

Biological-based control strategies for MBR membrane biofouling: a review

Yin Cui, Huan Gao, Ran Yu, Lei Gao and Manjun Zhan

ABSTRACT

Membrane bioreactor (MBR) technology has been paid extensive attention for wastewater treatment because of its advantages of high effluent quality and minimized occupation space and sludge production. However, the membrane fouling is always an inevitable problem, which causes high operation and maintenance costs and prevents the wide use of MBR technology. The membrane biofouling is the most complicated and has relatively slow progress among all types of fouling. In recent years, many membrane biofouling control methods have been developed. Different from the physical or chemical methods, the biological-based strategies are not only more effective for membrane biofouling control, but also milder and more environment-friendly and, therefore, have been increasingly employed. This paper mainly focuses on the mechanism, unique advantages and development of biological-based control strategies for MBR membrane biofouling such as quorum quenching, uncoupling, flocculants and so on. The paper summarizes the up-to-date development of membrane biofouling control strategies, emphasizes the advantages and promising potential of biological-based ones, and points out the direction for future studies.

Key words | biofouling, flocculants, MBR, quorum quenching, uncoupling

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HIGHLIGHTS

- Membrane biofouling is the most complicated among all types of membrane fouling.
- New physical and chemical methods may hurt membranes and environment.
- Cheaper enzyme extraction methods for enzymatic control of membrane biofouling need to be introduced.
- Environmental conditions and cost are the main limitations of biological strategies.
- The effects of biological methods on the microbial ecology need to be explored.

CAPSULE

Newly developed biological-based strategies for MBR membrane biofouling control are summarized. The unique advantages of the biological control strategies over physical or chemical ones, as well as their future research direction and possible challenges, are discussed.

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ABBREVIATIONS

MBR	Membrane bioreactor
SBR	Sequencing batch reactor
A ² O	Anaerobic/anoxic/aerobic
HRT	Hydraulic retention time
SRT	Solids retention time
EPS	Extracellular polymeric substances
SMP	Soluble microbial product
GO-CNC	Graphene oxide-cellulose nanocrystal

PVDF	Vinylidene fluoride
PMS	Peroxymonosulfate
AHLs	N-acyl-homoserine lactones
AI-2	Autoinducer-2
QS	Quorum sensing
QQ	Quorum quenching
MEC	Magnetic enzyme carriers
C ₈ -HSL	N-octanoyl-DL-homoserine lactone
C ₁₀ -HSL	N-(decanoyl)-DL-homoserine lactone
C ₁₂ -HSL	N-(dodecanoyl)-DL-homoserine lactone
BHL	N-butyryl-DL-homoserine lactone
CFS	Cell-free supernatant
DPD	4,5-dihydroxy-2,3-pentanedione
GCL	Gamma caprolactone
QSIs	QS inhibitors
OdDHL	N-(3-oxododecanoyl)-L-homoserine lactone
ATP	Adenosine triphosphate
PMF	Proton motive force
TCS	3,3',4',5'-tetrachlorosalicylic acid
LB-EPS	Loosely bound EPS
TB-EPS	Tightly bound EPS
DNP	2,4-dinitrophenol
OCP	O-chlorophenol
α -PLL	α -poly-L-lysine

INTRODUCTION

Until now, the activated sludge method is still the most commonly used biological treatment process in wastewater treatment. Due to the low solid-liquid separation efficiency of the traditional activated sludge wastewater treatment processes such as oxidation ditches, sequencing batch reactor (SBR), anaerobic/anoxic/aerobic (A²O), and the increasingly stringent effluent discharge standards, MBR technology has attracted more and more attention (Krzeminski *et al.* 2017; Meng *et al.* 2017). MBR technology is a new wastewater treatment process that combines the functions of both membrane separation and biological wastewater treatment. It is not only well known for higher solid-liquid separation efficiency than the traditional activated sludge processes, but also possesses many other advantages such as high-quality effluent, higher organic loading, less sludge yield, improved nitrification/denitrification performances and complete separation of hydraulic retention time (HRT) from solids retention time (SRT)

(Wang *et al.* 2014; Tan *et al.* 2019). Under normal circumstances, MBR traps particles and pathogenic bacteria such as *Escherichia coli* through a membrane with a pore size of 0.01–1 μ m. The separation process in the MBR technology can be enhanced by using a highly selective membrane with a pore diameter between 0.02 and 0.4 μ m (Xiao *et al.* 2014). The development history of MBR technology is more than 30 years (Yamamoto *et al.* 1988). By the end of 2020, there had been more than 104,000 papers about MBR. Patent publications have maintained an exponential growth trend (Figure 1; The data comes from Google Scholar, the search keyword is 'MBR'). The number of engineering applications has also continuously increased in China, the United States, Europe and other countries over the world (Krzeminski *et al.* 2017; Xiao *et al.* 2019).

Despite the popularities and unique advantages of MBR technology in the wastewater treatment system, membrane fouling is always the most important and depressing concern (Gil *et al.* 2010; Wang *et al.* 2014; Meng *et al.* 2017, 2019), which prevents MBR technology from wider applications. Membrane fouling is caused by complex physical and chemical interactions among the various fouling constituents in the feed, and between these constituents and the membrane surface (Guo *et al.* 2012). Mainly due to the deposits of the inorganic/organic substances in the sludge, as well as the adsorption or accumulation of extracellular polymeric substances (EPS) and soluble microbial product (SMP) on the membrane surface or inside the membrane, the membrane filtration resistance increases and the membrane flux decreases, resulting in membrane fouling during the MBR operation process (Yu *et al.* 2012; Yue *et al.* 2015; Meng *et al.* 2017). The main types of membrane fouling can be divided into four categories (Kochkodan & Hilal 2015): (1) organic fouling, which is mainly caused by organic compounds in the system, such as polysaccharides, proteins, and humic oils; (2) inorganic dirt, which is ascribed to the deposition of inorganic substances, mainly refers to metal salts such as calcium carbonate and calcium sulfate; (3) colloid fouling, which is emerged on account of colloids and suspended particles in the size range of a few nanometers to a few microns; (4) biofouling, which mainly refers to the biofilm formed due to the bacterial attachment to the membrane surface and then the combination with other compounds such as EPS. Biofouling accounts for more than 45% of the membrane fouling and is generally regarded as the most intractable for removal among these four fouling categories (Komlenic 2010; Aslam *et al.* 2018).

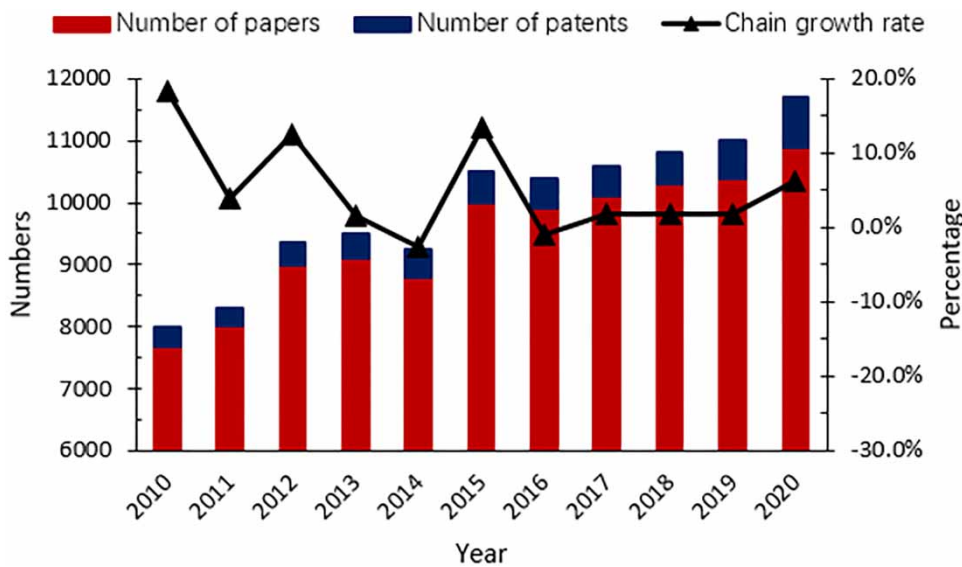


Figure 1 | Publication of papers and patents in the past ten years. (The data come from Google Scholar, the search keyword is 'MBR').

Membrane biofouling will reduce the membrane flux and cause higher energy consumption for membrane cleaning. To avoid these problems, the polluted membrane needs to be cleaned and replaced at regular intervals, resulting in higher costs for MBR application (Bao *et al.* 2019). Hence, the control strategies for MBR membrane biofouling need extensive attention.

The membrane biofouling control methods mainly include physical (e.g. membrane relaxation and backflushing, etc.), chemical (e.g. NaClO, NaOH cleaning) and biological (e.g. enzymatic agents and energy uncoupling) ones. Since the formation of membrane biofouling is a complex and difficult process, the traditional physical and chemical methods usually exhibit poor effects on biofouling control (Qasim *et al.* 2018; Wang *et al.* 2020a, 2020b). Also, the high cost and damage to the membrane are their limitations.

Until now, the physical and chemical methods for membrane biofouling control have been well documented and reviewed (Meng *et al.* 2017; Xiao *et al.* 2019), while less attention has been paid to the biological ones or the combination of physical/chemical techniques with biological ones for membrane biofouling. This review systematically introduced the formation mechanism and impact factors of membrane biofouling. The mechanisms, advantages, and challenges of biological-based strategies for membrane biofouling controlling are critically reviewed, which is expected to provide valuable information to scientists and engineers who engage in this field.

FORMATION AND IMPACT FACTORS OF MEMBRANE BIOFOULING

Membrane biofouling is caused by the deposition, growth and metabolism of microbial cells (bacteria, algae, fungi and protozoa) or flocs and the formation of biofilm on the membrane (Siddiqui *et al.* 2015). Current research manifests that the biofouling of membranes can usually be divided into the following processes (Kochkodan & Hilal 2015; Ishizaki *et al.* 2017) (Figure 2). (1) The formation of conditioning film. Organic materials are adsorbed onto the surface of the membrane in advance to form a conditioning film, which contains both organic macromolecules (polysaccharides, proteins, humus) and inorganic compounds. The conditioning film may promote the adhesion of bacteria. (2) The transport and attachment of suspended bacterial cells to the membrane. (3) The generation of EPS, SMP and biofilm. The attached bacteria continuously produce EPS and SMP during their proliferation process, which contributes to the integrity of the biofilm structure. (4) Cell detachment. Mature cells are separated from the biofilm matrix, and their subpopulations regenerate biofilms in new locations. The bacteria in the biofilm can be protected from the action of many antibacterial agents (Matin *et al.* 2011).

The first impact factor for formation of membrane biofouling is the surface characteristics of the film. The adsorption of organic materials and bacteria is the first and crucial step of biological fouling of membranes. Some

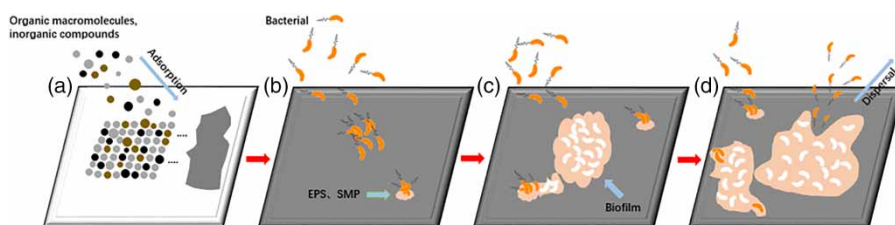


Figure 2 | Formation process of membrane biofouling: (a) The formation of conditioning film; (b) the attachment of bacteria; (c) generation of EPS, SMP and biofilm; (d) cell detachment.

surface properties of the membrane (hydrophobicity/hydrophilicity, zeta potential, surface roughness, and so on) exert significant influences on this step (Kochkodan *et al.* 2014; Lv *et al.* 2018). The second is operation conditions such as hydraulic conditions. Physical effects such as aeration intensity and hydraulic conditions also show great impacts on the adsorption stage of membrane biofouling (Saeki *et al.* 2017). The third is the types of microorganisms in the membrane system. Biofilm contamination is mainly caused by the microorganism associated with *Corynebacterium*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Flavobacterium*, *Aeromonas* and to a lesser extent by fungi such as *Penicillium*, *Trichoderma*, and other eukaryote microorganisms (Kochkodan & Hilal 2015). Last but not least, the generation and removal of EPS and SMP also have great effects on the membrane biofouling. The EPS and SMP will form a dense structure (biofilm) on the membrane with other dirt, leading to the great reduction in the permeability of the membrane and the effects of many antibacterial agents. Among the above factors, EPS and SMP are considered to be the main fouling impact factors for membrane biofouling (Lee *et al.* 2020).

MBR MEMBRANE BIOFOULING CONTROL STRATEGIES

When MBR membrane fouling, especially biofouling occurs, the system's operation and energy costs will be greatly increased. Consequently, many researchers are devoted to investigations to develop efficient technologies to solve the membrane biofouling problems. Until recently, physical and chemical methods were the most commonly used methods to control or eliminate membrane fouling. Those physical (e.g. backflushing and relaxation) and chemical (e.g. acid-base treatment and oxidation) methods have certain effects on the control of membrane fouling, but the shortcomings of these methods

are also addressed by many researchers. Physical methods usually can only remove reversible fouling, and some strict mechanical cleaning can cause membrane damage. If using aeration to alleviate membrane pollution, the best aeration conditions needs to be studied. Membrane fouling may be aggravated when the aeration intensity is too high (Sabouhi *et al.* 2020). Besides, frequent chemical cleaning will greatly shorten the service life of the membrane, reduce the permeability of the membrane, and cause the deterioration of the MBR effluent quality. Therefore, different chemicals and cleaning frequencies are required for wastewater with various properties to extend the service life of the membrane (Hacifazlıoğlu *et al.* 2019).

When using NaClO to chemically clean the membrane it cannot completely remove the MBR membrane biofouling, which might cause the remaining microorganisms to quickly form a new biofilm in the subsequent MBR operations (Cai & Liu 2016). In addition, the residual NaClO in the MBR can cause severe biological pyrolysis, and subsequently form toxic halogenated aromatic by-products, causing great threat to the water environment (Zhang & Liu 2019; Cai *et al.* 2020). Moreover, if chemical fungicides are used for a long time to remove a membrane's biological pollution, bacteria will gradually form a defense mechanism to reduce the effect of fungicide later (Matin *et al.* 2011). Although some new physical and chemical methods remedying the above-mentioned shortcomings have appeared in recent years (Table 1), there are still ineradicable problems such as higher costs and secondary environmental pollution. Compared with physical and chemical methods, biological ones can not only effectively remove the membrane's biological fouling, but also have less impact on the ecological environment and human health. Therefore, many biological-based strategies for membrane biofouling control have been developed rapidly in these years, such as the use of quorum quenching inhibitors and cell wall hydrolases.

Table 1 | Mechanism and limitations of new physical and chemical strategies

Classification	Methods	Mechanism	Limitations	References
New physical strategies	Ultrasonic cleaning	(1) Shear force, drag force, pressure difference and high-pressure shock wave; (2) Agglomerate small particles	(1) Decompose sludge into small particles, increase EPS adhesion; (2) Membrane damages	Borea <i>et al.</i> (2018); Qasim <i>et al.</i> (2018); Sui <i>et al.</i> (2008); Zheng <i>et al.</i> (2019)
	Electric field assistance	(1) Prevent sludge and colloids from depositing on the membrane surface; (2) Promote the microorganisms' metabolism; (3) Oxidation of H ₂ O	(1) Operation complexity; (2) High costs	Ma <i>et al.</i> (2015); Su <i>et al.</i> (2020); Xu <i>et al.</i> (2015); Yin <i>et al.</i> (2020a, 2020b)
	Membrane materials	Increase the hydrophilicity of the membrane	(1) High costs; (2) Membrane damages	Hir <i>et al.</i> (2017)
New chemical strategies	PMS Ferric hydroxide	Oxidize and degrade dirt (1) Increase the size of biomass flocs; (2) Enhance the microorganisms' activity	(1) Limited ability to remove membrane biofouling; (2) High costs of chemicals; (3) Risk to the ecological environment and human health	Wang <i>et al.</i> (2020a, 2020b) Huang <i>et al.</i> (2019)
	Ozone	(1) Reduce the zeta potential; (2) Increase the surface hydrophobicity of flocs		Tang <i>et al.</i> (2017); Wu & Huang (2010)

Development of physical and chemical membrane biofouling control strategies

Physical strategies

Ultrasonic cleaning is recently studied as a new physical flushing method, which can generate physical phenomena such as acoustic streaming, microstreaming, microjets and shock waves in heterogeneous solid-liquid systems. These physical phenomena reveal that the separation of dirt and membrane by shear force, drag force, pressure difference and high-pressure shock will generate a wave generated by unidirectional flow currents (Qasim *et al.* 2018). In addition, ultrasonic radiation can reduce the possibility of pore clogging by agglomerating small particles (Borea *et al.* 2018). However, the latest research found that ultrasound would decompose sludge into small particles, and thus increase the adhesion of EPS to the membrane to intensify the biofouling (Zheng *et al.* 2019). Besides, ultrasonic radiation may negatively affect bacterial activities and cause membrane damage (Sui *et al.* 2008).

Electric field assistance is an emerging membrane biofouling control technology with cost-effectiveness and low energy consumption. This method mainly controls membrane

biofouling through the following actions (Ma *et al.* 2015; Xu *et al.* 2015): (1) electric field force to effectively prevent negatively charged sludge and colloids from depositing on the membrane surface; (2) proper electric field intensity to promote the microbial metabolisms of the attached sludge, which may enhance the degradation of organic matters; (3) H₂O₂ generated in-situ in a bio-electrochemical system in MBR to oxidize membrane fouling. In several different studies, when the electric field was introduced into the MBR, the EPS content in the activated sludge was dramatically reduced from 52.8% to 90.6% (Su *et al.* 2020; Yin *et al.* 2020a, 2020b). At the same time, the EPS adsorption onto and their deposition from the membrane surface were delayed, which showed a good membrane biofouling control effect. The application limitations of the electric fields are mainly reflected in the operation complexity, the cost of materials under large-scale conditions, and the determination of the optimal electric field intensity value.

In addition to the two new physical methods mentioned above, others are available to optimize the characteristics of membrane materials. These methods are mainly aimed at the hydrophilicity of the membrane. Fixing a photocatalyst such as TiO₂ in the membrane matrix can reduce the membrane biofouling by increasing the membrane hydrophilicity

and inducing the free radicals' production to degrade pollutants (Hir *et al.* 2017). Besides, the addition of graphene oxide-cellulose nanocrystal (GO-CNC) composite material to vinylidene fluoride (PVDF) microporous membrane can reduce the EPS accumulation and alleviate the membrane biofouling. However, these methods are costly and may even cause damage to the membrane.

Chemical strategies

In view of the problems that traditional chemical cleaning methods also produce toxic and harmful by-products, researchers are looking for the environmentally friendly alternative chemical agents to control membrane fouling. As a strong oxidant without chlorine, peroxymonosulfate (PMS) is employed as a chemical cleaning agent for MBR. Under the same dosage, the dirt cleaning efficiency by PMS was equivalent to that by NaClO and reached as high as 82.2%. When PMS was combined with ferrous ions, not only the membrane fouling removal efficiency increased to 91% but also the applied chemical agent amount reduced by approximately 75% (Wang *et al.* 2020a, 2020b). The addition of ferric hydroxide could slow down the membrane fouling rate by about 35%. This was mainly because the iron could increase the size of biomass flocs and enhance the microbial activities, and consequently more organic matters were degraded (Huang *et al.* 2019). As a strong oxidant, ozone has also been used to clean membrane fouling (Tang *et al.* 2017). Ozone mainly expands the sludge flocs by reducing the zeta potential value and increasing the surface hydrophobicity of flocs, thereby increasing the permeability of the sludge suspension (Wu & Huang 2010). On the whole, the chemical membrane fouling control strategies mainly focus on oxidants and their effects are varied.

Although these emerging physical and chemical methods have overcome many traditional ones' shortcomings, the membrane biofouling control efficiencies are not significantly improved. In addition, the issues of the cost, the operational complexity, the damage to the membrane, and the impacts on the environment also need to be resolved. Therefore, the biological-based methods are attracting more and more attention mainly because of their environmental friendliness and lower energy consumption.

Biological-based strategies

Quorum quenching

Microbes can communicate with each other through signal molecules such as N-acyl-homoserine lactones (AHLs) and

autoinducer-2 (AI-2). The LuxI-type protein (AHL synthase) leads to the formation of an amide bond between S-adenosyl-methionine (SAM) and acyl-acyl carrier protein (acyl-ACP). Subsequently, the AHL autoinducers will be formed by the intermediate lactonized with the release of methylthioadenosine (Oh & Lee 2018). When the concentration of autoinducers reaches a threshold level proportional to cell density, it will bind to the receptor proteins (transcription factor, usually from LuxR family) and activate the transcription of specific genes to thus regulate the microbial communities' performances such as EPS production, biofilm formation, luminescence, and virulence (Davies *et al.* 1998; Shroff & Nerenberg 2012). This gene-based regulatory mechanism is called quorum sensing (QS). In the MBR system, QS plays a very important role in biological fouling formation. When AHL, one of the common auto-inducible factors is added to MBR, it may increase the membrane biofouling rate through promoting QS (Yeon *et al.* 2009a, 2009b). Quorum quenching (QQ) is a mechanism used to inhibit the communication between cells, which can occur through enzymatic activities, microbial metabolisms, or chemical reaction processes (Millanar-Marfa *et al.* 2020). The methods based on QQ have been proved to effectively reduce the membrane biofouling mainly through (1) preventing the production of auto-inducible factors, (2) interfering with the binding of signal molecules to the receptor, and (3) inactivating (destroying or degrading) auto-inducible factors (Kim *et al.* 2018; Turan & Engin 2018) (Figure 3). The main studied QQ based biological strategies for membrane biofouling control currently are: QQ enzymatic treatment, and QQ bacteria, bio-stimulant and QS inhibitor applications (Table 2).

The first commonly studied membrane biofouling control strategy derived from QQ is enzymatic treatment, which is based on the mechanism of degrading or altering AHL signal' structures. The three main kinds of enzymes are: (1) AHL lactonases for the hydrolysis of lactone ring; (2) AHL acylases for the hydrolysis of the amide bond;

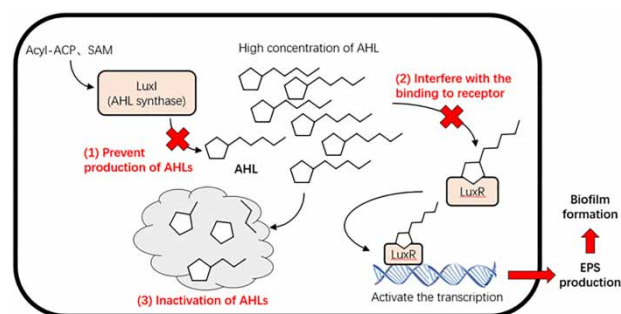


Figure 3 | QS (or QQ) control strategies.

Table 2 | Strategies based on Qs (or QQ)

Categories	Names	Source or active component	Mode of action	Targeted signal molecules	Biofouling mitigation capabilities	References
Enzymes	AHL-lactonase	<i>Halomonas</i> sp. strain 33; <i>Agrobacterium tumefaciens</i>	AHLs degradation	/	/	Dong <i>et al.</i> (2002)
	AHL-acylase (including fixed on the carrier)	<i>Tenacibaculum discolor</i> strain 20 J; <i>Hyphamonas</i> sp. DG895		C ₄ -HSL, C ₁₂ -HSL and C ₈ -HSL	Membrane fouling rate was reduced by 58.0–85.8%	Lee <i>et al.</i> (2017); Jiang <i>et al.</i> (2013); Yeon <i>et al.</i> (2009a, 2009b)
	AHL-oxidoreductase AHL-oxidase	<i>Burkholderia</i> strain GG4 <i>Bacillus megaterium</i>		3-oxo-C ₆ -HSL C ₄ -HSL and C ₁₂ -HSL	/ /	Chan <i>et al.</i> (2011) Chowdhary <i>et al.</i> (2007)
QQ bacteria	<i>Rhodococcus</i> sp. BH4	Lactonase	Signal molecules degradation	(C ₆ , C ₇ , C ₈ , C ₁₀ , 3-oxo-C ₆ , 3-oxo-C ₈)-HSL	Membrane fouling rate was reduced by 75.0–89.0%	Nahm <i>et al.</i> (2017); Maqbool <i>et al.</i> (2015)
	<i>Bacillus methylotrophicus</i> sp. WY	Lactonase		(C ₈ , C ₁₀ , C ₁₂ , C ₁₄ , 3-oxo-C ₆ , 3-oxo-C ₁₂)-HSL	The degradation of targeted signal molecules exceeds 90%. Membrane flux increased by 3-4 times	Khan <i>et al.</i> (2016)
	<i>Enterococcus</i> sp. HEMM-1	Lactonase		(C ₄ , C ₆)-HSL, BHL	Biofilm formation was reduced by 15–44%	Ham <i>et al.</i> (2018)
	<i>Serratia</i> sp. Z4	Unknown		C ₈ -HSL	C ₈ -HSL was reduced by 93%	Dong <i>et al.</i> (2020)
	<i>Acinetobacter</i> sp. DKY-1	Unknown		AI-2 (DPD)	Biofilm formation was reduced by about 81.5%	Lee <i>et al.</i> (2018)
	<i>Candida albicans</i>	Farnesol		AI-2 (DPD)	Anti-biofouling capability increased by 70%	Lee <i>et al.</i> (2016a, 2016b)
	<i>Pseudomonas nitroreducens</i> JYQ3	Acylase		C ₆ -HSL	Membrane flux increased by 19%	Kaur & Yogalakshmi (2018)
	<i>Pseudomonas</i> JYQ4	Acylase		C ₆ -HSL	Membrane flux increased by 22%	
	<i>Pseudomonas</i> sp. 1A1	Acylase		(C ₆ , C ₈ , C ₁₀ , C ₁₂ , 3-oxo-C ₁₂)-HSL	Membrane fouling rate was reduced by about 63.6%	Cheong <i>et al.</i> (2013)
	<i>Delftia</i> sp. T6	Acylase		C ₈ -HSL	Biofilm formation was reduced by about 76%	Gul <i>et al.</i> (2018)
<i>Bacillus</i> sp. T5	Acylase		C ₈ -HSL	Biofilm formation was reduced by about 85%		
Bio-stimulants	Gamma caprolactone (GCL)	Plants	Stimulating the growth of <i>Rhodococcus</i> species	Same with <i>Rhodococcus</i>	EPS secretion was reduced by 1/3–1/2	Yu <i>et al.</i> (2016)

(continued)

Table 2 | continued

Categories	Names	Source or active component	Mode of action	Targeted signal molecules	Biofouling mitigation capabilities	References
QS inhibitors	Halogenated Furanones	<i>Delisea pulchra</i>	Mimic AHL signals and inhibit gene expression	Inhibiting the production of homologous AHL molecules	/	Manefield <i>et al.</i> (1999)
	Vanillin	Vanilla beans extract (<i>Vanilla planifolia Andrews</i>)	Interferes with the binding of signal molecules to the receptor and prevents the production of auto-inducible factors	Inhibiting the production of (C ₄ , C ₆ , C ₈ -3-oxo-C ₈)-HSL	Both short-chain and long-chain AHLs were inhibited	Ponnusamy <i>et al.</i> (2009)
	6-gingerol and its analogs	Ginger	Interferes with the binding of signal molecules to the receptor	Inhibiting the production of short-chain AHLs	Permeate flux of biofilm increased 27–63%	Ham <i>et al.</i> (2019)
	<i>Piper betle</i>	<i>Piper betle</i> extract	Inhibit QS-mediated biofilm formation in <i>P. aeruginosa</i>	Af-2	Membrane fouling rate was reduced by 56.9%	Siddiqui <i>et al.</i> (2012)
	<i>Garlic</i>	<i>Garlic</i> extract	Interferes with expression of QS-controlled virulence genes in <i>P. aeruginosa</i>	/	/	Rasmussen <i>et al.</i> (2005)

and (3) AHL oxidoreductases for the modification of acyl chain (Iorhemen *et al.* 2017). The first extensively studied QQ enzyme is a porcine kidney acylase (Yeon *et al.* 2009a, 2009b), one of the AHL acylases, which can hydrolyze N-octanoyl-DL-homoserine lactone (C₈-HSL) (one of AHLs) and inactivate them to effectively reduce the EPS production and retard the membrane biofouling progress when added into the MBR. Since the added enzyme activity would generally decrease after one day of MBR operation, the strategies to immobilize the enzyme onto a carrier to reduce the loss of free enzyme have also been extensively investigated. In the early studies, when magnetic enzyme carriers (MEC) were used as the carrier of acyltransferase, the stability of the enzyme was greatly improved, which effectively mitigated the biofilm formation (Yeon *et al.* 2009a, 2009b). Sodium alginate and nano filter-capsules have also been used as enzyme carriers, and usually could reduce EPS and SMP levels by about 30% (Jiang *et al.* 2013; Lee *et al.* 2017). However, the enzyme treatment approach has great limitations since the enzymatic activities are sensitive to the environmental conditions (e.g. temperature and pH) and the dosage of enzymes required is too large when applied. In addition, when used in large-scale MBR, the enzymatic agents cannot mix well with pollutants so that the effect of membrane biofouling control was limited (Brepols *et al.* 2008). Accordingly, most studies have focused on the use of bacteria with QQ activity to inhibit membrane biofouling.

Many QQ bacteria species have been reported to effectively control the MBR membrane biofouling. After adding the QQ bacteria *Rhodococcus* sp. BH4 to the MBR, they effectively interfered with the QS through the quorum quenching, thereby inhibiting the membrane biofouling processes (Jahangir *et al.* 2012; Maqbool *et al.* 2015; Nahm *et al.* 2017). Wrapping BH4 in QQ beads (one of QQ media) can efficaciously prolong their microbial action time to up to four months when applied in two types of pilot-scale MBRs (Lee *et al.* 2016a, 2016b). *Bacillus methylotrophicus* sp. WY was isolated from the activated sludge of a wastewater treatment plant in China, and was found to degrade a variety of AHLs, such as C₈-HSL, N-(decanoyl)-DL-homoserine lactone (C₁₀-HSL), and N-(dodecanoyl)-DL-homoserine lactone (C₁₂-HSL) with a degradation rate as high as 90% and a membrane filtration time three times longer (Khan *et al.* 2016). Other HEMM-1 was also reported as QQ bacteria, which mainly degraded AHL with short acyl chains, such as N-butyryl-DL-homoserine lactone (BHL). Their cell-free supernatant (CFS) showed higher QQ activity than those of other QQ ones (Ham *et al.*

2018). Dong *et al.* (2020) isolated a strain of *Serratia* sp. from the sewage, which decreased the SMP levels by 75% within 8 days and the EPS levels by 37% within 12 days in an MBR, indicating their strong QQ ability potential. *Acinetobacter* sp. DKY-1 was isolated from an MBR. Unlike many other QQ bacteria, DKY-1 weakened QS by blocking or decomposing the AI-2 signaling molecule 4,5-dihydroxy-2,3-pentanedione (DPD) (Lee *et al.* 2018). Therefore, adding QQ bacteria to MBR will achieve high degradation rates of signal molecules such as AHL or AI-2, and effectively inhibit EPS and SMP production, which finally contributes to the alleviation of membrane biofouling.

The addition of the bio-stimulants to MBR is another way to promote the growth of indigenous QQ bacteria for membrane biofouling control. Gamma caprolactone (GCL) is one of the best studied bio-stimulants. It can enhance QQ by stimulating the growth of *Rhodococcus* species, which can degrade AHLs and reduce the membrane biofouling (Yu *et al.* 2016). In order to improve the survival rates and activities of QQ bacteria, Yu *et al.* (2019) designed core-shell structured quorum quenching beads, which imbedded a bio-stimulant in the core with QQ bacteria fixed in the shell, which displayed significant membrane pollution mitigation effects through enhancing the AHL degradation rates and reducing the EPS and SMP yields.

In addition, QS inhibitors (QSIs), which are non-enzymatic compounds, can also be used to control MBR membrane biofouling by interfering with QS signal transmission and reducing their generation or combination with the receptor (Choo *et al.* 2020). QSI is generally extracted from eukaryotes, such as halogenated furanones from *Delisea pulchra* (algae), vanillin from vanilla beans (plants), and farnesol from *Candida albicans* (fungi). Vanillin(4-hydroxy-3-methoxybenzaldehyde) is the first QSI compound used to alleviate membrane biofouling via the inhibition of the generation of both short-chain and long-chain AHLs (Ponnusamy *et al.* 2009). Since the chemical structure of the halogenated furanone is similar to that of AHLs, it can compete with the homologous AHL molecules for their receptor sites and interfere with the QS signal transduction (Manefield *et al.* 1999). Similarly, the chemical structure of 6-gingerol in ginger was found to be similar to that of N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), leading to competitive binding to the cognate receptors of the QS systems (Ham *et al.* 2019). Because curcumin can effectively control the production of short-chain AHLs, it can also be used as QSI and has been proved to significantly delay the occurrence of TMP in MBR while the removal efficiencies of nitrogen and phosphorus were not affected (Lade *et al.*

2017). Different from the QSIs mentioned before, the farnesol in *Candida albicans* fungus was reported to reduce the membrane biofouling process in the MBR by inhibiting the AI-2 QS (Lee *et al.* 2016a, 2016b). The combination of multiple QSIs is likely to achieve better membrane biofouling control effects. When vanillin was combined with cinnamaldehyde and attached to the membrane, the polysaccharide contents, microorganisms on the membrane surface, were significantly reduced during the operation of the system (Katebian *et al.* 2018). The current research on QSI stimulants is mainly for laboratory-scale membrane filtration systems. Their effects on larger-scale pilot systems and actual treatment systems need to be verified.

Enzymatic destruction of EPS

EPS and SMP are important substances in bio-cake during membrane biofouling formation, which cause degradation critical for effective alleviation of membrane biofouling. The structure and function of EPS are documented to mainly be jointly maintained by the key components, protein and polysaccharides, in the EPS. EPS concentration can be limited when these key components have been destroyed (Shi *et al.* 2017). Proteolytic enzymes (proteinase K, trypsin, subtilisin, etc.) and polysaccharide degrading enzymes (glucanase, cellulase, etc.) can effectively destroy the EPS structure and have been proven to help inhibit the biofilm formation (Molobela *et al.* 2010; Pei *et al.* 2010). Like QQ enzymes, these methods both utilize enzymes to control the membrane biofouling and face the limitations of high cost of enzyme extraction and poor stability due to the variation of temperature, pH, and salt concentration.

Energy uncoupling

Adenosine triphosphate (ATP) is the main energy source for microbial metabolisms. It is produced by consuming the proton motive force (PMF) produced by the coupling of electron transport and oxidative phosphorylation (Jiang & Liu 2012). As the important impact factor for membrane biofouling, EPS synthesis is highly dependent on ATP. The addition of uncoupling agents for electron transport or oxidative phosphorylation would inhibit ATP production and thus alleviate biological membrane pollution. The commonly used uncoupling agents have been summarized in Table 3. After adding 100 µg/L metabolic uncoupling agent 3,3',4',5-tetrachlorosalicylic acid (TCS) to the MBR, the secretions of not only loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), but also QS signal AI-2 and

Table 3 | Uncoupling agents

Abbreviation	Full name	Effect	References
TCS	3,3',4',5-tetrachlorosalicylic acid	The net synthesis of cell ATP was reduced by 75–90%; The formation rate of membrane fouling has been slowed down by about 2 times.	Xu <i>et al.</i> (2012); Jiang & Liu (2010)
NP	Nitrophenols	The ATP level were reduced by 81.8%; The release of EPS were reduced from 26.98 mg/g VSS to 20.52 mg/g VSS.	Liang & Hu (2012)
DNP	2,4-dinitrophenol	Low dosage of DNP accelerated membrane fouling while high dosage retarded membrane fouling.	Ding <i>et al.</i> (2019)
OCP	O-chlorophenol	Effect on MBR membrane biofouling is still unclear.	Fang <i>et al.</i> (2019, 2020)
CCCP	Carbonyl cyanide m-chlorophenylhydrazone	The biofilm formation was significantly inhibited.	Baugh <i>et al.</i> (2012)

AHLs (C₈-HSL) in MBR were inhibited. Besides, the membrane biofouling cycle is prolonged by more than 2 times with no adverse effects on the growth and catabolism of the activated sludge (Jiang & Liu 2010). Low TCS concentrations would lead to a significant decline in microbial attachment (mainly in the initial attachment phase) and subsequent biofilm development in MBR. These indicated that the inhibition mechanism of biofilm formation for uncoupling agent includes the depression of the EPS secretion as well as the motility of bacteria (Feng *et al.* 2020). The addition of the metabolic uncoupling agent 2,4-dinitrophenol (DNP) also showed a positive impact on relieving membrane biofouling stress. However, the addition of low dosage of DNP would not alleviate the membrane biofouling threat, and conversely resulted in more SMP release, which increased the resistance of the filter cake layer and aggravated the membrane biofouling. DNP with high dosages had a strong inhibitory effect on the production of proteins, polysaccharides, and so on, and effectively limited the formation of cake layer on the membrane surface (Ding *et al.* 2019). High DNP doses delayed the transition of the fouling model from pore clogging to filter cake layer. O-chlorophenol (OCP) is another common metabolic uncoupling agent. Recent studies have found that it would reduce the SMP formation. But due to its cytotoxicity, microorganisms secrete more EPS in order to protect themselves (Fang *et al.* 2019, 2020). Hence, OCP's effect on MBR membrane biofouling is still unclear.

In summary, the using of the uncouplers to inhibit the ATP synthesis should be feasible for membrane biofouling control. However, most ATP uncouplers, such as DNP,

CCCP, TCS, DCP, PCP, TCP, FCCP, are aromatic compounds and are recalcitrant/toxic, which in turn limits their applications.

Cell wall hydrolases

The cell wall hydrolase can simplify the structure of the fouling layer by hydrolyzing macromolecular EPS and SMP (Wong *et al.* 2015), and thus improves the membrane performances and reduces the membrane biofouling process. These enzymes can specifically bind to the cell wall of the target bacteria and cause cell lysis. In addition, they can prevent bacteria from accumulating on the surface of the membrane and play a key role in delaying the membrane biofouling (Bhagwat *et al.* 2020). Lysozyme could destroy bacterial cell walls to prevent microorganisms from forming biofilms (Xiong & Liu 2010). However, in recent years, there have been few studies on the use of bacterial cell wall hydrolase for MBR membrane biofouling. It is speculated that the cell wall hydrolase will depress the microbial activities in the activated sludge of the MBR and cause the deterioration of the wastewater treatment performance.

Biological or natural flocculant

The chemicals, such as iron-based flocculants (ferric chloride, ferric sulfate, ferric hydroxide, etc.), cationic polymers and other inorganic or organic flocculants can be used as flocculants to effectively mitigate membrane biofouling, but exert negative impacts on the environment and human health. Therefore, biological or natural flocculants

with less adverse impacts on the environment have been developed (Table 4). Salt-tolerant *Arthrobacter* was isolated from seawater as a biological flocculant and added into the MBR system to achieve the successful mitigation effects on membrane biofouling (Tan et al. 2017). Meanwhile, they reduced the levels of not only EPS and SMP levels, but also humic acid-like, fulvic acid-like and aromatic protein components. When the biopolymer flocculant α -poly-L-lysine (α -PLL) was utilized to collect *Chlorella ellipsoidea*, the membrane biofouling process was inhibited due to their inherent antibacterial activity (Noh et al. 2018). After two modified starches (CGMS and MGMS) were added to the MBR, respectively, the concentrations of macromolecules with MW (molecular weight) ≥ 100 kDa in the supernatant significantly decreased. The MBR with CGMS added displayed better membrane biofouling mitigation effects because the flocs formed were larger and fell more easily from the membrane surface (Ji et al. 2016). Deng et al. (2020) found that after adding a bio-flocculant (GemFloc™), the sludge suspension in the MBR with the bio-flocculant (G-MBR) contained less SMP content, and the content ratio of protein to polysaccharide (SMPP/SMPC) decreased too. Meanwhile, TB-EPS and the sludge's

floc size, zeta potential, and relative hydrophobicity increased, leading to the reduction of the membrane cake layer and pore blocking resistances. The microbial community in G-MBR displayed higher diversity, and there are more species (e.g. *Arenimonas* and *Flaviumibacter*) beneficial to membrane biofouling control. In addition, the introduction of algae into the MBR can reduce the membrane biofouling by 50%, mainly because algae would inhibit the overgrowth of filamentous bacteria and reduce the absolute value of sludge zeta potential to improve the flocculation and stability of the MBR system (Sun et al. 2018).

Bacteriophage

Bacteriophages are viruses that can infect and lyse host cells. The metabolic characteristics of bacteriophages to break down the host cells is related to two different life cycles: lytic and lysogenic cycles (Figure 4) (Harada et al. 2018). Currently, bacteriophages have been used as a new tool in water pollution control. The lysis ability of the phage indicates its application potential in membrane pollution control, which is mainly reflected in four aspects: (1) the phage can replicate where the pollution occurs to achieve

Table 4 | Biological or natural flocculant

Flocculant	Source	Dosage (g/day)	Membrane fouling rate (kPa/day)	Reduction ratio of fouling rate (compared with control)	References
Chitosan	Sea shrimp or crab shell	1.0	3.70	26.0%	Guo et al. (2010)
GBF (green bioflocculant)	Modified natural starch-based cationic flocculant	1.0 at first 10 days and then 0.5	0.04	Almost 100% (within 70 days)	Ngo & Guo (2009)
Gemfloc®	Patent of University of Technology Sydney (UTS)	1.0	0.067	87.0%	Deng et al. (2015), 2020)
		0.5	0.59	46.8%	
Marine <i>Arthrobacter</i> cells	Isolate from seawater	/	0.75	26.4%	Tan et al. (2017)
Algae	Secondary clarifier wall (Heilongjiang province, China)	Algae/sludge = 1:10	0.93	50.3%	Sun et al. (2018)
Diatomite	Produced from seas and lakes	/	1.1	76.6%	Liu et al. (2011); Yang et al. (2010)
		2.0	/	/	
MPE (membrane performance enhancer)	Cationic polymer-based substances	1.0	3.47	95.7%	Yoon et al. (2005)
		2.2	6.40	92.0%	
Attapulgitic clay	Hangzhou, China	18 g/L (only one time)	<0.49	-6.5% (but TMP was always lower than control)	Yi et al. (2013)

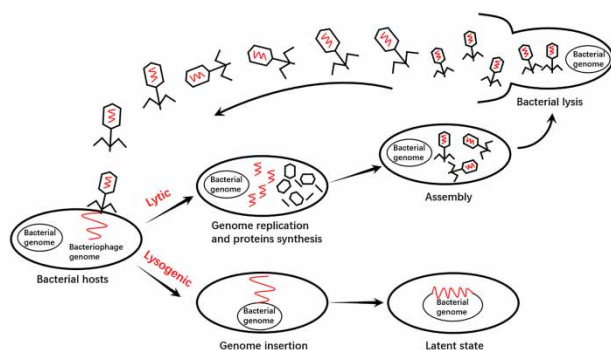


Figure 4 | The lysis process of bacteriophage.

in-situ pollution control; (2) the enzyme produced by the phage can hydrolyze biofilm polymer matrix; (3) the phage control method is compatible with others such as QSIs and QQ enzymes; (4) the phage is easier to apply on a larger scale (Wu *et al.* 2017). Ayyaru *et al.* (2018) used *E. coli* phage P2 on the modified nanocomposite membrane to increase the membrane flux by 57%. The use of a pyophage cocktail would effectively reduce the membrane biofouling by 45%, and inhibit bacteria-induced biofilm formations (Aydin & Can 2020). In addition, it is necessary to use phage mixtures and multivalent phages to expand their host range to improve the efficiency of membrane biofouling control (Mathieu *et al.* 2019). When QSI or QQ enzyme is combined with the phage, the sensitivity of bacteria to phage can be enhanced for better bacteriophage control efficiencies (Remy *et al.* 2018).

The main limitation of the bacteriophage control method lies in three points. First, the host range of a phage is usually narrow and not enough information is available to characterize the phage. Second, the bacteria in the system can gradually develop an immune system against the existing phage and may make the phage inactive (Chan *et al.* 2013). Third, excessive use of bacteriophages may lead to the destruction of useful bacteria for wastewater treatment in the system itself, resulting in deterioration of the treated water quality (Armon 2020). Therefore, further systematic investigations are still needed for the bacteriophage control method.

Predation of protozoa and metazoans

Protozoa and metazoans are the main bacteria consumers in the sludge. They can change the structure of the membrane cake layer and biofilm through predation, and affect the contents and compositions of EPS and SMP. Compared with protozoa, metazoans may play a more important role

in retarding the formation progress of the membrane cake layer due to their greater mobility and wider prey range (Derlon *et al.* 2013). Metazoans, nematodes (*Plectus aquatilis*) and oligovalves (*Aelosoma hemprichi*) were found to reduce membrane biofouling by 119–164 and 50%, respectively, mainly because the biofilm was destroyed from a uniform pie-like structure to an uneven porous structure (Klein *et al.* 2016). The addition of worms to the integrated MBR increased the SMP concentration, resulting in a higher membrane fouling rate (Menniti & Morgenroth 2010). Due to the worm predation, the size of the flocs in the system decreased, causing more serious membrane fouling (Navaratna *et al.* 2014). When separating the worm reactor from the MBR, a completely different situation arises. After the sludge passes through the worm reactor and then enters the MBR, the sludge flocs are more uniform, and the filamentous bacteria are inhibited. Besides, the possibility of biofouling created by SMP is reduced, thereby reducing the membrane biofouling rate (Wang *et al.* 2006; Navaratna *et al.* 2014). When the worms appear on the membrane, they will change the biofilm fouling structure from dense to an open and heterogeneous one through peristalsis and digging holes, thereby enhancing the filtration performance of the membrane (Jabornig & Podmirseg 2015; Klein *et al.* 2016).

CONCLUSIONS AND PERSPECTIVES

This article reviews the formation mechanisms of MBR membrane biofouling and emphatically summarized and discussed the biological-based strategies for membrane biofouling controlling (Figure 5). The following conclusions and perspectives have been drawn as below.

- (1) The future research on physical and chemical methods must pay serious attention to the prevention of secondary pollution to the environment and the damage to the membrane. When using the chemical methods to control the membrane fouling, it is necessary to fully consider the reaction among the compounds, and the sustainability of membrane permeability after chemical treatment.
- (2) It is vitally important to introduce cheaper enzyme extraction methods for enzymatic control of membrane biofouling since the high extraction cost of enzymes limits their application.
- (3) The dosage control of biopharmaceuticals is critical when using uncouplers, natural flocculants, and

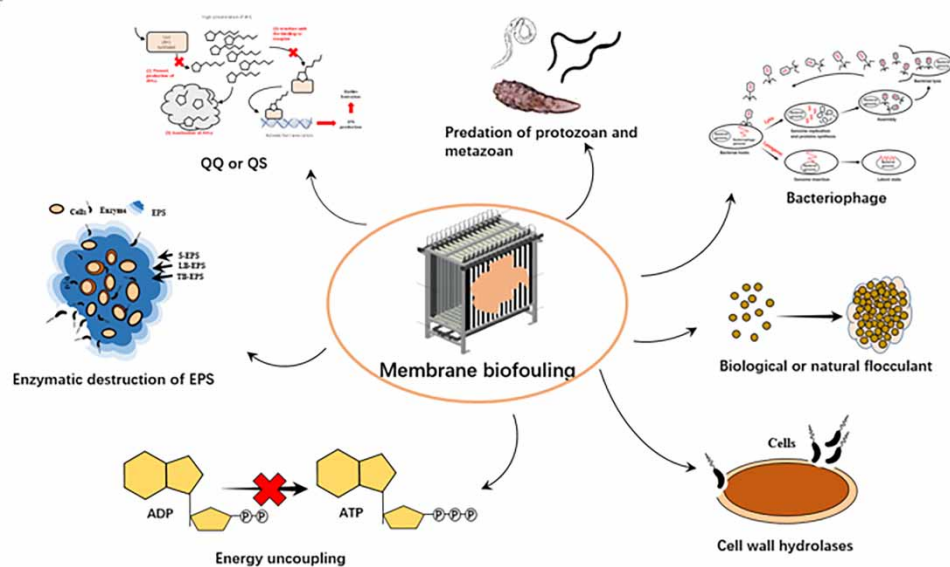


Figure 5 | Biological-based strategies for membrane biofouling.

protozoans/metazoans to mitigate membrane biofouling. A low dosage may have no effect while a too-high dosage may aggravate membrane biofouling.

- (4) The difficulty of using bacteriophages to control membrane biofouling lies in the narrow host range of bacteriophages and the gradually developed microbial defense strategy against bacteriophages. Therefore, the mixed use of multiple bacteriophages or the isolation of new bacteriophages that the host bacteria cannot resist are promising. The impacts of environmental conditions on bacteriophages also need to be considered in the future research.
- (5) So far, almost all the researches on biological control strategies for membrane biofouling are on the laboratory scale. A larger-scale pilot stage or the actual plant case need to be carried in order to verify the effectiveness and safety of these developed strategies.
- (6) Biological membrane biofouling control methods mainly depend on the use of enzymes, bacteria, or viruses. However, the treatment stability to the method adaptation to the environmental conditions, and the large biological agent demand, still need to be solved in the near future.
- (7) Biological membrane biofouling control methods will inevitably affect the microbial community compositions and their diversity in the MBR. Therefore, the impacts of using these methods on the MBR system performance still need to be carefully investigated.

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CONFLICT OF INTERESTS

There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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