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Development of microalgal biofilm for wastewater remediation: from mechanism to practical application

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Abstract

The high cost of biomass harvesting by centrifugation and high safety risks of collected algal biomass by chemical flocculation are serious problems jeopardizing the industrial implementation of microalgae bio-products. Recently, microalgae immobilization is regarded as a promising technology with the potential of lowering biomass production cost and ensuring biomass safety in the industry. Therefore, microalgal biofilms, an effective and affordable way to immobilize algal cells, are emerging into the limelight. This paper summarizes the progress achieved in biofilm system design and biofilm formation mechanisms. Newly designed algal biofilm systems are compared to demonstrate their advantages and weaknesses. Besides, mechanisms associated with the two steps –initial attachment of microalgae and biofilm thickening – of biofilm formation are discussed in this paper. Factors such as substratum material, algal strain and operational parameters, which could impact the formation and operation of algal biofilm, are demonstrated. Efforts devoted to the industrial application of algal biofilm to treat wastewater are discussed. The biotechnology of microalgal biofilm is currently at the critical stage of developing from fundamental research to industrial implementation. Undeniably, there are still many problems that limit the wide use of algal biofilm for biomass production and wastewater treatment. In this paper, we present some potential solutions to current problems and discuss the development trends of algal biofilm in the foreseeable future. It is expected that by addressing current problems microalgal biofilm will be widely used at the industrial scale.

Keywords: algae; environmental remediation; biomass; waste treatment; waste minimization

INTRODUCTION

As a category of unicellular microorganisms with good performance in the assimilation of nitrogen, phosphorus and carbon, microalgae have been widely used for wastewater remediation.[1,2]

Compared with traditional wastewater treatment, microalgae-based wastewater remediation has advantages in the aspects of biomass utilization and environmental protection. First, microalgae could assimilate nutrients in wastewater and synthesize high-value biomass. Algal biomass enriched with protein, polyunsaturated fatty acids and natural pigments can be further used as feedstock of animal feed, biofuel, bio-fertilizer and organic chemicals.[3,4] Hence microalgae-based wastewater remediation can be regarded as a value-added biotechnology for sustainable development. Second, microalgae-based wastewater remediation is a process of fixing carbon dioxide (CO_2) and releasing oxygen (O_2), while traditional wastewater treatment technologies, including aerobic digestion and anaerobic fermentation, produce a large amount of greenhouse gases (CO_2 and CH_4).[5,6] Owing to the aforementioned advantages, in recent years microalgae-based wastewater remediation has emerged into the limelight.[7]

Traditional methods of harvesting microalgae mainly include centrifugation, filtration, gravity-driven sedimentation and flocculation. The disadvantages of the aforementioned harvesting methods have been fully documented by previous studies.[8,9] For example, centrifugation, which is energy intensive and costly, could remarkably increase the total cost of algal biomass and limit the wide use of biomass in downstream industries.[10] In some cases, the cost of biomass harvesting could even account for 30% of the total cost of microalgae production. Also, gravity-driven sedimentation has a very low harvesting rate and flocculation may introduce metal ions, such as ferric ions and aluminum ions, into algal biomass.[11] In our view, these problems of harvesting methods have seriously jeopardized the sustainable development of microalgae-related industries, particularly microalgae-based wastewater remediation. Therefore, it is of importance to develop new and applicable technologies for microalgae harvesting.

Recently, the immobilization of algal cells for microalgae cultivation and biomass harvesting attracts the attention of researchers from academia and industry.[12,13] Construction of microalgal biofilm by attaching microalgae on the surface of certain substrata is a practically feasible immobilization method.[13] As the microalgae growth is performed on biofilm, biomass can be

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harvested by using a scraper in an efficient and cost-saving way. Besides, biomass collection from microalgal biofilm is a physical process, which will not introduce toxic or unfavorable chemical agents.[10] Hence the development of microalgal biofilm could overcome the technological problems of traditional harvesting methods.

Researchers have studied the formation mechanisms of microalgal biofilm and devoted much effort to the industrialization of this novel technology. This can be regarded as a promising technological upgrade in microalgae-related industry. This work introduces the designs of some microalgal biofilm systems and discusses the formation mechanisms of biofilm. Particularly, the roles of bacteria and extracellular polymeric substance (EPS) in the formation of microalgal biofilm are discussed in detail. In addition, factors, such as physical properties of biofilm substratum, algal species, nutrient concentration and illumination, which can influence the formation of microalgal biofilm, are summarized. Last, but not the least, the practical application of microalgal biofilm for nutrient recovery and biomass production in wastewater remediation is discussed. To promote the further development of microalgal biofilm, this work also identifies some major challenges of microalgal biofilm in applications and discusses the prospects of this novel technology.

RESEARCH INTERESTS RELATED TO MICROALGAL BIOFILM

Originally, the concept of microalgal biofilm might have been derived from the microbial mats observed in nature. In most cases, natural biofilm or mat is a complex matrix consisting of a variety of microorganisms, such as microalgae, bacteria and even fungi.[14,15] For example, Kublanovskaya *et al*. analyzed the biofilm formed on the White Sea coast and found that *Haematococcus lacustris* cells were located inn the upper layer of biofilm (photoautotrophic layer), while heterotrophic microorganisms were located in the matrix.[16] Compared with suspended microalgae grown in water, microbial biofilm could be collected or harvested in a much more efficient way. Therefore, the inspiration from nature may encourage researchers to realize the practical feasibility of using microalgal biofilm for biomass production in the industry.

Traditionally, microalgae cultivated in media or wastewater are suspended and then harvested by centrifugation, chemical flocculation or filtration.[10] In the operation of algal biofilm, biomass attached to substratum can be harvested in a very simple way by using scrapers. Compared with the cultivation of suspended microalgae, microalgal biofilm has advantages in energy consumption, production cost and biomass safety. First, centrifugation has a high energy consumption, ranging from 0.3 to 8 kW h m^{-3} based on the operation mode, and the high energy consumption limits the substantial commercialization of microalgal products.[17] With the use of scrapers to harvest biomass on biofilm, energy consumption can be dramatically reduced. Accordingly, the cost of biomass harvesting and microalgae production can be lowered. Second, some chemicals, such as aluminum ions and polyacrylamide, are added to the water phase for flocculation, but these chemicals or their degradation products may be toxic or unhealthy. Fortunately, no toxic chemicals are introduced into the biomass collected from algal biofilm. As a consequence, safety problems resulting the use of chemicals can be prevented. Owing to the aforementioned advantages, in recent years there have been an increasing number of studies on algal biofilm.

According to the summary of Wang *et al*., up to now many well-known universities and institutes, such as Wageningen University, University of Texas, University of Toronto, Chinese Academy of Sciences, Iowa State University, University of Valladolid, Tokyo Institute of Technology, Korea Research Institute of Bioscience and Biotechnology, and Academy of Sciences of Czech Republic, have focused on microalgal biofilm research and industrial implementation.[18] As shown in Table 1, the main research interests related to algal biofilm are microbial interactions of natural biofilm, structures of man-made algal biofilm, selection of substratum, microbial community in biofilm, optimization of operational factors, biomass productivity on biofilm and wastewater treatment by algal biofilm. The fundamental research mainly includes interspecific relationships between algae and other microbes attached to biofilm, EPS secretion by algae and bacteria, mechanisms of cell–substratum interaction, changes in microbial communities on algal biofilm during the operation period, effects of environmental conditions on algal metabolisms, etc. (Table 1). In addition, researchers and technicians have devoted efforts toward applied research, which mainly includes the design of a practically feasible biofilm system, optimization of operational factors for biomass production and nutrient removal, selection of appropriate materials as biofilm substratum, etc. (Table 1). In the view of the present authors, as great progress has been achieved, microalgal biofilm is transitioning from scientific research to industrial implementation.

DESIGN OF MICROALGAL BIOFILM SYSTEMS

Owing to the advantages of microalgal biofilm in the aspects of algae production and biomass separation, a variety of systems, such as attached-growth photobioreactor (AG-PBR), suspended-solid-phase photobioreactor (ssPBR), rotating algal biofilm (RAB) and algal biofilm membrane photobioreactor (BMPBR), have been designed and tested by previous studies (Table 2). Specific characteristics of the microbial biofilm systems are discussed and compared as follows.

Ozkan *et al*. designed a horizontal biofilm system, which was named 'algae biofilm photobioreactor system'.[40] As shown in Table 2, the whole biofilm system was placed under a light source and the recirculation and drip system provides microalgae with essential nutrients. The main advantages of this system are that the light-receiving area of the biofilm system was large, and microalgae could perform well in photosynthesis. Nevertheless, due to the horizontal structure, this biofilm system had a very large footprint, which may limit its application in the industry. For example, the biomass productivity of this biofilm system was only 0.71 g m^{-2} d^{-1}, which is much lower than that of some vertical biofilm systems (Table 2). Besides, as the biofilm surface was exposed to light source, evaporation loss rate was remarkably high, reaching 1.09 L m^{-2} d^{-1}.[40] In this case, about 1.54 L water would be evaporated for the production of 1 g algal biomass. In the view of the present authors, algae production at the expense of high evaporation loss of water cannot be regarded as a sustainable model. Therefore, large footprint and high evaporation loss rate are two problems jeopardizing the industrial implementation of horizontal biofilm system.

As shown in Table 2, many previous studies designed vertical biofilm systems for algae cultivation. Compared with the horizontal biofilm system, the vertical biofilm system has a smaller

Table 1. Research interests and objectives related to algal biofilm

Research interest	Main content	Research objective	References
Microbial interactions of algal biofilm in nature	(1) Study the dominant microorganisms (microalgae, bacteria, fungi, etc.) in natural biofilm (2) Study the synergistic relations between microalgae and other microbes in natural biofilm (3) Study the effects of environmental conditions on EPS concentration and microbial community in biofilm	(1) Identify the evolution of microbial community in algal biofilm exposed to outdoor environment (2) Provide tips to the operation of man-made algal biofilm in outdoor environment	16,19,20
Structures of man-made algal biofilm	(1) Design different types of algal biofilm systems according to the actual requirements (2) Test the effects of structures on biofilm formation and operation	(1) Identify the biomass productivity on different types of algal biofilm systems (2) Identify the strengths and weaknesses of each type of biofilm system	21-23
Selection of substratum	(1) Analyze the physical properties of materials used as substrata of algal biofilm (2) Compare the attachment performance of different materials used as biofilm substrata (3) Explore the relation between substratum properties and microalgae attachment	(1) Find out the most suitable materials for microalgae attachment (2) Accurately predict the suitability of materials as substrata according to the physical properties	24-26
Microbial community in biofilm	(1) Study the changes of microbial community during the operation of algal biofilm (2) Study the dominant microalgae or bacteria playing key roles in the formation and operation of algal biofilm	(1) Identify the dominant microalgae on biofilm (2) Develop strategies to control the microbial community on algal biofilm and maintain the efficient operation of biofilm system	27-29
Optimization of operational factors	(1) Study the effects of operational factors (temperature, illumination, nutrient concentration, etc.) on the biomass productivity on algal biofilm (2) Optimize the operational factors to improve the performance of algal biofilm in the operation	(1) Find out the factor with influential effects on biofilm productivity (2) Achieve high biomass yield by employing optimal conditions	30-32
Biomass productivity on biofilm	(1) Study the growth of different algal strains on substrata of biofilm (2) Explore the methods to increase biomass productivity of algal biofilm	(1) Convert the nutrients in culture media to algal biomass in an efficient way (2) Produce high-value algal biomass for downstream industry	23,24
Wastewater treatment by algal biofilm	(1) Study the nutrients removal by algal biofilm in wastewater treatment (2) Explore the roles of microalgae and bacteria attached on biofilm in nutrients removal	(1) Demonstrate algal–bacterial interactions on biofilm during wastewater treatment (2) Achieve high removal efficiency of nutrient in wastewater treatment	33-35

footprint. Because of this advantage, the vertical biofilm system is becoming a hot research topic. Liu *et al*. designed a single-layer vertical plate attached photobioreactor, of which the biomass productivity was 5.7 g m^{-2} d^{-1}.[39] To further improve microalgae productivity, multiple cultivation modules were inserted inside the glass chamber to construct a system with multiple layers of biofilm. Accordingly, biomass productivity of this system with multiple layers of biofilm was improved to 70.9 g m^{-2} d^{-1}.[39] The dramatic increase in biomass productivity is mainly attributed to the increase in number of biofilm layers. However, it should be noted that with the decrease in the distance between biofilm layers, microalgae growth on biofilm might be negatively impacted by insufficient illumination. For example, in the study of Zhang *et al*., when the distance between biofilm layers decreased from 8 to 2 cm, received light intensity decreased from 193.18 to 48.3 μmol m^{-2} s^{-1}.[21] Therefore, in practice, the distance between biofilm layers should be adjusted to ensure the high biomass productivity of vertical systems with multiple biofilm layers.

Some previous studies designed gas–liquid separation biofilm system to integrate CO_2 fixation and organic nutrient removal.[22,37] For example, Guo *et al*. designed a biofilm system consisting of liquid chamber and gas chamber, which were separated by a gas-permeable membrane.[22] In this system, when gas flow rate and liquid flow rate were set as 3 mL min^{-1} and 2 mL h^{-1}, CO_2 removal efficiency and NO_3^- removal efficiency reached 32.7% and 6.7%, respectively.[22] In our view, in a real-world application, such a biofilm system could not only be used for wastewater remediation, but also be employed to reduce the emission of greenhouse gas. Disk biofilm systems were also developed by some studies. Compared with some vertical or horizontal biofilm systems, disk biofilm systems had much higher biomass productivity (Table 2).

The wash-off of algal cells is unavoidable in the operation of biofilm systems, including vertical biofilm system, horizontal biofilm system and disk biofilm system, since a portion of microalgae might not strongly adhere to the substratum.[42] Boelee *et al*. reported that the amount of biomass washed out remained stable until the end of the quasi-steady-state period and the release of chunks of biofilm.[42] To minimize the wash-off of microalgae cells, previous studies have developed some methods. Guo *et al*. added stainless steel mesh to biofilm to induce additional interlaced grooves and enlarge the area for microalgae cell attachment, thus

Table 2. Systems designed for microalgae growth and biofilm operation

Biofilm system	Description	Biomass yield or productivity	References
Attached-growth photobioreactor (AG-PBR)	(1) AG-PBR (width: 0.10 m; length: 0.50 m; height: 0.60 m) was made of transparent acrylic plastic (2) Low-cost attached-growth media were made of used drinking-water bottles (3) Septic tank effluent provided nutrients to algae in AG-PBR	47 g m^{-2} d^{-1}	33
Vertical algal biofilm-enhanced raceway pond (VAB-enhanced raceway pond)	(1) VAB-enhanced raceway pond was built with 70 cm length, 40 cm width and 15 cm height (2) Some materials (coral velvet, pleuche, cotton linen, coarse linen, fine linen, cotton duct, gauze, organza, etc.) were tested to test their ability to support algae growth	6.95–8.11 g m^{-2} d^{-1}	21
Suspended-solid phase photobioreactor (ssPBR)	(1) Algae carriers were made into pom-pom with a diameter of 2.5 cm × 20 strings of cotton, linen or mohair (2) Attached microalgae had much higher protein content (50.1%) than suspended algae (3) Attached algae had lower biomass accumulation rate and oxygen-evolving activity than suspended algae	0.6–2.7 g m^{-2} d^{-1}	36
Rotating algal biofilm (RAB)	(1) Attachment material (with a surface area of 450 cm^2) was stretched around the shafts of RAB to form a triangular configuration (2) Algae grown on cotton duct had higher biomass productivity (1.08 g m^{-2} d^{-1}) than those grown on cotton rag, cotton denim, and cotton corduroy (3) Algae harvested from RAB had higher protein content (37.74%) than algae from flat panel photobioreactor	0.08–1.08 g m^{-2} d^{-1}	23
RAB	(1) Pilot-scale RAB reactors (2) A conveyor belt was stretched around drive shafts to form a vertical configuration (3) A liquid reservoir (2.43 m long × 1.83 m wide × 0.22 m deep) contained 1000 L wastewater	7.0 g m^{-2} d^{-1}	34
Gas-permeable membrane PBR integrated with additional rough surface (GMPBR-RS)	(1) Liquid chamber (150 mm × 30 mm × 6 mm) and gas chamber (150 mm × 30 mm × 4 mm) were made of PMMA and separated by gas-permeable membrane (2) Interlaced grooves induced by the stainless steel mesh could enlarge the area for microalgae attachment and reduce the washout of algal cells caused by fluid shear force	31.44 g m^{-2}	22
Biofilm cultivation system with gas–liquid separation	(1) Biofilm reactor (0.2 m × 0.08 m × 0.03 m) was made of PMMA (2) A selectively permeable membrane, PTFE, was employed to separate liquid chamber and gas chamber (3) Microalgae attached on the surface of PTFE and formed into biofilm under light illumination	25.65 g m^{-2} (2.57 g m^{-2} d^{-1})	37
Algal biofilm membrane photobioreactor (BMPBR) equipped with solid carriers and submerged membrane module	(1) The reactor consisted of two zones, including main zone (0.7 m × 0.4 m × 0.7 m) and outlet zone (0.3 m × 0.4 m × 0.7 m) (2) Flexible fiber bundles used as carriers for algae attachment were submerged in middle of the reactor (3) Advantages of flexible fiber bundle include large surface area, high adsorption capacity and low cost	0.072 g L^{-1} d^{-1} (attached microalgae: 0.052 g L^{-1} d^{-1})	38
Single-layer vertical plate-attached photobioreactor	(1) A 0.2 m × 0.4 m glass plate (3 mm thickness) was located vertically in the center of glass chamber (0.5 m × 0.3 m × 0.05 m) (2) Microalgae were filtered onto a cellulose acetate/nitrate membrane to form an algal 'disk' (3) Flow rate of culture medium was regulated to ensure good attachment of microalgae with minimum wash-off	5.7 g m^{-2} d^{-1}	39
Algae biofilm photobioreactor system	(1) The biofilm system consisted of a biofilm growth surface, a medium recirculation system, and an illumination system (2) Biofilm growth surface was a concrete layer (8 mm thickness) over a wooden support plate and had a cultivation area of 0.275 m^2	0.71 g m^{-2} d^{-1}	40
	(1) Inserted glass plates and attached algal film were regarded as a 'cultivation module'	70.9 g m^{-2} d^{-1}	39

Table 2. Continued

Biofilm system	Description	Biomass yield or productivity	References
Light dilution and multi-plate-attached photobioreactor	(2) Dimensions of the glass chamber were $w \times h \times l = 0.4 \times 0.1 \times 0.3$ m and of the inserted glass plate were 0.3×0.1 m (3) The gap between the adjacent glass plates was controlled between 0.02 m and 0.06 m		
Rotating biological contactor-based photobioreactor	(1) The system (21 L) consisted of a water-tight container, four disks and eight lamps (2) Disks were replaced in the container with 42% of the disk surface submerged (3) Disk materials (stainless steel woven meshes and sanded polycarbonate disk) were tested	20.1 g m^{-2} d^{-1}	41
Revolving algae biofilm (RAB) reactor	(1) RAB reactor consisted of a liquid container (1.5 L) and a rotating belt with a surface area of 0.13 m^2 for microalgae attachment (2) The belt rotated at a linear velocity of 4 cm s^{-1} (1.2 rpm) (3) RAB reactor was operated in a continuous operation mode and HRT was set as 3-day	NA	32
Algal biofilm photobioreactor	(1) Biofilm photobioreactor consisted of gas distribution system (A), columnar flotation system (B) and reaction system (C) (2) A membrane material with good light absorption and less reflection was used for algae growth (3) A special array of curtain membrane assemblies was applied to realize full utilization of light	7.37 g m^{-2} (1.474 g m^{-2} d^{-1})	2

HRT, hydraulic retention time; NA, not available; PMMA, polymethyl methacrylate; PTFE, polytetrafluoroethylene membrane.

reducing the washout of microalgae cells caused by fluid shear force.[22] Liu *et al*. gently controlled the flow rate of the culture medium to maintain the well attachment of microalgae on biofilm with minimum wash-off.[39] These strategies effectively minimized the wash-off of microalgae and maintained the operation of the biofilm system.

FORMATION MECHANISM OF ALGAL BIOFILM

Although the structures and shapes of microalgal biofilm systems can be very different, the core technology is construction of biofilm by microalgae attachment for biomass production. Generally, the formation of algal biofilm consists of two steps: the initial attachment of microalgae on substratum, and biofilm thickening (Fig. 1).[18]

Initial attachment of microalgae

The initial attachment of microalgae can be observed when the substratum is immersed into algae culture media.[18] Normally, adsorption of algal cells on substratum occurs when the repulsive electrostatic interactions are overcome by the attractive van der Waals and acid–base interactions.[27] Previous studies reported that the attractive van der Waals interactions are effective at shorter separation distance while the attractive acid–base interactions are dominant at larger separation distance.[27,43] It should be noted that the initial attachment is a reversible process since microalgae adsorbed on the surface of substratum might be washed off easily. Hence substratum materials with rougher surface and more binding sites should be employed for algal biofilm construction. In practice, physical parameters that are tested to judge the properties of substratum material include surface free energy, roughness and contact angle.[44]

In addition to physical properties of substratum material, external conditions could impact the initial attachment of microalgae. For example, Mohd-Sahib *et al*. discovered that the pH of culture media could determine the zeta potential and further influence the initial attachment of microalgae on certain substrata.[43] When pH values were 3, 5, 7 and 9, rates of formation of early attachment reached 1.87, 2.07, 1.68 and 1.02 mg g^{-1} min^{-1}, respectively, suggesting that the optimal pH value for microalgae attachment on polyurethane foam support material should be around 5.[43] The main mechanism for this phenomenon is that the pH value of culture media could directly determine the ionization of functional groups on the cell surface and the surface charge of algal cells. In some cases, initial attachment of microalgae can be impacted by the bacterial colony on the substratum. Hodoki reported that immigration of microalgae from water phase to substratum was proportional to the density of attached bacteria on all substrata.[45]

Therefore, in a real-world application, to promote the initial attachment of algal cells on substratum, researchers and technicians could select proper substratum materials, create favorable external conditions and/or regulate the microbial density on substratum.

Biofilm thickening

Biofilm thickening, which refers to the development of mature biofilm by microorganism reproduction, on substratum is involved with complex biochemical processes, such as bacterial colonization, algal–bacterial interaction and secretion of extracellular polymeric substances (EPS).[18,46] In the biofilm-based wastewater remediation at an industrial scale, it is not practically

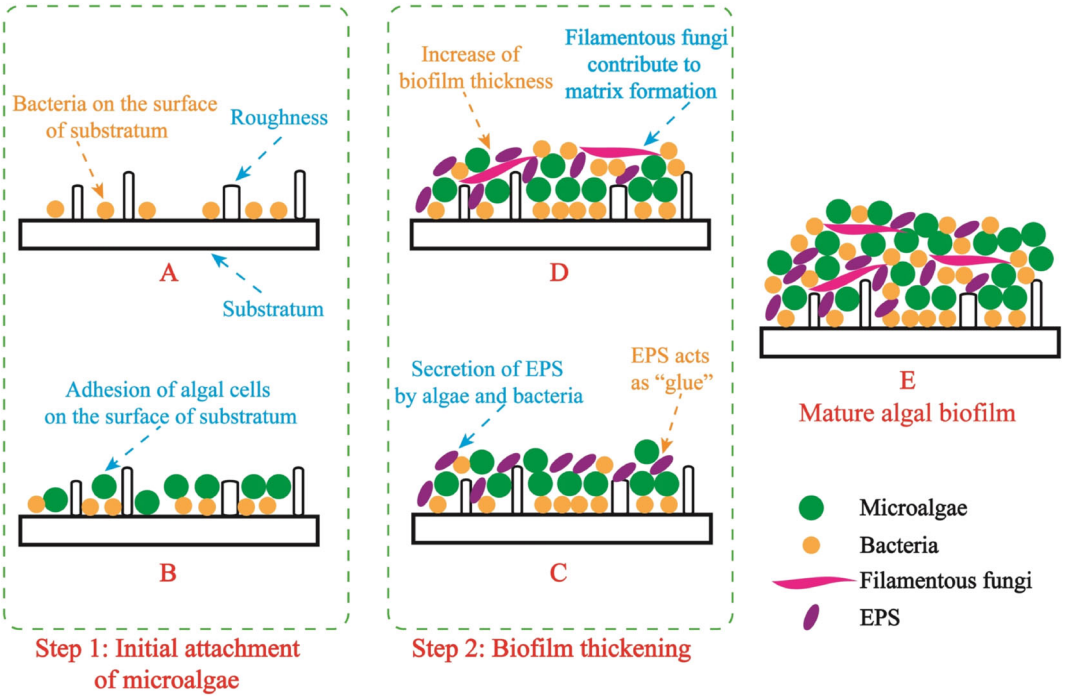

Figure 1. Formation of algal biofilm and associated mechanisms.

feasible to create an axenic environment. Thus algae growth on substratum is accompanied by bacterial colonization. Accordingly, interactions between algae and wastewater-borne bacteria and interactions between algae and airborne bacteria are unavoidable in the process of biofilm thickening. Therefore, the term 'microalgal biofilm' was defined as the microalgae-dominated biofilm, in which a small quantity of bacteria might exist as well.[18]

Microalgae and bacteria on substratum could secrete EPS, which might act as a 'glue' to promote the adhesion of algal cells. Main components of EPS are protein and polysaccharides, while sometimes nucleic acids and lipids are also included in EPS matrix.[28] The main functions of EPS are to construct a polymer network and maintain the mechanical stability of the matrix, promoting the formation of biofilm.[47] In some cases, algal cells protected by EPS exhibit resistance to environmental stress conditions, such as heat, dryness, decompression and ultraviolet rays. Recent studies have reported that components of EPS may play different roles in the formation and operation of algal biofilm.[47] For example, charged polysaccharides and protein can impact the sorption of organic compounds and inorganic ions, further determining the composition of algal biofilm. Besides, polysaccharides and protein influence the water retention and cohesion of biofilm, while lipid acts as surfactant.[47] Factors impacting the productivity and profile of EPS include bacterial strain, algal strain, illumination, temperature, nutrients in culture media, and so on. Accordingly, for the purpose of constructing algal biofilm, efforts should be devoted to algal strain screening, bacterial community control, illumination and temperature adjustment, and nutrient supply.

With the increase in biofilm thickness on substratum, microalgae located in different layers of biofilm may have different trophic models and metabolisms. As reported by Schnurr and Allen, microalgae located on the outer layer of biofilm perform photosynthesis under the condition of illumination, whereas microalgae on the inner layer of biofilm perform heterotrophic metabolism.[28] Hence both illumination and wastewater-borne nutrients contribute to the microalgae growth and reproduction and influence the formation of algal biofilm.[28] In addition, there might be a symbiotic relation between microalgae and bacteria in the formation of biofilm. Specifically, algae located on the inner layer and bacteria could degrade solid organics in culture media or wastewater, and release CO_2 via heterotrophic metabolism. At the same time, microalgae on the outer layer of biofilm capture CO_2 and produce O_2 by photosynthetic metabolisms. Synergistic relations based on the mass transfer between algae and bacteria are beneficial to biofilm thickening.

EPS secreted by microorganisms

As discussed above, EPS secreted by microorganisms attached to substratum plays a key role in biofilm thickening. To clearly demonstrate the microstructural change in the process of biofilm formation, previous studies identified the specific components of EPS and explored the inducing conditions for EPS secretion.[47,48] Mishra and Jha grew *Dunaliella salina* in media with different salinity gradients and discovered that the increase in salt concentration promoted the secretion of EPS.[48] When the concentration of salt was 3.0 mol L^{-1}, maximum percentages of monosaccharide, including glucose, fructose, xylose and galactose, in EPS were achieved.[48] In addition to *Dunaliella salina*, some other algal strains, such as *Odontella aurita*, *Porphyridium cruentum*, *Arthrospira platensis* and *Chlorella vulgaris*, are able to secret EPS.[49] Monosaccharide and uronic acid composition in the EPS of *Chlorella vulgaris* mainly included glucose, galactose, arabinose, rhamnose, mannose, fucose, xylose, galacturonic acid and glucuronic acid.[49] Previous studies identified some bacterial strains with high productivity of EPS. For example, *Serratia* sp. could produce loosely bound EPS at the rate of 2.45 g L^{-1} in 48 h fermentation.[50]

Therefore, strategies can be adopted to induce the secretion of EPS by microalgae and bacteria for biofilm formation.

FACTORS IMPACTING MICROALGAL BIOFILM FORMATION

In a real-world application, a variety of factors, such as substratum material, algal strain, temperature, illumination and pH, could influence the formation of algal biofilm. In this section, the effects of the aforementioned factors on microalgal biofilm formation are discussed. Besides, an in-depth discussion of the mechanisms, which can be employed to explain the formation process of algal biofilm, is provided.

Substratum

Selection criteria and important factors

Owing to the importance of cell–substratum interaction to the initial attachment of microalgae, previous studies tested the feasibility of various substratum materials, such as natural bio-products, chemical products, metal products, textile products and glass, in biofilm construction (Table 3). Deantes-Espinosa *et al*. stated that the materials, which are easy to obtain, non-toxic and reusable, with good attachment performance, could be used as substrata of algal biofilms.[51] In addition, cost and durability of substratum are important factors that should be considered for the wide application of algal biofilm.

As shown in Table 3, in the selection of proper substratum materials, properties that were taken into consideration mainly include surface roughness, contact angle, point of zero charge, surface free energy and hydrophilic/hydrophobic property. In the view of the present authors, surface roughness and hydrophilic/hydrophobic property play key roles in the algae–substratum interaction. First, surface roughness determines the quantity of binding sites on substratum. Thus algal cells are more likely to be attached to rough materials rather than smooth materials. There is a linear relation between biofilm productivity and substratum surface roughness.[24] Zhang *et al*. reported that with the increase in surface roughness from 0.07 to 18.98 μm, biomass productivity increased from 4.01 g m^{-2} d^{-1} (polymethyl methacrylate) to 10.92 g m^{-2} d^{-1} (pine sawdust).[24] The relation between algae productivity on biofilm and surface roughness was also identified by the study of Sekar *et al*., which discovered that the microalgae attachment decreased progressively with increasing smoothness.[53] Secondly, the hydrophilic/hydrophobic property determines the force between algal cells and substratum in the stage of initial adhesion.[54] Normally, hydrophilic materials possess a good liquid-holding capacity.[24,55] Microalgae with hydrophilic surfaces tend to attach to hydrophobic surfaces rather than hydrophilic ones.[25] In the study of Sekar *et al*. that used glass and metal materials (hard substrata) for biofilm construction, experimental results showed that there was a negative correlation between microalgae attachment and the material's wettability.[53] Therefore, to promote the formation of algal biofilm, substrata with hydrophobic surfaces should be selected for the attachment of hydrophilic microalgae. Excellent hydrophobic property is normally present in materials with low surface free energy, so surface free energy could also be considered as a factor in the evaluation of substratum material.[25]

Specific materials

Since lignocellulosic materials, such as sawdust, rice husk, bagasse, and cork, can be obtained at very low cost in agriculture, they are widely used as substrata for microalgae attachment (Table 3). Previous studies indicated that lignocellulosic materials showed advantages over some chemical products in biomass productivity on biofilm.[24,51] For example, biomass productivity on pine sawdust reached 10.92 g m^{-2} d^{-1}, whereas that on polymethyl methacrylate was only 4.01 g m^{-2} d^{-1}. Although lignocellulosic materials perform well in microalgae attachment, the recovery of microalgae attached on lignocellulosic substrata is difficult due to the debris and splitting of the material.[51] Besides, due to the substratum erosion caused by microorganisms, durability of lignocellulosic material used in algal biofilm is not good.

Textile products are a category of materials with a rough surface, which is suitable for microalgae attachment. Compared with stainless steel and glass, nylon has a much rougher surface.[26] Accordingly, algal cells attached to nylon could reach 8.6×10^3 mm^{-2}, whereas algal cells attached on stainless steel and glass were only 6.2×10^3 and 4.7×10^3 mm^{-2}, respectively.[26] Like lignocellulosic materials, textile products can be negatively impacted by the erosion occurring in biofilm development and operation. Accordingly, poor durability and reusability limit the wide use of textile products as substrata in algal biofilm systems.

To increase the duration of substratum, metal products, particularly stainless steel, can be used for microalgae attachment.[56] In the study of Tsavatopoulou and Manariotis, by the end of 16-day cultivation biomass yield on stainless steel reached 21.1 g m^{-2}.[25] However, one of the weaknesses of stainless steel is the material smoothness. In practice, mechanical treatment can be conducted to increase the roughness of metal products, further promoting the initial adhesion of algal cells on substratum.

Algal strain

On the substratum made of titanium, *Nitzschia amphibia* (about 20.0×10^3 cm^{-2}) had much higher cell density than *Chlorella vulgaris* (about 6.8×10^3 cm^{-2}) and *Chroococcus minutus* (about 7.5×10^3 cm^{-2}).[53] A similar phenomenon was observed by Cui *et al*., who grew microalgae on substratum made of stainless steel.[26] In the study of Cui *et al*., attached cells of *Scenedesmus dimorphus* and *Nannochloropsis oculata* on stainless steel reached 6.2×10^3 and 0.63×10^3 mm^{-2}, respectively.[26] The difference between these two algal strains in cell attachment suggests that the properties of algal cells are critical to the formation of biofilm.

Owing to the importance of algal strain to biofilm productivity, some studies worked on the screening of proper algal strains for biofilm development according to the actual requirements. Cheng *et al*. compared 12 algal strains belonging to three phyla – Cyanophyta, Chlorophyta and Euglenophyta – and found that *Chlorella pyrenoidosa* could efficiently remove nutrients in swine wastewater and produce algal biomass on biofilm.[57] Orandi *et al*. isolated a microbial consortium dominated by microalga *Ulothrix* sp. from acid mine drainage and used this consortium to construct algal biofilm for wastewater remediation.[58] During the operation of biofilm system, dominant microalgae may change in different phases. It was reported that dominant algae on biofilm in the initial phase, second phase and third phase were green algae, diatoms and blue-green algae, respectively.[28,29] It was discovered that initial colonization on algal biofilm was mainly composed of *Achnanthes minutissima*, *Chlorella vulgaris*, *Chlorococcum humicolo* and *Cocconeis scutellum*, whereas dominant algae on biofilm after 10-day operation were filamentous green algae and cyanobacteria, indicating the dramatic changes in the algal community during the operation period of biofilm.[29] In our view, filamentous green algae and cyanobacteria have

Table 3. Substratum materials tested for biofilm construction

Material category	Specific material	Material parameter	Algal strain	Biomass attachment parameter	References
Natural bio-products	Loofah sponge	Surface area: 5.06 $m^2\ g^{-1}$; contact angle: 91.65°	*Scenedesmus* sp.	Recovery of attached algae: 51.15 mg g^{-1}	51
	Pine sawdust	Surface roughness: 18.98 μm; groove width: 20.44 μm; groove depth: 49.33 μm	*Scenedesmus obliquus*, *Chlorella vulgaris*, *Oscillatoria tenuis*	Biomass productivity: 10.92 g $m^{-2}\ d^{-1}$	24
	Rice husk	Surface roughness: 10.01 μm; groove width: 47.85 μm; groove depth: 29.29 μm	*Scenedesmus obliquus*, *Chlorella vulgaris*, *Oscillatoria tenuis*	Biomass productivity: 7.32 g $m^{-2}\ d^{-1}$	24
	Oak sawdust	Surface roughness: 11.29 μm; groove width: 15.48 μm; groove depth: 39.31 μm	*Scenedesmus obliquus*, *Chlorella vulgaris*, *Oscillatoria tenuis*	Biomass productivity: 8.07 g $m^{-2}\ d^{-1}$	24
	Sugarcane bagasse	Surface roughness: 11.25 μm; groove width: 26.00 μm; groove depth: 16.68 μm	*Scenedesmus obliquus*, *Chlorella vulgaris*, *Oscillatoria tenuis*	Biomass productivity: 9.54 g $m^{-2}\ d^{-1}$	24
	Cork	Point of zero charge: 4; contact angle: 57.1°; surface free energy: 46.2 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 14.9 g m^{-2}	25
Chemical products	Polyurethane foam	Surface area: 6.12 $m^2\ g^{-1}$; contact angle: 90.05°	*Scenedesmus* sp.	Recovery of attached algae: 40.78 mg g^{-1}	51
	Polymethyl methacrylate	Surface roughness: 0.07 μm	*Scenedesmus obliquus*, *Chlorella vulgaris*, *Oscillatoria tenuis*	Biomass productivity: 4.01 g $m^{-2}\ d^{-1}$	24
	Polystyrene	NA	Mixed algal strains	Biomass productivity: 1.34 g $m^{-2}\ d^{-1}$	52
	Cellulose acetate	NA	Mixed algal strains	Biomass productivity: 2.08 g $m^{-2}\ d^{-1}$	52
	Silicone rubber	Point of zero charge: 6.4; contact angle: 66.4°; surface free energy: 39.5 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 19.1 g m^{-2}	25
	Polycarbonate	NA	Mixed algal strains	Biomass productivity: 1.25 g $m^{-2}\ d^{-1}$	52
Textile products	Denim	Point of zero charge: 6.8; contact angle: < 2°; surface free energy: 71.3 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 12.7 g m^{-2}	25
	Sponge towel	Point of zero charge: 6.8; contact angle: < 2°; surface free energy: 70.9 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 21 g m^{-2}	25
	Nylon	Surface roughness: R_a is 1264 nm, R_q is 68.1 nm, and R_z is 45.4 nm; contact angle: 51°	*Scenedesmus dimorphus*	Attached cells: 8.6×10^3 mm^{-2}	26
Metal products	Stainless steel	Wettability coefficient: 29.9 (hydrophobic)	*Chlorella vulgaris*	Algal cell density: 7.8×10^3 cm^{-2}	53
	Stainless steel	Point of zero charge: 6.6; contact angle: 49.2°; surface free energy: 50.6 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 21.1 g m^{-2}	25
	Stainless steel (1200SA)	Surface roughness: R_a is 383.6 nm, R_q is 70.9 nm, and R_z is 60.7 nm; contact angle: 73°	*Scenedesmus dimorphus*	Attached cells: 6.2×10^3 mm^{-2}	26

Table 3. Continued

Material category	Specific material	Material parameter	Algal strain	Biomass attachment parameter	References
Glass	Glass	Wettability coefficient: 66.6 (relatively hydrophilic)	*Chlorella vulgaris*	Algal cell density: 0.9×10^3 cm^{-2}	53
	Plexiglass	Point of zero charge: 4; contact angle: 70.9°; surface free energy: 35.0 mJ m^{-2}	*Scenedesmus rubescens*	Biomass productivity: 35.1 g m^{-2}	25
	Glass	NA	Mixed algal strains	Biomass productivity: 1.12 g m^{-2} d^{-1}	52
	Glass	Surface free energy: 69.3 mJ m^{-2}; roughness: 8.0 nm	Marine *Chlorella* sp.	Adhesion density: ~1500–2250 cells mm^{-2}	54
	Glass	Surface roughness: R_a is 32.4 nm, R_q is 0.37 nm, and R_z is 0.26 nm; contact angle: 34°	*Scenedesmus dimorphus*	Attached cells: 4.7×10^3 mm^{-2}	26

R_a is the average roughness over the entire sampling length of the surface; R_q is the mean surface roughness; and R_z is the sum of the height of the largest profile peak and the largest profile valley depth within a sampling length; NA, 'not available'.

better performance in attachment, thus having a lower probability of being washed off during operation of the biofilm. Hence, to maintain the functions of algal biofilm, researchers and technicians should not only select appropriate algal strains for inoculation, but also control the dominant algal strains during the operation period. Otherwise, functions and performance of algal biofilm will be dramatically changed with the shift in algal community on substratum.

To our knowledge, in the selection of proper algal strains, some critical factors should be taken into consideration. First, the attachment performance of algal cell is an important factor since algae–substratum interaction plays a key role in the initial adhesion.[18] Normally, microalgae perform well in EPS secretion and own filamentous structures can be regarded as good strains for biofilm development. Second, algal strains should be adaptable to different trophic modes in different stages of biofilm formation and operation. Algal cells perform autotrophic or mixotrophic metabolism in the stage of initial attachment, while the trophic mode of microalgae will be transferred to heterotrophic mode with the increase in biofilm thickness. Third, the characteristics of algal strains inoculated on biofilm should be in accordance with the functions of biofilm. The functions of algal biofilms may include biomass production, recovery of heavy metal, removal of organic nutrient, and so on. For example, Cheng *et al.* and Orandi *et al.* used different algal strains to construct biofilms for the removal of organics and heavy metals in wastewater.[57,58] Fourthly, the algal strains selected for biofilm construction should meet the actual requirement of downstream industry since the utilization of harvested biomass partly determines the economic value of algal biofilms.

Operational factors

Like the suspended microalgae grown in media, algal cells attached on biofilm are impacted by some operational factors, such as illumination, temperature, nutrient concentration and culture density. These operational factors not only determine the biomass productivity of microalgae but also impact the secretion of EPS and the profile of the microbial community.

Illumination, which directly impacts the algal photosynthesis, is of importance to the biomass productivity on biofilm. Specific illumination parameters mainly include light dilution rate, light wavelength and light intensity.[30,31] In addition, illumination also influences the abundance of microbial species on the biofilm. In the study by Chaiwong *et al.*, dominant algal species on biofilm under blue light and red light were *Chlorococcum* sp. (78%) and *Leptolyngbya* sp. (85%), respectively.[33] Chaiwong *et al.* reported that the dominant bacteria – Proteobacteria – on biofilm under blue light were subordinate on biofilm under red light.[33] Hence illumination could have an indirect effect on biofilm formation via changing the profile of the bacterial community.

Since the metabolism of microalgae and bacteria is dependent on nutrient supply, nutrient concentration is a critical factor influencing biofilm formation. First, it has been widely realized that insufficient nutrients may limit algae growth and result in low biomass productivity.[59] Second, nutrient concentration could partly determine the microbial community on biofilm. It was discovered that with increase in sulfate concentration from 1 to 4 g L^{-1}, the percentage of Proteobacteria in the bacterial community increased, whereas the percentage of Bacteroidetes dropped in 7-day culture.[32] Also, the ratio of organic carbon to inorganic carbon in the culture media can influence the abundance and proportions of algae compared to bacteria.[28] Normally, a low ratio

of organic carbon to inorganic carbon in culture media could result in a high proportion of algae in the microbial community on biofilm.[28,60] Third, Li *et al.* reported that EPS concentrations reached about 1.75 and 1.25 g L^{-1}, respectively, when the C/N ratios (molar mass ratios) were set at 12.82 and 0.96, indicating the significance of nutrient concentration to EPS secretion.[61]

When the culture density of microalgae increased from 2×10^2 to 2.3×10^5 cells mL^{-1}, microalgae attachment on both titanium and glass increased to about 23.0×10^3 and 17.0×10^3 cm^{-2}, respectively, suggesting that it is a practically feasible way to promote the formation of biofilm by increasing the inoculation density of algal cells.[53] The main mechanism for this phenomenon is that, as more algal cells are inoculated into the media, the possibility of algae–substratum interaction will increase.

Conflicts and uncertainties

Although some previous studies explored a couple of physical properties of substrata for microorganism attachment, there is no universal explanation for the attachment of different microbial species across different substrata surfaces.[62,63] Sometimes there are a few conflicts between different research studies. For example, Alexander and Williams stated that contact angle is not a good predictor of biological responses to materials, while some studies used contact angle as a critical factor in the evaluation of substrata properties.[62] In our view, the development of algal biofilm is determined by a variety of factors rather than only one factor or parameter. Although the hydrophobic property of substratum is of importance to the initial attachment of microalgae, its exact effects can vary from species to species since the algal adhesion is determined by both substratum property and algal property.[63] In addition, in different periods of biofilm formation, the critical factors influencing algae attachment and biofilm thickening are not the same. It was reported that the cell surface property influenced the initial adhesion of microalgae, while operational factors, such as illumination and nutrient supply, are important to the biofilm thickening.[25,64]

Therefore, the conflicts and uncertainties observed in previous studies are attributed to the complexity of algal biofilms. In the foreseeable future, more detailed studies will be conducted to fully reveal the algae–substratum interactions, algae–algae interactions and algae–bacteria interactions during the formation and operation of algal biofilm. A couple of important parameters that should be taken into consideration include the surface property of algal cells, surface property of substratum, extracellular polymers secreted by algae or bacteria, and so on.

PERFORMANCE OF ALGAL BIOFILM IN WASTEWATER REMEDIATION

The concept of biofilm-based wastewater remediation brings both economic benefit and environmental benefit. As wastewater is used as an alternative culture medium for microalgae growth, the cost of algal biomass can be lowered. In addition, nutrient removal by algal biofilm could solve the environmental problems caused by wastewater. Therefore, in recent years, efforts have been devoted to the fundamental research and applied research of biofilm-based wastewater remediation.

Biomass production

Microalgae cultivation based on nutrient recovery from wastewater has been proven to be a cost-saving way to produce microbial biomass.[65] In previous studies, biomass productivity of microalgae on biofilm reached 1.474 g m^{-2} d^{-1} in hog manure wastewater, 47 g m^{-2} d^{-1} in septic tank effluent and 7 g m^{-2} d^{-1} in sludge-thickening supernatant.[2,33,34] It was reported that the biomass productivity of suspended microalgae in a typical mixotrophic artificial medium (TAP medium) was about 0.367 g L^{-1} d^{-1} (maximum biomass yield of 1.1 g L^{-1} on the third day of cultivation).[66] Thus, in terms of biomass productivity, 1 m^2 algal biofilms grown in hog manure wastewater, septic tank effluent and septic tank effluent are equal to 4.02, 19.07 and 128.07 L mixotrophic artificial medium, respectively. Since algal biofilm can be constructed as a vertical system and wastewater can be obtained at very low cost, biofilm-based microalgae production in wastewater has great advantages in system footprint and production cost.

As presented above, biofilms in wastewater from different sources produced algal biomass at different productivities. The main reason for this phenomenon is that nutrient profiles of wastewater vary. Quan *et al.* operated algal biofilm in landfill leachate with different ratios of nitrogen to phosphorus (N/P) and discovered that the maximum biofilm density of 28.0 g m^{-2} was achieved when the molar ratio of N/P was 16:1.[35] In addition, the system parameters could partly determine the biomass productivity of algal biofilm. For example, when the vertical heights of algal biofilms were set as 0.9 and 1.8 m, biomass productivity (footprint) reached 4.5 and 6.5 g m^{-2} d^{-1}, respectively.[34] Therefore, to increase the biomass productivity, parameters of wastewater nutrient and algal biofilm should be optimized.

Nutrient removal by algal biofilm

Nutrient removal not only influences the biomass yield of algal biofilm but also determines the water quality of wastewater after treatment. Table 4 indicates that a couple of algal biofilms performed well in the treatment of real wastewater and synthetic wastewater. First, vertical algal biofilm-enhanced raceway pond removed 86.37% of total nitrogen (TN), 91.20% of chemical oxygen demand (COD) and 95.19% of total phosphorus (TP) in synthetic wastewater prepared based on the primary settled sewage.[21] High removal efficiency of nutrient suggests that the algal biofilm system could effectively utilize the wastewater-borne nutrients. Secondly, by the operation of an attached-growth photobioreactor, concentrations of TN, COD and TP in septic tank effluent after treatment were reduced to 11, 50 and 1.9 mg L^{-1}, respectively, meeting the discharge standards.[33] Therefore, both removal efficiency and residual concentration of nutrients are important concerns in biofilm-based wastewater remediation.

With the operation of biofilm, microalgae continuously shift between water phase and atmosphere. Hence microalgae attached on substratum not only assimilate organic carbon in wastewater but also capture carbon dioxide. This can be regarded as a representative characteristic of algal biofilm in wastewater remediation. In addition, since some algal biofilms consist of algal–bacterial consortia, nutrient removal is attributed to both microalgal activity and bacterial activity. In most cases, the algal–bacterial consortia show more advantages over pure microalgae for nutrient removal in wastewater remediation. For example, microalgae and bacteria attached on the substratum may establish a cooperative relation in the nutrient removal process. Microalgae could fix carbon dioxide and release oxygen via photosynthesis and, at the same time, oxygen is essential to bacterial metabolism. Accordingly, the exchange of oxygen between microalgae and bacteria is favorable to microorganism growth and nutrient removal in wastewater.

Table 4. Nutrient removal by algal biofilm in wastewater remediation

Wastewater	Biofilm system	Removal of TN	Removal of COD	Removal of TP	Remarks	References
Hog manure wastewater	Algal biofilm photobioreactor	69.55% (initial content: 143 mg L^{-1})	95.67% (initial content: 1126 mg L^{-1})	64.40% (initial content: 16.24 mg L^{-1})	About 100 μmol m^{-2} s^{-1} light intensity around the membrane; 5-day operation	2
Septic tank effluent	Attached-growth photobioreactor (AG-PBR)	77% (final content: 11 mg L^{-1})	72% (final content: 50 mg L^{-1})	71% (final content: 1.9 mg L^{-1})	Blue light; 90-day operation	33
Septic tank effluent	Attached-growth photobioreactor (AG-PBR)	65% (final content: 17 mg L^{-1})	60% (final content: 70 mg L^{-1})	66% (final content: 2.3 mg L^{-1})	Red light; 90-day operation	33
Synthetic wastewater prepared based on primary settled sewage	Vertical algal biofilm-enhanced raceway pond	86.37% (ANR: 0.73 g m^{-2} d^{-1})	91.20% (ANR: 22.31 g m^{-2} d^{-1})	95.19% (ANR: 0.14 g m^{-2} d^{-1})	2 cm operation distance between biofilm layers	21
Synthetic wastewater prepared based on primary settled sewage	Vertical algal biofilm-enhanced raceway pond	89.06% (ANR: 0.76 g m^{-2} d^{-1})	97.18% (ANR: 22.47 g m^{-2} d^{-1})	96.82% (ANR: 0.14 g m^{-2} d^{-1})	6 cm operation distance between biofilm layers	21
Simulated secondary effluent	Algal biofilm membrane photobioreactor (BMPBR)	TIN: 82.5% (RR: 6.19 mg L^{-1} d^{-1}); NH_4^+-N: 96.0% (RR: 2.40 mg L^{-1} d^{-1})	NA	85.9% (RR: 0.35 mg L^{-1} d^{-1})	20-day operation	38
Synthetic wastewater mimicking acid mine drainage	Revolving algae biofilm (RAB) reactor	Ammonia: 69.52% (RR: 15.53 mg L^{-1} d^{-1})	93.98% (RR: 125.3 mg L^{-1} d^{-1})	97.62% (RR: 3.58 mg L^{-1} d^{-1})	1 g L^{-1} sulfate in the influent	32
Synthetic wastewater mimicking acid mine drainage	Revolving algae biofilm (RAB) reactor	Ammonia: 68.33% (RR: 15.26 mg L^{-1} d^{-1})	92.16% (RR: 123.2 mg L^{-1} d^{-1})	98.56% (RR: 3.62 mg L^{-1} d^{-1})	2 g L^{-1} sulfate in the influent	32
Synthetic wastewater mimicking acid mine drainage	Revolving algae biofilm (RAB) reactor	Ammonia: 52.11% (RR: 11.64 mg L^{-1} d^{-1})	95.53% (RR: 127.4 mg L^{-1} d^{-1})	98.96% (RR: 3.62 mg L^{-1} d^{-1})	4 g L^{-1} sulfate in the influent	32
Synthetic wastewater	Microalgal biofilm	NO_3-N concentration < 2.2 mg N L^{-1}	NA	PO_4^3-P concentration < 0.15 mg P L^{-1}	15-day continuous operation with loading rates of 1.0 g NO_3^--N m^{-2} d^{-1} and 0.13 g PO_4^{3-}-P m^{-2} d^{-1}	42
Sludge thickening supernatant from municipal wastewater treatment facility	RAB	RR: 2300 mg m^{-2} d^{-1}	NA	RR: 660 mg m^{-2} d^{-1}	RAB with 1.8 m height; 1.3-day HRT	34
Sludge thickening supernatant from municipal wastewater treatment facility	RAB	RR: ~420 mg m^{-2} d^{-1}	NA	RR: ~90 mg m^{-2} d^{-1}	RAB with 1.8 m height; 7-day HRT	34

Table 4. Continued

Wastewater	Biofilm system	Removal of TN	Removal of COD	Removal of TP	Remarks	References
Landfill leachate	Microalgal biofilm integrated with ozonization treatment	81.6%	64.3%	100%	N/P molar ratio of 16:1	35
Anaerobically digested swine wastewater	Microalgal biofilm	NH_3-N: 82.2% (initial content: 578.27 mg L^{-1}) NO_3-N: 84.3% (initial content: 63.03 mg L^{-1})	74.8% (initial content: 385.77 mg L^{-1})	70.3% (initial content: 39.12 mg L^{-1})	8-day operation	57
Synthetic wastewater prepared based on the piggery wastewater	Vertical algal biofilm-enhanced raceway pond	82.00% (ANR: 3.08 g m^{-2} d^{-1})	86.81% (ANR: 85.03 g m^{-2} d^{-1})	93.84% (ANR: 1.88 g m^{-2} d^{-1})	2 cm operation distance between biofilm layers	21
Synthetic wastewater prepared based on the piggery wastewater	Vertical algal biofilm-enhanced raceway pond	81.69% (ANR: 2.96 g m^{-2} d^{-1})	97.17% (ANR: 123.19 g m^{-2} d^{-1})	95.38% (ANR: 2.86 g m^{-2} d^{-1})	6 cm operation distance between biofilm layers	21

ANR, average nutrient removal rate; HRT, hydraulic retention time; NA, not available; RAB, rotating algal biofilm; RR, removal rate; TIN, total inorganic nitrogen.

Like the suspended microalgae, attached microalgae are impacted by a variety of physical factors, such as illumination and temperature, in the removal of nutrients.[30,67,68] Based on the modeling analysis, Tenore *et al*. stated that light is confirmed as the most significant factor in the ecology of phototrophic–heterotrophic biofilms.[68] In the study of Chaiwong *et al*., algal biofilms operated under red light and blue light removed different contents of nutrients in wastewater.[33] In addition, light intensity and light–dark cycle could influence the content of algal biomass, which further determine the performance of biofilm in nutrient removal.[30] Not only physical factors, but also algal–bacterial interactions could impact the biofilm-based wastewater remediation.[69] Katam *et al*. found that algal–bacterial cooperation is beneficial to carbon and nutrient removal by algal biofilm in domestic wastewater.[67]

CHALLENGES AND POTENTIAL SOLUTIONS

As shown in Table 4, only a few recent studies have successfully used algal biofilm to treat real wastewater in laboratory research, while many studies have worked on testing algal biofilm in synthetic wastewater. Besides, the implementation of algal biofilm at an industrial scale in wastewater treatment plants is rare. In the view of the present authors, the industrial implementation of microalgal biofilm is hindered by some challenges related to bacterial contamination, biomass utilization, nutrient removal and ecological disturbance (Fig. 2). In this section, solutions to the aforementioned challenges and prospects for microalgal biofilm in the foreseeable future are discussed in detail.

Challenges

Bacterial contamination

Table 4 demonstrates that many studies used synthetic wastewater in the operation of algal biofilm. For example, algal biofilm systems have been used to treat synthetic wastewater prepared based on primary settled sewage, secondary effluent, acid mine drainage and piggery wastewater.[21,32,38] However, to our knowledge, there are a couple of differences between synthetic wastewater and real wastewater. One of the differences is that the bacterial community of real wastewater is much more complicated than that of synthetic wastewater. Besides, in the industrial implementation of algal biofilm, microalgae are exposed to the outdoor ambient environment, which usually contains higher density of bacteria or fungal spores than the laboratory environment. Hence bacterial contamination caused by the direct contact of algal biofilm with airborne bacteria and wastewater-borne bacteria should be considered as a challenge in the industrial implementation of algal biofilm.

Bacterial contamination on algal biofilm may result in the failure of microalgae growth and the low quality of algal biomass. First, there is intensive competition between bacteria and microalgae for nutrients in wastewater. In wastewater without sufficient nutrients, fast growth of bacteria may lower the concentration of nutrients available to microalgae, resulting in the failure of algae growth. Second, some pathogenic or toxic bacteria may grow on biofilm with microalgae together in the wastewater treatment. In this case, harvested biomass from algal biofilm can be pathogenic or toxic. As a consequence, the utilization of biomass in downstream industry will be a serious challenge.

Biomass utilization

At present, the production of high-value algal biomass enriched with bioactive compounds is regarded as a promising development trend in the microalgae industry. Previous studies have successfully integrated wastewater remediation with the production of high-value algal biomass. For example, food industry effluents such as molasses, dairy wastewater, brewery effluent and meat processing wastewater, with no toxic compounds, have proven to be good media alternatives for the production of high-value microalgae.[59,70,71] Harvested biomass has the potential for use as ingredients of animal feed and agricultural bio-fertilizer.[72]

Now, however, such a value-added process may not be applicable in the implementation of algal biofilm. First, some algal strains, including *Dunaliella salina* and *Haematococcus pluvialis*, enriched with high-value compounds have not been widely used as dominant microorganisms to construct algal biofilm. In other words, algal biofilms to produce certain types of algal bioactive compounds, such as astaxanthin, β-carotene and polyunsaturated fatty acids, have not been developed. Second, most previous studies mainly focused on the biomass yield or productivity on algal biofilm, but neglected the nutritional values of algal biomass. Hence, although some high-value algal strains were observed in microbial consortia on biofilm in previous studies, methods to induce the biosynthesis of bioactive compounds in microalgae attached on biofilm have not been fully studied. Third, as discussed above, potential bacterial contamination occurring in the wastewater-based algal biofilm operation may threaten the safety of harvested biomass, limiting the use of microalgae in downstream industry.

Nutrient removal

Nutrient removal efficiency of algal biofilm in some real wastewater is low, suggesting that the concentrations of residual nutrients in treated wastewater might be high (Table 4). For example, the removal efficiency of NH_3-N, COD and TP in anaerobically digested swine wastewater reached 82.2%, 74.8% and 70.3%, respectively.[57] In this case, the concentrations of residual nutrients, NH_3-N, COD and TP, in treated wastewater (8-day treatment by algal biofilm) were 102.93, 97.21 and 11.62 mg L^{-1}, respectively.[57] Accordingly, the treated wastewater could not be directly discharged or reused. One of the main reasons for this challenge is that microorganisms, including microalgae and bacteria, on biofilm could not fully degrade the solid organics in wastewater. In the wastewater treatment process, microorganisms on biofilm could directly assimilate the dissolved nutrients, but the degradation of solid organics is a more complicated process. Normally, microalgae and bacteria should secrete extracellular enzymes to convert solid organics into low-molecular-weight nutrients. Then, the assimilation of low-molecular-weight nutrients by microalgae will contribute to the water cleaning. In this process, a high proportion of nutrients with poor biodegradability will result in the low removal efficiency.

Ecological disturbance

As reported by previous studies, the washout of algal cells from biofilm frequently occurs in the continuous operation of biofilm system.[22,42] Although some research efforts attempted to solve this problem by modifying the system structure and reducing the fluid shear force, the washout of algal cells could not be absolutely prevented. Under this situation, in the continuous operation of algal biofilm system, a large quantity of microalgae will enter wastewater, further posing a threat to the ecological balance in nature after the discharge of effluent. Although sterilization could rule out the negative effects of algae and bacteria on ecological balance, this method is costly and would not be practical in the industry. In this case, if algae and bacteria detached from biofilm flow into natural waters with the discharge of effluent, they may become inducers of algae bloom in eutrophic waters and seriously disturb the ecological balance.

Potential solutions

The solutions to the aforementioned challenges or problems are of importance to the wide application of algal biofilm at an industrial scale. In the view of the present authors, both fundamental research and applied research should be conducted to further promote the industrialization of algal biofilm.

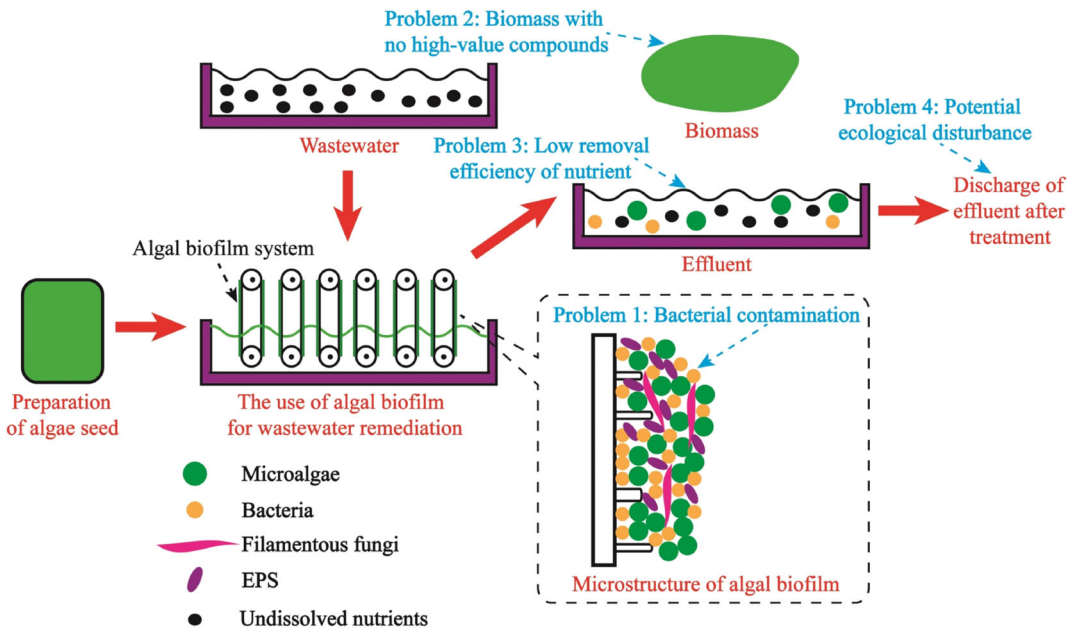

Figure 2. Problems in the employment of algal biofilm for wastewater remediation.

First, changes in bacterial community on the biofilm during continuous operation should be fully revealed. In particular, in-depth studies of the relations between microalgae and bacteria in nutrients assimilation should be conducted. Previous studies reported that bacteria may cooperate with microalgae for nutrient removal in wastewater remediation.[69,73] Such a cooperation model between bacteria and microalgae can be regarded as a synergism. If the methods promoting the development of synergistic relation on biofilm can be identified, the failure of microalgae growth caused by bacterial contamination can be avoided. This will be beneficial to the industrial application of algal biofilm.

Second, algal strains with high-value compounds should be screened and employed for biofilm construction. In fact, a few studies have devoted efforts to the development of biofilm with high-value microalgae. For example, Cheng *et al*. used *Chlorella pyrenoidosa* enriched with protein for biofilm-attached culture and harvested algal biomass composed of 57.30% proteins.[57] Besides, amino acids in the proteins of *Chlorella pyrenoidosa* grown on biofilm contained 21.73% essential amino acids.[57] In the study of Thanh-Tri *et al*., *Haematococcus pluvialis* with high content of astaxanthin was immobilized on a twin-layer porous substrate photobioreactor (TL-PSBR). Thus the biofilm system produced astaxanthin-rich biomass (astaxanthin content: 2–3% of dry weight).[74] Therefore, with the wide use of high-value algal strains, the operation of biofilm for biomass production will become a value-added process.

Third, strategies to improve nutrient removal in biofilm-based wastewater treatment mainly include pretreatment of wastewater and development of algal–bacterial cooperation. By appropriate pretreatment, such as anaerobic digestion or chemical oxidation, nutrients in wastewater will become more biodegradable. For example, in anaerobic digestion, organic carbon in wastewater is converted to volatile fatty acids, which can be assimilated by algal cells in an efficient way. In addition, development of algal–bacterial cooperation is favorable to nutrient removal in wastewater. In the cooperation, bacteria degrade nutrients in wastewater and generate CO_2, which can be captured by microalgae via photosynthesis. At the same time, O_2 produced by microalgae in photosynthesis is an essential component for the heterotrophic metabolism of bacteria.

Fourth, potential effects of industrial application of algal biofilm on the environment should be fully assessed. For example, the profile of the microbial community in wastewater after treatment by algal biofilm should be analyzed. Microorganisms with the potential of causing biological invasion should be killed before the discharge of wastewater. In a real-world application, environmental safety of algal strains inoculated on biofilm should be evaluated. In this way, ecological risks caused by the discharge of wastewater with microalgae or bacteria can be reduced.

CONCLUSIONS

Development of microalgal biofilm is a promising way to produce algal biomass and treat wastewater. Based on the fundamental research of biofilm formation and the roles of algae and bacteria, major mechanisms associated with the formation and operation of algal biofilm have been revealed. In addition, factors including substratum material, algal strain and operational factors, which impact the properties of the algal biofilm, were optimized. The practical feasibility of employing algal biofilm for biomass production and wastewater remediation was fully evaluated by previous studies. At present, the biotechnology of algal biofilm is at a critical stage towards industrial implementation. In the view of the present authors, major problems jeopardizing the wide application of algal biofilm are related to bacterial contamination, biomass utilization, nutrient removal and ecological disturbance. It is expected that by addressing these problems algal biofilm will be widely used for the efficient wastewater remediation and high-value biomass production.

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