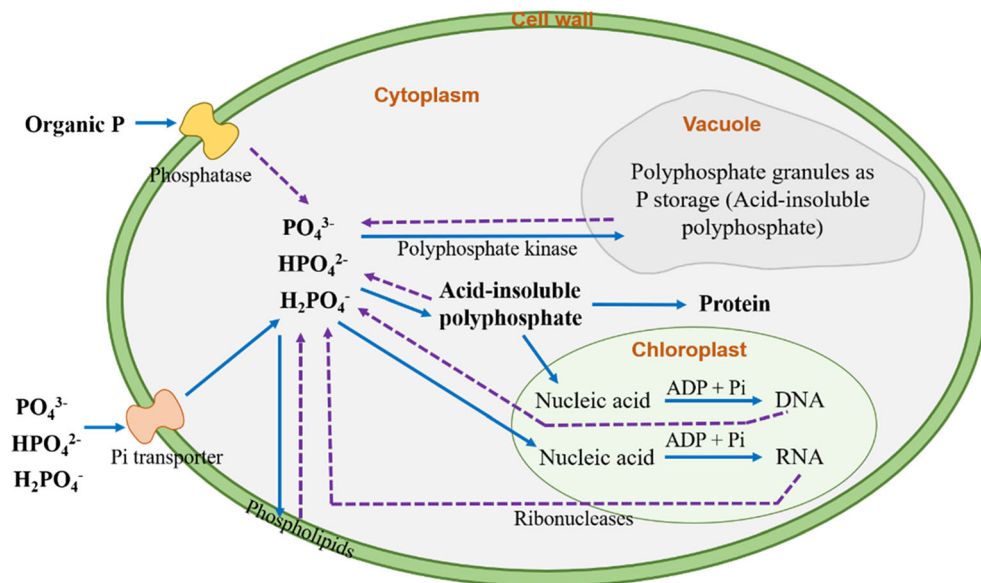
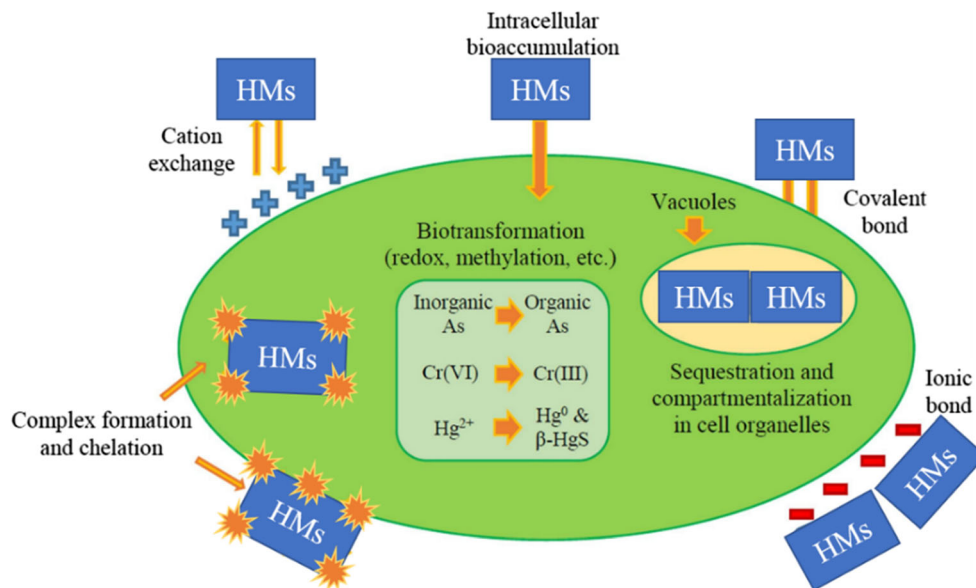


Fig. 5 Mechanism of heavy metals (HMs) biosorption and detoxification by microalgae. Modified from Leong and Chang [81]



induced by the active ingredients produced by the algae under the action of light [95, 97].

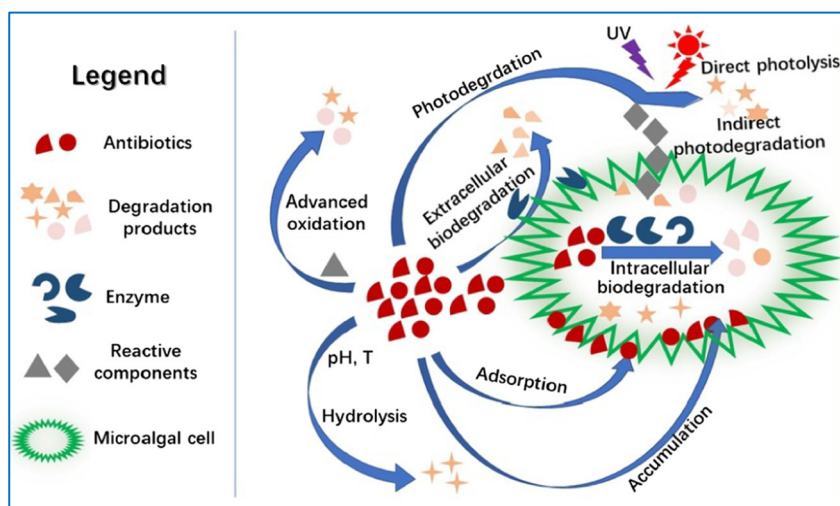
Antibiotics removal is highly dependent on microalgae strains [95]. The removal mechanism of levofloxacin by *Chlorella vulgaris* is bioaccumulation and cellular internal biodegradation [98], but the main mechanism of *Phaeodactylum tricornerutum* to remove oxytetracycline is biosorption [99]. de Godos et al. [100] investigated the removal of tetracycline in a high-rate algal pond in which synthetic wastewater is treated by a combination of *Chlorella* bacteria and found that photodegradation was the main removal mechanism, and biosorption also played a certain role. At present, antibiotic removal technology based on microalgae is still in its infancy. The potential of microalgae to remove antibiotics in actual wastewater remains to be explored; the simultaneous removal mechanism of multiple antibiotics and the toxicity of

intermediate products in the antibiotic degradation process also need to be further clarified.

Influencing Factors of Microalgae on the Removal of Nutrients and Pollutants

There are many factors that affect the removal of nutrients and pollutants by microalgae, such as microalgae species, the characteristics of wastewater, light, and temperature. It has been found that *Chlorella* sp. and *Scenedesmus* sp. can effectively remove nutrients, heavy metals, and antibiotics in agricultural and industrial wastewater, and *Chlorella* is the most widely used. In addition, many species of algae can be used in municipal wastewater treatment, such as *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Spirulina maxima*, *Arthrospira platensis*, *Botryococcus*

Fig. 6 Schematic diagram of the mechanism of antibiotics removal by microalgae [95]



braunii, *Dunaliella salina*, *Haematococcus pluvialis*, *Isochrysis galbana*, and *Neochloris oleoabundans* [9••, 95•].

The physical and chemical characteristics (chromaticity, pH, C/N ratio, N/P ratio, nutrients, and pollutants content) of different types of wastewater are different, which have a great impact on nutrient removal rate and microalgae growth [4••]. Municipal wastewater and animal manure wastewaters are the two most widely used wastewaters for the cultivation of microalgae. The microalgae strains all have the optimal nutrient conditions for their growth, while the nutrients in the actual wastewater rarely match with the optimal conditions for microalgae growth. Currently, in order to solve this problem, the following two methods are adopted. One is to screen or train microalgae to adapt to wastewater, and the other is to pre-treat wastewater to meet the growth conditions of microalgae [9••]. The N/P ratio and C/N ratio of wastewater are usually unbalanced for the conditions required for microalgae growth, which will affect the biomass productivity and the removal rate of nutrients. The low nitrogen content in wastewater reduces the removal of phosphorus by microalgae, but the removal of nitrogen is not limited by the phosphorus content [101]. The optimal value of N/P ratio for freshwater algae growth is 6.8–10; lower than this value would cause nitrogen limitation [9••]. It can be solved by mixing other types of wastewater, and this method is also suitable for situations where the C/N ratio is high. For example, the C/N ratio of brewery wastewater is relatively high, while that of swine wastewater is relatively low, and mixing the two types of wastewater can balance the C/N ratio. Zheng et al. mixed swine wastewater (C/N = 1.0) and brewery packaging wastewater (C/N = 30.5) in a ratio of 1:5, and treated the mixed wastewater with *Chlorella vulgaris*, and found that the removal rate of nitrogen, phosphorus, and COD was improved [102].

In addition, light intensity, light quality, light-dark ratio, and temperature are also the main factors affecting the removal of nutrients from wastewater by microalgae. Generally, higher light intensity and longer light time can improve the removal rate of nutrients. And under the same light intensity, the growth rate of microalgae under red light and blue light is higher than other light qualities (such as yellow, green light) [76•, 103]. In terms of temperature, as the temperature increases, the physiological metabolism of microalgae and its ability to remove nutrients are enhanced, and the removal rate reaches the highest when the cultivation temperature is close to the optimal value for microalgae growth [21].

Overall, microalgae can convert carbon, nitrogen, and phosphorus in wastewater into biomass, and in addition, pollutants can be removed by means of biosorption, bioaccumulation, and biodegradation. The microalgae species, the characteristics of wastewater, light, and temperature all affect the removal efficiency of pollutants.

The Microalgae Growth Modes and Cultivation System

Microalgae Growth Modes

Microalgae can adopt different growth modes for metabolism according to different environmental conditions, including autotrophic, heterotrophic, and mixotrophic modes. The different nutrition and energy sources used by microalgae result in variation of growth characteristics and cell composition different [104]. Understanding the characteristics of the microalgal growth mode is of great significance to the development of the technology of microalgae cultivation using wastewater.

The autotrophic metabolism of microalgae accumulates biomass through photosynthesis by using light sources and inorganic carbon (such as CO₂) as energy and carbon sources, respectively, which helps reduce global carbon dioxide. Outdoor large-scale cultivation of microalgae (such as open ponds) takes advantage of this condition of photoautotrophic cultivation. However, the biomass concentration of microalgae cultivated under phototrophic conditions is low. According to reports, under optimal conditions, the microalgae growth rate of some autotrophic species is 0.2 g·L⁻¹·d⁻¹ [105].

Heterotrophic cultivation is that microalgae use organic compounds (such as glucose, glycerol, and acetic acid) as energy sources and do not need light to maintain their normal growth and metabolism. Some microalgae strains can grow under both autotrophic conditions with light and heterotrophic conditions with lack of light. It has been shown that certain microalgae under heterotrophic cultivation can obtain higher biomass and lipid yields than autotrophic cultivation [106]. For example, for *Chlorella protothecoides*, compared with autotrophic cultivation, its lipid content increased by 40% under heterotrophic conditions [107]. In addition, heterotrophic cultivation can avoid the phenomenon that the light restricts the growth of high-density microalgae in large-scale photobioreactors [108]. However, the cost of heterotrophic cultivation is relatively high due to the need for organic carbon sources, and the use of organic matter in wastewater can solve this problem. In addition, due to the presence of organic matter, this cultivation mode is susceptible to contamination, so the closed photobioreactor is more suitable for its large-scale production [109].

Microalgae use both inorganic and organic compounds as carbon sources for growth and metabolism under light conditions, which is called mixotrophic cultivation. This growth mode is similar to the combination of autotrophic and heterotrophic, and there are also pollution problems that may be caused by organic matter [110]. Therefore, it is suitable to use enclosed photobioreactors for scale-up process, which can provide a light source and reduce the risk of pollution.

In addition, for many microalgae strains, mixotrophic cultivation can obtain higher biomass than autotrophic and heterotrophic cultivation individually [106].

Microalgae Cultivation System

At present, the large-scale cultivation system of microalgae is mainly divided into two types: open cultivation system and closed cultivation system. Open cultivation is a method of large-scale cultivation of microalgae in an open space (Fig. 7a, b). Because the construction capital and operating cost of an open cultivation system are relatively low, it is more suitable for industrial-scale applications. However, due to the greater impact of climate and the relative susceptibility to other microorganisms (such as bacteria), the biomass productivity of an open cultivation system is usually lower than that in a closed cultivation system [111, 112]. Closed cultivation system (photobioreactors) is more effective in controlling cultivation conditions (Fig. 7c, d).

Open Cultivation System

Open cultivation system has the advantages of low construction and operation costs, high production capacity, and easy cleaning after cultivation, generally including circular pond (Fig. 7a) and raceway pond (Fig. 7b) [111, 113, 114]. As the most common cultivation system in the photoautotrophic cultivation of microalgae, the raceway pond is continuously stirred by the stirring wheel to keep the algae suspended and promote the flow of water and the transfer of gas and liquid [115]. Montalvo et al. [116] successfully used a raceway pond

(12×3×0.4 m) to cultivate *Arthrospira maxima* OF15 in diluted sugarcane vinasse (30%, v/v), and after 15 days of cultivation, the removal rates of COD, TN, and phosphate in the wastewater were 81, 61.7, and 50%, respectively, and the algal biomass obtained could reach up to 2.25 g·L⁻¹.

However, the open cultivation system still has some shortcomings, such as large amount of water evaporation, large land area, low utilization rate of light by microalgae cells, relative susceptibility to other microorganisms, cultivation efficiency, and yield are greatly affected by environmental factors [114, 117]. In order to solve the abovementioned problems of open cultivation system, current researches focus on the development of suitable closed cultivation systems.

Closed Cultivation System

The enclosed photobioreactor is a relatively closed system with light sources, which can ensure the growth of microalgae in a relatively stable environment, where the cultivation conditions are easy to control and are not easily interfered by external microorganisms, so in such a system microalgal biomass with high purity and high density can be obtained in high reliability [118, 119]. However, the high operating cost of the enclosed photobioreactors is still an obstacle to its commercialization, and the use of wastewater as a nutrient source and water source for microalgae cultivation makes the process cost-effective [120].

At present, the enclosed photobioreactors mainly include tubular, vertical-column, and flat-plate bioreactors [114, 121]. Tubular photobioreactor (Fig. 7c) is the most common kind of closed cultivation system, and it consists of a series of straight,

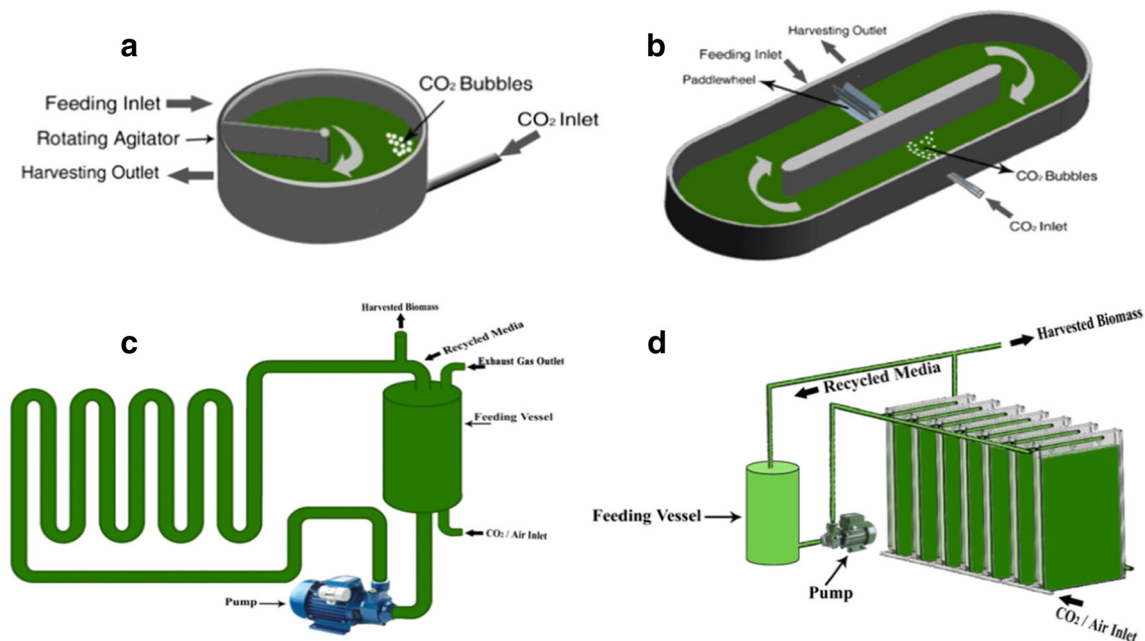


Fig. 7 Different types of photobioreactors (a circular pond, b raceway pond, c tubular photobioreactor, d flat-plate photobioreactor) [128]

coiled, or circular transparent tubes with glass or plastic as the main material, which is more suitable for large-scale outdoor cultivation [121–123]. In the tubular photobioreactor, mass transfer and microalgae circulation are usually accomplished by mechanical pumps or airlift pumps [124]. Although the tubular photobioreactor can be installed with a thermostat to adjust the culture temperature, it is costly and difficult to realize. In addition, photoinhibition and adhesion of cells on tube wall often occur in the reactor [114]. The vertical-column photobioreactor can be simply regarded as the vertical placement of the tubular photobioreactor, and it is divided into bubble-column and airlift photobioreactor [106, 125]. It is reported that the biomass concentration and specific growth rate obtained by these two vertical-column photobioreactors (the largest diameter is 19 cm) are equivalent to those usually obtained in narrow tubular photobioreactor [126].

The flat-plate photobioreactor (Fig. 7d) is composed of two transparent or translucent glass sheets and has the characteristics of large specific surface area exposed to light, short light path, and small land area. It can be placed vertically or tilted at a certain angle to maximize the use of solar energy [127, 128]. Compared with the horizontal tubular photobioreactor, the accumulation of dissolved oxygen concentration in the flat-plate photobioreactor is relatively low, and the modular design is easy to scale up, so it is widely used in the large-scale production of microalgae indoor and outdoor [121, 127, 129]. Several studies have shown that the use of flat-plate photobioreactors can achieve higher photosynthetic efficiency, but the device magnification requires large amounts of separation and support materials, the cultivation temperature is difficult to control, and the phenomenon of microalgae adherence to the wall is inevitable [106, 114, 130]. Table 3 summarizes the advantages and disadvantages of open ponds and enclosed photobioreactors.

In summary, open cultivation system and closed cultivation system are still the two most common microalgae cultivation systems. Open ponds are greatly affected by the seasonal

climate and are susceptible to other microorganisms, while enclosed photobioreactors have high operating costs and are relatively difficult to achieve large-scale industrial applications.

Methods of Microalgae Biomass Recovery

Microalgae biomass recovery (microalgae harvesting) is an important foundation for the downstream processing of algal biomass after the use of wastewater to cultivate microalgae. Currently, the major recovery methods include gravity sedimentation, centrifugation, filtration, flotation, and flocculation [131–133]. Centrifugation is the most commonly used method for rapid harvesting of microalgae, and its yield can reach 98% [133]. The harvested biomass can be safely used for the development of value-added products. The main disadvantages are the high energy cost and the large shear force generated during the process, which may cause cell damage [134].

Sedimentation is the process of increasing the concentration of microalgae biomass through gravity. This process is slow, and the settling rate is limited by the density and size of the algae cells. Gravity sedimentation is not suitable for the harvesting of microalgae with a particle size of 4–5 μm due to its small settling rate, while it is suitable for the harvesting of large microalgae with a particle size of more than 70 μm [135]. In addition, the settling efficiency of microalgae with high cell density is better than that of low-density microalgae. Although the operating cost of sedimentation is relatively low, the harvesting efficiency is limited and the speed is slow, so it is generally used in combination with flocculation, centrifugation, and other technologies to reduce sedimentation time and improve harvesting efficiency [136]. Flotation is a process in which microbubbles are used as carriers to adsorb and drag microalgae cells to the surface of liquid for enrichment

Table 3 Advantages and disadvantages of open ponds and enclosed photobioreactors

Cultivation systems	Advantages	Disadvantages
Open ponds	Low operation cost; low energy input; easy to clean after cultivation; easy to maintain	Water evaporation; large land area; low utilization rate of light by microalgae cells; easily polluted; easily interfered by the weather
Tubular photobioreactor	Large light surface area; suitable for outdoor cultivation; high biomass productivity	High cost compared to open ponds; poor mass transfer; photoinhibition; adhesion of microalgae cells on tube wall
Column photobioreactor	High mass transfer; reduced photoinhibition and photooxidation; compact; easy to operate; easy to adjust the light-dark cycle; easy to sterilize	High cost compared to open ponds; small light surface area
Flat-plate photobioreactor	Large light surface area; suitable for outdoor cultivation; high biomass productivity; low dissolved oxygen accumulation; easy to sterilize	Difficult to control culture temperature; difficult to scale-up; low photosynthetic efficiency

and harvesting, and it is suitable for harvesting low-density microalgae [137].

Filtration is a method of separating solid and liquid through porous membrane, which can be divided into dead-end filtration, vacuum filtration, pressure filtration, and tangential flow filtration (such as macrofiltration, ultrafiltration, microfiltration, nanofiltration, and reverse osmosis) [137, 138]. For suspended algae with a small cell volume, tangential flow filtration is considered to be more feasible than dead-end filtration [139]. Membrane filtration has a high harvesting efficiency, but membrane fouling and replacement of the filter membrane undoubtedly increase the cost.

Flocculation harvesting of microalgae is mainly through charge neutralization, bridging, and net trapping to gather microalgae cells together and then through solid-liquid separation to complete the purpose of microalgae harvesting. At present, the commonly used flocculation methods mainly include chemical flocculation (inorganic flocculant and organic flocculant) [140], physical flocculation (electric flocculation, magnetic flocculation) [141, 142], and biological flocculation [143]. Among them, biological flocculation has the advantages of simple operation, energy-efficient, safety, and nontoxicity, which has huge development potential [140].

In brief, each harvesting method has its advantages and disadvantages. In order to achieve efficient harvesting of microalgae, more efficient harvesting techniques can be continuously developed, or multiple methods can be integrated according to the specific application of different types of microalgae.

Application of Microalgae Biomass from Wastewater

Microalgae can be used to produce value-added products due to the high content of proteins, carbohydrates, lipids, vitamins, and antioxidants [144]. Currently, the harvested microalgae biomass can be used as the feedstock for the production of biofuels, animal feed, biofertilizers, and bioactive compound extraction [145–148]. Based on existing reports, it can be seen that the development cost of microalgae biological products is still relatively high. If the nutrients in wastewater can be used to cultivate microalgae while avoiding possible secondary pollution from pollutants, the production cost of algal biomass will be greatly reduced, and the microalgae biomass production industry will be brought into great leaps forward.

Biofuels

Biofuels, as a clean and renewable energy source, have always been considered alternatives for fossil fuels. As a third-generation biofuel, microalgae have the advantages of fast growth, simple cultivation conditions, no occupation of arable

land, and alleviation of the greenhouse effect [41]. Besides, the use of microalgae cultivated in wastewater to produce biofuels (such as biodiesel, bioethanol, biogas, biohydrogen) is extremely cost-effective and in line with concept of sustainable development of wastewater treatment [149]. It is reported that many types of microalgae have high lipids content, including triacylglycerides, which are high-quality raw materials for biodiesel [150]. At present, the development of potential biodiesel for photosynthetic microalgae mainly focuses on improving the lipid content and biomass production.

Animal Feed

Microalgae biomass contains high nutrients such as proteins, vitamins, and unsaturated fatty acids, so it is often used as a feed additive, which can improve the physiological functions of animals and enhance immune responses and antibacterial and antiviral capabilities [151, 152]. At present, microalgae biomass has been added to the diets of many animals such as fish, shrimps, pets and cattle, pigs, chickens, and so on [151, 153]. Fuentes-Grünwald et al. [154] cultivated *Chlorella vulgaris* and *Scenedesmus obliquus* in the anaerobic digestion of animal manure, and the results showed that the protein content of the harvested biomass exceeded 45%, which is very suitable for animal feed. Generally, a diet composed of a mixture of several algae species can provide balanced nutrition for animals, which is more conducive to the growth and development of animals [155]. Costa et al. [156] demonstrated that *Spirulina platensis* and *Chlorella pyrenoidosa* have the potential to replace tropical grasses with low protein content as feed for ruminants and increase the average daily gain of steers. However, it is worth noting that microalgae biomass harvested from wastewater may accumulate heavy metals and some toxic substances, and their safety needs to be further evaluated when used as feed [151].

Biofertilizers

In recent years, microalgae biomass has been regarded as a promising source of biofertilizers, which can adjust the mineral composition of demineralized soils, improve soil fertility, stimulate plants to secrete growth hormones, and increase plant yields [157, 158]. Castro et al. [159] used algal biomass harvested from a high-rate algal pond containing meat processing wastewater as fertilizer for millet growth and found that the yield of millet was not significantly different from applying urea as fertilizer, indicating that microalgae biofertilizer can be used to replace synthetic nitrogen fertilizer. Silambarasan et al. [146] reported that the combined application of de-oiled algal biomass waste and inorganic fertilizer can increase the growth and yield of tomato plants compared with applying inorganic fertilizer alone.

In short, although in the context of circular economy, it is meaningful to use the algal biomass cultivated in wastewater as the feedstocks for biofuels, animal feed, and biofertilizers, the pollutants in the wastewater may be transferred to the microalgae. The pollutants adsorbed on the algae surface can be desorbed to purify the biomass, while the pollutants accumulated in the cells cannot be removed. Therefore, the safety of microalgae biomass resource utilization needs to be further evaluated. At present, many countries have formulated regulations on the existence of various contaminants in feed or food [160], which is of great significance for the beginning of discussions on whether wastewater can be used to cultivate microalgae for animal feed production.

Challenges and Future Prospects

Although the use of wastewater from different sources such as municipal wastewater, agricultural wastewater, and industrial wastewater to cultivate microalgae has been extensively studied, most of the research mainly stays on the lab-scale, and recently some pilot studies have gradually developed. For example, Posadas et al. [161] evaluated the effectiveness of three semi-industrial outdoor raceway ponds to treat secondary domestic sewage and found that the removal rates of COD, TN, and TP can reach 84, 79, and 57%, respectively. Zurano et al. [162] used a pilot-scale thin-layer cascade photobioreactor to treat urban primary wastewater. After 10 months of uninterrupted operation, the results showed that although the composition of the wastewater and environmental conditions are different, the microalgae-bacteria system still shows great stability. However, after the process is expanded to an industrial scale, the performance may be different, so under actual operating conditions, its feasibility and economy are still urgent needs for future research.

Therefore, the key tasks in the future still include screening and improving dominant algae suitable for different types of wastewater, designing and developing systems or equipment that can be used for large-scale cultivation, optimizing cultivation conditions, and developing cost-effective microalgae separation and capture technologies. Moreover, positioning the secondary pollution after the absorption and transformation or adsorption of primary pollutants and the development of safety assurance technologies for the utilization of microalgae biomass generated after wastewater purification will also become the focus of the future. These breakthroughs can effectively help the future development of the technology.

Conclusion

The coupling technology based on wastewater biological treatment and the production of microalgae biomass and

value-added products has positive application prospects. It can simultaneously achieve multiple purposes such as wastewater purification, nutrient recovery, and production of high-valued microalgal biomass. This article reviews the research progress of microalgae in the treatment of municipal wastewater, agricultural wastewater, and industrial wastewater and introduces the mechanism and influencing factors of microalgae to remove nutrients and pollutants in details. In addition, the microalgae cultivation system, the recovery method of algae biomass, and its application are discussed. Overall, most of the current wastewater treatment and biomass production technology based on microalgae still remain on the laboratory scale, and there are still many challenges in large-scale applications, which are worthy of further study. Moreover, there is still a broad space for research on whether the pollutants in wastewater are safe for the resource utilization of algal biomass.

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Declarations

Conflict of Interest The authors declare no competing interests. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Human and Animal Rights and Informed Consent This article does not contain any study with human and animals performed by any of the authors.

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- Of major importance

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