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Review

Microalgal–Bacteria Biofilm in Wastewater Treatment: Advantages, Principles, and Establishment

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Abstract: The attached microalgal–bacterial consortium (microalgae–bacteria biofilm, MBBF) has been increasingly recognized in wastewater treatment for its superior pollutant removal efficiency, resilience to toxic substances, and improved harvesting performance. This review initially discusses the advantages of MBBFs compared to activated sludge and suspended microalgal–bacterial consortia. These advantages stem from the coexistence of pollutant removal pathways for the bacteria and microalgae in MBBFs, as well as the synergistic interactions between the microalgae and bacteria that enhance pollutant removal and resilience capabilities. Subsequently, the establishment of the MBBF system is emphasized, covering the establishment process, influencing factors of MBBF formation, and the utilization of photobioreactors. Lastly, the challenges associated with implementing MBBFs in wastewater treatment are deliberated. This study aims to present a detailed and comprehensive overview of the application of MBBFs for wastewater treatment and biomass production.

Keywords: wastewater; microalgal–bacterial consortium; biofilm; photobioreactor; pollutant removal

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1. Introduction

The application of microalgal–bacterial consortium (MBC)-based wastewater treatment has gained recognition as a potent method for pollutant elimination, offering competitive advantages in pollutant removal, carbon neutrality, and the potential for valuable chemical production [1,2]. The symbiotic interplay between microalgae and bacteria involves the exchange of metabolic byproducts, diverse pathways for pollutant removal, and the reinforcement of structural stability. These synergistic interactions enhance the metabolic activity and environmental tolerance of microorganisms, simultaneously improving pollutant removal and biomass harvesting. For instance, the incorporation of symbiotic bacteria resulted in a 22.1% improvement in chemical oxygen demand (COD) removal efficiency, a 20% increase in total nitrogen (TN) removal, and an 8.1% enhancement in total phosphorus (TP) removal by *Chlorella*. Additionally, concentrations of chlorophyll a, b, and carotenoids exhibited respective elevations of 35.7%, 20.9%, and 11.2% [3]. However, considering the intricate nature of wastewater composition, elevated pollutant concentrations, and the presence of various toxic substances in contemporary wastewater treatment processes, conventional MBCs face challenges in maintaining stable and efficient treatment capacities under such complex wastewater conditions.

MBCs exist in suspended, granular, and biofilm configurations [4]. In a suspended MBC, the biomass is relatively dispersed due to the small size of the microorganisms' cells and the negative surface borne by the microalgae. These factors induce cell repulsion,

resulting in a lower proportion of microorganism biomass and susceptibility to outflow losses. Therefore, maintaining stable sewage treatment efficiency and managing downstream microalgae harvesting costs pose challenges for the suspended MBC [5]. A granular MBC effectively overcomes the limitations of a suspended MBC. However, its broad implementation encounters hurdles such as the complexity of MBC particle formation and granulation, along with the high expenses of embedding substrates [4]. An alternative approach is an attached MBC, which entails selectively adsorbing bacteria and microalgae onto an inert carrier surface to establish a microalgae–bacteria biofilm (MBBF). The MBBF mitigates the light attenuation caused by the high biomass in a suspension MBC. Increased secretion of extracellular polymeric substances (EPSs) bolsters the MBC's resistance to external disruptions, thereby enhancing sewage treatment efficacy. Moreover, the ease of biomass harvesting in an MBBF also contributes to lowered energy consumption during subsequent biomass separation [6].

Compared to conventional activated sludge processes and suspended MBCs, the symbiotic relationship between microalgae and bacteria in an MBBF confers notable advantages in biomass harvesting and pollutant removal. This is primarily attributed to the photosynthetic activity of microalgae and the oxidative decomposition facilitated by bacteria. To be more specific, firstly, MBBFs exhibit heightened biomass levels and wastewater treatment efficacy. The metabolic byproducts of the bacteria supply carbon sources and growth hormones for microalgae growth, thereby sustaining robust growth and metabolic activity [4]. Simultaneously, the diverse pollutant removal pathways inherent in MBCs efficiently reduce the concentrations of various pollutants (carbon, nitrogen, and phosphorus) to exceedingly low levels [7]. Secondly, MBBFs exhibit superior resilience against shock loads. The EPSs secreted by the bacteria facilitate MBCs' adhesion to the carrier surface (e.g., the polyvinyl chloride of plastic materials and polypropylene of synthetic fibers), forming a protective barrier that enables the MBBF to withstand harsh environments (e.g., dry conditions, extreme pH levels, and temperature fluctuations) and counteract toxic agents [8]. Thirdly, MBBFs demand lower energy input. The microalgae's photosynthesis yields O_2 for bacterial metabolism, thereby curtailing aeration energy consumption. In turn, the bacteria provide CO_2 to the microalgae, lessening the necessity for additional carbon sources. Furthermore, unlike suspended MBCs, where light attenuation restricts light exposure to only one-third of cells, the formation of MBBFs ensures adequate illumination for all cells [9].

However, the efficiency of MBBFs is frequently impeded by an extended initiation phase, with material characteristics and external factors significantly influencing the duration of MBBF formation. For instance, excessive light intensities might impede microbial synthesis metabolism, thereby delaying the formation of the MBBF [10]. Additionally, negatively charged carriers might resist microbial attachment [11]. In light of the effective and reliable pollutant treatment capacity of MBBFs, it is essential to comprehensively grasp its principles and structure, laying the foundation for broader-scale applications and ongoing improvement in the future. Therefore, the purposes of this study are as follows: (1) Thoroughly expound on the advantages of MBBFs compared with other wastewater treatment strategies, highlighting the competitiveness of MBBFs in pollutant treatment and energy recovery. (2) Delve into the principles underpinning the use of MBBFs for wastewater treatment, including various pollutant removal pathways (carbon, nitrogen, and phosphorus) and the synergistic interactions between bacteria and microalgae. (3) Introduce the strategy of establishing MBBFs, detailing the biofilm formation process, fundamental principles, influential factors, and the utilization of photobioreactors (PBRs). (4) Address the challenges encountered in the application of MBBFs and outline future research priorities.

2. Advantages of MBBFs in Comparison with Other Wastewater Treatment Technologies

Compared to traditional wastewater treatment strategies, the presence of bacteria in MBBFs amplifies the metabolic activity of microalgae and enhances their adaptability to complex wastewater compositions, thus endowing them with the capacity to break down emerging pollutants such as antibiotics and heavy metals. Furthermore, due to the heightened aggregation of microorganisms in the biofilm structure, it simplifies the collection process without significant losses, as compared with suspended MBCs. The extracted valuable compounds, such as fucoxanthin and fatty acids, from the increased biomass not only serve as raw materials for biofuels, animal feed, and other applications, but also elevate the economic benefits associated with this technology. Additionally, the utilization of MBBFs for carbon sequestration is gaining attention as a viable approach for handling high-concentration CO_2 emissions from flue gases.

2.1. Emerging Pollutant Removal

MBBFs have demonstrated remarkable efficacy in the removal of emerging pollutants such as antibiotics and heavy metals, highlighting their broad applicability in wastewater treatment. Table 1 summarizes the elimination capacities and mechanisms underpinning the removal of emerging pollutants when employing MBCs. Kuang et al. [12] developed an electroactive biofilm composed of electroactive bacteria (Geobacteraceae and Pseudomonas) and marine microalgae (Pseudooceanicola and Hoeflea) to treat seawater aquaculture wastewater, achieving remarkable simultaneous removal efficiencies of 99.25% for sulfamethoxazole and 98.25% for Cu^{2+}. In another study, a mixotrophic photoelectroactive biofilm reactor was operated which incorporated light intensity regulation for microalgae and extracellular electron extraction for bacteria, effectively achieving enhanced nutrient removals (65% NH_4^+-N, 95% PO_4^{3-}-P, and 52% sulfamethoxazole) [13]. Moreover, MBBFs effectively eliminated a range of heavy metals from wastewater streams, such as chromium, copper, and cadmium, through the biological processes of adsorption and bioaccumulation [14]. A similar observation was also achieved when applying an MBBF to treat Se-rich aquaculture wastewater, which successfully reduced the Se level from 115 μg/L to 18.7 μg/L, and 21.8 mg/kg of Se was accumulated by the MBBF [15]. The increased adaptability of MBBFs to antibiotics could be attributed to the following factors: (1) Microorganisms engage in metal ion absorption through biological adsorption mechanisms. This process entails metal ions adhering to cell surfaces via physical adsorption, van der Waals forces, ion exchange, chelation, or inorganic microprecipitation mechanisms [16]. Approximately 21.8 mg/kg of Se was attached onto the cell surface by the MBBF before being assimilated by cells through binding transporters [15]. (2) The EPS secreted during MBBF formation establishes a shielding barrier against toxic substances, effectively averting direct contact between microorganisms and harmful compounds. The diffusion coefficient of antibiotics in polysaccharides or glycoproteins is only 36–76% compared to that in water [17]. Antibiotic-degrading bacteria, such as *Pseudomonas*, are shielded by the EPS produced by the microalgae microbiota, thereby ensuring the stability of antibiotic removal processes [18]. (3) Microalgae play a pivotal role in assisting bacteria in the breakdown and metabolism of antibiotics. Upon external stimulation, microalgae release extracellular enzymes dedicated to degrading antibiotics into smaller, more easily metabolizable molecules absorbed by bacteria [19]. For instance, subsequent to the initial degradation of norfloxacin by *Chlorella vulgaris*, the ATP levels and norfloxacin removal rates of activated sludge escalated significantly by 1.26 times.

Table 1. Removal of emerging pollutants by, and mechanisms of, MBCs.

Microalgae–Bacteria Consortium	Emerging Pollutants Types	Pollutant Concentration	Removal Efficiency	Removal Mechanism	Reference
Mud from Sanyuan Lake and *Scenedesmus obliquus FACHB-12*	Chlortetracyc (CTC)	80 mg/L	CTC: 79.7 ± 2.2%	Biosorption and enzymatic biodegradation	[12]
C. vulgaris and *B. licheniformis*	Oxytetracycline (OTC) and enrofloxacin (EFX)	OTC < 5 mg/L, EFX < 1 mg/L	OTC: 97.84~99.76% EFX: 42.68~42.90%	Photodegradation and biological effects	[13]
Sediments mixed with the aquaculture waster for the formation of the MBBF	Se	115 ± 5 μg/L	Se: 83.74%	Sulfate pathway	[15]
H. pluvialis and activated sludge	Sulfamethoxazole (SMX), Tetracycline and Erythromycin (ERY)	ERY (100 mg/L), SMX (100 mg/L) and TET (37.3 mg/L)	SMX: 97.08% ERY: 98.15% TET: 89.73%	Biosorption	[20]
Scenedesmus almeriensis biomass was harvested from an HRAP	Tetracycline (TTC), ciprofloxacin (CPF), sulfadiazine (SDZ) and sulfamethoxazole (SMX)	100 μg/L	TTC: 99.9% CPF: 78.0% SDZ: 52.6% SMX: 5.0%	Biosorption and biodegradation	[21]
Chlorella sorokiniana and *Brevundimon*	Cephalexin (CEP) and Erythromycin (ERY)	50 μg/L	CEP: 96.54 ± 5.31% ERY: 92.38 ± 3.13%	Biodegradation	[22]
Chlorella sp., *Spirulina* platensis and *Artemia* sp.	Ketoprofen	16 mM	degraded up to 16 mM ketoprofen	Biodegradation	[23]
Photo-rotating biological contactor: *Ulothrix* sp.	Cu	80–100 mg/L	Cu: 50%	Biosorption	[24]
Chlorella sp. and *B. tropica*	Hg	0.041 mg/L	Hg: 86%	Biosorption	[25]

2.2. High Value of Microalgal–Bacterial Biomass

The microalgal–bacterial biomass of MBBFs has been emphasized as a sustainable and abundant biological resource, boasting the potential for biofuel production and high-value chemical extraction [26]. On the one hand, MBCs synthesize more substantial lipid reserves during their growth as compared with many terrestrial plant species [27], rendering them a promising source of biofuel. Additionally, the use of MBBFs present advantages in terms of reduced harvesting costs and higher biomass yields than suspended MBCs, positioning it as a potentially ideal strategy for biofuel production [7]. Ge et al. [28] showed that under mixed nutrient conditions in wastewater treatment, the simultaneous enhancement of nutrient removal and lipid production in *Chlorella vulgaris* underscored the potential for utilizing the microalgal–bacterial biomass cultured in wastewater as biofuel feedstock. MBCs exhibit the capacity to yield diverse types of sustainable biofuels, including biodiesel derived from microalgae lipids, biohydrogen generated by photobiological reactions, biomethane, and bioethanol produced through an anaerobic digestion process. Among these, the biodiesel synthesis through the ester exchange reaction of algal oil stands out as a highly effective method for biofuel production. Glycerol plays the predominant component of biodiesel, comprising up to 1 kg per every 10 kg of biodiesel [29]. Biodiesel not only curtails CO_2, hydrocarbons, and carbon monoxide, but also functions as a direct alternative for compression ignition engines or as a supplementary component to conventional fossil diesel. Certain unicellular green microalgae exhibit the capability to produce hydrogen through

photolysis, using water and sunlight as energy sources. In anaerobic conditions, anaerobic bacteria stimulate the microalgae hydrogenase to reverse and generate hydrogen [30]. The cell wall of an MBC contains a significant amount of cellulose and starch, which serve as substrates for anaerobic digestion aimed at producing methane and ethanol [31]. Nevertheless, the dense structure of the cell wall poses challenges for its hydrolysis and acidification processes.

On the other hand, the microalgal–bacterial biomass holds notable edible and medicinal value attributed to the presence of diverse antioxidant compounds, such as carotenoids, phenols, and vitamins [32]. The consumption of antioxidant-rich foods shields the body against oxidative stress damage induced by reactive oxygen species, potentially augmenting human longevity [33]. Qiu et al. [34] unveiled that microalgae cultivated in wastewater possessed remarkable antioxidant properties, making them an effective alternative feed for fruit flies. Astaxanthin, a potent carotenoid, exerts effective inhibition against lipid peroxidation induced by free radicals, manifesting antioxidant, anti-aging, anti-tumor, and preventive attributes concerning cardiovascular and cerebrovascular diseases. Its ability to scavenge free radicals surpasses that of carotenoid by more than 10 times and eclipses vitamin E's potency by over 100 times. *Rainy red* microalgae contain substantial astaxanthin content, constituting 4–7% of the cells' dry weight, positioning them as the premier biological source for natural astaxanthin production [35]. Another recently researched carotenoid, fucoxanthin, exhibits promise in mitigating oxidative damage induced by reactive oxygen species and hydrogen peroxide. Its anti-obesity, anti-tumor, and anti-inflammatory properties designate it as a highly anticipated contender for drug development [36]. *Phaeodactylum tricornutum* has been shown to achieve the simultaneous synthesis of medium-chain fatty acids and fucoxanthin while concurrently removing nitrogen during wastewater treatment processes [37].

2.3. Carbon Capture

The utilization of microalgae in MBBFs for capturing industrial CO_2 has been extensively documented in the literature, with the potential to reduce carbon emissions by up to 50% [38]. Theoretically, one hectare of microalgae could sequester a maximum of 513 tons of CO_2 per year to produce 280 tons of dry biomass, with a carbon capture rate of 1.83 tons per ton of biomass, positioning microalgae platforms as the most suitable technology for carbon capture [39]. Valdovinos et al. [40] achieved a notable CO_2 capture rate of 102.13 tons per year per hectare through the cultivation of *Chlorella* in waterway ponds. Within MBBF systems, the microalgae release dissolved organic carbon for bacterial consumption, while the bacteria mineralize sulfur, nitrogen, and phosphorus to facilitate the continued growth of the microalgae [41]. Compared to other carbon capture technologies, the utilization of MBBFs for CO_2 capture is deemed an optimal approach due to their capacity in resource recovery, utilization of wastewater as a nutrient source, and enhanced CO_2 absorption properties [15].

In open systems, microalgae have the ability to maintain atmospheric CO_2 concentrations at 400 ppm and even survive in smoke containing up to 150,000 ppm of CO_2 [42]. Their exceptional tolerance enables them to thrive in direct exposure to flue gas emitted by thermal or coal-fired power plants. Currently, microalgae carbon capture technology has been extensively employed for the large-scale purification of industrial flue gases. For instance, in a carbon capture project in Inner Mongolia, microalgae were utilized to absorb CO_2 from the flue gas of coal-fired power plants, sequestering 20,000 tons of CO_2 annually and generating 600,000 tons of methanol and biodiesel [39]. Another carbon capture facility, based on a pilot-scale microalgae system, boasted a total CO_2 capture capacity of 320,000 tons per year [43].

3. Pollutant Removal Pathways and Collaborative Mechanism of MBBFs

3.1. Multiple Pollutant Removal Pathways

As a promising technology for achieving low-carbon wastewater resource recovery in the future, MBBFs necessitate a comprehensive understanding of their pollutant removal mechanisms and the microbial interactions within biofilms for industrial applications. Figure 1 summarizes the diverse metabolic pathways through which MBCs remove pollutants, as mentioned in previous studies, including assimilation (nitrogen and phosphorus removal) and photosynthesis (carbon removal) by microalgae as well as nitrification–denitrification (nitrogen removal) and polyphosphate aggregation (phosphorus removal) by bacteria [4,7]. Through the synergistic effects of multiple metabolic pathways and the interactive dynamics between bacteria and microalgae, MBBFs exhibit the capability to diminish pollutants to exceptionally low levels.

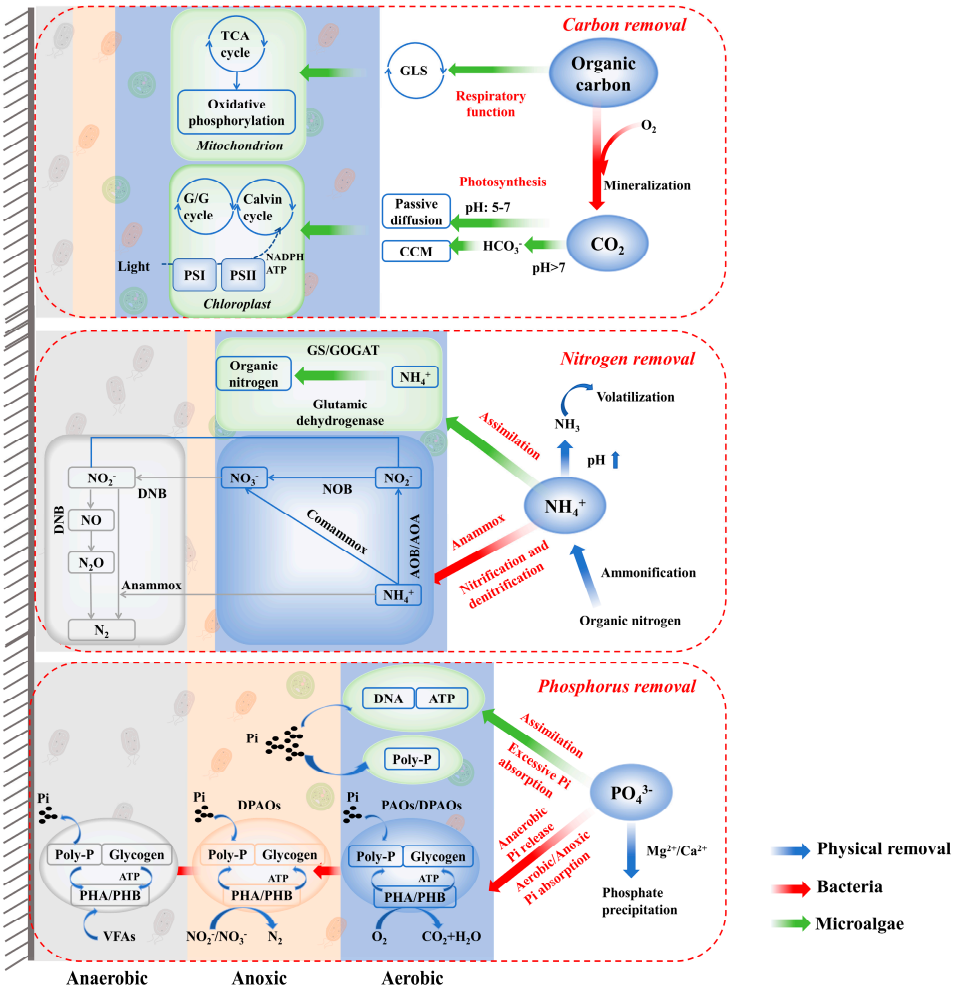

Figure 1. Pollutant removal pathways of MBBFs, including carbon removal, nitrogen removal, and phosphorus removal. AOB: ammonia-oxidizing bacteria; AOA: ammonia-oxidizing archaea; NOB: nitrite-oxidizing bacteria; DNB: Denitrifying bacteria; Comammox: complete ammonia oxidation; Anammox: anaerobic ammonia oxidation; PAOs: polyphosphate-accumulating organisms; DPAOs: denitrifying phosphate-accumulating organisms; Poly-P: polyphosphates; PHA: polyhydroxyalkanoates; PHB: polyhydroxybutyrates; VFAs: Volatile fatty acid; GLS: glycolysis; CCM: CO_2 concentration mechanism; GS/GOGAT: glutamine synthetase/glutamine oxoglutarate aminotransferase; TCA cycle: Tricarboxylic acid cycle.

3.1.1. Carbon Removal

In MBBFs, the removal of carbon pollutants primarily occurs through oxidative decomposition by heterotrophic bacteria and heterotrophic and mixotrophic microalgae, as well as through the activities of microalgae photosynthesis. On the one hand, microalgae utilize both inorganic and organic carbon sources for growth. In environments with neutral to acidic conditions (pH = 5–7), inorganic carbon predominantly exists as CO_2, which microalgae absorb into cells through passive diffusion driven by osmotic pressure. The absorbed CO_2 is subsequently converted into organic compounds, such as glucose, through the Calvin cycle during the dark reaction stage, facilitated by ribulose-1,5-bisphosphate carboxylase/oxygenase [44]. However, the pH of the wastewater gradually rises due to the ongoing photosynthesis by the microalgae. Inorganic carbon primarily exists in the form of HCO_3^- when the pH exceeds 7, leading to an alkaline environment. Microalgae employ extracellular carbonic anhydrase to actively transport HCO_3^- into the cells, where it is converted to CO_2 before entering the Calvin cycle [45]. Regarding organic carbon sources, glucose proceeds to the tricarboxylic acid cycle (TCA) after glycolysis, while acetate is transformed into Acetyl-CoA that subsequently enters the glyoxylate cycle, tricarboxylic acid cycle, and fatty acid synthesis pathway. Conversely, heterotrophic bacteria employ organic carbon pollutants as electron donors and O_2 as electron acceptors to oxidize and decompose carbon-containing organic matter into CO_2 through mineralization. This process enhances the photosynthetic efficiency of microalgae. Moreover, versatile microalgae could simultaneously conduct photosynthesis and respiration, enabling the removal of both CO_2 and organic carbon from wastewater [46].

3.1.2. Nitrogen Removal

The nitrogen removal pathways in MBBFs encompass assimilation by microalgae, nitrification–denitrification, and anammox by bacteria. Wastewater typically contains two forms of nitrogen: inorganic nitrogen and organic nitrogen, both of which could be utilized by MBCs. Inorganic nitrogen consists of three primary forms: NH_4^+-N, nitrate (NO_2^--N), and nitrite (NO_3^--N). In alkaline environments (usually pH > 8), a portion of NH_4^+-N is converted into ammonia and eliminated through volatilization [47]. While physical removal methods effectively eliminate a significant amount of nitrogen, particularly in cases when the wastewater has a high NH_4^+-N concentration (>1000 mg/L), biological processes are still essential for further reducing nitrogen levels to the desired target.

Microalgae play a significant role in nitrogen assimilating, converting both organic and inorganic nitrogen into amino acids. Prior to microalgae assimilation, all forms of organic and inorganic nitrogen are converted into NH_4^+-N. Enzymes such as nitrate reductase and nitrite reductase facilitate the conversion of NO_2^--N and NO_3^--N into NH_4^+-N, while organic nitrogen compounds like urea and proteins are mineralized into NH_4^+-N through intracellular urease or extracellular periplasmic amino acid-supported transformations [46,48–50]. Subsequently, microalgae convert NH_4^+-N into amino acids via two pathways: the glutamate dehydrogenase (GDH) pathway and the glutamine synthetase/glutamine oxoglutarate transaminase (GOGAT) cycle [4].

Bacteria in MBBFs facilitate nitrogen removal through nitrification–denitrification and anaerobic ammonia oxidation. Various types of bacteria are involved in these processes, including ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), nitrite-oxidizing bacteria (NOB), denitrifying bacteria (DNB), complete ammonia-oxidizing bacteria (comammox), and anaerobic ammonia-oxidizing bacteria (anammox). These microbial communities play crucial roles in the nitrification–denitrification process within the system. Specifically, in the nitrification process, AOB/AOA oxidize NH_4^+-N to NO_2^--N under aerobic conditions, which is further oxidized by NOB to NO_3^--N. In contrast, comammox bacteria have the ability to directly convert NH_4^+-N to NO_3^--N. Comammox bacteria demonstrate superior environmental adaptability compared to AOB/AOA, thriving in extreme conditions such as high temperatures (up to 56 °C), low levels of dissolved oxygen (~0.2 mg/L), and low NH_4^+-N concentrations, making them a promising alternative for

nitrogen removal [4]. In the denitrification stage, DNB convert NO_3^--N into nitrous oxides (NO, N_2O, and N_2) through denitrification in the anoxic regions of the biofilm, requiring organic carbon for energy metabolism [51,52]. Apart from the conventional nitrification–denitrification, the unique metabolism, namely anammox, independently carries out the nitrogen treatment process. With its elaborate enzyme system, anammox bacteria convert NH_4^+-N into NO_2^--N under anaerobic conditions and directly produce N_2 [53]. This metabolism is garnering more recognition for its oxygen-independency, minimal need for additional carbon sources, reduced sludge generation, and substantial decrease in energy consumption [54].

3.1.3. Phosphorus Removal

The biological pathways for phosphorus removal in MBBFs primarily involve microalgae assimilation and phosphorus accumulation by polyphosphate-accumulating organisms (PAOs). Phosphorus assimilation by microalgae consists of two pathways: Firstly, direct absorption of phosphorus for the synthesis of essential biochemical substances. Phosphorus plays a crucial role in the metabolic processes of microalgae, particularly PO_4^{3-}-P, which is actively transported into microalgae, phosphorylated, and assimilated into microalgae biomass (e.g., DNA, RNA, and lipids), as well as for ATP synthesis [55,56]; Secondly, polyphosphate (Poly-P) accumulation in microalgae. When exposed to high phosphorus concentrations, microalgae tend to absorb excess phosphorus. Instead of utilizing for growth, excess PO_4^{3-}-P is preferentially stored within the cells in the form of Poly-P to help microalgae cope with phosphorus-stressed environments [57]. Notably, *Chlorella vulgaris* and *Chlamydomonasreinhardtii* exhibit exceptional phosphorus accumulation capabilities, demonstrating higher phosphorus absorption capacities compared with phosphorus-accumulating bacteria of equivalent mass [58].

PAOs follow common pathways for phosphorus removal by absorbing phosphorus under aerobic/anaerobic conditions to produce Poly-P, which is subsequently released under anaerobic conditions [4,7]. Specifically, in the absence of dissolved oxygen or NO_3^--N, PAOs actively uptake fatty acids, amino acids, and glucose into the cell to synthesize intracellular carbon storage compounds like polyhydroxyalkanoates (PHAs) or polyhydroxybutyrates (PHBs). The necessary energy is derived from the hydrolysis of Poly-P and fermentation of intracellular sugars, resulting in the release of phosphates. In aerobic conditions, PAOs regain activity and store excess phosphorus in the form of Poly-P beyond growth requirements. The energy for phosphorus absorption and Poly-P synthesis is generated through the oxidative metabolism of PHAs/PHBs, storing high-energy bonds in Poly-P to remove PO_4^{3-}-P from water.

Moreover, two highly efficient bacteria, denitrifying polyphosphate-accumulating organisms (DPAOs) and heterotrophic nitrification aerobic denitrification (HN-AD), have gained attention for their ability to concurrently eliminate nitrogen and phosphorus. In contrast to conventional PAOs which solely rely on O_2 as the electron acceptor, DPAOs utilize NO_3^--N/NO_2^--N as electron acceptors in the absence of O_2, using intracellular PHAs for denitrification and phosphorus removal simultaneously under hypoxic conditions [59]. DPAOs have been shown to reduce COD and aeration energy consumption by 50% [60]. Another type of phosphorus removal bacteria, HN-AD, incorporates the process of heterotrophic nitrification and aerobic denitrification under aerobic conditions, employing O_2, NO_3^--N, or NO_2^--N as electron acceptors to oxidize organic substances and promote phosphorus absorption, achieving excess phosphorus removal alongside nitrification–denitrification [61]. For instance, the HHEP5 strain has demonstrated proficiency in simultaneous nitrification–denitrification and phosphorus removal (SNDPR), achieving nitrogen and phosphorus removal rates exceeding 90% [62]. However, the limited abundance of DPAOs and the continuous need for an aerobic environment of HN-AD impede their emergence as the source of the predominant metabolic reactions in MBBFs.

3.2. Interactions between Microalgae and Bacteria in MBBFs

The diverse microalgae and bacteria communities in MBBFs foster intricate interactions, including synergistic interactions wherein bacteria and microalgae mutually enhance growth activities and resistance, as well as antagonistic interactions characterized by nutrient and space competition. These interactions are instrumental in establishing stable community structures and upholding effective pollutant removal functionalities.

3.2.1. The Synergistic Interactions between Microalgae and Bacteria Enhance Growth Activity and Resistance

The synergistic interactions between microalgae and bacteria are crucial in enhancing metabolism and growth activities, ensuring the stability of biofilm systems and pollutant removal. Figure 2 shows the primary mechanisms underlying this synergy, including cross-feeding based on environmental and biological metabolites, transmission of signaling molecules, and horizontal gene transfer [4,7,63]. On the one hand, nutrient exchange between microalgae and bacterial metabolites is a pivotal mechanism for fostering microbial growth. The metabolites engaged in cross-feeding within MBBFs are categorized into two groups: environmental chemicals and biological chemicals. Environmental chemicals comprise O_2 released through microalgae photosynthesis and CO_2 through bacterial respiration, small organic compounds derived from bacterial degradation of complex molecules, and EPS. Specifically, bacteria utilize the O_2 produced during microalgae photosynthesis for respiration, subsequently releasing CO_2, nitrogen, and phosphorus that enhance microalgal assimilation [64]. Conversely, microalgae (e.g., *Chlorella vulgaris*) supply organic carbon (carbohydrates and proteins) to facilitate bacterial growth (e.g., AOB), leading to a notable increase in the nitrogen rate to 294.5 mg N/L/d [64]. Moreover, EPSs contain abundant functional groups, like carboxyl, amino, hydroxyl, and carbon groups, which capture toxic chemicals (e.g., organic pollutants, heavy metals, and nanoparticles) providing stronger stability for MBBFs [65,66]. Biological chemicals encompass vitamins, growth hormones, and trace elements secreted by both bacteria and microalgae. For instance, vitamin deficiency is a common issue among microalgae, particularly in the case of vitamin B_{12}, which plays a critical role in the function of methionine synthase in microalgae. Approximately 50% of identified microalgae species experience a deficiency in vitamin B_{12}. Research by Croft et al. [67] revealed that halophilic bacteria supplied vitamin B_{12} to *Amphidinium operculatum*, and *Thalassiosira pseudonana* and its associated bacteria participate in the cross-feeding of sulfur and vitamin B_{12} [68]. Moreover, *Marinobacter* sp. associated with *Scrippsiella trochoidea*, generated a low-affinity iron chelator known as ferritin (VF), significantly enhancing microalgae Fe uptake by 20 times [69].

On the other hand, the exchange of signaling molecules between bacteria and microalgae could serve as a potential mechanism for enhancing the metabolic activity and resistance of MBBF. Signal molecules are substances that convey signal between cells, controlling the transcription of functional genes by binding to specific receptors on the cell surface or inside the cell to regulate the MBC behavior [70]. The primary signaling molecule in MBBF is indole-3-acetic acid (IAA), an endogenous plant hormone present in microalgae that can also be secreted by bacteria [71]. For instance, the microalgae *C. sorokiana* released tryptophan (Trp) and thiamine in return for IAA produced by the bacterium *A. brasilense* [72]. Amin et al. [73] discovered that sulfur bacteria possessed the ability to transform Trp released by diatoms into IAA by utilizing transcriptomics and targeted metabolite analysis, consequently facilitating diatom cell division. Moreover, *N*-acyl homoserine lactone (AHL) serves as another potent signaling molecule. According to Zhou et al. [74], AHL released by bacteria interacted with homologous receptors close to bacterial receptors (such as DNA-binding transcription factors known as R proteins) in the *Chlorophyta*. This interaction stimulated the microalgae activity for synthesizing aromatic proteins, promoting self-aggregation and aiding in biofilm formation. Ou et al. [75] further verified that AHL mediated the initial adhesion of biofilms by altering the properties of

electron donors on microalgae surfaces, including extracellular protein (PN) secretion, PN secondary structure, and PN amino acid composition.

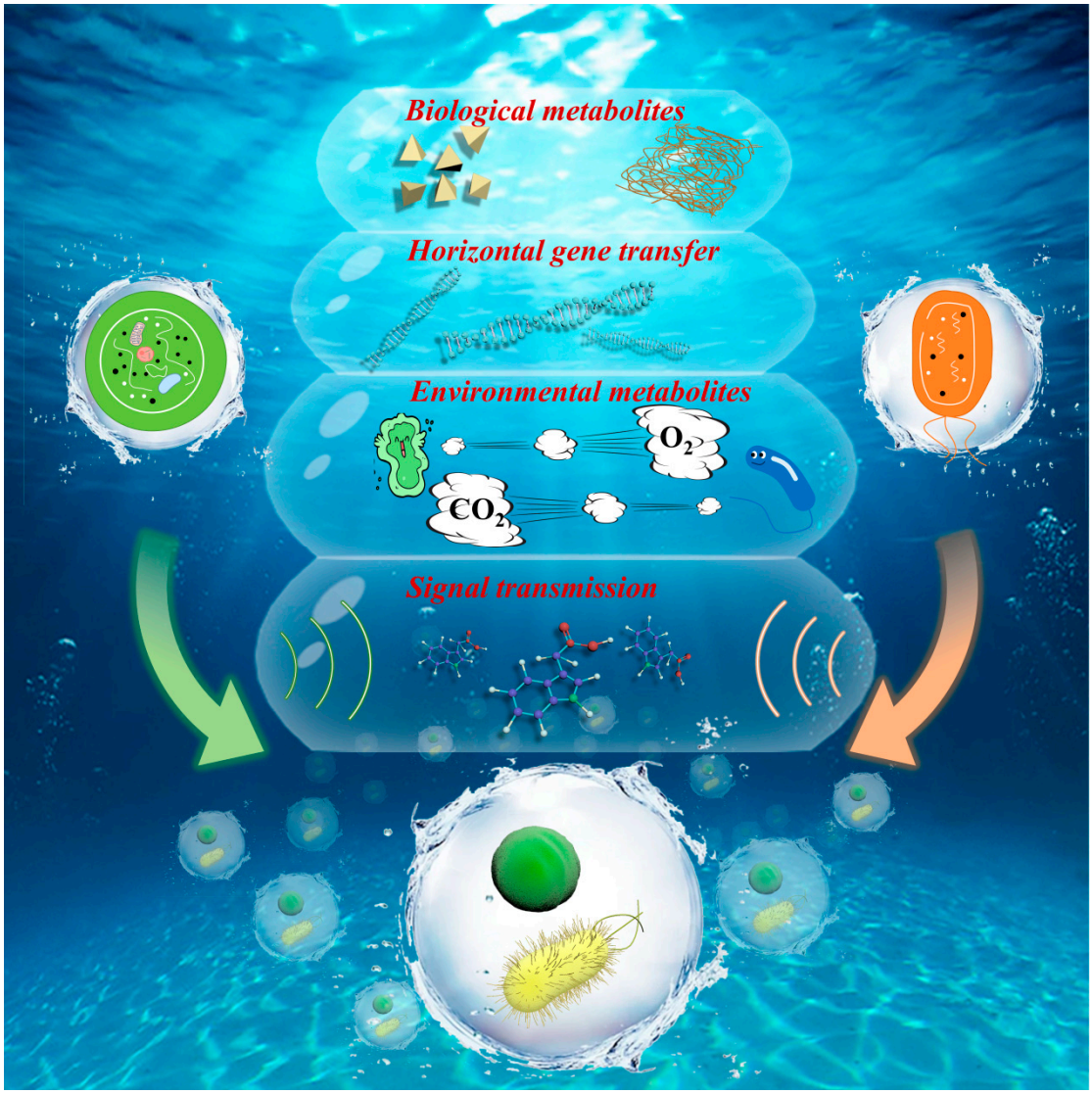

Figure 2. The synergistic mechanism between microalgae and bacteria, including cross-feeding (environmental and biological metabolites), signal transmission, and horizontal gene transfer.

In addition to nutrient exchange and signal transmission, horizontal gene transfer (HGT) is also thought to boost the resistance of MBBFs. Multi-species systems possess a broader array of genes compared to individual organisms, allowing microorganisms to acquire novel gene functions through gene transfer and adapt to environmental challenges [76]. Through extended co-cultivation, microalgae acquire certain genes from bacteria (e.g., encoding ferritin uptake, enzymes associated with the ornithine urea cycle, and metal detoxification) to enhance their ability to adapt to Fe, nitrogen deficiency, and heavy metal settings [6]. *Zygnematophyceae* acquired PYP/PYL/RCA-like ABA genes from bacteria via HGT, enabling it to thrive in arid environments. The integration of bacterial antibiotic and heavy metal tolerance genes into the genome through HGT has become a common mechanism for microalgae to develop resistance to harmful substances. Wang et al. [77] suggested that under conditions of high temperature and a high concentration of blue microalgae, plasmids carrying antibiotic resistance genes were transferred from *Escherichia coli* (donor strain) to blue microalgae cells (recipient strain), thereby augmenting the system's antibiotic resistance. Similarly, Hirooka et al. [78] discovered through genomic

analysis that *Chlamydomonas reinhardtii* acquired the arsenate reductase and arsenate efflux transporter genes from bacteria via HGT, thereby bolstering its resistance to arsenic and markedly improving the ability of MBBFs to withstand heavy metals.

Therefore, the cross-feeding of metabolites between bacteria and microalgae diminishes extra energy consumption while bolstering system stability. Moreover, the reciprocal impact of signaling molecules and HGT heightens the metabolic activity and resistance of MBCs by modulating gene function, showcasing the potential of MBCs for pollutant removal across diverse wastewater settings.

3.2.2. Competition and Antagonism between Microalgae and Bacteria

In situations of malnutrition, bacteria and microalgae engage in competition for nutrients and living space, and may even antagonize each other. This competitive dynamic between bacteria and microalgae is often fueled by nutrient limitations and confrontational interactions. For instance, Gonzalez et al. [79] found that AOB and microalgae competed for NH_4^+-N, resulting in reduced metabolic activity of microalgae. Under conditions of carbon limitation, competition for CO_2 may arise between nitrifying bacteria and microalgae. Additionally, certain bacteria and microalgae release specific toxic substances to hinder the growth of other microorganisms and secure their survival in challenging environments. On the one hand, certain metabolites of microalgae, like chloramphenicol, exhibit bactericidal properties against both Gram-positive and Gram-negative bacteria [47,80]. The malyngolide produced by *Lyngbya* spp. inhibited bacterial quorum sensing [81]. On the other hand, some bacteria release toxic compounds such as alkaloids (e.g., quinolone derivatives) and functional enzymes (e.g., β-glucanase), which impact the transcription of photosynthesis-related genes and impede electron transfer in microalgae. For instance, quinolone derivatives hindered microalgae respiration, DNA, and protein synthesis, whereas β-glucanase disrupted the cell wall of microalgae [82].

In conclusion, comprehending the synergistic and competitive mechanisms in MBBFs aid in devising strategies to boost the metabolic activity and resistance of both bacteria and microalgae. Enhancing the synergistic interactions within MBBFs and alleviating the effects of antagonistic interactions set the stage for establishing more stable and efficient MBBF-based wastewater treatment systems.

4. The Principle and Process of Establishing an MBBF

4.1. Establishment of an MBC

Establishing an ample MBC marks the initial stage of the MBBF membrane formation process. This step directly influences the duration required and the initial structure for MBC formation. In situ cultivation and artificial inoculation are the two primary methods for establishing MBCs [4]. In situ cultivation involves exposing the target wastewater directly to light, thereby selectively enriching and cultivating MBCs that are best suited for the particular wastewater [83]. By directly exposing the wastewater to light, it is possible to screen and enrich in situ microbial populations that possess strong adaptation and pollutant removal capabilities for the specific qualities of the target wastewater. However, this approach is constrained by the inadequate nutrient concentration within the wastewater, necessitating a longer duration to cultivate a sufficient microbial population. Zhang et al. [84] undertook almost 3 years of light adaptation treatment and finally obtained an MBC from rare earth element tailings wastewater.

Comparatively, artificial inoculation allows for the rapid acquisition of an ample amount of MBCs with high pollutant removal capacity and a rapid growth rate [85]. This approach involves enriching and cultivating specific strains of microalgae and bacteria with high metabolic activity and resistance in an artificial culture medium. The enriched MBC is then introduced into the target wastewater using adaptive laboratory evolution (ALE) to attain a relatively stable diversity and performance. In general, ALE comprises three strategies: (1) Inoculating the enriched MBC directly into the target wastewater for cyclic cultivation until stable pollutant removal and biomass growth are achieved. Sun et al. [86]

applied ALE to cultivate and acclimate *Schizochytrium* sp. to high salinity conditions, achieving excellent growth and lipid production. (2) Gradually increasing the pollutant loading to enhance the adaptability of microorganisms to wastewater. Qiu et al. [83] gradually elevated the NH_4^+-N loading by increasing centrate wastewater proportions, achieving impressive nutrient removals and biomass growth. (3) Modifying the cultivation environment, such as by optimizing culture medium and adjusting operating conditions, effectively enhancing the adaptability of the MBC. Pang et al. [87] achieved an MBC with markedly enhanced nitrogen (96%) and phosphorus (79%) removal efficiency by progressively augmenting light intensity and shaking speed. Although numerous ALE strategies have been documented, further research is still required to establish standardized and effective adaptation methods.

4.2. MBBF Formation Process

MBCs utilize adhesive EPSs as a framework to adhere to carrier surfaces and establish biofilm. The formation process of an MBBF generally includes three stages: adhesion, attachment, and proliferation (Figure 3).

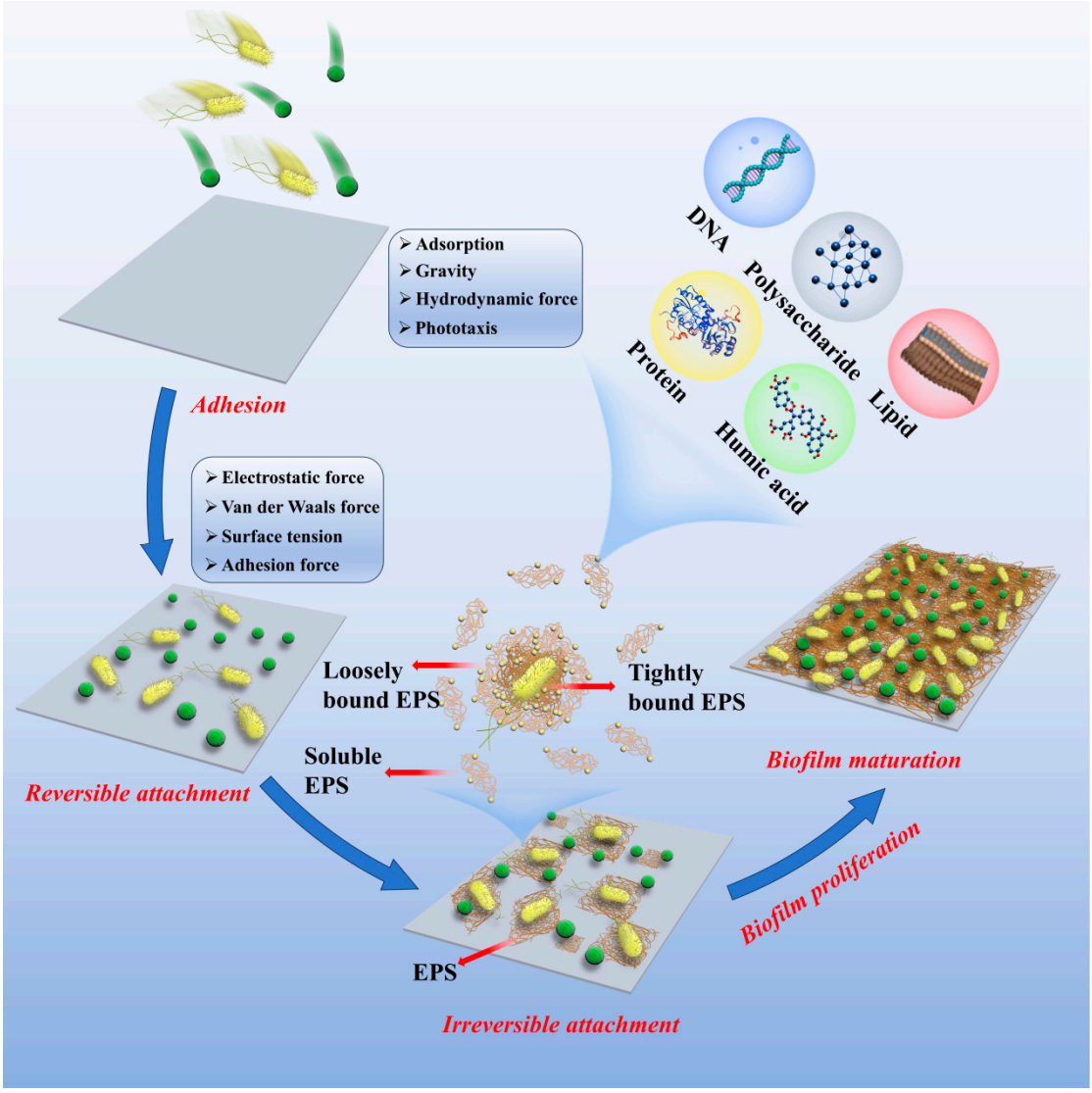

Figure 3. Microalgal–bacterial biofilm formation process, including adhesion, attachment phase (reversible and irreversible attachment), and biofilm maturation.

4.2.1. Establishment of Biofilm on Carrier Surface

Adhesion stage. The adhesion represents the primary stage, whereby suspended bacteria and microalgae are propelled towards the surface of the carrier through a combination of directional adsorption, gravity, hydraulic dynamics, and microalgae phototaxis. The inclination of microorganisms towards favorable environments, such as light sources and nutrients, can enhance their adhesion to carriers [88]. For instance, Fu et al. [89] leveraged the phototaxis of MBCs and employed a light guide plate as a carrier to convert a linear light source into a surface light source. This approach not only heightened the cell adhesion rate but also optimized light transmission within the reactor.

Attachment stage. Microorganisms progress to the attachment phase upon reaching the carrier surface. Microalgae, bacteria, and carriers engage in interactions with one another, such as electrostatic interactions, leading to sequential reversible and irreversible attachment. In reversible attachment, the cell membrane proteins of membranes and microbial organelles (e.g., flagella and cilia) adhere to the carrier surface relying on various forces such as electrostatic forces, van der Waals forces, surface tension, and adhesion forces. However, this type of attachment is susceptible to external forces and detaches due to the weak adhesion. Comparatively, the subsequent irreversible attachment involves the attached MBC tightly adhering to the carrier by secreting EPSs, forming a primary biofilm that is not easily detached or affected by the external environment.

Proliferation stage. The proliferation stage of the MBBF commences after the microorganisms have attached to the carrier. Based on the characteristics of the microbial community structure, the MBBF's growth stage is segmented into the EPS-developing stage and the biomass proliferation stage. Ultimately, the microalgae and bacteria in the MBBF establish a mature and stable MBBF network connected by EPSs [90].

4.2.2. EPSs Form the Backbone of Biofilms

EPSs, composed of polysaccharides, proteins, nucleic acids, humic acids, lipids, and other substances secreted by microorganisms, play a crucial role in the formation of MBBFs (Figure 3) [78]. EPSs are classified into the soluble EPSs (S-EPSs), loosely EPSs (L-EPSs), and tightly EPSs (T-EPSs) based on their binding mode with microorganisms [91]. Due to the solubility, S-EPSs facilitate the transfer of nutrients and metabolites between microorganisms, thereby promoting intercellular interactions. The polysaccharides in L-EPSs and T-EPSs possess high viscosity to capture cells, and the proteins in them are believed to enhance the resistance of the MBC through aromatic accumulation and hydrophobicity, which are essential for the stability and tolerance of MBBFs [4]. Therefore, EPSs serve the functions of aggregating, connecting, and protecting microorganisms.

5. Factors Affecting the Formation of MBBFs

It is crucial to understand the influencing factors and process parameters to accelerate biofilm formation and maintain the system stability of MBBFs. As such, this section discusses the factors that affect the formation of MBBFs, including environmental factors, biological factors, and carrier factors.

5.1. Environmental Factors

The primary environmental factors that influence the growth and adhesion of MBBFs include the light [92], pH [93], CO_2 concentration [94], and water flow rate [95].

Light. Firstly, light is a critical limiting factor in the growth process of algae through light wavelength, intensity, and duration. The thickness and photosynthetic rate of an MBBF increase as light intensity increases before reaching the light saturation point (LSP) [96]. Further increases above the LSP hinder the efficiency of pigment absorption and light energy conversion in microalgae, leading to a final decreased biomass [97]. Similarly, excessive light intensity suppressed bacterial activities such as AOB and NOB, resulting in the variation of nitrogen removal pathways with nitrite accumulation [98]. Moreover, the light cycle also influences the growth and reproduction of algal cells. Andrea et al. [99] posited

that intermittent light enhanced the growth of MBBFs by 8.9% compared to continuous light exposure, which was ascribed to the flashlight effect.

pH. The optimal pH for diverse microbial communities within MBBFs varies. In particular, an environment with pH at 4.0–8.0 promotes MBCs' aggregation to form biofilms, whereas both extreme acidic and alkaline conditions inhibited the activities of MBBF [9]. When the pH $\leq$ 6, microalgae exhibit a negative zeta potential while bacteria exhibit a positive charge, and the acidic environment promotes bacteria EPS secretion, which both facilitate the formation of MBBFs [100]. However, highly acidic pH levels (<4.0) markedly inhibit MBC activities, primarily by causing strong acid damage to the cell walls of microalgae [101]. It is worth noting that the absorption of HCO_3^- by microalgae photosynthesis increases the pH above 10.0, which inhibits the Calvin cycle activity in converting inorganic carbon into organic carbon [102].

CO_2 concentration. While CO_2 serves as the vital inorganic carbon source for MBBFs, excessive concentrations of CO_2 inhibit MBBF metabolic activity. Blanken et al. [103] demonstrated that CO_2 concentrations from 4% to 10% did not lead to significant changes in microalgae growth, suggesting a saturation point in the utilization of CO_2 by microalgae. More seriously, only specially cultivated microalgae strains could adapt to CO_2 concentrations exceeding 15% [100]. In addition, owing to the faster growth rate of bacteria (0.5/h) in comparison to microalgae (0.2/d), O_2 deficiency often occurs, highlighting the importance of maintaining a delicate balance between O_2 and CO_2 to sustain the symbiotic relationship between microalgae and bacteria [104]. On the other hand, the methods of CO_2 gas supply impact the stability of the MBBF. In PBRs utilizing non-submerged and semi-submerged carriers (partially exposed to air), the MBBF directly absorbs CO_2 from the atmosphere via gas–solid phase transfer, where gas transfer to the biofilm surface only needs to pass through a thin liquid film, therefore achieving low mass transfer resistance and high transmission efficiency [105].

Water flow rate. Water flow velocity is a crucial factor influencing the formation, biofilm development, and stability of MBBFs. At low flow rates, microbial communities are easily attached to carrier surfaces due to weak hydraulic shear forces. In contrast, high flow rates lead to denser biofilms with stronger adhesion to the carrier, stemming from the squeezing effect of water flow [106,107]. Therefore, the water flow rates should be adjusted according to the various stages of biofilm formation. During the early stage of biofilm formation, lower flow rates enhance microbial adhesion, while higher flow rates generating larger shear forces are not conducive to biofilm adhesion on the carrier surface [108]. In the maturation stage, the flow rates should be appropriately increased to promote substrate and gas mass transfer, as well as facilitate automatic biofilm renewal, while excessively low flow rates may lead to the formation of thick biofilms, resulting in increased light blockage and mass transfer resistance.

5.2. Biological Factors

The choice of microalgae species and the inoculation ratio of bacteria to microalgae are additional critical factors.

Microalgae species. Eukaryotic microalgae are typically preferred as the inoculum for MBBFs over blue–green microalgae for the following reasons [9]: (1) The growth of eukaryotic microalgae is more manageable compared to blue–green microalgae. (2) Algal toxins released by blue–green microalgae are harmful to bacteria. (3) The contents of high-value substances in blue–green microalgae are notably lower than those in eukaryotic microalgae. The selection of microalgae species should adhere to the principles of high productivity, strong pollution removal capabilities, and stable dominance within MBBFs [9]. Currently, *Chlorella vulgaris* and *Scenedesmus* are among the most frequently utilized microalgae species due to their capacity to generate significant quantities of valuable byproducts like protein, vitamins, and fatty acids, while effectively eliminating pollutants [9].

Inoculation ratio. Given the reliance of MBBF formation on bacteria-secreted EPSs, a higher inoculation ratio of bacteria to microalgae is advantageous for MBBF development.

Nguyen et al. [109] proved that the MBC biomass rose with the increase of ratios of activated sludge (bacteria) to microalgae from 1:9 to 1:3, as activated sludge supplemented carbon sources and enhanced the symbiotic relationship between the microalgae and bacteria. However, when the inoculation ratio exceeded 1:3 and reached 1:1, the total MBC biomass exhibited a declining trend as the high concentration of activated sludge impeded light penetration and microalgae photosynthesis.

5.3. Carrier Factors

The carrier plays a vital role as the site for MBCs to develop biofilms, and its surface properties have a substantial influence on the formation and metabolic activity of MBBFs [110].

Carrier materials. The carriers used for MBBFs can be categorized into inorganic materials (such as ceramic particles, glass, stainless steel plates, and limestone), natural organic polymer materials (including calcium alginate, agar, cotton, and wooden carriers), and synthetic organic polymer materials (like polytetrafluoroethylene (PVDF), polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), nylon, polyurethane (PU), and polystyrene), based on the material composition [11,111]. These carriers all possess favorable pore structures and larger surface areas, which facilitate the attachment of microorganisms and the formation of biofilms.

Surface properties. In the selection of carriers, factors such as charge, hydrophilicity, and biodegradability should also be taken into account: (1) Carriers with a positive charge are more conducive to microbial adsorption as, during the adhesion stage, carriers primarily adsorb microorganisms through electrostatic forces. In typical aquatic environments, the pH generally exceeds the isoelectric point of microorganisms (around 3.5), resulting in a negative charge on the surface of microorganisms due to the ionization effect of amino acids. Therefore, MBBFs are more likely to form on the surfaces of carriers with a positive charge [112]. (2) Hydrophobic carriers exhibit stronger and irreversible surface adsorption compared to hydrophilicity due to the existence of water exclusion mechanism [113]. Ozkan et al. [114] proved that within the first 4 min of the experiment, the adhesion rate of *Chlorella* on hydrophobic surfaces was approximately three times higher than that on hydrophilic surfaces, and the achieved adhesion density was also 2.7 times greater after 320 min. (3) The carriers should be resistant to degradation by microorganisms and exhibit scarce toxic effects on MBCs. Natural organic polymer materials like cellulose and paper are prone to degradation by microorganisms, leading to a shortened service life of the carrier [115]. Metal materials such as copper and aluminum plates, while not subject to degradation, release excessive metal ions that are toxic to microorganisms. Consequently, synthetic organic polymer materials that are resistant to degradation and non-toxic, such as PE, PVC, and foam materials, are regarded as ideal carriers.

6. Photobioreactor Suitable for Implementing MBBF Applications

In recent years, various types of PBRs based on MBBFs have been developed for wastewater treatment. Based on whether the reactor directly interacts with the external environment, PBRs are categorized into open PBRs and closed PBRs (Figure 4). An open PBR allows direct exchange of CO_2/O_2 and other substances with the external environment, while a closed PBR cannot facilitate gas exchange with the external environment. The following section offers a comprehensive overview of the types and features of these two PBR categories.

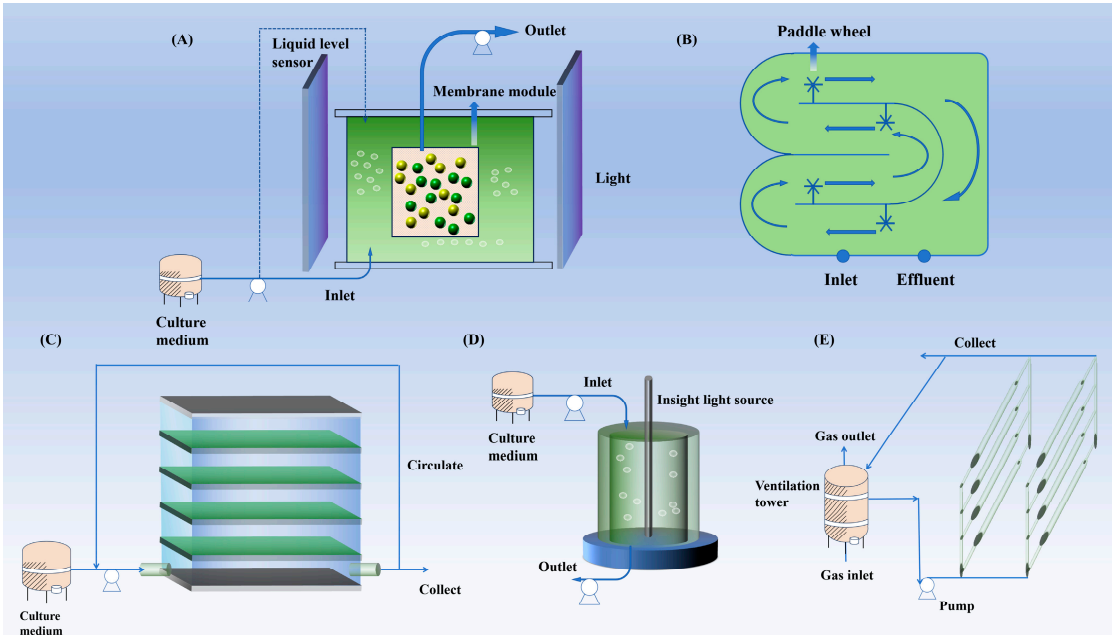

Figure 4. Types of photobioreactors, including open photobioreactors: (**A**) membrane photobioreactor [116] and (**B**) runway photobioreactor [117], and closed photobioreactors: (**C**) plate photobioreactor [118], (**D**) column photobioreactor [119], and (**E**) tubular photobioreactor [120].

6.1. Open Photobioreactor

An open PBR is exposed to the external environment, boasts a simple design, and occupies minimal space. Microorganisms within this system are highly influenced by external factors, leading to an unstable community structure and pollutant removal efficiency.

6.1.1. Membrane Photobioreactor

A membrane photobioreactor (MPBR) leverages the adsorption capability of MBCs to create biofilms on solid culture media, making it an efficient reactor for retaining MBC biomass. The additional membrane components allow the MPBR to autonomously regulate hydraulic retention time (HRT) and solid retention time (SRT), thereby preventing biomass washout and attaining higher biomass concentrations. Luo et al. [121] implemented lower HRT and SRT in a MPBR to elevate the nutrient load, successfully achieving 79% removal of NO_3^--N and 78% removal of PO_4^--P. Gao et al. [122] also proposed the integration of a microfiltration membrane module as a solid–liquid separator in a novel MPBR to enable operation at higher culture medium flow rates, resulting in enhanced pollutant removal efficiency (4.13 vs. 0.59 mg nitrogen/L/d and 0.43 vs. 0.08 mg phosphorus/L/d) and microalgae biomass production (39.93 vs. 10.36 mg/L/d) compared to a conventional PBR.

Similar to other membrane bioreactors, membrane fouling is a significant limitation in MPBR production. It not only decreases the lifespan of the membrane but also raises operational expenses. Due to the formation of MBBFs requiring the skeleton of viscous substances such as EPSs, membrane fouling of MBBF-based MPBRs is almost inevitable. Currently, there are limited studies on MBBFs in MPBRs, highlighting the necessity for additional experiments and research in this area.

6.1.2. Runway Photobioreactor

A runway photobioreactor (RPBR) utilizes natural light as a light and heat source, and mixes the microorganisms within the pool by a pump or paddle wheel. In comparison to a closed PBR, a RPBR offers several advantages: simple structure, ease of large-scale cultivation, low construction and operating costs, and relatively lower production expenses. Ketheesan et al. [123] developed a 23 L air lift RPB for diatom cultivation which

achieved a maximum biomass dry weight of 0.19 g/L/day and a maximum CO_2 utilization rate of 33% at a CO_2 by utilizing the concentration gradient to realize a self-driven microorganism solution.

Currently, the primary challenges hindering the advancement of RPBRs include the high energy consumption associated with disturbing the microalgae solution, light energy losses, and low efficiency in CO_2 utilization. Radmann et al. [124] discovered that the photosynthetic capacity of spirulina decreased when the microalgae concentration reached 0.4–1.0 g/L in an RPBR, which was attributed to the shadowing effects caused by high concentrations. Researchers have suggested various methods to enhance light energy and CO_2 utilization efficiency while minimizing energy consumption. In the research of Mendoza et al. [125], a pit was excavated in the flow channel at the front end of a webbed wheel in a 50 m $\times$ 2 m RPBR and CO_2 was injected from the pit to enhance its contact time with the microalgae solution, thereby improving CO_2 utilization efficiency. David et al. [126] observed that the energy consumption was lower when the RPBR was driven by a pump compared to paddle wheels and propellers, which was attributed to the more uniform distribution of microorganisms in the vertical direction.

6.2. Closed Photobioreactor

A closed PBR incorporates isolation measures both internally and externally to support the sterile cultivation of microalgae, with three common types: flat plate, column, and tubular PBRs. Compared to the open PBR, the primary advantage of the closed PBR is the ability to precisely regulate the temperature and growth conditions of relevant microorganisms with minimal external influence, thus achieving pure cultivation of MBCs.

6.2.1. Plate Photobioreactor

A plate photobioreactor (PPBR) is a flat medium made of materials like glass, resin, and other transparent substances that facilitate the attachment and growth of microalgae. A sufficient surface area to volume ratio and optimal light utilization make PPBRs suitable for MBBF applications [127]. Koller et al. [128] successfully cultivated *Scenedesmus ovalternus* in a PPBR, achieving a highest production rate of 1.7 gCDW/L/d and biomass concentration of 7.5 g CDW/L. Shi et al. [129] immobilized microalgae on a double-layer porous plate PPBR to minimize the loss of microalgae, which obtained 3 times and 2 times the removal efficiency of nitrogen (1.3 mg/L) and phosphorus (1 mg/L), respectively, than that of an open pond system.

Owing to material strength constraints and challenges in scalability of single-layer PPBRs, researchers have enhanced MBC biomasses by incorporating multi-layer PPBR reaction units [129]. However, in practical applications, the shadow effect caused by a multi-layer PPBR and the high concentration of the MBBF lead to a significant reduction in light penetration depth [130]. Researchers proposed using optimized light sources to solve the non-uniformity of light. Jung et al. [131] implemented a 10-layer PPBR and addressed uneven light distribution by integrating a plate waveguide layer with embedded light scatterers, leading to an eight-fold enhancement in biomass cultivation efficiency. Sun et al. [127] introduced hollow PMMA tubes into a PPBR, to boost the average light intensity in the internal region by 2–6.5 times, resulting in a 23.42% increase in biomass concentration and a 12.52% improvement in photosynthetic efficiency.

6.2.2. Column Photobioreactor

A column photobioreactor (CPBR) is designed with a vertical column configuration, which offers effective gas–liquid mass transfer, promotes biomass production, and provides control over light/dark cycles [132]. CPBRs are categorized into bubble-type and lift-type CPBRs based on the intake mode [119]. Compared to bubble types, lift-type CPBRs have a shorter mixing time, leading to enhanced gas mass transfer efficiency. Nayak et al. [133] indicated that *Anabaena* sp. PCC 7120 exhibited higher light utilization efficiency and

CO_2 fixation rate in a lift-type CPBR than in a bubble type, resulting in a higher biomass concentration (1.13 vs. 0.71 g/L).

The feature of positioning the light source inside the cylinder is deemed an attractive configuration of CPBRs. Internal illumination is more effective in utilizing incident light and minimizing the distance between light sources within bioreactors compared to external illumination, as it helps alleviate the negative effects of dark areas and light attenuation. Furthermore, internally illuminated PBRs exhibit higher energy ratios (generated energy to energy input) compared to other reported PBRs [134]. Hu et al. [132] installed a series of blue and red LEDs in a 28 L CPBR to ensure uniform light distribution, resulting in a peak microalgae density of 1.88×10^3 cells/L. Murray et al. [135] utilized internal illumination through a freely floating wireless light source in an immersion light CPBR, achieving 1.18 g biomass/mol *C. vulgaris* and 1.15 g biomass/mol *H. pluvialis*. In contrast, external illumination at the same light intensity only yielded 0.78 and 0.05 g biomass/mol of biomass. Although CPBRs provide advantages such as high mass transfer efficiency, uniform mixing, low shear force, low energy consumption, and ease of operation, they also have certain limitations when deployed on real sites, including their small size, high cost, and challenges related to scaling up.

6.2.3. Tubular Photobioreactor

The structure of a tubular photobioreactor (TPBR) consists of curved, horizontal, vertical, and spiral configurations arranged in arrays or layers, which offers benefits such as a large light exposure surface area, high microalgae biomass production, suitability for outdoor cultivation, and adequate contact time for gas–liquid mass transfer. Kang et al. [136] employed a microalgae-based curved TPBR, namely a periphyton photobioreactor, to effectively remove up to 77% and 68% of nitrogen and phosphorus, as well as 86.4% of personal care products. Binnal et al. [137] similarly utilized a curved TPBR to culture *Chlorella vulgaris*, achieving the highest biomass (1.96 g/L) and CO_2 concentration (78.03%) under optimal conditions.

However, one drawback of TPBRs is the suboptimal mixing conditions caused by poor water flow in longer pipes, leading to uneven distribution of nutrients, pH, and CO_2. To address this issue, Zhang et al. [138] incorporated a spiral mixer into the TPBR system to homogenize water quality, resulting in a 37.26% increase in biomass productivity. Gómez-Pérez et al. [139] proposed a novel twisted TPBR design with a geometric shape that induced vortex formation, reducing energy consumption by 77% at a flow rate of 0.2 m/s, reducing energy consumption while maintaining water quality homogeneity.

Closed PBRs excel in controlling MBC cultivation conditions, minimizing contamination, and accommodating a broader spectrum of microorganisms. Open PBRs enable large-scale cultivation of microalgae at reduced costs. In future research, the integration of closed and open PBR hybrid reactors, along with the advancement of innovative PBR designs, is poised to emerge as a trending topic in the field [140,141].

7. Challenges and Future Perspectives of MBBF Coupled Wastewater Treatment Systems

7.1. Challenges of MBBF Coupled Wastewater Treatment Systems

While MBBFs present notable benefits, such as maintaining high biomass concentrations, reducing land footprint, enhancing pollutant removal, conserving energy, and demonstrating resilience to environmental toxicity when compared to traditional sewage treatment methods, multiple challenges, such as efficient start-up and a prolonged adaptation stage, should not be overlooked. Firstly, the prolonged start-up cycle and elevated operating costs of an MBBF system are likely ascribed by the extended time required in the MBBF's start-up phase for enrichment of sufficient biomass, as well as the difficulty of biofilm formation by multiple interfering factors hindering the adhesion and aggregation of microorganisms [141]. While strategies such as carrier modification [142], operational induction techniques [143], and the addition of cationic substances [144] have been developed to facilitate the formation of microalgal or bacterial biofilms, it is worth noting

that the implementation of effective strategies to expedite the start-up of MBBF systems still remains limited. Additionally, an MBBF has to undergo an adaptation stage before it attains a superior and stable pollutant removal capacity in the target wastewater, posing the challenge of achieving the desired performance. For example, the growth cycle for most microalgae undergoing adaptive evolution spanned 100 to 500 generations [145]. Qiu et al. [84] noted that the microalgal system used for purifying snow water required 16 days for adapting to return to its original pollutant removal efficiency after altering the cultivation conditions.

7.2. Future Perspectives of MBBF Coupled Wastewater Treatment Systems

In response to the above challenges, rapid MBBF enrichment and domestication strategies need to be proposed. Understanding the mechanisms behind biofilm formation and identifying the key factors involved are critical for the swift start-up and effective application of MBBF systems. For example, enhancing the light-stimulated secretion of extracellular proteins and polysaccharides in MBBFs could be a promising strategy for improving biofilm formation [141,146]. Additionally, to enhance the widespread application of MBBF systems for treating a variety of wastewater types, it is important to strengthen their capacity for removing specific pollutants. This could be achieved by screening and introducing microalgae and bacteria with targeted removal functions to regulate the community structure of MBBFs, thereby fostering effective synergistic interactions and quorum sensing to ultimately improve microbial tolerance to specific pollutants. In summary, the use of MBBFs has the potential to be an effective and widely applicable wastewater treatment strategy, warranting ongoing attention and research.

8. Conclusions

Multiple studies have highlighted the significant potential of MBBFs in wastewater treatment and resource recovery. Compared with other traditional wastewater treatment technologies, MBBFs offer a broader range of applications including the removal of emerging pollutants, extraction of high-value products, and carbon capture. MBBFs also exhibit enhanced pollutant removal capabilities and resistance due to the collective effect of multiple metabolic pathways such as pollutant degradation, encompassing assimilation, nitrification–denitrification, phosphorus accumulation, and synergistic interactions between microalgae and bacteria (i.e., cross-feeding based on environmental and biological metabolites, the transmission of signaling molecules, and horizontal gene transfer). Additionally, environmental factors, biological factors and carrier factors are essential for the formation and maintenance of the MBBF biofilm, as well as for the overall stability of the system. These diverse advantages position the use of MBBFs as a promising solution for addressing the challenges of wastewater treatment and resource recovery in sustainable environmental management.

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