Immobilized microalgal system: An achievable idea for upgrading current microalgal wastewater treatment

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1. Introduction

Negative impacts of aqueous pollution have acted as prominent and urgent livelihood issues in many countries [1,2]. According to the United Nations World Water Development Report [3], over 2212 km³ of wastewater is generated per annum worldwide, corresponding to 56% of the global water withdrawals (FAO’s AQUASTAT). Many risks, including human health threats, ecosystem disruption, and oxygen depletion in the aquatic environment, are associated with the direct discharge of untreated wastewater into the environment [4,5]. Moreover, extra chemicals and energy consumption, as well as greenhouse gases emissions from the conventional wastewater treatment (including anaerobic and aerobic processes), have plagued scientists for decades. Especially in China, with the implementation of higher effluent standards Class 1-grade A of wastewater treatment in recent years, the consequently delayed aeration processes and additional operating units increased the operating costs and chemical consumption significantly [6,7]. Biological treatment processes dominated by various forms of activated sludge process mainly consist of oxidation ditch and anaerobic/anoxic/oxic (AAO), and sewage sludge (SS) production is inevitable [8]. Furthermore, SS is being produced at an alarming rate (over 65 million tons year⁻¹ by the end of 2020) with the development of leading wastewater treatment capacity and remarkable technological innovation capacity [6]. As reported, in the past decade, over 30 million tons of SS are produced annually, with about 70% not managed properly [9]. These sludges, with dual attributes of “pollution” and “resource”, can return to the environment and become another source of pollution if not handled safely [6,10]. And greenhouse gas emissions from wastewater treatment have also been highlighted in recent years [10]. The situation of wastewater treatment currently is still worrying, and there is still a long way to go in the future [11–13]. Developing
sustainable wastewater treatment becomes urgent.

Researchers have never stopped exploring attempts at wastewater treatment techniques [14–16]. “Photosynthetic wastewater treatment” by using the predominant contributor, microalgae, which carries out about 40% of photosynthesis on Earth, has been recently regarded as a new green way to vigorously promote nutrient recovery and industrialize wastewater reclamation simultaneously [17,18]. Cultivating microalgae in wastewater is recognized as an energy-saving and low-technical required technology since it combines nutrients removal from wastewater with the inherent advantages of microalgae, including high photosynthetic efficiency, rapid growth rate, and notable adaptability and ability to simultaneously produce various value-added compounds, such as pigments, amino acid, polysaccharide, biofuel, bio-fertilizer, etc. [19–21]. However, the metabolic activity of the frequently used suspended microalgae is often largely influenced by adverse environmental conditions and the presence of various hazardous compounds. Many elements related to the nutrient supply and growth conditions have been further considered and investigated [22–26]. For instance, regarding light is essential for obligate photosynthetic organisms like microalgae. There are two processes in photosynthesis, i.e., light-dependent reactions and light-independent reactions. Generally, in the presence of light, microalgae capture and transfer light energy through light-harvesting antennae, photosynthetic system II, and other major photosynthetic complexes and convert light energy into chemical energy ATP and biochemical reductant NADPH [27]. These formed products are essential for the photo-fixation of inorganic carbon CO₂ [28]. Further, light has the most obvious effect on carbon and carbon-related metabolisms of microalgae since microalgae predominantly utilize CO₂ as their primary carbon source in the photoautotrophic mode and use the organic carbon source, such as glucose, during the Embden-Meyerhof Pathway; while under darkness, the available carbon sources for microalgae to uptake is the organic carbon via the Pentose Phosphate Pathway [26]. This also means that light is the key factor in switching photoautotrophic and heterotrophic metabolism in microalgae. Normally, the photosynthetic activity of microalgae increases with light intensity under nutrient-abundant conditions. However, photo-inhibition and photoprotective pigments increase when light intensities exceed the saturation point [29]. It is well known that the metabolic networks of living organisms are intricate. Carbon metabolisms are also closely related to the metabolism of elements such as N and P. Researchers have found that the maximum and minimum uptake of NO₃ or NO₂ assimilation occur under irradiation and dark, respectively [30]. Glutamine synthetase (GS) enzyme, which catalyzes NH₄ incorporation into the carbon skeleton of glutamate, loses its activity under dark conditions and can be reactivated upon reillumination [26]. Additionally, emerging micro-pollutants, such as pharmaceuticals and personal care products (PPCPs) [24], nanoparticles [22,25], and antibiotics [23], also aroused much attention. Briefly, the complex and unknown environments bring many challenges to microalgae growth and the removal performance of target contaminants. Furthermore, microalgal cells are usually tiny and have low-density distribution within wastewater, making it difficult and energy-intensive to be harvested and separated from the treated units during familiar suspended cultivation [31,32]. According to a rough estimate, 20–30% of microalgal biomass production cost is spent on biomass recovery [33]. For instance, the most commonly employed harvesting routes, such as centrifugation, filtration, and flotation, account for 90% total cost in the open ponds system. Besides, several harvesting methods, such as flocculation, pose potential risks to downstream product production. It was regarded that the potential health risks of adding chemical flocculant can introduce into the production such as biodiesel [31]. Yin et al. [31] discussed the different available harvesting methods for microalgae. Unfortunately, among all the objects under discussion, no current approach has been demonstrated to be suitable for large-scale microalgal production and harvesting simply at low cost. Hence, creating a relatively mild living micro-environment for microalgal cells and selecting a suitable harvesting process is essential to safeguard pollutant removal efficiency and recover biomass resources. Bio-technology based on immobilized microalgae undoubtedly can provide new ideas for solutions to these issues.

“Immobilized microalgae” conception derived from the mostly accepted concept of “immobilization” [34–36]. It can be standardized simply as “a biotechnology that uses natural or artificial, physical and chemical means to prevent the living free microalgal cells from moving independently in its original location, and the microalgal cells located in a limited space can retain some desired biological activity and be reusable in an aqueous phase system”. The high-population density properties of microalgal immobilization allow integration of the high-efficient treatment and the subsequent separation, considerably lowering carbon footprint and accelerating treatment efficiency without other extra energy-intensive recycling operations. Also, immobilized microalgal cultivation exhibits a similar potential for nutrient recovery with suspended one and better economic value in the chemical and agricultural industries. Immobilized cultivation is currently considered a promising approach for remediating the aqueous environment and upgrading sustainable biological wastewater treatment [37–39].

The earliest wastewater treatment process using immobilized microalgae can be traced back to the 1900s (Fig. 1a) [38,40]. Since then, immobilized microalgae techniques have been recognized and diversely used in macronutrient removal and biomass growth. Especially in the recent ten years, wastewater treatment concept has shifted from pollutant removal to collaborative pollutant removal and sewage resource recovery, greatly stimulating the research attention on microalgal and immobilized microalgal wastewater treatment (Fig. 1b). However, apparently, the development of microalgal immobilization is relatively delayed. The gap between microalgal immobilization and wastewater treatment is required to be filled. The existing literature has not been systematized for developing immobilized microalgae for wastewater treatment and investigated the bottlenecks of microalgal immobilization moving towards practical applications. There is only some fragmented information available now about the immobilization technology and attempts on pollutants removal. As far as we know, up to now, only very limited reviews have mentioned the applicability of immobilized microalgae for treating wastewater (Fig. 1c) [37,39,41]. It is ambiguous or conclusive whether immobilized microalgae own potential for wastewater treatment. The main mechanisms employed for microalgal immobilization technologies and the pollutants removal of immobilized microalgae are not comprehensive, which may hinder the applicability of introducing the immobilized microalgal system for further upgrading the microalgal wastewater treatment process. In particular, a comparatively systematic, detailed, and updated review on the development of microalgal immobilization toward wastewater treatment over the last ten years is currently lacking.

Based on general literature analysis, this review aims to discuss the feasibility of employing immobilized microalgae as a promising microalgal wastewater treatment technology. Firstly, the benefits and drawbacks of immobilized microalgae were systematically evaluated. Meanwhile, combined with evaluating results with better pollutant removal efficiency and more positive effects on immobilized microalgal cells, developing immobilized microalgal technology has been proven to implement wastewater treatment
and microalgal biomass recovery simultaneously. After, the main technical approaches used for microalgal immobilization were analyzed in-depth. Additionally, mechanisms currently related to the immobilized microalgal system for pollutant removal were reviewed and discussed. Furthermore, the emerging approaches for large-scale fabrication of immobilized microalgal systems were introduced in detail subsequently, which may present a huge potential for accelerating immobilized microalgal systems for engineering applications. Several research recommendations are proposed for the current knowledge gaps. Together, we believe this new idea for thinking in the next generation of microalgal wastewater treatment can be empathized and cause immediate interest among peer researchers.

2. Why use immobilized microalgal wastewater treatment systems?

The immobilization of microorganisms is inspired by the extraordinary characteristics and ability of most natural microorganisms. They present self-aggregation capabilities and can adhere to different kinds of biotic or abiotic surfaces, or within porous structures, by self-produced extracellular compounds [42]. Artificial microalgal immobilization can reproduce the ecological advantages of biofilms (i.e., shelter, homeostasis, metabolic cooperation, nutrient availability, and specific physiologic status) and remove nutrients (e.g., ammonium) from wastewater. More importantly, without immobilization, the practical limitation of harvesting microalgal cells from treated wastewater is problematic, largely hindering the applicability of microalgae-based wastewater treatment [43,44].

2.1. Benefits and drawbacks of immobilized microalgae

Microalgal cells under immobilized and suspended cultivation exhibit varied physiological and physicochemical properties owing to the different growth environments. Similarly, the existence format of the scattered and aggregated microalgae corresponding to suspended and immobilized cultivation also shows diverse differences in both resource utilization and pollutant removal [36].

Microalgal cells encapsulated in gel beads or affixed to the non-suspended carriers’ surface exist as an aggregation with a relatively higher partial cell density than those in a free-suspended system. Meanwhile, higher cell growth potential with satisfactory pollutants removal can be obtained in an immobilized microalgae system due to the well-organized structure and improved light utilization [45,46]. For example, the marine cyanobacterium Synechococcus elongatus and the microalgae Scenedesmus sp. immobilized in chitosan capsules and Loofa matrix exhibited better C, N, and P removal efficiencies than those in a suspended system [47,48]. The collective collaborative effects of the immobilized system can help microalgal cells to tolerate and adapt to environmental stresses or toxicity obviously [49,50]. Immobilization has an obvious effect on reducing the toxicity of inorganic mental oxide nano-adsorbents and carriers in the treatment of N and P wastewater, promoting
resistance to cell growth disruption, and helping to avoid the photo-inhibition and reduce cellular toxicity [51,52]. Additionally, bigger immobilized microalgal beads or immobilized carriers can make traditional harvesting and dewatering processes much easier and more energy-saving. Moreover, from the perspective of practical wastewater treatment, immobilized microalgal cultivation could reduce the interference of introducing alien microorganisms to the original ecosystem because the beads may inhibit the liberation of the immobilized microorganisms into the wastewater [50].

However, microalgal immobilization also has brought some drawbacks. Polymers or carriers in immobilization systems may inhibit mass transfer and resource absorption [36]. Besides, the effects of immobilized carriers and reagents for immobilization on the downstream processing (e.g., bioenergy production, acquisition, and processing) are still to be specific. The stability and repeatability of immobilized microalgae must be further verified in actual wastewater treatment. Furthermore, the additional operating procedure on microalgal immobilization may result in higher operation costs and a higher requirement for operation control staff compared with a suspended system. More importantly, potential secondary environmental pollution and risks caused by the immobilization materials and the microalgal leakage that could occur with a prolonged operation period might also hinder the applicability of the microalgal immobilization system.

2.2. Pollutants removal by immobilized microalgae

Recently, it has been recognized that wastewater could act as a vital nutrient provider for microalgae, witnessing the potential of using microalgae against wastewater treatment [23,53,54]. Owing to unique biological characteristics, microalgae often could grow well in various wastewater with high nutrient utilization and efficiently convert these nutrients into various value-added biomolecules [35–57]. Moreover, immobilized microalgae may provide an idea for further upgrading microalgae-based biological wastewater treatment mainly due to their easy-harvest and higher environmental resistance. Zhuang et al. [41] comprehensively reported and reviewed, and summarized the progress of non-suspended microalgae-based wastewater biorefinery by analyzing 120 groups of pollutant removal rates with the distribution patterns from more than 60 reports (Fig. 2). It conveyed that the pollutants removal rate of COD, TN, TP, NH₄⁻N, and NO₃⁻N by non-suspended microalgae varied greatly from 0% to 100%, with the medians rate ranged from 78.2 to 93.2%. Centralized distribution of higher removal rate of COD, TP, and NH₄⁻N suggested that the removal of COD, TP, and NH₄⁻N was easier in most cases. Instead of regular nutrient removal, some microalgae also seem to be capable of dealing with other pollutants like PPCPs [58], plastics [59], heavy metals [60,61], dyes [62], antibiotics [63,64], and pharmaceuticals [65–67].

2.3. Effects of immobilization on the physiological activity of microalgae

Compared with the suspended system, immobilized microalgae may exhibit varied performance due to the selected immobilized materials. For instance, some synthetic foams and resins used for microalgal immobilization have been demonstrated as highly toxic due to the possible leftover pre-polymers [38]. In addition to the immobilized material toxicity, immobilization operation processes, such as immobilization or encapsulation of microalgae in polymers, also exert significant impacts on the microorganisms because of chemical interactions and limitations between the immobilization matrix and cells [37]. Possible cellular toxicity may also occur due to metabolite accumulation within the matrix. Mass transfer limitations caused by the immobilization matrix are considered a key factor of physiological changes for the immobilized microalgae, which can usually be observed by a concentrated cell distribution at the immobilized matrix surface [36]. Matrices thickness, inner metabolic by-product accumulation, light, and resistance of mass transfer of CO₂ are the possible key causes.

Encouragingly, increasingly natural non-toxic polymers are being used in immobilization. Research has revealed that immobilization can create a beneficial physical barrier for microorganisms against diverse and complex wastewater conditions [49,50,68]. Hence, it is essential to understand the effect of immobilization on the physiological activity of microalgae to further develop microalgal immobilization toward wastewater treatment. Also, it responds to the feasibility of why the immobilized microalgal system can be considered an idea for microalgal wastewater treatment.

2.3.1. Enhancement of cell growth and morphology

Immobilization or encapsulation of microalgae in polymers or carriers is beneficial to cells in most cases. Cell growth and morphology can act as two basic indicators to examine the impact of an immobilization system on microorganisms. Homburg et al. [69] scrutinized how hydrogel structure, bead size, and biomass loading affected the behavior of microalgae entrapped in a lens-shaped silica hydrogel and found that the entrapped cells exhibited a suitable bio-compatibility with considerable growth. Benstein et al. [70] designed a systematic process using the immobilized dinoFLAGATE Symbiodinium vormutum grown on a two-layer packed bed column reactor for isolating carotenoid peridinin. This process exhibited a significantly higher growth rate and maximal biomass yield than the suspension culture, demonstrating its potential applicability.

Based on the obvious effects of immobilization on microalgal growth, enhancing the immobilization efficiency and developing more related technologies also attracted much attention. For example, co-immobilization of microalgae with other microorganisms has also been proven a functional strategy for increasing the microalgal population [71–73]. Gonzalez et al. [73] co-immobilized microalga C. vulgaris with a plant-growth-promoting bacteria, Azospirillum brasilense, in alginate beads. The results showed that co-immobilization enhanced microalgal proliferation, pigment production, and microalgal biomass. It was speculated that phytohormones like IAA (indole-3-acetic acid) produced by A. brasilense might play a vital role in stimulating microalgal growth. Homburg et al. [74] entrapped the microalga C. reinhardtii in a low-sodium and low-propylamine silica hydrogel to improve viability and growth. Similar to cells entrapped in calcium alginate, the entrapped cells in silica hydrogel maintained the PSII quantum yield above 0.3 with a specific growth rate of 0.23 ± 0.01 d⁻¹.
Moreover, considering the high effectiveness of sodium tripolyphosphate (TPP) for chelating calcium ions, researchers successfully deposited a TPP layer over the calcium alginate beads to form a strong interaction between the TPP ions and calcium that could help improve their cell holding capacity and microalgal cell growth ability [75]. Interestingly, Krujatz et al. [76] put forward a directional-designed immobilization process called “Green Bio-printing (a technology that fabricates photosynthetic algae-laden hydrogel scaffolds by using 3D plotting)” for entrapping microalgae, which improved microalgal viability and maintained a growth rate of 0.4–0.7 d⁻¹. This clearly liberates researchers from immobilized microalgal operations relying on the repetitive tedious manual steps, largely enhancing the applicability of using immobilized microalgae for wastewater treatment.

2.3.2. Positive responses of metabolism, physiology, and productivity

Liu et al. [77] reported that immobilization could alter the cell growth behavior, carbon utilization, and nitrogen uptake of Chlorococcum vulgaris under mixotrophic conditions; however, the synthesis of amino acids associated with ammonia assimilation can remain stable. Calderon et al. [78] found that the microalga Botryococcus braunii can be immobilized using a non-toxic and recyclable material made of polyester wadding, subsequently achieving a higher biomass yield (1.05 ± 0.05 g L⁻¹) with similar cellular components when compared with a suspended culture (0.734 ± 0.003 g L⁻¹). More interestingly, immobilization was reported to improve the microalgal lipid yield and fatty acid composition as compared to free cells, which may apply to the biofuel industry [79]. Abu Sepian et al. [79] discovered that the microalgal lipid content increased to 51.6% and exhibited a satisfactory fatty acid methyl ester (FAME) profile by combining a matrix system of sodium alginate and sodium carboxymethylcellulose in the immobilized method. Huang et al. [80] co-immobilized microalgae and bacteria to illustrate that both the anabolic activity of microalgae and the dye decolorization capacity were significantly promoted.

2.3.3. Protection of cells against environmental stresses or toxicity

Immobilized cells could present systematic growth and biocompatible activity in the carrier even under environmental stresses or toxicity [81]. For example, the immobilized cells Chlorella pyrenoidosa reinhardtii maintained a higher rate of nitrate uptake and viability in a wider range of pH (5.5–8.0) and temperature (25–38 °C) compared with suspended cells [82]. Wang et al. [83] compared the growth and antioxidant responses of free and immobilized microalgae, Selenastrum capricornutum, under combined toxicity of polycyclic aromatic hydrocarbons and heavy metals. They found that immobilized systems can protect microalgal cells against the toxic contaminants and change the synergistic effect of co-contaminants on cells to antagonist effects. Huang et al. [80] used immobilized Chlorella with calcium alginate to disclose that the immobilization positively affected both microalgal growth and physiological activity. However, owing to heterogeneous cell distribution inside the immobilization carrier, the relatively low mass transfer of CO₂ or nutrients may limit the growth and cell division of the immobilized microalgae. Luckily, many researchers have reported that microalgae can overcome the imposed damage or space limitation caused by toxicity, high cell density, or uneven cell distribution after a long operation [73,83,84]. Besides, stock culture management of functional microalgae species is a vital part of microalgal wastewater treatment, which is also another embodiment of the application of microalgal immobilization technology. For example, algal spores encapsulated with alginate have been proven to maintain normal physiological activities and faster growth than non-encapsulated spores after 1.5 months of culture [85]. It also creates favorable conditions for self-protection and subsequent recovery of functional microalgae species under external adverse pressure such as climate change, radiation, and corrosion [85,86]. Notably, Syiem and Bhattacharjee [87] demonstrated that various biological functions of microalgal immobilized within calcium alginate could be well maintained for a long period (three years) in dehydrated and dark conditions and showed that microalgal cells in immobilized carriers could be regenerated after a certain amount of time, with cellular functions, such as photosynthesis, respiration, and enzyme activity, being well preserved. A similar study evaluated the viability of Synechococcus elongatus immobilized with alginate after cold storage; in surprise, cells continued growing even faster and producing new cells, even after 3.4 years of cold storage [49].

3. Main technical approaches employed for microalgal immobilization

Currently, the technical theories used for microalgal immobilization can be mainly divided into the following two types (Fig. 3): adsorption or attachment onto a matrix and entrapment within a porous matrix.

3.1. Adsorption or attachment onto a matrix

Many microorganisms can naturally attach and then proliferate on a matrix surface [42,88,89]. This unique biological phenomenon has inspired us to deeply explore the possibility of using immobilization techniques to fix microalgal cells on different types of carriers [90–92]. These matrix-fixed microorganisms like microalgae (Fig. 3a), may promote cellular activity and volumetric biosorption ability, which may present considerable potential for ecological applications, such as intensifying wastewater treatment and carbon capture. To achieve this goal, selecting an appropriate matrix is of great importance. Finding a matrix with satisfactory strength, higher porosity, and better environmental resistance to withstand the possible natural shear stress and effectively transport nutrients to cells is necessary. In the case of microalgal immobilization, several aspects should be considered when selecting an ideal matrix. Specifically, the matrix should: (1) retain the microalgal viability, biocatalytic activity, and operational stability over a prolonged period; (2) ensure the smooth transportation of gases and nutrients; (3) maintain the matrix structure with the lowest possible microalgal cell leakage; (4) have a large space and suitable surface functional groups for cell attachment and proliferation; (5) exhibit low microalgal toxicity and high light transmittance; (6) maintain high stability under varied circumstances, and environmental shear stress; (7) be easy-to-harvest and have high recyclability; and (8) be cost-effective and highly applicable in practice.

Currently, synthetic and natural carriers are the two main matrix categories used for microalgal immobilization (Fig. 4). The attachment mechanism of the microalgal cells to these two matrix surfaces may occur through natural gravity, hydraulic collision, electrostatic adsorption or chemical binding [89]. Several synthetic carriers with hydrophilic and refractory characteristics, such as porous glass, ceramics, polyurethane foam, polyvinylidene fluoride, polycrylonitrile, and polysulfone, have been successfully used for microbial immobilization [93]. However, obstacles, including potential microalgal toxicity, relatively high cost, limited light penetration, and biofilm failure under shear stress, may affect the treatment efficiency and further hinder the applicability [37]. To overcome these bottlenecks, the directional design of new materials using scaffolds associated with green adhesive coating techniques is required. For instance, Bernal et al. [94] devised a
technique for assembling cells using an external electric field to fabricate a biocomposite coating of cyanobacteria on flexible polyester sheets (PEs), which resulted in a more efficient and compact cell packing on the surface. By sandwiching cells between light-transmitting polyelectrolytes and attaching them onto porous substrates that allowed transportation of nutrients and gas, this flexible multilayered cell-based photo-absorbing biomaterial could serve as a complete simulated “bionic leaf” for converting carbon dioxide into fuels or chemicals using solar energy. Moreover, Pannier et al. [95] successfully deposited the microalgae-containing sodium alginate thin layers onto glass carriers. Subsequently, they gelled them using amino-functionalized silica sol to obtain a reinforced alginate hydrogel, which may be much better at resisting in the high salt environment of mariculture wastewater treatment.

Natural biomass-based scaffold materials present more options as well. The biomass-derived natural carriers, referred to as a bio-matrix, usually comprise loofah [90,91,94,95,100], corn cob [61], pine bark [91,98], sugarcane bagasse [99], or cotton cloth pieces [100]. The open network of fibrous support in these natural materials allows them to quickly contact microalgal cells and then establish a

Fig. 3. Main technical approaches employed for microalgal immobilization: a, adsorption or attachment onto a matrix; b, entrapment within a porous matrix.

Fig. 4. Synthetic and natural matrix for microalgal immobilization. Adapted from Refs. [61,90,91,94,95,100] with permission, Copyright, 2013, Elsevier; 2020, Springer Nature; 2017, American Chemical Society; 2014, The Royal Society of Chemistry; 2020 and 2016, Elsevier.
robust immobilization system [90]. The high void volume, permeability, and low cost of these fibrous matrices make them particularly attractive [90]. Among them, the loofa sponge is one of the most popular candidates for microalgal immobilization owing to its high binding capacity with cells, low manipulating cost, and satisfactory stability during long-term operation [101]. Saeed and Iqbal [90] showed that immobilized cells in a loofa sponge displayed a better growth performance than suspended cells. Moreover, the loofa-immobilized cell systems can potentially treat various pollutants such as heavy metals, dyes, inorganic/organic matter, and chlorinated compounds [90]. More importantly, after treating wastewater, the immobilized biomass within the matrix could be fully converted into value-added products, including alcohols, organic acids, enzymes, and secondary metabolites [90].

In short, the microagal immobilization system based on adsorption or attachment is intuitively attractive. However, the relatively low bioburden of the attached carriers and the potential risks of biofilm failure associated with the natural biological life-cycle could cause insufficient cell loading on the matrix, which may result in low environmental adaptability, further causing a decreased pollutants treatment efficiency [42,89]. Additionally, not all microalgae are amenable to biofilm formation [88]. Based on preliminary techno-economic analysis, fibrous skeletons like loofah-based biocarriers were demonstrated as a more suitable and available approach for a large-scale system.

3.2. Entrapment within a porous matrix

Entrapping microalgal cells within a porous matrix is another universal immobilization technique usually achieved by permitting cell diffusion into a porous matrix or by cross-linking cells in situ using a porous matrix [37,102]. Usually, to offer the surroundings for immobilized biomass within the matrix, which could be fully converted into value-added products, including alcohols, organic acids, enzymes, and secondary metabolites [90].

Owing to the natural structure of alginate, the gelation mechanism between monovalent alginate salts (e.g., Na-alginate) and polymeric materials (such as gelatin, collagen, and polyvinyl alcohol). Among them, alginate is the most commonly employed material due to its low cost, minimal toxicity, high transparency, and satisfactory stability. Alginate constitutes a family of unbranched binary copolymers of 1→4-linked-b-D-mannuronic acid and α-L-guluronic acid in different proportions and sequences [38]. Here, the immobilization method of entrapped microalgae with alginate is discussed emphatically. For other matrices, the process for cell immobilization is similar.

Owing to the natural structure of alginate, the gelation mechanism between monovalent alginate salts (e.g., Na-alginate) and microalgal cells occurs within a few minutes in a solution containing gel-forming ions (e.g., Ca²⁺) (Fig. 3b). Specifically, microalgal cells are first added to an aqueous gelling solution with appropriate mixing. Subsequently, this cell-containing gel solution is extruded as droplets via a nozzle or orifice into a cation-containing solution for gel formation via polymerization or another cross-linking mechanism. Then, immobilized beads with entrapped microalgal cells are obtained after a short period of stabilization. However, in order to further achieve higher resistance toward real wastewater, a detailed optimization of the obtained alginate-immobilized microalgal system is still highly necessary and should include: optimizing the addition of microagal beads against various wastewater treatment volumes and types [105]; selecting high-stress tolerant species together with immobilized beads [106]; choosing the suitable alginate materials [107]; directional fabrication of the bead size [108]; and enhancing environmental tolerance to factors such as pH, alkalinity and salinity through structural modification [109].

4. Mechanisms of pollutants removal by immobilized microalgae

To promote the applicability of immobilized microalgae for wastewater treatment, understanding the corresponding mechanisms behind pollutant removal is important. Currently, research is mainly carried out from two perspectives: primary elimination pathways and specific degradation processes. As for exploring the elimination pathways, biosorption may also be crucial during biological pollutants removal [110,111]. Extracellular polymeric substances (EPS) released from microalgae are widely recognized as the main contributor to the spontaneous aggregation of microalgal cells in aquatic environments [41]. A complex interaction between the micro-environment of a non-suspended system (concentration gradient and light intensity) and the microalgal physiology (trophic type, division, cellular components, and EPS release) would be dynamically changed, which may promote cell growth along with increased EPS release. Authigenic and exotic polymers may act as a buffer, establishing a concentration gradient between the outside environment and the immobilized microalgal cells, evidencing contaminants were absorbed and held in this polymeric matrix and gradually transported into microalgae [41]. Upon closer exploration of the critical role of microalgae, researchers found that enzymes are the main triggers of pollutant biodegradation. For instance, after analyzing the enzymatic activities involved in nitrogen metabolism, de-Bashan et al. [112] found that the ammonium absorption capacity of microalgae was positively correlated to the cellular activities of both glutamate dehydrogenase (GDH) and glutamine synthetase (GS). Subsequently, Meza et al. [113] observed the relationship between intracellular ammonium accumulation and the activities of GS and GDH using the alginate-immobilized microalga C. vulgaris with either of two wild-type strains or their corresponding indole-3-acetic acid (IAA)-attenuated strains. Intriguingly, it found that IAA produced from the strains enhanced the GS and GDH activities and participated in intracellular ammonium uptake and assimilation of microalgae.

From the perspective of substance transformation, interpreting the microalgal degradation by-products of the target pollutants is another approach to exploring specific degradation processes of pollutant removal. Rasoul-Amini et al. [114] reported the biotransformation of monoterpenes by characterizing and identifying biotransformation by-products through GC/MS after incubation with substrates, confirming that metabolic activities varied with the addition of the substrate. Using immobilized microalgae, Wang et al. [66] investigated the removal and degradation of the frequently-detected endocrine disrupter 17b-estradiol (E2). It was theorized that the E2 removal pathway in the immobilized cells, inferred through HPLC and LC-HRMS, involved hydroxylation, o-methylation, glycosylation, dehydrogenation, and decarboxylation. In addition, Xie et al. [65] revealed the mechanism in bacterial and microalgal communities during biological sulfamethoxazole (SMX) degradation by an immobilized microalgal-bacterial consortium, discovering that SMX was bio-degraded through oxazole ring breakage, mononitration, and breakage of S−N bonds and C−N bonds. A process called “assimilation” turns inorganic N into organic N into peptides and proteins, converting it into chlorophyll, energy-transfer molecules (ADP and ATP), and nucleic acids. Accordingly, a newly established method for deriving information on pollutant removal mechanisms is with the aid of metabolic processes directly linked to nitrogen metabolism. Liu et al. [115] comprehensively analyzed ammonium’s removal possibility and conversion routes using both suspended and immobilized microalgal grown in sewage. It demonstrated that ammonium assimilation was the crucial removal route and resulted in protein synthesis. Due to their relationship with photosynthesis and
respiration, monitoring the gas fluxes of oxygen and carbon dioxide may be another way to assess microalgae’s metabolic characteristics during different trophic modes. Zhang and Perre [116] described the relationship between gas production/consumption and the varying biomass obtained from immobilized C. vulgaris during different trophic modes. The results indicated that the cultivation mode affected the cell growth rate and the colony morphology, resulting in different metabolic reactions occurring in the colony.

From above, possible involving mechanisms of the immobilized microalgal system for pollutants removal are distinguished by microalgal cell wall as the extracellular and intracellular parts, as shown in Fig. 5. Firstly, cells build and enter the immobilization system for pollutant removal. Fig. 5.

**5. Can an immobilized microalgal system be applied in real wastewater treatment?**

Indeed, immobilized microalgae may present a certain potential for dealing with wastewater. However, achieving a cost-effective and stable large-scale microalgal immobilization system for treating actual wastewater still has some insurmountable bottlenecks. For instance, how to achieve the large-scale immobilized microalgal production goals in applications of practical wastewater treatment? How about the feasibility of immobilized microalgae in multifarious real wastewater? What about the effectiveness of upgrading the existing wastewater treatment technologies with an immobilized microalgal system?

**5.1. High-tech technologies promote large-scale production**

Recently, Malik et al. [122] successfully achieved large-scale fabrication of microalgae-laden hydrogel membranes using a multi-material pneumatic extrusion system connected to the end effector of a robotic arm, thereby providing an economically feasible strategy for industrial applications in areas of microalgal bioremediation, bioenergy, and bioremediation (Fig. 6a). An immobilization technology developed for 3D-printing microalgae with excellent viability and superior growth even at adverse temperature conditions was proposed and named as “Green Bio-printing” (Fig. 6b) [76]. Similarly, the silk protein hydrogels fabricated by 3D-printing have also been introduced to host microalgae, discover that the long-term cell survivability, steady photosynthetic activity, and outstanding cell performance appeared in this immobilized system could fulfill the genuine need for microalgal-based aquatic cleanup (Fig. 6c) [123]. Furthermore, Lee et al. [124] used drop-on-demand inkjet printing to immobilize spores of the
Microalgae Ecklonia cava within alginate microparticles, suggesting that inkjet printing is suitable for immobilizing microalgae and that it can accurately control the size and number of encapsulated spores (Fig. 6d). Finally, Trampe et al. [125] co-immobilized a chemical nano-sensor with green microalgae as the bio-ink in order to map the cell metabolism and spatiotemporal dynamics of their chemical microenvironment in a 3D-printed structure (Fig. 6e).

5.2. Application potentials in diverse wastewater and bioreactors

Although many studies have focused on the large-scale production feasibility of immobilized microalgae, the systematic evaluation of its applicability, especially for wastewater treatment, is still lacking. Considering the complex environment of actual wastewater, immobilized microalgae might present variable performances when scaling up. As shown in Table 1, studies have been conducted using different wastewater qualities aiming to treat different pollutants. A majority of efficient on-site pretreatments are also included. For example, nitrification of anaerobic digestate using a consortium of microalgae and nitrifiers was performed in an open photobioreactor with moving bed carriers, subsequently presenting a significantly higher nitrifying activity [126]. Beads of alginate-immobilized microalgae were used to remove nutrients from wastewater in a compact reactor and facilitate microalgal harvesting for biorefinery. The fluidized-bed reactor containing entrapped C. vulgaris or Scenedesmus abundans was used for purifying secondary effluent, with nearly complete removal of both TP and NH₃-N after 30 days of operation [127]. Orandi and Lewis et al. [128] reported that a photo-rotating biological contactor inoculated with indigenous microalgae was successfully scaled. They efficiently removed various common heavy metals (Cu, Mn, Mg, Zn, Ca, Na, Ni) and trace elements (Fe, Al, Cr, Co, Se, Ag, Mo) from multisynthetic acid mine drainage. Additionally, Lee et al. [129] used agar–alginate-immobilized cyanobacteria (Dermocarpella sp.) arranged as tubular chains to efficiently treat swine wastewater.

Currently, microalgal immobilization is usually associated with a photobioreactor for treating wastewater. For instance, to relieve the stress of severe loading and a low wastewater treatment efficiency caused by the direct discharge of high-concentration meat processing wastewater into a municipal sewage system, integration of an ozone pretreatment with co-immobilized microalgae-activated sludge bacterial symbiosis significantly improved biodegradability, achieving efficient on-site treatment of MPW, with 25.7% sCOD, 16.1% TN, and 14.3% TP increased in removal efficiency [130]. Zamalloa et al. [131] proposed an in situ two-stage treatment system using chemical–biological flocculant in conjunction with microalgal biofilm in nutrients utilization in a roof-mounted parallel plate reactor, achieving effective removal of 74% COD, 82% SS, 67% TN, and 96% TP. Similarly, the two-stage dairy effluent treatment with a high organics content using immobilized C. pyrenoidosa exhibited complete removal of NH₄-N and 98% removal of PO₄³⁻⁻P within 96 h [132]. These results suggested that the integration units (e.g., the two-stage treatment system), which have a relatively low operation cost, may be applied as a decentralized domestic wastewater treatment system.

5.3. Downstream applications lead upgradation closer

Although microalgal biomass is regarded as “energy-rich waste”, there are considerable challenges in achieving a viable energy balance in microalgal cultivation operations since the microalgal biomass in dilute suspended cultivation are only around 0.02–0.05% dry weight [140,141]. And this number may be even lower in the system where wastewater is used as the medium. However, compared with the suspended system, immobilized microalgae in wastewater are advanced for higher cell density and biomass, which is advantageous for microalgal biomass harvest and downstream application [142]. Currently, there are three main downstream directions of immobilized microalgae: (1) bioenergy production, such as hydrogen [143–145] and biodiesel [79]; (2) bioactive compounds production, including carbohydrates, proteins, carotenoids, and fatty acids [146–148]; and (3) other recovery and reuse [149–151]. However, little information is available so far on simultaneous wastewater treatment and microalgal biomass conversion, which should be further investigated to make this technology more viable. In the works of Bhatia et al. [139], recent advances in pretreating microalgal biomass and conversing algal bioenergy resources are fully summarized (Fig. 7). The same applies to immobilized microalgal biomass.

6. Conclusion and future research recommendations

Although using microalgal immobilization to treat wastewater has been proposed for some time, researches related to the scale-up or long-term operation are still limited, largely hindering its applicability. To provide new ideas for upgrading current microalgal wastewater treatment and microalgal wastewater treatment, the merits of using immobilized microalgae to treat wastewater are
comprehensively discussed, focusing on the systematic comparison between suspended and immobilized systems, summarizing the positive effect on the physiological activity of microalgae and pollutants removal efficiency. Furthermore, the main theories related to immobilized microalgae are discussed. Mechanisms involving pollutant removal by immobilized microalgae are thoroughly deliberated. Notably, possibilities on immobilized microalgae for upgrading the current wastewater treatment have been discussed, involving a whole process from the technical feasibility of scale-up engineering applications and successful attempts at various water quality treatment to perfect coupling with a variety of bio-reactors and treatment processes, as well as downstream applications. Eventually, the current bottlenecks and future research recommendations for accelerating the development of microalgal immobilization for upgrading microalgal-based wastewater treatment are proposed as follows:

(1) Current immobilization technologies for microalgal wastewater treatment are mostly attachment immobilization oriented, with cheaper, easily accessible biological matrix materials [41,103]. They are suitable for the mild hydrodynamic environment owing to the relatively weak cell-substrate adhesion. Exploiting the entrapping methods is recommended for microalgal immobilization since the better system stability for long-term operation;

(2) Leakage of entrapped or attached immobilized microalgal cells commonly occurs with a prolonged cultivation period [46,152], which may harm the ecosystem. Thus, determining the cause of leakage and discovering new materials for better fitting the microalgal immobilization demand is strongly encouraging;

(3) A limited number of contaminants have been investigated by employing the immobilized microalgal system [37,39]. As for pollutants removal efficiency and microalgal energy-substances accumulation, the current rhetoric that whether the immobilized system is superior to suspended culture is still controversial [43,127]. More surveys addressing the complicated joint effects of various pollutants and environmental factors while focusing on the feasibility of microalgal immobilization in different wastewater will be indispensable. In addition to focusing on the removal effect of target pollutants, the removal rate should also be placed in an important position.

(4) It is controversial whether immobilization is more beneficial for microalgae. On the positive side, immobilization provides

| Category                  | Wastewater Property                        | Species                          | Immobilized type/ method                                  | Initial concentration (mg L⁻¹) | Nutrient removal rate (%) | System                  | Ref.    |
|---------------------------|-------------------------------------------|----------------------------------|-----------------------------------------------------------|-----------------------------|--------------------------|-------------------------|---------|
| Agricultural              | Untreated piggery wastewater               | C. sorokiniana                   | Attached on carriers (sponge, activated carbon)           | COD: 5000 –10000 mg L⁻¹; BOD: 1500 –4500 mg L⁻¹; TN: 500 –700 mg L⁻¹; TP: 150 –250 mg L⁻¹; COD: 1168.92 ± 3.14 mg L⁻¹; TN: 55.04 ± 0.39 mg L⁻¹; TP: 27.16 ± 1.24 mg L⁻¹; | COD: 95.7%; BOD: 99.0%; TN: 94.1%; TP: 96.9%; COD: 98.47 ± 0.69%; TN: 98.87 ± 0.07%; TP: 98.49 ± 0.73%; SMX: undefined; COD: 72.12 ± 1.34%; SMX: 99.0 ± 0.2%; N: 40–86%; P: 26–72%; E2: 85–99% | Semi-batch cultivation | [133]   |
| Municipal                 | Actual anaerobically digested centrate     | C. vulgaris                      | Alginate-entrapped (microalgae-bacterial consortium & PAC) | 17β-estradiol(E2); 1 mg L⁻¹; | 72%–82%; NaClO pretreatment; an orbital shaking incubator | Batch PBRs               | [65]    |
| Raw domestic wastewater   | Desmodesmus sp.                            | Alginate-entrapped               | Cr: 50 mg L⁻¹; | 72%–82%; NaClO pretreatment; an orbital shaking incubator | Cr: 60% | Flasks | [66]    |
| Industrial                | Chromium ions among industrial effluents   | C. sorokiniana                   | Alginate-entrapped | NH₄⁺-N: 28.35 mg L⁻¹; | NH₄⁺-N: 90%; | Mootrophic conditions; a glass tube; | [62]    |
| Textile wastewater        | C. vulgaris and C. sp.                     | Alginate-entrapped               | NH₄⁺-N: 81%; Cr: 72%; | NH₄⁺-N: 90%; | COD: 75%; | Mootrophic conditions; a glass tube; | [62]    |
| Meat processing wastewater| Scenedesmus obliquus, C. Vulgaris and C. sorokiniana & activated sludge bacteria | Alginate-entrapped & co-immobilized microalgae/bacteria | COD: 4458 mg L⁻¹; Color: 920 mg L⁻¹; COD: 1868 ± 2 mg L⁻¹; TN: 1546 ± 8.3 mg L⁻¹; | 72%–82%; NaClO pretreatment; an orbital shaking incubator | 78%–80%; TP: 68–72%; BaA: 87–95%; BaP: 57–83% | Four mini-bioreactors | [134]   |
| Benzo(a)anthracene (BaA) and benzo(a)pyrene (BaP) containing wastewater | Selenastrum capricornutum and Scenedesmus acutus | Alginate-entrapped               | TP: 126.9 ± 0.4 mg L⁻¹; BaA: 266 µg L⁻¹; | 78%–80%; NaClO pretreatment; an orbital shaking incubator | 78%–80%; TP: 68–72%; BaA: 87–95%; BaP: 57–83% | Four mini-bioreactors | [135]   |
| Natural                   | Synthetic multitrophic wastewater          | Maß-flocs collected from a settling tank of a municipal sewage treatment plant | Microalgae-bacterial flocs entrapped into PVA-alginate beads | NH₄⁺-N: 100 mg L⁻¹; PO₄³⁻: 10 mg L⁻¹; | NH₄⁺-N: 61%; PO₄³⁻: 82%; | Multitrophic microreactor | [136]   |
| Eutrophic wastewater      | C. sorokiniana                             | Modified mussel shell powder for microalgal immobilization | N: 18 mg L⁻¹; | N: 95 ± 2.61%; | Conical flask | | [137]   |
| Marine water              | Scenedesmus bijugatus                      | Alginate-entrapped               | Alginate-entrapped | NH₄⁺-N: 900 µM; NH₄⁺-N: 29.89 mg L⁻¹; PO₄³⁻: 0.61; | NH₄⁺-N: 81; 89%–94%; Orbital shaker | Conical flask | [138]   |

Table 1: Applications of immobilized microalgae on different wastewater.
a beneficial barrier for cells against various complex and changing wastewater environments [50,83]. However, it cannot be ignored that immobilized substrate restricts the entrance of nutrients, thereby lowering the treatment efficiency [77]. Clarifying the mechanisms of substances transfer and conversion is vital. Integration of experimental studies and modeling explanation is promising for helping to unravel the immobilized growth and nutrient removal of immobilized systems.

(5) Techno-economic and life cycle impact assessments are urgently needed for helping to comprehensively understand the system's removal effectiveness and energy conversion potentials. Further focusing on the energy-intensive and most costly steps, targeted research and investment areas for increasing productivity and decreasing energy requirements are thought to accelerate the next step for this technology moving towards practical application.

(6) To broaden and enhance the practical applicability, attempts to integrate microalgal immobilization with other biological technologies using activated sludge, symbiotic bacteria, or constructed wetlands is extremely necessary. Additionally, intersection subject involving many fields like intelligent microalga farming incorporating with Internet-of-things, Big Data, and artificial intelligence is also extremely encouraged for promoting the wide application of this system in the future.

Author contributions

Meina Han: Conceptualization, Formal analysis, Investigation; Writing — original draft. Chaofan Zhang: Conceptualization, Investigation. Shih-Hsin Ho: Supervision, Project administration, Writing - review & editing.

Declaration of competing interest

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

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Fig. 7. Schematic illustration of valorization of algae biomass: a, valuable products; b, algal biomass treatment and product production. Reprinted with permission [139], Copyrights, 2022, Elsevier.
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