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REVIEW

Immobilized microalgae: principles, processes and its applications in wastewater treatment

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Abstract
Microalgae have emerged as potential candidates for biomass production and pollutant removal. However, expensive biomass harvesting, insufficient biomass productivity, and low energy intensity limit the large-scale production of microalgae. To break through these bottlenecks, a novel technology of immobilized microalgae culture coupled with wastewater treatment has received increasing attention in recent years. In this review, the characteristics of two immobilized microalgae culture technologies are first presented and then their mechanisms are discussed in terms of biofilm formation theories, including thermodynamic theory, Derjaguin-Landau-Verwei-Overbeek theory (DLVO) and its extended theory (xDLVO), as well as ionic cross-linking mechanisms in the process of microalgae encapsulated in alginate. The main factors (algal strains, carriers, and culture conditions) affecting the growth of microalgae are also discussed. It is also summarized that immobilized microalgae show considerable potential for nitrogen and phosphorus removal, heavy metal removal, pesticide and antibiotic removal in wastewater treatment. The role of bacteria in the cultivation of microalgae by immobilization techniques and their application in wastewater treatment are clarified. This is economically feasible and technically superior. The problems and challenges faced by immobilized microalgae are finally presented.

Keywords Immobilized microalgae · Wastewater treatment · xDLVO · Cross-linking

Introduction

The increasing demand for energy and the environmental pollution resulting from fossil fuel usage are significant concerns (Vohra et al. 2021). Additionally, climate change control measures restrict fossil fuel extraction (Welsby et al. 2021). These factors have spurred the search for clean, sustainable, and green alternative energy sources (Tutak and Brodny 2022). Microalgae emerges as promising candidates in this context. As a renewable raw material source (Tazikeh et al. 2022), microalgae offer an eco-friendly and sustainable pathway to bioenergy. They efficiently convert solar energy into bioenergy and boast a high lipid content. Furthermore, microalgae are highly adaptable and can survive in a variety of environments (Abdullah et al. 2019). They hardly competes with arable land suitable for food production (Langholtz et al. 2016).

Extensive studies have been conducted on the use of microalgae for biomass and bioenergy production in recent decades. These studies involve various aspects, such as microalgae cultivation, harvesting, oil extraction, and conversion processes (Bauer et al. 2023; Kumar et al. 2023; Neag et al. 2023; Rossi et al. 2023; Rossignol et al. 1999). It has been observed that suspended microalgae are small, typically several micrometers in size, and have a low scattered density, less than 1%, in the culture media (Tan et al. 2015). The cultivation process requires a significant amount of water and nutrients. This is not economically feasible for large-scale microalgae cultivation and complicates the traditional harvesting process. Various harvesting techniques have been proposed for harvesting microalgae including flocculation, flotation (including floating bead flotation), filtration, centrifugation, or a combination of these

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technologies (Kumar et al. 2023; Xu et al. 2021). However, in large-scale cultivation, these techniques continue to face the disadvantage of high energy consumption, leading to increased production costs (Xu et al. 2021). Interestingly, microalgae exist not only in a suspended state but also as biofilm (passive immobilization) in nature. This presents an advantage in harvesting because a large number of microalgae are concentrated on a substrate. They can be easily harvested by scraping off the substrate (Hu et al. 2021).

To reduce the cost of cultivation, microalgae culture is generally coupled with wastewater treatment (Vo et al. 2020) because microalgae can utilize the mineral nutrients in wastewater (Wang et al. 2021). This also provides a possibility for dealing with the large amount of wastewater generated in the production and living processes of modern society. These wastewaters contain a large amount of nutrients, such as nitrogen and phosphorus, heavy metals such as lead, and emerging pollutants of concern. Traditional wastewater treatment processes include aerobic activated sludge process, nitrification denitrification, and chemical precipitation. The advantage of these processes is that they can effectively remove pollutants from wastewater, but they also generate a large amount of sludge, causing secondary pollution. Meanwhile, these processes have drawbacks such as high energy consumption, long process flow, and increased carbon emissions, which do not conform to the concept of carbon neutrality in sewage treatment plants (You et al. 2022). Microalgae, especially microalgae biofilms, have been applied in some wastewater treatment processes due to their low energy demand, low cost-effectiveness, nutrient recyclability, greenhouse gas suppression, and the use of useful biomass for nutrient recovery (Huang et al. 2023).

However, wastewater with complex composition and high nutrient concentration may lead to failure of biofilm growth or low biomass quality (Hu et al. 2021). To solve this problem, active immobilization is proposed, where the microalgae are encapsulated in a substrate and placed in a medium for growth (Zhuang et al. 2020). In this way, cells will be able to tolerate higher concentrations of pollutants as they do not come into direct contact with them. Immobilized microalgae came to favor the harvesting of microalgae. In one report, at the end of the incubation, the sedimentation rate of microalgae beads was 1.9 cm/s, which was significantly faster than that in the suspension system (<0.0002 cm/s). In addition, more than 98% of the microalgal cells could be harvested with gauze or mesh sieves (Mathimani and Mallick 2018). Meanwhile, compared to suspended microalgae, the density of microalgae cells is higher, so the required space is smaller (Roostaei et al. 2018). Due to these benefits offered by immobilized microalgae, related studies are appearing more and more, especially in the field of wastewater treatment (Han et al. 2022). At the same time, bacteria-microalgae co-culture system in the wastewater treatment also received widespread attention. Microalgae provide the oxygen required for bacteria to degrade organic pollutants through photosynthesis. During this process, CO_2 released by bacteria can be absorbed by microalgae during photosynthesis. Additionally, bacterial extracellular polymers (EPS) containing polysaccharides, proteins, and phospholipids can improve the properties of the substrate surface, thereby accelerating the attachment of microalgae cells, which is beneficial for the formation of biofilms. Currently, only two reviews on the concept of immobilized microalgae and the application to wastewater are summarized (de-Bashan and Bashan 2010; Moreno-Garrido 2008). However, there is a lack of mechanistic description of immobilized microalgae and the role of bacteria, while new technologies and results in the last 10 years need to be reviewed.

In this paper, the latest research progress of immobilized microalgae technology is reviewed, two main immobilization mechanisms of immobilized microalgae culture are analyzed, and the main factors affecting the growth of immobilized microalgae are reviewed. It also reviews the role of bacteria in the immobilization culture of microalgae and discusses the application of immobilized microalgae in wastewater treatment. The aim of this review is to improve the understanding of the mechanism of immobilization in microalgae culture, to guide and inspire researchers in solving wastewater treatment problems, and to provide ideas for the large-scale production of microalgae.

Immobilization techniques and mechanism for microalgae

Immobilization techniques

Microalgae immobilization techniques can be mainly divided into two categories: "passive" (biofilm) and "active" immobilization (Moreno-Garrido 2008). Biofilms are associations of microorganisms that develop on solid surfaces. Microorganisms are embedded in EPS, forming a complex structure. Once the microalgae have accumulated a mature microalgal biofilm on the carrier, it can be lifted from the water surface (separating the algae from the water). Then the microalgae biomass can be mechanically harvested (Moreno-Garrido 2008; Zhuang et al. 2018).

As mentioned above, passive immobilization culture of microalgae mainly refers to the microalgal cells attachment on the carrier surface and formation of a biofilm. For the exploration of the mechanism of this immobilization technology, we mainly focus on the formation process of biofilm. In fact, a biofilm is a highly structured and dynamic microalgal community. The formation process of biofilm includes a series of complex biological, physical and chemical processes, which is manifested as the proliferation and growth of microalgal cells in a specific environment after adhering to the surface of the carrier, and developing into a biofilm with a certain organization and complete performance (Wang et al. 2018).

Active immobilization, also known as gel trapping and embedding, uses polymers for cross-linking, and the principle is to trap microalgae cells in the network space of water-insoluble gel polymer pores through polymerization/precipitation/ion crosslinking (Lee et al. 2020). Both artificial polymers (polypropylene phthalamide, polyurethane and epoxy resins) and natural gels (agar, sodium alginate, carrageenan, chitosan, carrageenan) have been considered as embedding materials. They do not negatively affect the viability of encapsulated cells, allow diffusion of small molecules (such as nutrients, glucose, and oxygen), and are highly biocompatible. Sodium alginate (SA) show significant promise due to its simplicity of beads-making operations (de-Bashan and Bashan 2010).

Mechanism

Biofilm formation generally is composed of four stages (Fig. 1). First, the suspended cells reach the carrier surface by the motion of flagella, hydrodynamics or Brownian motion under gravity (Fig. 1a). Second, through the organelles such as flagella, cilia, and the outer membrane proteins of the cell membrane, they attach to the surface of the carrier under the action of electrostatic force, van der Waals force, surface tension and adhesion, which is the initial irreversible attachment process (Cui and Yuan 2013) (Fig. 1b). In the third phase, cells on the surface of the carrier generate EPS during reproduction, which connects the dispersed cells into a lamellar colony on the carrier surface and adheres them to the surface of the carrier (Fig. 1c) (Schnurr and Allen 2015). When this ability becomes stronger, it is irreversible attachment, which is the basis of biofilm formation (Wang et al. 2018). In the fourth stage, cells grow and reproduce, spreading to form a mature biofilm with certain complex structures (Fig. 1d).

Among them, the main theories considering initial adhesion between microalgal cells and substrate surface in the second stage of biofilm formation include: thermodynamic theory, DLVO (Derjaguin-Landau-Verwei-Overbeek) theory, and theoretical models such as xDLVO. When microalgae cells approach the carrier surface in a liquid, three

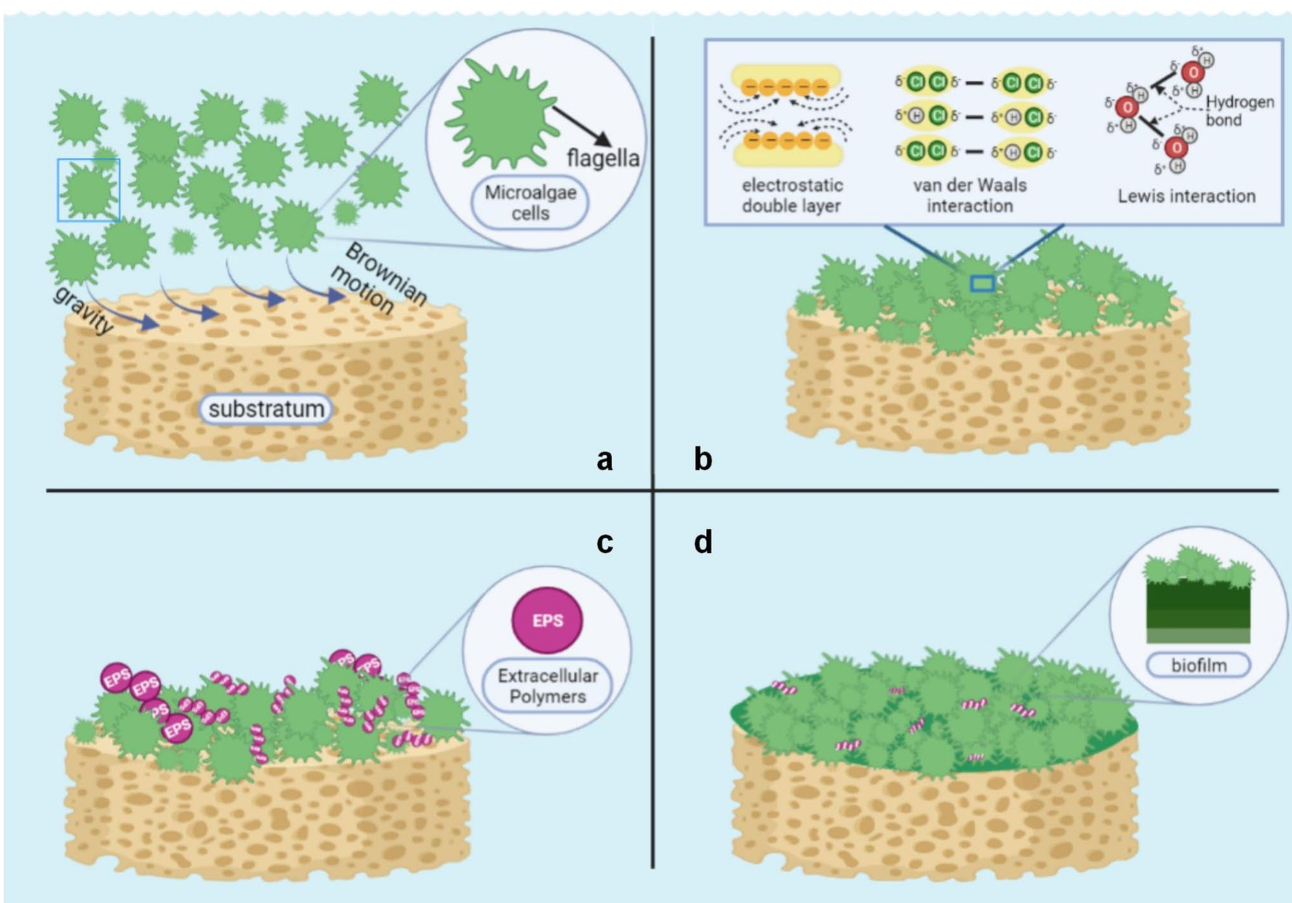

Fig. 1 Mechanism of microalgae attachment (**a** Algal cell transport,**b** Initial irreversible adhesion,**c** Irreversible adhesion,**b** Biofilm thickening.)

interfaces are involved: the microalgae cell-liquid interface, the carrier-liquid interface and the microalgae cell-carrier interface. Assuming that the charge effect can be neglected during the adhesion of microalgal cells to the carrier and there is no chemical bonding between the microalgal cells and the carrier at the early stage of adhesion, the adhesion process between microalgal cells and solid surfaces can be described by the thermodynamic theory (Eq. 1). Microbial cell adhesion behavior was evaluated by analyzing the work of adhesion (ΔG_{adh}) of cells before and after adhesion to the material surface. When ΔG_{adh} is negative, cells easily adhere to the material surface. When ΔG_{adh} is positive, it is difficult for cells to adhere to the surface of the material (Gusnaniar et al. 2017). ΔG_{adh} is also equivalent to the sum of the Lewis acid–base ($\Delta G^{AB}{}_{adh}$) and van der Waals components ($\Delta G^{LW}{}_{adh}$) of the adhesion free energy. These two parameters can be calculated by measuring the contact angle and zeta potential of two target objects. It requires fewer parameters to be measured and specific values can be calculated. However, the existence of assumption in this theory leads to a rough estimation. Therefore, the classical DLVO theory based on van der Waals interactions and electrostatic interactions compensates for this limitation. In DLVO theory, U_{DLVO} consists of the contributions of Lifshitz-Van der Waals interaction and electric double layer interaction, which lead to the mechanisms of biofilm adhesion processes (Eqs. 2). However, the theory ignores the effects of microorganisms binding water, spatial miles, hydrophobic gravitational forces, and hydrophilic repulsive forces during adhesion (Bos et al. 1999). Therefore, the x-DLVO theory proposed by Van Oss adds the Lewis acid–base interaction (Eqs. 3) (Busscher et al. 2010).

$$\Delta G\text{adh} = \gamma^{ms} + \gamma^{ml} + \gamma^{sl} \tag{1}$$

where, γ^{ms}, γ^{ml} and γ^{sl} are the interfacial free energies of microalgal cell- substances, microalgal cell-liquid, and substances -liquid, respectively. The interfacial free energies are determined by the contact angle and the surface tension between the interfaces (Gusnaniar et al. 2017).

$$U_{DLVO} = U_{LW} + U_{EL} \tag{2}$$

$$U_{xDLVO} = U_{LW} + U_{EL} + U_{AB} \tag{3}$$

where, U_{LW} is the Lifshitz-Van der Waals interaction, U_{EL} is the electrostatic interaction, and U_{AB} is the Lewis acid–base interaction. U_{LW} is related to the radius (or equivalent radius) of the microalgae cells, the separation distance between the studied objects and $\Delta G^{LW}{}_{adh}$. When the targets are two spheres, there is a negative correlation with radius and ΔG and a positive correlation with separation distance. U_{EL} is dependent on zeta potential, bilayer thickness, algal cell radius, and separation distance. U_{AB} is correlated with the radius, the separation distance, and the associated length of the molecules in the liquid medium.

Alginates are unbranched binary copolymers of 1–4 linked *β*-D-mannuronic acid (M) and *α*-L-guluronic acid (G) that can be isolated from algae (Kube et al. 2019). Alginate is composed of G-G blocks, G-M blocks and M-M blocks. These blocks are present in different proportions and different molecular weights in alginate formulations, which give them different physical and chemical properties (Paredes Juárez et al. 2014). Therefore, there are many types of alginates. In the field of encapsulation, alginates are divided into high G alginates, medium G alginates and low G alginates (Kube et al. 2019). To form pellets, alginate is usually dropped in a solution containing a high concentration of cations.

Calcium chloride ($CaCl_2$) is one of the most commonly used reagents for ionically crosslinking alginates, and it usually causes rapid gelation due to its high solubility in aqueous solutions. Ca^{2+} acts as a binder to crosslink alginate polymers to form solid beads (Ahmad Raus et al. 2021). It has been pointed out that divalent cations bind only to the guluronic acid (G) block of the alginate chain because the structure of the G block allows for a high degree of coordination of the divalent ion. The G blocks of one polymer then form linkages with the G blocks of adjacent polymer chains, which is known as a cross-linked egg-box model, resulting in a gel structure (Lee and Mooney 2012). Therefore, the selection of alginate also affect the cross-linking and the growth of microalgae (Kube et al. 2019). Schematic diagram of making microalgae beads is shown in Fig. 2.

Factors affecting the growth of immobilized microalgae

Although the mechanisms of these two types of immobilized cultured microalgae are different, the factors involved in the growth of algae are basically the same. These factors affecting the growth of immobilized microalgae include: microalgae strains, immobilized carrier, culture conditions (Ngene et al. 2010).

Microalgae strains

Microalgae strains, morphology and cell surface physicochemical properties all have general effects on the growth of microalgae (Yuan et al. 2009). These factors have an effect on the growth of microalgae in suspension and are more prominent for the growth of passively immobilized microalgae.

Most cells have the property of adhering to the wall and thus forming biofilms (Table 1). It has been shown that *Chlorella vulgaris* is more suitable for adherent growth and can achieve more biomass on its biofilm than the other five freshwater microalgae in the control group (Shen et al. 2014).

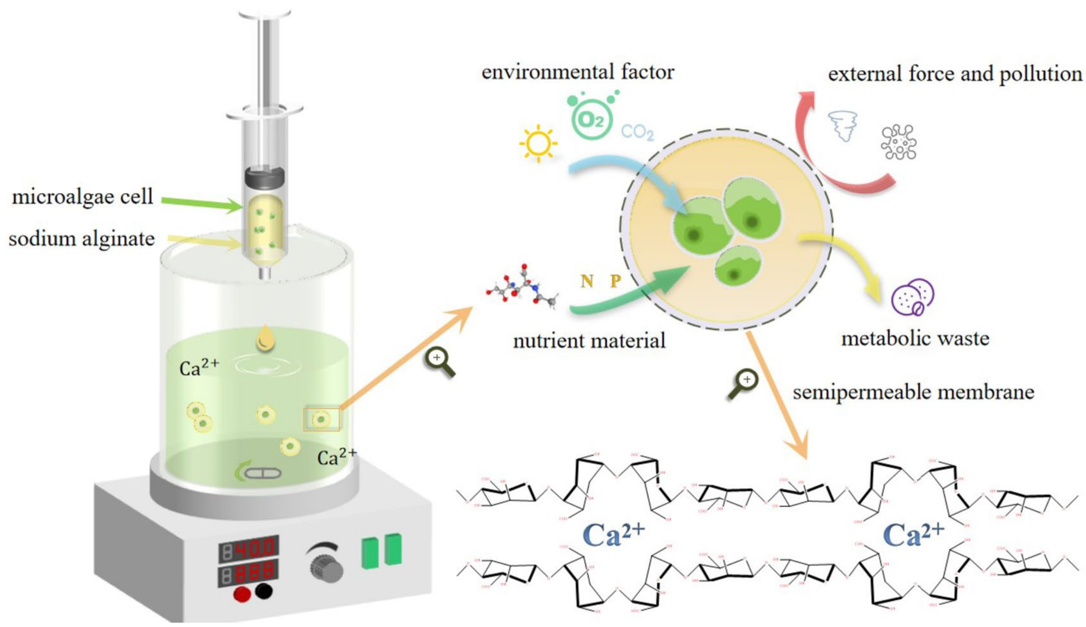

Fig. 2 Schematic diagram of microalgae beads preparation

Table 1 Common algae species in immobilized culture

Microalgal strains	Immobilization	Immobilized carrier	Carrier Characteristics	Bead size (mm)	Biomass	References
C. vulgaris	active	SA	Highly porous, transparent and biocompatible	2	0.67 mg/bead	Lam and Lee (2012)
C. sorokiniana	active	PVA + SA	High biocompatibility, transparency and mechanical strength	4.96 ± 0.24	–	Jeong and Jang (2021)
Chlamydomonas reinhardtii	active	SA	Highly porous, transparent and biocompatible	2.38 ± 0.42 3.80 ± 0.5 5.11 ± 0.60	35 ± 1.4 mg/mL 33 ± 0.7 mg/mL 27 ± 0.1 mg/mL	Lee et al. (2020)
C. vulgaris	active	SA	Highly porous, transparent and biocompatible	2.12 ± 0.36 3.72 ± 0.39 5.08 ± 0.56	33 ± 1.3 mg/mL 29 ± 0.6 mg/mL 24 ± 0.2 mg/mL	Lee et al. (2020)
C. vulgaris	passive	cellulose ester membrane	Strong hydrophobicity and low wettability	–	12.64 g/m^{2}/d	Rincon et al. (2017)
S. obliquus, C. vulgaris, Oscllatoria tenuis	passive	lignocellulose materials	Hydrolysate promotes high-value microalgal biomass content	–	10.92 g/m^{2}/d	Zhang et al. (2017)
Chlorella pyrenoidosa	passive	filter paper	Rough surfaces and large pores	–	5.03 g/m^{2}/d	Wood et al. (2022)
Cyanobacteria	passive	cotton rope	Rough surfaces and large surface areas	–	4.8 g/m^{2}/d	Cheng et al. (2017b)

SA sodium alginate, *PVA* polyvinyl alcohol

Algae with different cell shapes have different growth characteristics; for example, filamentous algae are more likely to aggregate into clusters and grow attached to surfaces. In addition, charge properties of microalgal cell surface and microscopic forces (such as molecular, ionic forces) can also affect cell aggregation and adhesion (Ozkan and Berberoglu 2013). The differences in the hydrophobic properties of the surfaces of microalgae can be attributed to the differences between their cell wall structure and the surface groups present on the cell walls. The presence of anionic and cationic groups (carboxyl, phosphate, or amine groups) and hydrophobic domains partially control the ability of microorganisms to flocculate or adsorb (Xia et al. 2016).

There are no special requirements for microalgae species in active immobilization. Microalgae and cell surface physicochemical properties of microalgae were not specifically studied in active immobilization, probably because both were encapsulated in carriers and were not significantly affected during incubation. Microalgae species with high growth rates, nutrient removal rates, and lipid productivity under photoautotrophic culture conditions are generally used for active immobilization, for example, *Chlorella. vulgaris* (Lam and Lee 2012), *C. sorokiniana* (Jeong and Jang 2021), *Chlamydomonas reinhardtii* (Lee et al. 2020), *Scenedesmus rubescens* among others (Zamani et al. 2012). Common immobilized cultured algal species are shown in Table 1. In wastewater treatment, immobilized *Scenedesmus rubescens* MCCS 018, *Chlamydomonas* sp. MCCS 026, and *Chroococcus dispersus* MCCS 006 had the highest PO_4^{3-}-P removal efficiency in 10 microalgae (Zamani et al. 2012).

Immobilization carrier

The carrier is a basic element of the immobilized microalgae culture system. For biofilms, a review by Schnurr and Allen (2015) noted differences in biofilm growth across materials without quantifying material properties and found differences in growth rates. In a subsequent study, it was shown that the roughness (Zhang et al. 2020), wettability (Zheng et al. 2016), surface energy (Cui and Yuan 2013) and biotoxicity of the carrier were the main influencing factors on the immobilization of microalgae.

The rougher carrier surface has more asperities, which promotes the interception and retention of algal cells, enhancing the strength of cell adhesion, as well as further promoting dense seeding and the formation of strong and strengthened biofilms. This ultimately increases the indirect biomass production. In addition, some reports linked wettability to colonization time, and indicated that microalgal cells on hydrophobic materials are more likely to form biofilms due to water-repellent mechanisms (Genin et al. 2014). For example, Zheng et al. (2016) used a polytetrafluoroethylene emulsion to alter the surface wettability of the material, and the results showed that the biomass yield of *Scenedesmus* on the surface with a contact angle of 64° increased to 122.03 g/m^2 compared to the harvest of *Scenedesmus* on the untreated surface. Regarding surface energy, Cui &Yuan (2013) established a mathematical model to understand the surface free energy of solid supports and algal cells when attached to five materials including nylon, stainless steel, polycarbonate, polypropylene and glass. The results showed that the attachment of microalgae to materials with higher dispersive surface energy but lower polar surface energy would be more favorable. Notably, to mitigate the biological toxicity in the wastewater medium, a dual carrier approach (with activated carbon and sponge) was used to obtain better protein content (61.1%), protein productivity (0.48 g/L/d), lutein content (4.56 mg/g) and lutein productivity (3.56 mg/L/d) (Chen et al. 2021).

The choice of carrier material is also critical when culturing microalgae by embedding (i.e., active immobilization). Among them, polyacrylamide is not suitable for the cultivation of microalgae due to strong biological toxicity. Agar, gelatin, and carrageenan are all used by dissolving in hot water (40 ~ 70 °C) and then cooling to form a gel insoluble in cold water during the embedding process, which has a negative impact on the activity of microalgae. Therefore, they are not commonly used for fixation of microalgal cells. However, sodium alginate (SA) realizes the immobilization of microorganisms by cross-linking with calcium ions to form water-insoluble gel spheres. The reaction conditions are mild, and it can retain a large amount of water, which has little effect on the activity of microorganisms. Therefore, SA is generally considered to be a better embedding material (Xie et al. 2018; Zhang Yu and Khademhosseini 2017). Additionally, different grades of sodium alginate can have an effect on the growth of microalgae (Kube et al. 2019). In addition, polyvinyl alcohol (PVA) can also be used as a potential material for embedding carriers, because it is cheap and has good mechanical strength. However, it has poor light transmission ability, which can affect the growth of algal cells. Recent studies have improved the light transmission of PVA by mixing it with SA which is beneficial to the microalgae cultivation (Liang et al. 2022). It has also been studied that the incorporation of optical fiber into PVA material to improve its light transmission improved the nutrient uptake efficiency of microalgae (Jeong and Jang 2021).

Culture conditions

Light

Light is a key factor for the growth of algae. Microalgae can convert light energy into chemical energy (biomass) through photosynthesis (Li et al. 2019a). Light quality (Izadpanah et al. 2018; Yuan et al. 2020), light intensity (Seo et al. 2017; Sun et al. 2018) and light–dark ratio (Blanken et al. 2017a) are often the subject of research. Studies have shown that light intensity controls not only growth rate (Das et al. 2011), but also storage and structural lipid distribution (Khotimchenko and Yakovleva 2005) and pigment synthesis (Ma et al. 2018b). The effect of light wavelength on growth varies by species, because of differences in metabolic pathways, pigmentation, and photoreceptors between species. Spectra had a significant effect on microalgal cell size and biomass yield. For example, the smallest cells were observed under red light (Izadpanah et al. 2018). In another study the highest microalgal biomass production was shown under red light (Chang et al. 2022). In Blanken`s study, it was determined that biofilms did not affect light utilization efficiency at the tested light–dark ratios in both diurnal and continuous lighting regimes (Blanken et al. 2017b).

Compared to suspension culture, microalgae in passive immobilization systems have a fixed location in the biofilm. As a result, cells far from the surface may be light-confined, while those on the surface may be light-inhibited all the time (Huang et al. 2016). When the photons emitted by the external light source pass through the carrier in the reactor, they are introduced into the biofilm to provide energy for the biochemical reactions of microorganisms. Due to the absorption of light by intracellular pigments, the scattering of light by cells and the mutual occlusion between cells, the light intensity in the biofilm along the light transmission direction decays exponentially, that is, the phenomenon of light attenuation. This leads to uneven light exposure of cells in the biofilm, and even the underlying biofilm is completely in the dark area. Of course, this phenomenon can be improved by increasing the light intensity inside the biofilm. However, when the photon flux density (PFD) of surface microalgae exceeds the light saturation point of microalgae, it will inhibit the growth of surface microalgae or even lead to death (Schnurr et al. 2016). To maximize productivity, photon penetration into biofilms needs to be enhanced (Schnurr et al. 2016). A possible way to do this is to illuminate the biofilm from both sides with optimal light intensity (Mantzorou and Ververidis 2019). This light system requires high transparency of the immobilization carrier. The higher the transparency of the carrier, the more favorable the growth of biofilm. In the early stages of adherent culture, light penetrates only a small fraction of the depth of the algal biofilm due to a sharp decrease in light intensity caused by the high pigment content of individual cells. However, as the number of days in culture increased, almost 100% of the cells within the immobilized biofilm were effectively exposed to light (Wang et al. 2015).

For active immobilization, the density of microalgal cells on the bead surface is higher due to the availability of sufficient light. This leads to a shading effect that can have an impact on the growth of cells inside the microbeads (Ruiz-Marin et al. 2010). Smaller sized beads have a greater specific surface area compared to biofilms, which can mitigate the degree of self-shading to some extent (Lee et al. 2020). However, it can still limit the growth of microalgae (Lau et al. 1997). In order to improve the mechanical strength of the beads, some studies mixed sodium alginate and polyvinyl alcohol (PVA) in a certain proportion, but this sacrifices a certain degree of light transmittance. To overcome this shortcoming, Jeong & Jang (2021) embedded the optical fiber in the gelatinous sphere, which not only transmitted light from the light source to the end of the fiber, but also emitted light along itself, both of which enhanced the lighting conditions of the algal cells inside the gelatinous ball.

CO_2

Carbon dioxide (CO_2) is one of the indispensable raw materials in the photosynthesis process of microalgae. An important factor in obtaining optimal growth conditions is adequate CO_2 supply. However, volume fraction of atmospheric CO_2 is 0.03%, which limits the photosynthesis of suspended microalgae. Concentrated CO_2 streams are often used to grow microalgae. A common CO_2 stream is flue gas, which has high concentration of CO_2 and can be used in closed microalgae culture systems to help microalgae growth and environmental protection. Although many researchers have investigated the effect of CO_2 concentration on planktonic algal growth, the effect on algal biofilm growth has been scarce. For example, for most algae growing in suspension, the increase of the CO_2 concentration (to 5–7% v/v) significantly increases the growth rate until too high concentration negatively affects growth (Ryu et al. 2009). However, in Blanken's experiment, the increase of the CO_2 concentration from 0.625% to 1.25% only improved the growth of algal cells on the biofilm to a certain extent, while as the CO_2 concentration was further increased from 4 to 10%, the microalgae growth was not significantly improved (Blanken et al. 2017b). Specific studies on the culture of microalgae by encapsulation in relation to carbon dioxide concentration are yet to be investigated.

The aforementioned methods of increasing CO_2 concentration also have limitations, because the mass transfer efficiency of CO_2 into the neutral medium is not high (de Godos et al. 2014). It is estimated that only 10% of the CO_2 is eventually captured when the high concentration of CO_2 from the flue gas is injected directly into the medium. Obviously, even when high concentrations of CO_2 are provided, microalgae cannot utilize them efficiently. To solve it, a new cost-effective culture method which does not require the use of concentrated CO_2 input was proposed. Interestingly, this method only utilizes atmospheric CO_2 and a medium with high alkalinity, resulting in sustained high yields of microalgae in outdoor raceway ponds (Vadlamani et al. 2019).

Nutrients

Nutrients are the main chemical elements and compounds presented in the environment. They are divided into macronutrients such as carbon, nitrogen, phosphorus compounds, and micronutrients such as trace metals and vitamins (Razzak et al. 2017). Microalgae generally use inorganic salts as nutrients. The most common nutrients are nitrogen and phosphorus compounds such as nitrates, nitrites, ammonia, organic nitrogen and phosphates. Studies have shown that N and P concentrations can significantly increase the accumulation and overall growth rate of microalgae biofilm biomass. But excessive nutrient loading is also harmful to algal cells (Boelee et al. 2011). Nitrogen and phosphates limitation in the medium may decrease biomass production but increase lipid production (Yaakob et al. 2021). During the culture of active immobilized microalgae, the nutrient is present as a nutrient concentration gradient. Appropriately increasing external nutrient concentration will improve this situation, but this concentration varies with algal species and embedding materials. Once the maximum nutrient requirement (1/2N) for cell growth of immobilized algal beads was met, higher nutrient concentrations (1N) did not contribute significantly to cell numbers (Fig. 3) (Jin et al. 2011). Therefore, it is necessary to maintain the stability of wastewater properties when applied to wastewater treatment.

Generally, photoautotrophic microalgae do not need additional carbon in their culture medium. However, many microalgal species have the adaptation to switch from photoautotrophic to facultative or heterotrophic growth, which can be achieved by changing the nutrient carbon source in the culture medium (Razzak et al. 2017). Carbon sources can directly or indirectly affect the secretion of EPS, which can enhance the attachment of microalgal cell communities and thus help to maintain the stable structure of algal biofilms (Zhuang et al. 2018). Qian et al. (2023) found that denser biofilms and maximum attached biomass were obtained with the addition of 1000 mg C L^{-1} of concentrated glycerol during incubation, with attached biomass concentrations as high as about 97 g m^{-2}.

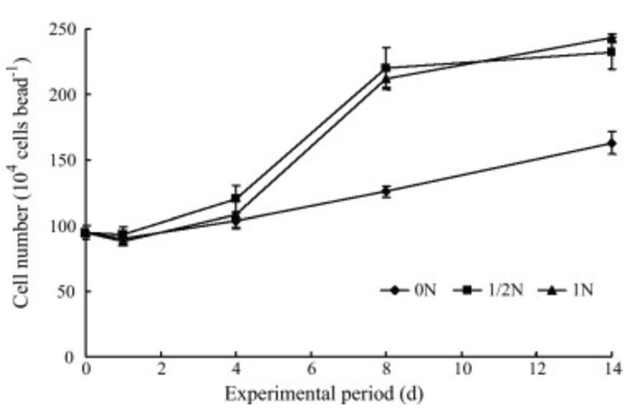

Fig. 3 Growth of *Chlorella vulgaris* cells in alginate immobilized beads with different concentrations of nutrients (1N means the 100% nutrients as the Bristol medium; 1/2N means the half nutrients as the Bristol medium; 0N is the control and without any nutrients supplemented) (Jin et al. 2011)

pH and temperature

In addition to the above three main factors, pH and temperature also play a certain role in the growth of microalgae.

pH is one of the important factors affecting the growth of microalgae. It affects the availability of CO_2 in algal photosynthesis, enzyme activities, and the absorption of nutrients (Sajjadi et al. 2018). The microalgae embedded within the beads release more oxygen due to photosynthesis than the oxygen released externally due to diffusion, which inhibits Rubisco. Since this is an enzyme associated with photosynthesis, it will affect the photosynthesis process. Due to diffusion inside and outside the bead, a pH gradient is created. This is advantageous because the high pH inside the beads facilitates the absorption of CO_2 (Timm et al. 2016). pH also affects the form of nutrients. For carbon sources, the dominant form is HCO_3^- at pH 6.36–10.33, with H_2CO_3 dominating below pH 6.36 and CO_3^{2-} dominating above pH 10.33. NH_4^+ and NH_3 will convert at pH between 8 and 10. pH is also associated with the formation of PO_4^{3-} species. Microalgae cultures for production purposes have a pH between 7 and 9 which is best for nutrient uptake. The optimum pH is 8.2 to 8.7 (Beltrán-Rocha et al. 2017). Compared with active immobilization, pH plays a greater role for passive immobilization, as lower pH can induce self-flocculation of algal cells. Liu et al. (2014) pointed out that lowering pH to slightly below the isoelectric point can promote the self-flocculation of microalgae. It was also noted that the mechanism may be that when the pH is lowered, the negatively charged self-flocculating microalgal cells become positively charged and then attract the negatively charged target algal cells to form flocs. The oxygen released by the microalgae embedded inside the beads due to photosynthesis is higher than the outside due to diffusion. Higher concentrations of oxygen inhibit ribulose. This results in a pH gradient in the beads. This may be advantageous as the high pH inside the beads will favor CO_2 uptake.

Temperature directly affects the solubility of nutrients in water and the enzymatic activity of microalgal cells, thus affecting algal growth rate and species composition in algal biofilms. Most microalgae are capable of performing photosynthesis and cell division in a wide temperature range, usually between 15 and 30 °C, but optimal conditions are between 20 and 25 °C (Li 1980). Numerous studies have demonstrated that effects below the optimal growth threshold are more favorable than that slightly above the optimal growth temperature (Ras et al. 2013). This was demonstrated in a study, where the article pointed out that temperatures below the maximum growth rate temperature would favor lipid accumulation. Especially when the temperature was decreased from 25 to 20 °C, lipid content increased by 170%, although there was a slight effect on growth rate (8% loss) (Xin et al. 2011).

Immobilized microalgae in wastewater treatment

Nitrogen and phosphorus removal

Nitrogen (N) and phosphorus (P) are both important constituents of cellular material. Proteins, enzymes, energy-transporting substances (including adenosine diphosphate (ADP) and adenosine triphosphate (ATP)), and genetic material in microalgae cells contain large amounts of N and P. According to the molecular formula of algae ($C_{106}H_{263}O_{110}N_{16}P$), some researchers theoretically calculated that the mass of N and P required to accumulate 1 g of algal biomass are 0.063 g and 0.009 g, respectively (Li et al. 2019b). It can be seen that microalgae have a high demand for N and P. In 1957, Oswald and Gotaas (1957) first proposed the concept of the application of algal cells for N and P removal in wastewater. Since then, the use of algal cell culture technology to treat sewage has received attention. Wastewater is extremely high in N and P and can provide sufficient nutrients. The total nitrogen (TN) concentration of the wastewater was in the range of 40–3000 mg/L and the total phosphorus (TP) concentration was in the range of 20–300 mg/L. However, the TN and TP concentrations in BG11 medium, which is commonly used for laboratory microalgae cultures, are about 35 mg/L and 2 mg/L, respectively. High N and P concentrations in wastewater are toxic to algal cells. And dilution of wastewater will require a large amount of water, which results in wastage of water resources. However, in active immobilization systems, microalgae can avoid direct exposure to high concentrations of nutrients due to the diffusion effect present in immobilized microalgae.

Nitrogen removal by microalgae mainly relies on the assimilation of the cell body. Inorganic nitrogen mainly exists in the form of nitrate, nitrite and ammonia nitrogen, which are used as nitrogen sources for photoautotrophic growth of microalgal cells, and are finally synthesized in algal cells to substances such as amino acids and proteins (Qie et al. 2019). Algae also have a heterotrophic mode., where organic nitrogen can be utilized by algae through heterotrophic growth, such as urea and amino acids. Some algae even fix nitrogen in the atmosphere (Taştan et al. 2012). P concentration in wastewater affects the mechanism of P uptake in algal cells. At low concentrations, P is directly assimilated by algal cells (Cai et al. 2013). However, when the phosphorus concentration in the wastewater is too high, the mechanism of P removal is changed, and the excess P is absorbed and stored in the cells in the form of PO_4^{3-} precipitates by algal cells (Powell et al. 2009). This precipitation is more facilitated when the pH is alkaline.

Compared with suspension culture, immobilized microalgae have better performance in nitrogen and phosphorus removal. It has been reported that the maximum absorption capacity of nitrogen and phosphorus in wastewater by microalgal biofilms can reach 1.0 g/m^2/day and 0.13 g/m^2, respectively (Boelee et al. 2011). Algal cells embedded with sodium cellulose sulphate /poly-dimethyl-diallyl -ammonium chloride (NaCS-PDMDAAC) can remove high concentrations of nitrogen and phosphorus (113.90/102.48 mg/L) in wastewater. The removal rates for TN and PO_4^{3-}-P were 12.56 and 10.24 mg/g/d, respectively (Zeng et al. 2012). The effect was significantly better than that of the suspension control group of *Chlorella* with the same initial concentration. This is because high microalgae biomass in immobilized systems consumes more nitrogen and phosphorus. However, the high efficiency in the actively immobilized system cannot be attributed solely to the function of the microalgae, but is also related to the adsorption of the immobilized carriers. N cations and anions (i.e., NH_4^+ and NO_3^-) can be reduced with ions in the matrix polymer by ion exchange (Banerjee et al. 2019). The removal of PO_4^{3-}-P is attributed to the release of calcium ions from the polymer (Mohsenpour et al. 2021). But immobilized microalgae also have limitations. Due to the shading effect and the fact that the density of microalgae cells within alginate beads reaches a plateau, the nutrient removal efficiency usually decreases, which leads to a gradual decline in nutrient removal efficiency. Therefore, microalgae beads need to be replaced regularly. Moreover, the application of immobilized cultured microalgae to wastewater had the added benefit, that is, it operates at shorter hydraulic retention times and can efficiently remove nutrients from wastewater (Kube et al. 2020; Whitton et al. 2018).

Heavy metals removal

Heavy metal concentrations in industrial wastewater are generally high and can pose a threat to organisms in the ecosystem. Therefore, removal of heavy metals from wastewater is necessary. Methods such as chemical precipitation (carbonate, hydroxide and sulfide precipitation), chemical oxidation and reduction, solvent extraction, reverse osmosis ion exchange, electrodialysis and adsorption have all been used for the removal of heavy metals (Cheng et al. 2019). Recently, microalgae have been found to have the potential to remediate various heavy metals (Samal et al. 2020) because of their high metal biosorption capacity. Since they show higher removal efficiency through biosorption and bioaccumulation mechanisms, microalgae can be used as alternative biosorbents for heavy metal remediation (Leong and Chang 2020). The remediation processes are extracellular precipitation/accumulation of heavy metals by living cells, complexation or cellular adsorption in living and dead cells, and cellular internalization requiring microbial activity or metabolic processes (Goswami et al. 2022a). Yang et al. (2021) reported that in algal bacterial granular sludge, the reduction of Cr(VI) can reach 99% in a relatively acidic environment, while the total Cr removal rate can reach 89% in weak acid conditions (Yang et al. 2021). Similarly, *Chlorella* grew in lead (Pb)-containing medium for 14 days and then a 92% reduction in Pb(II) concentration was reported; at the same time, the lipid content of the algal cells was improved (Nanda et al. 2021).

The above examples do demonstrate that microalgae are good candidates for wastewater removal. It should be pointed out that high concentrations of heavy metals may lead to the death of algal cells. However, some researchers point out that both live and dead algal cells can remove heavy metal ions from wastewater (Cheng et al. 2017a). The cell walls of dead algal cells have functional groups that bind heavy metals in water. Therefore, dead cells can also adsorb heavy metals (Suresh Kumar et al. 2015). But the adsorption capacity is limited. Another solution is to immobilize microalgae, which can resist the toxicity of high concentrations of heavy metals to algal cells to a certain extent, reduce the mortality of algal cells, and improve the removal rate of heavy metals. Also, enclosing the fixed microalgae cells in alginate beads helps to maintain a greater density of algae in the reactor, which allows for rapid removal of heavy metals (Kube et al. 2020). The biofilm cultures showed higher uptake and efficiency under high Cu^{2+} stress conditions with a copper content of 1.5 mg/L compared to the suspension system (Yousefi et al. 2023). This can prove that biofilm structures can be used in stressful situations and highly polluted wastewater. A study by (Moreno-Garrido et al. 2005) used sodium alginate to encapsulate the screened microalgae with better toxicity tolerance to remove Cd and Cu from seawater. The results showed that the immobilized microalgae removed 20% and 100% of Cd and Cu, respectively. Of these, both the embedding material and algal cells contributed to the removal of both heavy metals. Akhtar et al. (2003) cultured *Chlorella. sorokiniana* (LSIBCS) for Cr (III) removal using a loofah sponge as a passively immobilized carrier. The results showed that the cadmium removal efficiency of immobilized *Chlorella* from 10 mgL^{-1} solution was 97.9% (Akhtar et al., 2003).

Another disadvantage of suspended microalgae for heavy metal removal is that the algal biomass has small particle size, and low mechanical strength, making it difficult to separate algal biomass from wastewater. Again, immobilizing microalgae might solve this problem. It can be easily separated from wastewater after adsorbing heavy metals, and the obtained biomass can be used as a raw material for bioenergy. Therefore, the removal of heavy metals from wastewater with immobilized microalgae is a sustainable method. The sodium alginate carrier does increase the cost of wastewater treatment. But it can be partially compensated by its cost in the harvesting stage. The possible solution may be to reduce costs by finding low-cost materials or reusing carriers. For example, it has been noted that food-grade alginate is less costly (Kube et al. 2019). It has also been noted that 70% of alginate can be reused (Murujew et al. 2021). More example of immobilized microalgae removal from wastewater are shown in Table 2.

Removal of toxic substances

Some industrial wastewaters (e.g., pharmaceutical wastewater, dye wastewater, and agricultural wastewater) contain large amounts of toxic substances that lead to pollution of neighboring water bodies due to improper discharge (Goswami et al. 2022b; Rashid et al. 2021). Bioremediation is considered as a potential remediation method due to its economic efficiency and environmental friendliness (Rosli et al. 2020). Microalgae are considered as potential candidates for removal of toxic substances as they can effectively remove surrounding toxic substances through various trophic modes (Mustafa et al. 2021). However, the removal of toxic substances relies on microalgal strains with specific properties. Furthermore, the strong concentration of toxic substances requires advance acclimatized of microalgae prior to remediation.

Immobilized microalgae can help to overcome toxic or shock loads. So, it provides an interesting technique for removing toxic pollutants. In pharmaceutical wastewater, the immobilization technique was effective in protecting microalgae from carbamazepine (CBZ) toxicity and improving CBZ removal (84%) at high concentrations (> 50 mg/L) (Liang et al. 2022). In another study, microalgae immobilized in alginate pellets exhibited higher kinetic removal rates of endocrine disrupting compounds (bisphenol AF, bisphenol F, and 2,4-dichlorophenol) than suspended microalgae (Solé and Matamoros 2016). In the field of dye wastewater, microalgae immobilized in polyurethane foam proved to be effective in removing color, COD and nitrogen, as well as high biomass productivity. Chitosan-alginate microbead immobilized microalgae system effectively removes dyes and pollutants while creating a stable environment for microalgae growth. The addition of a fungus (*Aspergillus niger*) promoted the self-fixation of *Chlorella* to form bioparticles. This particle was very effective against pesticides, reducing the concentration of 17 pesticides (Hultberg and Bodin 2018). A study has shown that the removal of pesticides and antibiotics by microalgae has a lot to do with the hydraulic retention time (Ferrando and Matamoros 2020). The increase of HRT will reduce the decay of insecticides in the free microalgae reactor. However, the immobilized microalgae reactor can enhance the adaptability of microalgae system to HRT reduction. A significant improvement in pesticide and antibiotic removal was observed at a HTR of 8 days in continuous feed mode of operation. Therefore, immobilization is also considered to be an excellent method for removing pesticide contaminants. In addition, we can

Table 2 Immobilization of microalgae for removal of heavy metal

Microalgae	Heavy metal source	Experimental conditions	Microalgae immobilization	Carrier	Initial concentration of heavy metals	Heavy metal removal	References
Stichococcus bacillaris Strains CCAC 1898 B),	Zinc contaminated leachate from an abandoned mine dump near Braubach	T: 23.5 ± 2.5 ℃, LI: 110–140 μmol photons m^{-2} s^{-1}, L:D: 14/10 h, CT: 96 h	Passive	The Twin-Layer sheets were designed from two types of non-woven polyesters	Zn(II): 3.3 mg·L^{-1}	Zn(II):93.94% reduction	Li et al. (2015)
Diatoms, Green algae, Bacteria *(Gammaproteobacteria and Bacteroidia)*	Artificial wastewater	T: 30 ℃, LI: 2500 lx, L:D: 14/10 h	Passive	Spiral polyethylene pipe	Cu: 2 mg·L^{-1}, Cu: 10 mg·L^{-1}	Cu:99% reduction, Cu:98.2% reduction	Ma et al. (2018a)
Cyanobacteria, Green algae, Diatoms, Acid reducing bacteria	Wastewater produced by dissolving $NiSO_4$ $6H_2O$ based on the levels commonly reported in industrial effluents	T: 25 ℃, LI: 110–120 μmol photons m^{-2} s^{-1}, L:D: 14/10 h, IT: 504 h, CT: 168 h	Passive	The belt at revolving algal biofilm (RAB) reactor	Ni: 5000 mg·L^{-1}	Ni: > 90% reduction, 534 mg·L^{-1}·day^{-1} Ni removal rate	Zhou et al. (2021)
Chlorella sorokiniana	Artificial wastewater	T: 27 ℃, LI: 150 μmol photons m^{-2} s^{-1} CT: 24 h/ 8-10 h	Passive	sulfur-based copolymer	Cd (II): 50 mg·L^{-1} Cd (II) + Cu(II): 8 mg·L^{-1}	Cd (II): 90% reduction Cd (II): 95% reduction Cu(II): 90% reduction	Leon-Vaz et al. (2023)
S. obliquus FACHB-12	Artificial preparation of aqueous solution of $CdCl_2$	T: 25 ± 1℃, pH: 6, LI: 4000 lx, IT: 2 weeks, CT: 120 min	Passive	Luffa sponge	Cd: 3 mg·L^{-1}	Cd: 92.7% reduction	Ma et al. (2021)
Scenedesmus quadricauda	Artificially formulated heavy metal ion solution	T: 22 ℃, pH: 5, IT:168 h, CT: 120 min	Active	Calcium alginate	Cu(II): 600 mg·L^{-1}, Zn(II): 600 mg·L^{-1}, Ni(II): 600 mg·L^{-1}	Maximum Adsorption capacities: Cu(II): 75.6 mg/g, Zn(II): 55.2 mg/g, Ni(II):30.4 mg/g (mg metal ions/g adsorbent)	Bayramoğlu and Yakup Arıca (2009)

T temperature, *LI* light intensity, *L:D* night:day-cycle, *IT* incubation time, *CT* contact time immobilize multiple microalgae at the same time or allow the wild bacteria to develop naturally, and even add some pesticide-resistant bacteria to the microalgae for co-cultivation to form in immobilized algae system to better remove toxic substances in wastewater.

The role of bacteria in the immobilized microalgae

Interestingly, the presence of bacteria have been found to often promote the initial adhesion of microalgal cells onto the substrate surface (Schnurr and Allen 2015). Bacterial EPS containing polysaccharides, proteins and phospholipids can improve the properties of the substrate surface, thereby accelerating the attachment of microalgal cells (Xiao and Zheng 2016). Due to this facilitation, some studies have introduced bacteria into the culture medium (e.g. addition of wastewater or sludge) to shorten the duration of the initial adhesion of microalgae (Katam and Bhattacharyya 2019). Many researchers have shown that the presence of bacteria, and the resulting symbiotic relationship, is highly beneficial for the formation and overall growth of algal biofilms. Guo et al. (2011) first analyzed the correlation between the hydrophilic and hydrophobic bacterial communities in sludge and flocculation, and the results showed that the two bacterial communities were very different, and the hydrophobic colonies had better flocculation effect. It was also pointed out that the increase in hydrophobicity of granular sludge (AGS) resulted from changes in the community and EPS. Perera et al. (2022) demonstrated that adding bacterial-secreted EPS to the medium doubled the biomass of both microalgae. In addition, a study has shown that selective invasion of growth-promoting bacteria in microalgal algae results in increased microalgal biomass and productivity, which can eliminate other microalgal growth-inhibiting bacteria for microalgal culture (Cho et al. 2015).

In the process of active immobilized culture of microalgae, bacteria can be embedded in sodium alginate beads together with microalgae, or bacteria (or activated sludge) can be placed in a medium that only embeds microalgae beads (Mujtaba and Lee 2017). The effect of these two immobilization methods on culturing microalgae to obtain biomass is not very clear, but the mechanism of action of bacteria is obvious. This is quorum sensing between bacteria and microalgae (Zhou et al. 2016), and this sensing is mainly expressed as signaling molecules. Similar to indole acetic acid (IAA), they can stimulate or inhibit the growth of microalgae and bacteria. For example, indole acetic acid (IAA) produced by bacteria can significantly increase the yield of microalgae (Chang et al. 2022). The main reason why the latter can achieve this purpose is that the sodium alginate gel spheres are porous materials that allow small molecules to enter and can be utilized by microalgae embedded in the gel spheres (Mujtaba et al. 2018). Horizontal gene transfer (HGT) occurs in each symbiont and between symbionts and organisms of other species, respectively. In the bacterial-algal association system, HGT occurs between microalgae and bacteria in order to adapt to their environment (Dorrell et al. 2023). Some scholars began to study the horizontal gene transfer of target genes under specific factors (Li et al. 2023). This has positive significance for microalgae modification at the gene level. The bacterial-algal interactions in the bacterial-algal system are shown in Fig. 4. Shen et al. (2017) found that the addition of *Pseudomonas putida* to co-immobilize microalgae in gelatinous spheres significantly increased the cell density of *Chlorella*. At the same time, higher ammonium, phosphate and COD removal rates were also found. While most bacterial-algal biofilm systems show favorable results, not all bacteria favor the growth of microalgae. There are many factors that determine the interaction between microalgae and bacteria, including microalgae and bacterial strains (since interactions are species-specific) and microalgal growth stage. For example, some members of the families *Prasinophyceae* and *Bacillariophyceae* can secrete antimicrobial substances to inhibit the growth of co-cultured bacterial species. Many antibacterial metabolites have been characterized, including different types of fatty acids (e.g. eicosapentaenoic acid), glycosides, chlorophenes, terpenoids and chlorophyll alpha derivatives (Hom et al. 2015). Growth stage is another important factor affecting the interaction between microalgae and bacteria. Microalgae-bacteria interactions are not static, but often transit from symbiotic to parasitic according to developmental cues (growth stages) (Guo and Tong 2014). Therefore, it is necessary to explore bacterial strains that are helpful for the growth of microalgae based on the species-specific combination of microalgae and bacteria. Examples of microalgal-bacteria interactions that have a positive effect on microalgal growth or accumulation of valuable compounds are shown in Table 3. Although the interactions via chemical signals between bacteria and microalgae are apparent as described above, how algae and bacteria secrete different signaling molecules and their importance in cell-to-cell interactions remains unknown.

Life cycle assessment and economic evaluation

Life cycle assessment (LCA) is a means of evaluating the total environmental impact of a product or a class of facilities from cradle to grave. It is used to calculate the impacts and effects of a product, process or activity throughout its life cycle, from extraction to utilization and reuse to environmental sinks. Global Warming Potential (GWP) is widely reported in LCA studies on algae processes. GWP estimates the potential greenhouse gases emitted by the system. In the literature on suspended microalgae wastewater treatment systems, the GWP is basically in the range of 1100–2160 g CO_2 Eq./m^3 (Arashiro et al. 2022; Gowd et al.

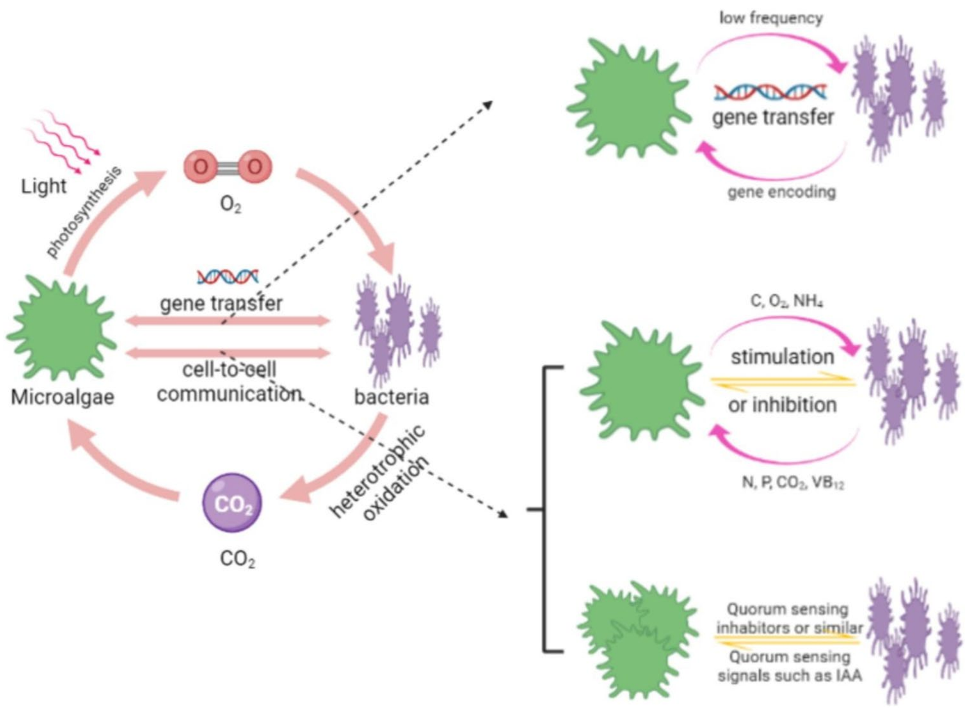

Fig. 4 Bacterial-algal interactions in a bacterial-algal system

Table 3 Immobilized microalgae-bacteria system promotes microalgal growth or accumulation of valuable compounds

Microalgae species	Bacteria species	Immobilization	Growth and nutrition of algal cells	References
Scenedesmus sp. 336	*Proteobacteria, Bacteroides, Firmicutes* in activated sludge	Bacterial algal biofilm	Total lipid: light: 26.56% higher than that of algae alone, dark: 5.31% higher than that of algae alone	Chen et al. (2019)
Chlorella vulgaris	*Proteobacteria, Bacteroidetes, Brevundimonas, Acinetobacter* in anaerobic fermentation broth	Calcium alginate immobilized *Chlorella vulgaris* + bacteria + PAC (powdered activated carbon)	Lipid: 372.4 ± 2.15 mg/g,,higher than that of algae alone, Protein: 324.7 ± 1.03 mg/g, higher than that of algae alone	Xie et al. (2018)
Chlorella vulgaris FACHB-30	*Pseudomonas putida*	Calcium alginate	Algal cell concentration: 6.654×10^6 cellsd·mL^{-1}, higher than that of other groups	Shen et al. (2017)
Chlorella vulgaris AG30007	activated sludge	alginate	Algal cell concentration: 0.58 g L $^{-1}$, higher than that of algae alone	Mujtaba and Lee (2017)
Cyanobacteria	*Alphaproteobacteria, Gammaproteobacteria*	Bacterial algal biofilm	Algal biomass: 8 times higher than algae cultured alone	Abed (2010)

2023). Passively immobilized microalgae culture systems are believed to significantly reduce the water and energy requirements of the culture process (Morales et al. 2020). Abinandan et al. (2020) demonstrated through a life cycle assessment that active immobilized microalgae can reduce fossil energy consumption by up to 50% when treating acidic mine wastewater. Table 4 illustrates the treatment of wastewater by immobilized algae through life cycle assessment and cost analysis.

The annual income from microalgae production in an open pond system (suspension system) in Portugal was €619,100. However, after removing the fixed capital

Table 4 Life Cycle Assessment and Economic Analysis of Suspended and Stationary Algae in Wastewater Treatment Systems

Microalgae species	Wastewater type	Cultivation method	Immobilization carrier	Life Cycle Assessment	Economic Analysis	References
Arthrospira platensis	municipal wastewater	suspension	–	GWP: 1154 kg CO_2 Eq./m^3	–	Gowd et al. (2023)
mixed *cyanobacteria*	municipal wastewater	suspension	–	GWP: 1155 kg CO_2 Eq./m^3	–	Arashiro et al. (2022)
Unknown microalgae	From open-lagoon wastewater treatment plant	passive immobilization	Cotton fiber	GWP: 30.4 gCO_2 Eq./MJ (67% reduction compared to petroleum diesel)	–	Barlow et al. (2016)
Tetraselmis Suecica	Artificial wastewater	passive immobilization	polypropylene	Water consumption: 30% reduction; Environmental impact: 20% reduction Compared to suspended systems	–	Morales et al. (2020)
Desmodesmus sp. MAS *and Heterochlorella* sp. MAS3	Acid mine drainage	Active immobilization	Alginate beads	GWP: 51.53 kgCO_2 Eq./m^3 reduction from transport; 3.397 kgCO_2 Eq./m^3 reduction from coal-fired power generation	50% reduction in fossil fuel consumption	Abinandan et al. (2020)
Desmodesmus sp. MAS1 *and Heterochlorella* sp. MAS3	Acid mine drainage	Active immobilization	Alginate beads	80% reduction in CO_2 emissions	Renewable energy reduction rate reduced by 9%	Kuppan et al. (2022)

GWP Global Warming Potential, *HRAP* High-rate ponds, *RABR* rotating algal biofilm reactor

investment, annual operating costs, the NPV is about -1.3 million Euros, making the project economically unviable. However, given the cost of treating wastewater, it may be economically viable (Nobre et al. 2024). A study has demonstrated that the break-even selling price of algal biomass in a wastewater treatment system is $0.549/kg to cover operating costs. Under optimal conditions, the cost of producing 1 L of biocrude is $0.96 (Fathima and Chatterjee 2022). One study creatively combines biofilms with suspended algae systems to treat wastewater (Rodrigues de Assis et al. 2020). This system has not only achieved a 2.6-fold increase in production and a fivefold increase in harvest efficiency, but also improved wastewater treatment. This allows the system to increase revenue while reducing operating costs (mixing and harvesting). Hybrid systems are expected to be a promising technology for large-scale microalgae cultivation. However, specific economic analyses and life cycle evaluations are lacking. The carrier material cost and fabrication cost increase the total cost of wastewater treatment due to the active immobilization system. The annual cost of the beads would be 85% of the total operating cost, limiting the economic attractiveness of the technology. However, it has been found that alginate from immobilized algal reactors can be reused. Adding a small amount of new sodium alginate to supplement the carrier can reduce the net operating cost by 60%, which is economically beneficial (Murujew et al. 2021). More research should focus on improving alginate recovery. Since operating costs can be reduced by 80% if the recovery rate can be increased to 90%. This contributes to the cost-effectiveness of active immobilization applications for large-scale wastewater treatment.

Conclusion and future perspective

In this review, two main immobilization mechanisms of immobilized microalgae culture, the main factors affecting the growth of immobilized microalgae and the efficiency of removing pollutants from wastewater were analyzed. The role of bacteria in immobilized culture of microalgae was discussed. Relevant LCA and economic analyses are also summarized. Compared with suspended microalgae, immobilized microalgae has advantages in terms of harvest economy and resistance to external environment. Therefore, this microalgae culture method, combined with wastewater treatment, is considered a renewable and sustainable technology.

Although there are many advantages in immobilized microalgae, there are also great challenges. In terms of immobilization culture mechanism, the active immobilization mechanism is less studied than the passive immobilization mechanism. Alginate has been widely used as an embedding material, but the cross-linking characteristics of other embedding materials (agar, carrageenan, chitosan, carrageenan, polyvinyl alcohol, polyacrylamide, polyurethane and epoxy resin) are still unclear. Regarding the factors affecting the growth of microalgae, the exploration of immobilized microalgae is still not enough, and more comprehensive exploration is needed to further optimize the growth conditions of microalgae, in order to obtain high-quality biomass energy more cost-effectively. Recently, co-cultures of bacteria and microalgae have received special attention, but immobilization-based co-cultures are not yet common and their mechanisms have not been investigated. The cultivation method coupled with wastewater treatment does not take into account the downstream processing of microalgal biomass. Applicability and feasibility in different types of wastewater sources have been explored, but most of them are still at laboratory scale, and the performance may be quite different after scale-up to pilot scale or larger. In addition, the life cycle evaluation and economic analysis of immobilized systems in wastewater treatment systems are still relatively few. More LCA and economic analysis will help optimize the immobilized system.

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Declarations

Competing interests The authors declare no competing interests.

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