The Roles of Bacteriophages in Membrane-based Water and Wastewater Treatment Processes: A Review

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Abstract:

Membrane filtration processes have been widely applied in water and wastewater treatment for many decades. Concerns related to membrane treatment effectiveness, membrane lifespan, and membrane fouling control have been paid great attention. To achieve sustainable membrane operation with regards to low energy and maintenance cost, monitoring membrane performance and applying suitable membrane control strategies are required. As the most abundant species in waters and wastewaters, bacteriophages have shown great potential to be employed in membrane processes as (1) indicators to assess membrane performance considering their similar properties to human pathogenic waterborne viruses; (2) surrogate particles to monitor membrane integrity due to their nano-sized nature; and (3) biological agents to alleviate membrane fouling because of their antimicrobial properties. This study aims to provide a comprehensive review of the roles of bacteriophages in membrane-based water and wastewater treatment processes, with focuses on their uses for membrane performance examination, membrane integrity monitoring, and membrane biofouling control. The advantages, limitations, and influencing factors of bacteriophage-based applications are reported. Finally, the challenges and prospects of bacteriophage-based applications in membrane processes for water treatment are highlighted.

Key words: Biofouling control; Membrane filtration; Membrane integrity; Surrogate particles; Virus indicator.

Outline

1. Introduction

Bacteriophages (also known as phages) are virus that have a capability only to infect and kill bacteria (Duckworth and Gulig, 2002; Haq et al., 2012). Bacteriophage performs antimicrobial roles either by directly causing lysis of bacteria or by extruding and replicating its genome inside of bacterial cells before undergoing lysis of bacteria under deteriorated conditions (Campbell, 2003; Nobrega et al., 2015). After bacteriophages were first discovered by Frederick Twort in 1915 and Félix D'Hérelle in 1917, the bacteriophages were initially applied to treat pathogenic bacterial infections in the medical field, i.e., bacteriophage therapy (Campbell, 2003; Duckworth and Gulig, 2002; Nobrega et al., 2015). Recently, the other potential applications of bacteriophages have received enormous attention, for example, as an additive in food products for conservation, as predators against plant pests/bacteria, as vehicles for vaccines delivery etc. (Campbell, 2003; Haq et al., 2012).

Bacteriophages are the most abundant life forms on earth, especially in the ocean and in fresh water resources (Hanlon, 2007; Wommack and Colwell, 2000). It is therefore not at all surprising that the application of bacteriophages has been extended to the environmental field. An early study emphasized that bacteriophages were useful alternatives to other microbiological and chemical tracers in modelling surface water due to their non-toxic nature (Martin, 1988). With the development of membrane filtration technology such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), and forward osmosis (FO) in the water and wastewater treatment processes, the roles of bacteriophages in membrane processes have been paid more attentions. In this regard, due to their viral nature, monitoring indigenous bacteriophages have been conducted in the pilot and full-scale water treatment plants in order to evaluate human enteric virus removal in membrane processes. Also, several types of bacteriophages are used as model tracers to assess the effectiveness of a membrane separation process. Because of their nano-sized

property, bacteriophages could be applied as surrogate particles in the membrane processes to examine membrane integrity. In addition, bacteriophages display antimicrobial properties, thus they could also be considered as biological agents for membrane biofouling control.

This review summarizes the recently reported literature on the roles of bacteriophages in membrane-based water treatment processes, with a focus on membrane performance examination, and membrane integrity monitoring, and membrane fouling control (Figure 1). Noticeably, the uses of bacteriophages, especially as tracers or antimicrobial agents, in membrane processes are mainly performed in the bench-scale or lab-scale systems till now. Thus, the advantages and limitations of bacteriophage-associated techniques are critically reviewed. In particular, the influences of operating conditions of membrane processes on the performance of bacteriophages are highlighted. The technological challenges are carefully evaluated and the breakthroughs required of bacteriophage-associated techniques are suggested. Finally, the prospects and research directions for bacteriophage-associated techniques in membrane processes are proposed.

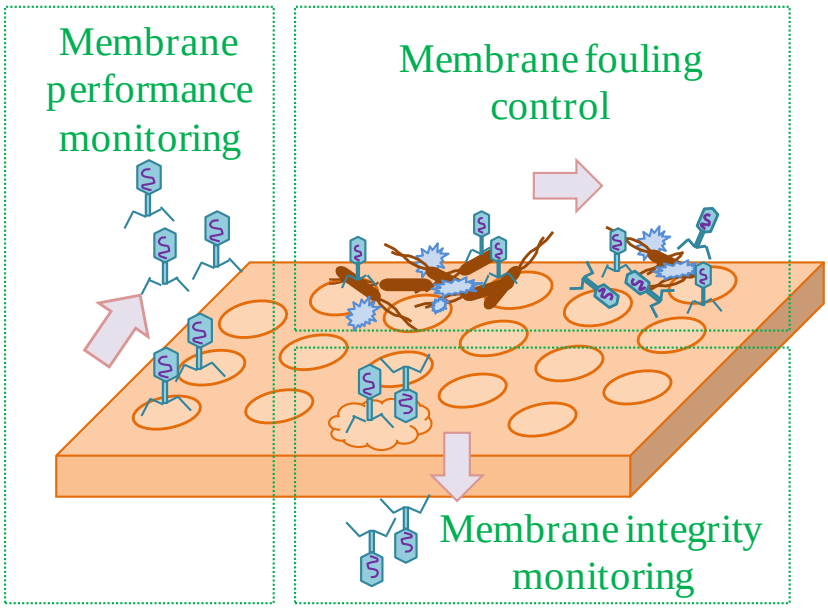

Figure 1. A diagram illustrating the roles of bacteriophages in membrane-based water and wastewater treatment processes.

2. The role of bacteriophages in evaluating membrane performance

2.1. Examination of membrane performance by monitoring indigenous bacteriophages

In the membrane process for drinking water production and wastewater reclamation, the removal of human pathogenic waterborne viruses (especially human enteric viruses) is a critical parameter to evaluate the membrane treatment efficiency (Kopecka et al., 1993). To directly detect human enteric viruses, either the fecal indicator bacteria methods or molecular methods (such as reverse transcription, polymerase chain reaction, and hybridisation etc.) are typically used. Compared to conventional fecal indicator bacteria methods, molecular techniques are more sensitive, specific, and rapid, but more expensive and cumbersome (Ebdon et al., 2012; Francy et al., 2012; Havelaar et al., 1991).

An alternative approach is to use indigenous bacteriophages as indicators to determine the presence of human enteric viruses considering the properties of bacteriophage. Specifically, the structure, composition, size, and replication features of indigenous bacteriophages are comparable to human enteric viruses. For example, FRNA bacteriophages have sizes of 25 nm and isoelectric point (IEP, i.e., the pH value at which the electrophoretic mobility of the particle equates zero) of 3.9, which are similar to those of human enterovirus (22-30 nm, IEP 4.0–6.4) and hepatitis A (27-28 nm, IEP 2.8) (Branch et al., 2016; Michen and Graule, 2010). At neutral pH (typical operation conditions for membrane-based water and wastewater treatment processes), the low IEP of typically-used indigenous bacteriophages appears to avoid membrane adsorption due to their negatively-charged surfaces. Moreover, indigenous bacteriophages are either positively correlated with the presence of enteric viruses in waters and wastewaters or more conservatively removed by membranes than the enteric viruses (Cromeans et al., 2005; Leclerc et al., 2000; Otaki et al., 1998). Importantly, bacteriophage

assay technique is much simpler and cheaper than any of the human enteric virus detection methods (Leclerc et al., 2000).

To identify reliable indicators for detection of human enteric viruses, microbiologists have made many efforts to select suitable bacteriophages. Leclerc et al. (Leclerc et al., 2000) summarized the reported bacteriophages that have been proposed as indicators of human enteric viruses, namely, somatic coliphages, male-specific RNA coliphages, and phages infecting *Bacteroides fragilis*. Recent studies that are involved in using these reliable indigenous bacteriophage indicators to investigate membrane performance have been summarized in Table 1. It is well noted that each group of bacteriophages suffer from their own limitations, such as lack of specificity, relatively low concentration, and bacteriophage resistance, etc. (Leclerc et al., 2000).

Nowadays, researchers are attempting to quantitatively explore the relationship between indigenous bacteriophages and human enteric viruses in order to screen the best indicator and improve the accuracy of prediction. However, inconsistent conclusions have been drawn in the reported studies. For example, Ebdon et al. (Ebdon et al., 2012) have proved that the phages infecting *Bacteroides* GB-124 were positively correlated to the human adenovirus and norovirus in municipal wastewaters. Francy et al. (Francy et al., 2012) illustrated that somatic coliphage and F-specific coliphage well represented the removal of viruses in the MBR and post-MBR disinfection process respectively. This reveals that the water characteristics and membrane process configuration are important parameters to influence indigenous bacteriophage removal efficiency. Thus, the quantitative relationship between indigenous bacteriophages and human enteric viruses appears to be case-dependent.

164 **Table 1. Monitoring indigenous bacteriophages to evaluate human enteric virus**
165 **removal in membrane-based water and wastewater treatment processes**

Type of bacteriophage	Feed water	Reactor scale	Membrane specification	Removal efficiency	Reference
Indigenous coliphages	River water	Pilot-scale MF/UF	Monolith type α-Alumina (0.2 μm)	40-90% removal for *E.coli* K12 phage; 98-100% for *E.coli* C phage;	(Otaki et al., 1998)
			Hollow fibre PE (0.1 μm)	88-99% removal for *E.coli* K12 phage; 99-100% for *E.coli* C phage;	
			Hollow fibre PAN (13 kDa)	100% removal for both phages	
Indigenous coliphages	Sewage wastewater	Bench-scale MBR	Flat sheet PE (0.4 μm)	2.3-5.9 log removal for Indigenous coliphages	(Ueda and Horan, 2000)
Indigenous coliphages	Municipal wastewater	Pilot-scale MBR	Flat sheet PE (0.4 μm)	5 log removal	(Oota et al., 2005)
Somatic and F-specific coliphages	Sewage wastewater	Pilot-scale MBR	Flat sheet PE (0.4 μm)	3.08 log removal for somatic coliphages; 3.78 log removal for F-specific phages	(Ottoson et al., 2006)
Male-specific (F+) coliphage	Sewage wastewater	Pilot-scale MBR	Hollow fibre PE (0.4 μm)	3.7 log removal	(Tam et al., 2007)
Somatic and F-specific coliphages	Sewage wastewater	Pilot-scale MBR	Hollow fiber PVDF (0.04 μm)	3.1-5.8 log removal for somatic coliphages; 3.3-5.7 log removal for F-specific phages	(Zhang and Farahbakhsh, 2007)
Somatic and F-specific coliphages	Sewage wastewater	Pilot-scale MBR	Flat sheet PE (0.4 μm)	2.6-5.6 log removal for both phages	(Marti et al., 2011)
Somatic and F-specific coliphages	Sewage wastewater	Full-scale MBR	Flat sheet PE (0.4 μm)	2.67-4.04 log removal for somatic coliphages; more than 4.58-6.0 log removal for F-specific phages	(Francy et al., 2012)
Somatic coliphages, F-specific bacteriophages, and bacteriophages infecting *B. fragilis*	Sewage wastewater	Full-scale MBR	Flat sheet PE (0.4 μm)	4.4 log removal for somatic coliphages; 5.8 log removal for F-specific phages; 3.7-4.1 log removal for bacteriophages infecting	(De Luca et al., 2013)

				B. fragilis	
FRNA bacteriophage	Sewage wastewater	Full-scale MBR	Hollow fibre PVDF (0.1-0.2 μm)	More than 4.65 log removal	(van den Akker et al., 2014)
F+ coliphage	Sewage wastewater	Full-scale MBR	Hollow fiber PVDF (0.04 μm)	5.4-7.1 log removal	(Chaudhry et al., 2015b)
Somatic coliphages and F-specific bacteriophage, and bacteriophages infecting *B. fragilis*	Sewage wastewater	Full-scale MBR	Hollow fiber PVDF (0.04 μm)	5.34 log removal for somatic coliphages; 3.5 log removal for F-specific bacteriophages; 3.8 for bacteriophages infecting *B. fragilis*	(Purnell et al., 2015)
Somatic coliphages and F-specific bacteriophages	River water	Pilot UF	Hollow fiber PVDF (0.04 μm)	3.8 log removal for somatic coliphages; 3log removal for F-specific bacteriophages	(Ferrer et al., 2015)

166 Abbreviation: polyacrylonitrile (PAN); polyethylene (PE); polyvinylidenefluoride (PVDF).

167 *2.2.Examination of membrane performance by monitoring added model bacteriophages*

168 2.2.1. Model bacteriophages

169 As the concentrations of indigenous viruses are very low in waters, especially in underground
170 and surface waters, accurate analysis of these indigenous viruses in membrane processes is
171 not practically feasible (Leclerc et al., 2000). An alternative approach is to spike large
172 quantities of model bacteriophages, such as MS2-like (genogroup I), Qβ-like (genogroup II),
173 GA-like (genogroup III), SP-like (genogroup IV) etc. into membrane processes to predict the
174 removal of human enteric viruses. This is mainly motivated by the facts that the sizes (20-30
175 μm) and structures of these surrogates are comparable to that of human pathogenic viruses
176 and safe for humans (Langlet et al., 2008).

177 It is worth noting that Antony et al. (Antony et al., 2012) have reviewed the studies reported
178 before 2010 on the removal efficiency of model viruses in membrane processes and the
179 impact of operating conditions on virus removal. In this review, we focus on recently-

180 published literature (after 2010) on the use of model bacteriophages in membrane-based
181 water and wastewater treatment processes for virus elimination examination.

182 **Table 2. Summary of the reported studies (after 2010) involved in using the**
183 **model bacteriophages as surrogates in membrane-based water and wastewater**
184 **treatment processes**

Type of bacteriophage	Feed water	Reactor scale	Membrane specification	Added concentration (PFU/mL)	Removal efficiency	Reference
MS2	Synthetic surface water	Bench-scale coagulation–filtration	Flat sheet PES (100 kDa)	10^{7}-10^{8}	6.19-6.78 log removal at 1 mg/L of Al^{3+} (pH 6-8)	(Guo and Hu, 2011)
T4, Qβ	River water	Bench-scale coagulation-filtration	Flat sheet PC (50 nm)	10^{11}	Almost completely removed for T4; 1-4 log removal at 54-108 mg/L of Al^{3+}	(Matsushita et al., 2011)
MS2 andMS2 grafted with enzymatic probes	Ultrapure water	Bench-scale filtration	Flat sheet PES (100 kDa)	1.75×10^{8}	NA	(Soussan et al., 2011)
MS2	Tap water; tap water containing 5 g/L NaCl; distilled water containing 1 and 9 g/L NaCl and PBS	Bench-scale filtration	Hollow fibre CA (100 kDa)	10^{8}	5.7-6.4 log removal for tap water; 5.6-5.7 log removal for tap water containing 5 g/L NaCl; 5.6-6, 5.1-5.6, and 5 log removal for distilled water containing 1 and 9 g/L NaCl, and PSB respectively	(Pierre et al., 2011)
MS2, Qβ and GA	River water	Pilot-scale filtration	Hollow fibre PVDF (0.03 µm, 200 kDa) and PES (100 kDa)	10^{6}	Above 4 log removal for MS2 and Qβ; 1.6 log removal for GA in the presence of pre-treatment (clarification and sand filtration)	(Boudaud et al., 2012)
MS2	Synthetic surface water	Bench-scale coagulation–filtration	Flat sheet PVDF (0.22 µm) and PES (100	10^{7}-10^{8}	> 4 log removal under batch conditions at 5 mg/L of Al^{3+} for MF; > 5	(Guo and Hu, 2012)

			kDa); hollow fibre PVDF (0.2 µm)		log removal at 1 mg/L of Al^{3+} for UF; > 5 log removal under continuous conditions at 5 mg/L of Al^{3+}.	
MS2	Secondary wastewater effluent; Filtered Secondary wastewater effluent; Sodium phosphate-based model water	Bench-scale filtration	Hollow fibre PVDF (0.1 µm)	$(4.8\pm1.6)\times10^{6}$	2.1-3.0 log removal for secondary wastewater effluent; 0.8 to 1.7 log removal for filtered secondary wastewater effluent; 1.0 log removal for sodium phosphate-based model water	(Huang et al., 2012)
MS2, φX174	Mixture of tap water and deionised water (1:1); surface water	Bench-scale and lab-scale filtration	Hollow fibre PES (0.02 µm)	10^{6}-10^{7}	2.5-6.0 log removal for MS2; 2.5-4.5 log removal for φX174	(Kreißel et al., 2012)
MS2	Secondary wastewater effluent	Full-scale filtration	Hollow fibre PVDF (0.04 µm)	4.6×10^{4}-5.9×10^{5}	1.18-3.96 log removal	(Regel et al., 2012)
MS2	Real and synthetic surface water	Bench-scale coagulation-filtration	Flat sheet PVDF (0.22 µm)	10^{7}-10^{8}	1.0-1.5 log removal for real surface water and 4-6.5 log removal for synthetic wastewater removal at an iron dosage of 13 mg/L as Fe	(Tanneru and Chellam, 2012)
MS2, φX174	Deionised water at different pH levels	Bench-scale filtration	Hollow fiber PVDF UF	10^{6}-10^{7}	3.7 log removal for MS2 at pH 7.6; 3.7 log removal for φX174 at pH 6.5; 2.5 log removal for φX174 at pH 9.4	(ElHadidy et al., 2013)
Qβ and MS2	River water	Bench-scale coagulation-filtration	Monolithic ceramic membrane (0.1 µm)	10^{8}	More than 2-6 log removal at different coagulant (Al^{3+} and Fe^{3+}) doses	(Matsushita et al., 2013)
MS2	Surface water	Bench-scale coagulation-filtration	Flat sheet PVDF (0.22 µm)	10^{8}	5.5-6.0 log removal at aluminium concentration of 30 mg/L	(Tanneru et al., 2013)

MS2	PBS buffer	Bench-scale filtration	Composite PAN/PET/ CNF nanofibrous membrane	10^6	> 4.0 log removal	(Wang et al., 2013)
F2	Tap water	Lab-scale photocatalytic membrane reactor	Flat sheet PVDF (0.20 μm) and PAN (0.05 μm)	1.35×10^7	3.88 log removal for PVDF membrane and 6.40 log for PAN membrane	(Zheng et al., 2013)
MS2, φX174	Surface water	Bench-scale filtration	Hollow fiber PVDF UF (2-56 nm)	10^6-10^7	3.5-6 log removal for MS2; 3-5.9 log removal for φX174	(ElHadidy et al., 2014)
P22	NaCl solution at different pH levels	Bench-scale filtration	Nanoporous iron oxide ceramics	10^7	~3 log removal	(Fidalgo de Cortalezzi et al., 2014)
MS2, φX174, fr	Wastewater mixed liquor sludge	Lab-scale MBR	Hollow fiber PVDF (0.04 μm)	10^5-10^8	1.7 log removal for MS2; 2.3 log removal for φX174, 4.2 log removal for fr	(Chaudhry et al., 2015b)
P22	Ultrapure water	Lab-scale photocatalytic membrane reactor	TiO_2 tubular ceramics (0.8 μm)	5×10^5	~5.0 log removal	(Guo et al., 2015)
MS2 and GB124(B-14)	Sewage wastewater	Full-scale MBR	Hollow fiber PVDF (0.04 μm)	MS2:2×10^{12} B-14: 1×10^8	2.25 and 2.3 log removal for MS2 and 2.3 and 8.0 log removal for B-14	(Purnell et al., 2015)
F2	Tap water	Lab-scale photocatalytic membrane reactor	Flat sheet PVDF (0.15 μm)	10^5–10^6	> 5.0 log removal	(Zheng et al., 2015)
MS2	Synthetic salt water	Lab-scale RO	New and aged polyamide RO membrane	10^5–10^6	More than 6.3 log removal for new RO membrane; 2.8-4.1 for aged RO membrane	(Antony et al., 2016)
FRNA	Sewage wastewater	Lab-scale MBR	Hollow fibre PVDF (0.04 μm)	10^5 -10^6	4.1-7.3 log removal	(Branch et al., 2016)
MS2	Synthetic salt water; pre-filtered	Lab-scale RO	New and aged polyamide	10^8	~5.8 log removal for new RO membrane; 4-5 log removal for	(Pype et al.,

	secondary effluent		RO membrane		aged RO membrane	2016b)
MS2	Swimming pool water	Bench-scale filtration	Monolithic SiC (350 nm)	1.16-2.27×10^7	0.95-1.12 log removal	(Skibinski et al., 2016)

Abbreviation: cellulose acetate (CA); cellulose nanofiber (CNF); polyacrylonitrile (PAN); polycarbonate (PC); polyethersulfone (PES); polyethylene terephthalate (PET); polyvinylidenefluoride (PVDF); Regenerated cellulose (RC).

In these reported studies (Table 2), the bacteriophages such as MS2, F2, φX174, Qβ, GA, and F2, are the mostly-used model surrogates to simulate human enteric viruses in membrane-based water and wastewater treatment processes. Although a few studies have compared virus removal efficiencies for various tested bacteriophages in the membrane processes (Boudaud et al., 2012; Chaudhry et al., 2015a; ElHadidy et al., 2013, 2014; Kreißel et al., 2012; Soussan et al., 2011), the findings are not similarly conclusive. For example, MS2 was removed more than φX174 in the experiments performed by Kreiβel et al. (Kreißel et al., 2012) and ElHadidy et al. (ElHadidy et al., 2013), but other researchers observed their comparable removal efficiencies (ElHadidy et al., 2014) or less removal for MS2 (Chaudhry et al., 2015b). This is attributed to the fact that the comparison of these surrogates was performed under completely non-identical experimental conditions. This raises concerns for the factors that influencing bacteriophage removal in membrane-based water and wastewater treatment processes. A detailed discussion of this issue is presented in the following section (Section 2.2.3).

2.2.2. Bacteriophage-membrane interaction

Generally, membranes reject bacteriophages mainly by size exclusion, adsorption of bacteriophages on membranes, and electrostatic repulsion between membranes and bacteriophages (ElHadidy et al., 2014; Jacangelo et al., 1995). Bacteriophage sizes normally range from 20 to 30 nm, which are smaller than the pore sizes of MF membranes, comparable

to those of UF membranes, and greater than those of NF and RO membranes. When the sizes of bacteriophages are greater than membrane pores, size exclusion will be the major removal mechanism. However, when bacteriophages are smaller than or comparable to membrane pores, the immobilization of bacteriophages depends on both bacteriophages and membrane surface properties (such as surface charge, hydrophobicity), relative size of the bacteriophages to the membrane pore, and bacteriophage shape/aggregation situation (ElHadidy et al., 2014).

In the membrane-based water and wastewater treatment processes, bacteriophage interactions with environmental surfaces such as fecal material, clays, and biological flocs, and water chemistry are also important in determining their removal (Chaudhry et al., 2015a; Huang et al., 2012). In addition, inactivation of the viruses due to existing bacterial extracellular enzymes, added chemicals (coagulants/flocculants), and predation in the wastewater processes was considered to contribute to bacteriophage elimination (Chaudhry et al., 2015a). Therefore, the size and interfacial characteristics of viruses, characteristics of the membranes (pore size, materials, surface properties), membrane fouling situation, environmental conditions (such as pH, the presence of activated sludge and natural organic substances) are major factors that could influence the efficiency of virus removal in membrane-based water and wastewater treatment processes.

2.2.3. The factors that influencing bacteriophage removal in membrane-based water and wastewater treatment processes

2.2.3.1. Effect of bacteriophage types on removal efficiency

Generally-used surrogate particles, such as Qβ, MS2, SP, GA, φX174 bacteriophages, have similar size ranged at 20-30 nm, except B-14, a double-stranded DNA virus with a size of ~65 nm (Langlet et al., 2008; Purnell et al., 2015). It is usually admitted that generalization to the behaviours of these bacteriophages has not been concluded due to their discrepancies in

surface properties. Although all reported model bacteriophages have negatively-charged surfaces at neutral pH condition, their hydrophobic nature and isoelectric points are different (e.g., isoelectric point is 3.1 for MS2, 2.1 for GA, 2.7 for Qβ, and 2.1 for SP (Langlet et al., 2008); 3.8 to 5.5 for B-14 (Purnell et al., 2015)). The surface properties of bacteriophages determine their aggregation tendency, adsorption by natural organic compounds/sludge flocs, and interaction with membrane surfaces, which impact bacteriophage removal efficiency in natural waters and wastewaters (Boudaud et al., 2012; Chaudhry et al., 2015a; ElHadidy et al., 2013, 2014; Kreißel et al., 2012). For example, in natural waters, the bacteriophages with greater hydrophobicity are more efficiently rejected by membranes possibly due to their stronger interaction with natural organic substances and membrane surface (Boudaud et al., 2012; Kreißel et al., 2012). In contrast, in MBRs, bacteriophage removal efficiency was associated with the attachment capability of bacteriophages to activated sludge floc in certain cases, rather than electrostatic interactions with membranes (Chaudhry et al., 2015a).

2.2.3.2. Effect of operating conditions on bacteriophage removal efficiency

(1) Feed water variation:

In real water and wastewater treatment processes, feed water is complex and variable. The composition of feed water, such as its organic content, nutrient amount, ionic strength, and the presence of toxic compounds etc., substantially influences bacteriophage elimination in a membrane filtration process.

A few previous studies have shown an increase of bacteriophage retention with increasing organic contents in the feed water (Huang et al., 2012; Kreißel et al., 2012). The enhancement of bacteriophage removal was mainly attributed to (1) the accumulated fouling layers on the membranes due to rejected large-sized organics (similar to a ‘dynamic membrane’), and (2) the greater pore constriction by the small organics that adhered to the membrane matrix. However, such phenomenon was not clearly observed by other researchers as it would

depend on the feed water composition and membrane used. Branch et al. (Branch et al., 2016) added glucose and glutamic acid into the feed water to increase COD in MBRs and found insignificant increase in bacteriophage removal. They found that although membrane separation enhanced bacteriophage rejection by the greater amount of accumulated fouling layers on the membranes, the entrained bacteriophages within the activated sludge flocs were reduced. In addition, a comparable removal efficiency of bacteriophages in clean water and in organic-contented water (such as surface water, filtered secondary wastewater effluent) was noticed (Huang et al., 2012; Kreißel et al., 2012). Possibly, under such experimental conditions, insignificant membrane fouling occurred due to the limited organic substances, which led to lack of improvement of bacteriophage rejection.

As membrane and bacteriophage surfaces are generally charged, ionic strength could influence the interaction of bacteriophages with the membrane surface. In the absence of organic substances, an increase of ionic strength could reduce electrostatic repulsion between the bacteriophages and like-charged membrane surfaces, leading to decreased bacteriophage retention. Especially, at similar ionic strengths, divalent ions were more effective than monovalent ions in suppressing the electrostatic repulsion between the membrane and bacteriophages, lessening the contribution of electrostatic repulsion to bacteriophage elimination (Huang et al., 2012). In the presence of organic substances and activated sludge, increased ionic strength could reduce the amount of adsorbed bacteriophages within negatively-charged activated sludge (Branch et al., 2016). On the other hand, the presence of organic substances and activated sludge could cause formation of membrane fouling layers or pore blocking, which benefits bacteriophage rejection. Therefore, the overall bacteriophage removal should necessarily be considered in view of the combined effects.

Whether toxic substances in the feed water could significantly influence bacteriophage removal efficiency tends to be case-dependent. For instances, increasing nutrients (such as

NH_3) loading in MBRs could lower bacteriophage rejection because increased pH level could limit bacteriophage aggregation. The addition of 2, 4-dinitrophenol into MBRs did not significantly affect bacteriophage removal. This is associated with the fact that the fewer bacteriophages interacted within the activated sludge flocs, but more serious fouling promoted bacteriophage immobilization (Branch et al., 2016).

(2) Addition of coagulants/flocculants:

In water and wastewater treatment processes, coagulation/flocculation is generally adopted as a pretreatment prior to MF/UF treatment in order to (1) alleviate membrane fouling as the coagulants/flocculants facilitate neutralizing the charge of foulants and enlarging foulant size (Gao et al., 2011; Leiknes, 2009); (2) improve trace organic contaminant removal by combined effects of charge neutralization, entrapment, adsorption, and complexation with coagulant ions into insoluble masses (Alexander et al., 2012).

Recent studies also pointed out that coagulation/flocculation and electrocoagulation pretreatment can significantly enhance the bacteriophage rejection by MF/UF (Chellam and Sari, 2016; Matsushita et al., 2011). It has been found that the coagulants (such as Al- and Fe-based coagulants) dose amount, coagulation duration, pH level, and the natural organic substance amount in the feed water influenced the membrane rejection efficiencies of spiked model bacteriophages (Guo and Hu, 2011; Guo and Hu, 2012; Matsushita et al., 2011; Matsushita et al., 2013; Tanneru and Chellam, 2012; Tanneru et al., 2013; Tanneru et al., 2014). The improved removal efficiencies are ascribed to two major mechanisms, such as (1) physical adsorption by coagulants (Tanneru et al., 2014); and (2) virus inactivation by coagulation because intermediate polymers formed during hydrolysis of the coagulants could adsorb on the viruses and physically interfere with their infectivity of host cells (Matsushita et al., 2011).

(3) Membrane and membrane module specification:

It should be recalled that bacteriophage rejection mechanisms are related to membrane pore size and surface characteristics. Theoretically, lower retention of bacteriophages would occur when membrane pore size is significantly greater than bacteriophage size under non-aggregated conditions. Pore size distribution would also be important if the membrane has a fraction of larger pores that could allow passage of bacteriophages. When membrane pore size is comparable to bacteriophage size, the membrane properties, such as hydrophobic and electrostatic interactions, play crucial roles in determining bacteriophage rejection efficiency (Antony et al., 2012). For instance, a hydrophobic UF membrane tends to retain more bacteriophages than a hydrophilic UF membrane (Boudaud et al., 2012), as noted earlier, possibly due to adsorption.

Due to RO membrane nature, bacteriophage rejection by RO membrane predominantly depends on size exclusion (Antony et al., 2016). Theoretically, a given virgin RO membrane has the capacity to completely reject bacteriophages (Pype et al., 2016a; Shannon et al., 2008). However, periodically chemical cleaning (for removing RO foulants) may physically damage the RO membrane function layer and allow increased bacteriophage passage, as a result, deteriorating bacteriophage removal efficiency (Antony et al., 2016; Pype et al., 2016b). While, membrane ageing usually results in a more negatively charged membrane and also in a higher hydrophobicity, which lead to the increased adsorption of bacteriophages (Pype et al., 2016b). Therefore, both physical and chemical changes of the function layer of the aged RO membrane determine the rejection effectiveness of bacteriophages. Besides this, on a basis of RO membrane module, the failure of module assembly components (such as damaged, degraded, and rolled O-rings) and unfavourable membrane operating conditions (such as water hammer, passage of sharp debris) are the major causes influencing bacteriophage rejection efficiencies (Antony et al., 2016; Jacangelo and Gray, 2015; Surawanvijit et al., 2015).

(4) Membrane fouling situation:

The occurrence of membrane fouling is normally associated with the blockage of membrane pores and the accumulation of cake layer foulants on the membrane surface. A few studies have illustrated that there was a statistically positive correlation of bacteriophage removal efficiency with an increase of fouling potential (Chaudhry et al., 2015b; Chaudhry et al., 2015a; ElHadidy et al., 2014; Huang et al., 2012; Kreißel et al., 2012; Madaeni et al., 1995; Marti et al., 2011). It has been elucidated that bacteriophages could be retained in accordance with either (1) the membrane pore blocking that narrows membrane pores and closes the largest in the pore size distribution (Madaeni et al., 1995; Marti et al., 2011) or (2) irreversible fouling, accumulated on the membrane that cannot be removed by physically cleaning (Chaudhry et al., 2015b; Chaudhry et al., 2015a; ElHadidy et al., 2014).

Whether cake layer fouling (i.e. reversible fouling) could benefit to reject bacteriophages remains uncertain. Some researchers proved that the buildup of protective cake layers did play a crucial role to improve bacteriophage removal (Chaudhry et al., 2015b; Chaudhry et al., 2015a; Yin et al., 2016), but other researchers reported almost no improvement of bacteriophage removal (Marti et al., 2011) or only slightly increased removal (ElHadidy et al., 2014). In particular, some studies emphasized that cake layer fouling began to affect bacteriophage removal only after irreversible fouling had accumulated on membranes, which was possibly attributed to the change of membrane surface charge and/or hydrophobicity because of irreversible fouling (ElHadidy et al., 2014).

On the other hand, a few studies reported an opposite finding that the TMP and/or the permeability of the membrane and the bactriophage rejection were not always correlated (Ferrer et al., 2015). For example, under serious fouling conditions (e.g., 100% increase in TMP), fouling layers did not further substantially improve bacteriophage rejection compared to those under moderate fouling conditions (i.e. 50% increase in TMP) (ElHadidy et al.,

2014). These dissimilar observations are thought to be related to the nature of the various cake layer foulants for different reactor scales, types of bacteriophages, membrane specifications, and operating conditions, and so on.

As fouling is inevitable in the membrane filtration process, it hints that the time for dosing and sampling the model bacteriophages in membrane systems appear to be practically crucial. Periodically physical/chemical cleaning is generally performed in the full-scale membrane treatment processes, after which membrane filterability is expected to be fully or partially recovered. Therefore, dosing and sampling model bacteriophages after membrane cleaning may be more appropriate to accurately evaluate bacteriophage removal capabilities of the membrane systems.

(5) Membrane operation conditions:

Permeate flux is a key operating parameter of a membrane filtration process. In a study of virus removal by MF, Madaeni et al. (Madaeni et al., 1995) observed that *poliovirus* removal was highest at a low flux (and applied pressure) and in the presence of stirring. They also observed that virus rejection followed a transient profile with very high initial removal, due to adsorption to the membrane, followed by a drop and then a gradual increase. The increase was attributed to the combined effects of flux decline and slow pore closure. Similar behaviours of MS2 and phiX174 were noticed by Kreiβel et al. (Kreißel et al., 2012) when the tested nature water with higher organic contents (dissolved organic substances ranged at 1.57-2.28 mg/L) was filtrated with UF membranes. However, the permeate flux did not influence the removal efficiencies of MS2 and phiX174 if less organic substances (0.61 mg/L) were present in the tested water. These findings highlight the difficulty in comparing data unless the experimental protocol is ‘standardised’. In addition, Yin et al. (Yin et al., 2016) emphasized that the flux relaxation and physical-backwashing in a MBR could cause a decrease of virus removal. They found that higher backwashing flux led to more significant

drop of virus removal than a longer backwashing period did. Similarly, chemical-backwashing in a MBR also resulted in a decreased virus rejection by the membrane (Tam et al., 2007; Wu et al., 2010). It is believed that membrane fouling performed a key role in rejection of virus in these studies and resulted in overestimation of virus elimination capability of a membrane.

2.3. Bacteriophages for membrane performance examination - challenges and prospects

As a simplistic procedure to evaluate virus retention capability of a given membrane, the use of bacteriophages has proven to be useful and feasible. However, it is worth noting that a few challenges need to be carefully addressed. First, the concentrations of indigenous bacteriophages are relatively low in water samples and analysis methodologies for some indigenous bacteriophages are not well developed (Leclerc et al., 2000). Enrichment of indigenous phages is generally used when direct plating measurement is applied (i.e., double layer agar method, which provides account of virus particles expressed as plaque forming units). As an alternative detection method, quantitative RT-PCR has successfully been used to detect bacteriophages, however, the accuracy and confidence of the testing still needs to be further developed (Langlet et al., 2009). Therefore, spiking large quantities of model bacteriophages into membrane processes to predict the removal of human enteric viruses have been extensively investigated in the bench-scale and lab-scale membrane systems. However, full-scale membrane challenge testing with model bacteriophages is rarely attempted, although a few recently-reported studies have proven the feasibilities on the use of bacteriophages to examine membrane virus removal efficiencies (Purnell et al., 2015; Regel et al., 2012).

Second, bacteriophages tend to perform self-aggregation or interact with surrounding environments for given sets of physico-chemical compositions of waters and wastewaters,

e.g., at pH below isoelectric point, the presence of organics/activated sludge/coagulants. On the one hand, accurate *in situ* measurement of bacteriophage aggregates size is not feasible owing to the complicated surrounding environment present. On the other hand, the increase in size by aggregation or adsorption largely improves bacteriophage elimination in membrane processes, therefore leading to a possible overestimation of membrane rejection performance (Langlet et al., 2008). Especially, in membrane-based wastewater treatment processes, much higher concentrations of colloidal, particulate, and flocculated particles in the suspensions than those in drinking water treatment processes. The adsorption of bacteriophages on these particles and membrane fouling layer could contribute majorly to their elimination efficiencies.

Third, the membrane and membrane module scenarios are strongly associated with bacteriophage removal efficiencies. It is well reported that the membrane fouling (irreversible and cake layer fouling) facilitates rejecting bacteriophages. However, the occurrence of membrane and membrane module failure due to mechanical, physical, or chemical effects could deteriorate bacteriophage removal effectiveness. Accordingly, it is difficult to accurately evaluate the removal of bacteriophages by membrane processes under such situations. Thus, selecting appropriate bacteriophage spiking and sampling time and ensuring membrane integrity during spiking are crucial issues should necessarily be considered.

Fourth, researchers have pointed out that such bacteriophage indicators used are not always associated with human enteric viruses. An early study illustrated that somatic coliphage and F-specific RNA bacteriophage are indexes of sewage contamination rather than faecal contamination, although they display different behaviours from human enteric viruses (Havelaar et al., 1991). Ottoson et al. (Ottoson et al., 2006) found that somatic coliphage and F-specific bacteriophage removal in a pilot MBR was at log 3.08 and 3.78 respectively, but human virus genomes were not as efficiently rejected as bacteriophages, with log 1.8 and 1.1

for enteroviruses and noroviruses, respectively. The differences are more or less pronounced depending on different detection methods, i.e., phages are cultured, while human viruses are detected with RT-PCR. RT-PCR could detect the virions which are not necessarily capable of causing infection or growing, resulting in underestimating infectious virus removal (Purnell et al., 2015).

As current challenges emphasize the limitations of accuracy of bacteriophage detection methods and the difficulties of inline assessment of the bacteriophage retention in membrane processes, research work in these directions should attract great attention. To improve bacteriophage detection limitation, a bacteriophage-based biosynthetic tracer has been developed by Soussan et al. (Soussan et al., 2011). This new tracer was designed by grafting enzymatic probes on the surface of an MS2 bacteriophage, which allows it to be directly and quickly quantified by spectrophotometry, fluorometry or amperometry. In addition, to overcome cumbersomeness in artificially propagating bacteriophages, Matsushita et al. (Matsushita et al., 2013) attempted to prepare virus-like particles, which consist of an artificially expressed norovirus capsid protein and therefore are morphologically and antigenically the same as native norovirus particles.

On the other hand, as the behaviours of some bacteriophage indicators are not always associated with those of human enteric viruses, examination of several bacteriophage groups may offer a practical and conservative way to assess human enteric virus removal efficiency, especially in full-scale membrane processes.

3. The role of bacteriophages in examining membrane integrity

3.1. Examination of membrane integrity by monitoring added model bacteriophages

In membrane processes, concerns regarding membrane integrity have been raised because compromised membrane integrity could allow unfavourable matter (especially pathogens) to

pass through the membranes and lead to lower permeate quality. Membrane integrity loss could happen due to physical damage and chemical attack (Ferrer et al., 2013; Ferrer et al., 2015). Therefore, periodically membrane integrity is monitored during operation, so that detecting and repairing membrane defects in the drinking water and wastewater treatment plants are crucial in order to achieve the designed membrane performance (Brehant et al., 2010). Non-invasive direct tests (sonic or acoustic sensing, porosimetry, pressure-based tests, etc.) and indirect tests (turbidity monitoring, particle counting, surrogates-based tests etc.) have been successfully applied to monitor membrane integrity in pilot-scale and full-scale plants (Antony et al., 2012; Guo et al., 2010).

Among these approaches, the use of model bacteriophages as surrogates to monitor membrane integrity has been considered as an online indirect test because (1) human virus seeding is hardly feasible or safe in water and wastewater treatment and (2) naturally occurring bacteriophages are thought to be the most suitable surrogates for enteric viruses to monitor membrane performance (Ferrer et al., 2015). The bacteriophage-based membrane integrity test displays a relatively high detection sensitivity compared to most of the integrity test approaches. It has been reported that under some cases, bacteriophages may pass through the membranes with small pin-holes and macro-pores, even though the membrane exhibits acceptable pressure decay (Pontius et al., 2011).

Although the traditional double-layer agar method is simple and cost-effective in examining bacteriophage amount, a long testing time (1-2 days) and prevention of bacteriophage aggregation is necessary. This could delay the detection time and reduce the detection accuracy. Alternatively, advanced molecular biological analysis such as the quantitative real-time polymerase chain reaction (RT-PCR) has recently been widely employed as an alternative method to monitor bacteriophages in membrane filtration process due to its fast response time and is routinely customized (Antony et al., 2012).

If the membrane removal efficiency of the dosed bacteriophage indicator is comparable to that of naturally occurring bacteriophages analyzed in routine sampling, this indicates that membrane integrity has not compromised or the properties of the membranes that affect the bacteriophage removal have not varied (Ferrer et al., 2015; Mi et al., 2004). Thus, the bacteriophages used in membrane performance examination are appropriate to membrane integrity monitoring. It has been elaborated that the surrogate bacteriophages with a lower rejection rate in membrane processes is suggested to be a better candidate for determination of membrane virus removal in worst case scenarios (Langlet et al., 2008). For membrane integrity tests, the bacteriophage having greater size than the tested membrane pore size is thought to be a better choice as size exclusion plays a predominant role rather than adsorption and static interactions (Ferrer et al., 2015). This guarantees that a small leakage of the tested membrane could be identified.

3.2. Bacteriophages for membrane integrity monitoring - challenges and prospects

It is worth noting that the factors that influencing bacteriophage removal in membrane processes (discussed in section 2.2.3) could also interfere with the membrane integrity test. On one hand, this means that besides size exclusion, other mechanisms such as adsorption and electrostatic interactions could perform inevitable interference in determining bacteriophage monitoring efficiency. Therefore, the variations of real feed composition and membrane surface properties due to fouling layer development inevitably affect the evaluation of membrane integrity. On the other hand, any existing fouling (especially irreversible fouling) may block the damaged part of the membrane, facilitating rejection of bacteriophages. While, the failure of module assembly components (such as damaged, degraded, and rolled O-rings) could provide passages for bacteriophages (Jacangelo and Gray, 2015). Accordingly, the failure of membrane integrity could not be accurately detected. We have to recognize that the use of bacteriophages to test membrane integrity in full scale plant

is not economically and technically feasible due to the cost in obtaining enough seeding bacteriophages, the sensitivity of detection equipment, and complex situations during operation (Ferrer et al., 2015; Pype et al., 2016a), although a few pilot-scale bacteriophage-based testing has been illustrated (Jacangelo and Gray, 2015).

It is noted that the bacteriophage method displays a lower detection limit compared to other online membrane integrity monitoring methods, therefore, researchers are attempting to integrate the developed mathematic models and bacteriophage monitoring technique to further improved membrane integrity prediction and sensitivity (Brehant et al., 2010; Mi et al., 2004). As hydrodynamics near the membrane surface have not been fully considered in the current bacteriophage-based methods for membrane integrity assessment, Pontius et al. (Pontius et al., 2011) attempted to develop a Lagrangian particle-tracking model to describe the movement of bacteriophages with space and time in membrane processes. This predictive model is in good agreement with the findings in bacteriophage challenge tests, which provides a possibility to use this model to accurately predict membrane integrity. In addition, the improvement of bacteriophage detection limitation could lead to more accurate membrane integrity detection. For example, it is well known that fluorescent particles can be used as surrogates in membrane processes (Surawanvijit et al., 2015), and Gitis et al. (Gitis et al., 2006) have proposed a new integrity probe by labelling bacteriophages with fluorescent dye, which could effectively detect nanometric scale breaches of UF membranes (i.e., single parts per billion).

4. The role of bacteriophages in controlling membrane biofouling

4.1. Mechanisms of bacteriophage action in biofilm control

Microorganisms are naturally present in all water resources. Inevitably, the deposition/growth of microorganisms and accumulation of microbial products (such as extracellular polymeric

substances, EPS) on membranes will occur in membrane-based water and wastewater treatment processes, leading to membrane biofouling (Wu and Fane, 2012). Membrane biofouling is a major drawback of membrane processes which leads to decreased membrane performance and increased maintenance cost.

Conventional physico-chemical biofouling control methods have been widely used in membrane processes, such as employment of pretreatment, optimization of operation conditions, application of biocides (such as chlorine, ozone, UV), and periodically physical and chemical cleaning (Al-Juboori and Yusaf, 2012; Matin et al., 2011; Wu et al., 2011a, 2011b). In addition, researchers have developed novel anti-biofouling membranes by modification of membrane surfaces and incorporation of nanomaterials into the membrane matrix (Ng et al., 2013). Recently, biological-based membrane biofouling control strategies, such as inhibition of quorum sensing, dispersal by use of nitric oxide, enzymatic disruption of extracellular polymeric substances, inhibition of microbial attachment by energy uncoupling, and disruption of biofilm by bacteriophages have been developed and received great attention. A few review articles have summarized recently-reported biological-based strategies in the control biofilm growth and membrane biofouling (Malaeb et al., 2013; Siddiqui et al., 2015; Xiong and Liu, 2010).

Among these biological-based biofouling control strategies, the bacteriophage-based method is starting to attract researchers' interests, especially when the continued emergence of antibiotic resistant bacteria has been recognized. This concept is initially derived from the "bacteriophage therapy" technique, which has been widely applied in many areas, such as the medical industry, food industry, and agriculture (Chan et al., 2013; Duckworth and Gulig, 2002; Nobrega et al., 2015).

The mechanisms of bacteriophages breaking down the host cells are associated with two different life cycles (i.e., lytic and lysogenic cycles, Figure 2) (Campbell, 2003; Kingwell,

2015). The lytic bacteriophages are known as virulent bacteriophages that synthesize and assemble new phage particles in the infected cells and then lyse the host cells. The released new phages in turn infect adjacent fresh host cells. The lysogenic bacteriophages are also named as temperate bacteriophages, which either undergo a lytic cycle or integrate their genome with the bacterial genome (i.e., prophage). The host cell that harbours a prophage is named as a lysogenic host, which can multiply and transfer the prophage through many generations. Prophages could be released from their host cells and re-enter lytic cycles under certain conditions (such as UV irradiation, mutagenic compounds, and unfavourable temperatures, etc.) (Campbell, 2003; Obeng et al., 2016). Recent evidence has shown that the temperate phages can promote bacterial hosts to respond rapidly to fluctuated surrounding environments (Obeng et al., 2016).

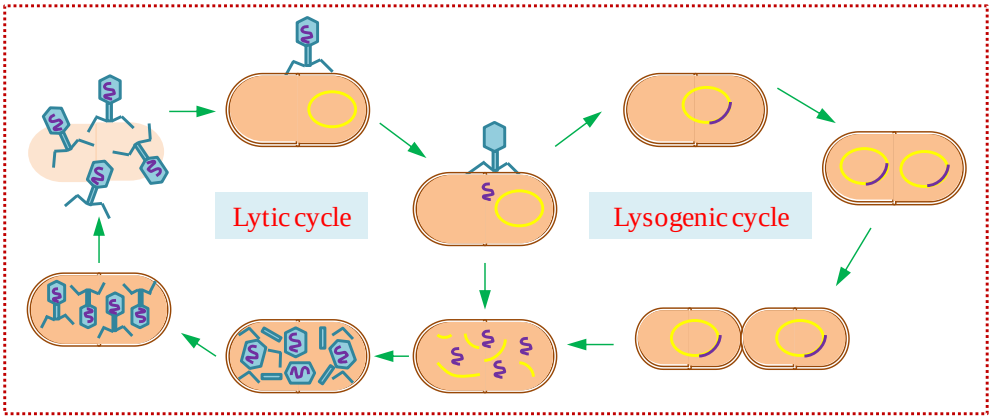

Figure 2. A schematic diagram illustrating the two life cycles of bacteriophages (Campbell, 2003; Kingwell, 2015).

Such natural properties of bacteriophages provide a possibility to control biofilm development, namely (1) bacteriophages replicate directly at the site of infection and are strongly associated with viable bacterial hosts, which could achieve *in situ* biofilm control; (2) bacteriophages produce enzymes that could hydrolyze biofilm polymeric matrix; (3) bacteriophages have total compatibility with other biofouling control strategies; (4) isolation and large-scale production of bacteriophages is potentially feasible, which allows production at an industrial scale (Balcão et al., 2014; Campbell, 2003).

Various model bacteriophages infecting pure culture-formed biofilms have been extensively investigated as bacterial population-size controllers. However, the reported natural host range is still limited. Importantly, the research work related with the bacteriophage-based membrane biofouling alleviation concept is still at a very early stage. So far, only one study has been reported by Prof Armon's group (Goldman et al., 2009). In this study, specific lytic bacteriophages that can infect *P. aeruginosa*, *A. johnsonii* and *B. subtilis* were selected and added into the feed water at a concentration of 6×10^5 CFU/100 mL in a bench-scale UF filtration system. It was observed that the dosed bacteriophages lessened the biofouling layer formation on the membrane surfaces, which allowed 40-60% higher membrane permeability. Furthermore, compared to the morphology of bacteria in the biofouling layer matrix without adding bacteriophages (as control experiment), the bacteria seemed "wrinkled" in the presence of bacteriophages as a result of the infection (Goldman et al., 2009).

It has been noticed that some bacteriophage excrete enzymes (called 'protein antibiotics') and are capable of causing rapid cell wall lysis or interfering cell wall formation, therefore preventing growth of the target bacterium. Bacteriophage lysins (phage encoded peptidoglycan hydrolases) are a kind of well-known enzyme that could break down the bonds in the peptidoglycan layer of the bacterial cell wall. Generally, at the terminal stage of the phage reproduction cycle, bacteriophage-encoded holins (a kind of small hydrophobic proteins) create holes in the cytoplasmic membrane of the host cell by oligomerization, which allows the bacteriophage-released lysins to enzymatically degrade the peptidoglycan layer in the infected bacterial cell wall (Loessner, 2005; Meng et al., 2011; Schmelcher et al., 2012).

More interestingly, lysins are capable of killing susceptible microorganisms when applied exogenously as recombinant proteins, which makes it potentially possible as anti-microbial agents. It is important to note that lysins can directly access Gram-positive cells due to the absence of outer cell membranes. For Gram-negative cells with outer membranes, lysins can

601 only perform lysis roles after the outer lipopolysaccharide layer is disrupted by additional
602 chemicals (ethylenediamine tetraacetic acid, detergents, etc.) (Loessner, 2005; Schmelcher et
603 al., 2012). In addition, some bacteriophages (such as Qβ, ϕX174) with small, single-stranded
604 nucleic acid genomes do not produce such proteins with muralytic activity, but produce
605 single proteins that interfere with murein (proteins that form the cell wall) biosynthesis and
606 assembly (Bernhardt et al., 2001; Young et al., 2000). Such protein antibiotics are believed to
607 perform anti-microbial behaviour (Loessner, 2005).

608 *4.2. Bacteriophages for biofouling control - challenges and prospects*

609 Although bacteriophage-based techniques are well recognized as effective biofilm control
610 solutions, they still lack real application in membrane fouling control. This may be attributed
611 to a few limitations of this technique. Firstly, we still lack sufficient information for well-
612 characterizing bacteriophages. At present, only ~500 complete bacteriophage genomes have
613 been sequenced. Thus, the host range of bacteriophages often consists of only a subset of
614 strains making up a single bacterial species, appearing to be relatively narrow (Chan et al.,
615 2013). This is the reason that the research efforts on bacteriophages controlling biofilms
616 mainly focus on single bacteriophage systems for the pure culture model host cells. A
617 knowledge gap between lab research findings and real applications of bacteriophages requires
618 further studies before bacteriophages can be considered as suitable candidates. In particular,
619 how and to what extent do environmental conditions influence bacteriophage-based
620 membrane biofouling control needs to be determined.

621 Secondly, bacteria appear able to potentially develop defence strategies (i.e., bacterial
622 immune systems) to existing bacteriophages and phage-encoded virulence genes that can
623 incorporate into the host bacterial genome (Obeng et al., 2016). The bacterial immune system
624 might inactivate bacteriophages. Nevertheless, it is conceivable that bacteriophages are more

refractory to bacterial resistance development than antibiotics. Although bacteriophages themselves could also evolve to overcome such bacterial resistance (Carrolo et al., 2010; Obeng et al., 2016), the ability to evolve may raise serious safety issues. It is worth noting that mutual interactions between bacteriophages and host bacteria have also shaped their co-evolution (Obeng et al., 2016). For instance, when a sufficient number of cells in a biofilm are lysogenized, the biofilm indeed tends to be more prolific and stable (Carrolo et al., 2010; Obeng et al., 2016).

To reduce the development of phage resistance, a feasible solution is to isolate new bacteriophages which cannot be resisted by the host bacteria. In addition, researchers have attempted to engineer bacteriophages that exert minimal evolution pressure. These engineered bacteriophages could not only enhance killing phage-resistant bacteria and antibiotic-resistant bacteria (Lu and Collins, 2009), but also improve biofilm disperse by disrupting the extracellular polymeric substances matrix (Lu and Collins, 2007). Alternatively, a combination of different bacteriophages (i.e., a phage cocktail) or bacteriophages combined antibiotics may also reduce the prevalence of bacteriophge resistance (Chan et al., 2013; Gu et al., 2012). More interestingly, certain antibiotics at sub-lethal concentrations could stimulate the host bacterial cell's production of some virulent phages, as a result, promoting the killing efficiency (Comeau et al., 2007; Ryan et al., 2012).

Thirdly, the predation relationship between bacteriophages and bacteria is rather complex. To achieve effective infection, the ratio of bacteriophages to viable cells should be within an optimal range. Also bacteriophages display high specificity against their target bacteria, whereas in the real world, the bacterial composition is complex, therefore requiring the development of phage mixtures.

Fourthly, the direct addition of bacteriophages into membrane processes to control membrane fouling may not be practically feasible, especially in large-scale membrane processes. A potential solution is to integrate bacteriophages with the membrane functional layer. Considering the fragile properties of bacteriophages, directly embedding bacteriophages into the membrane matrix poses a challenge. However, encapsulation of bacteriophages before mixing with the membrane materials, aiming to maintain their full structural and functional stabilization with decreased bulk size, may be feasible. In a recent research effort, immobilization of bacteriophage entities was achieved via entrapment within porous polymeric matrices of alginate and agar (Balcão et al., 2013). In addition, integration of bacteriaophage particles within lipid nanovesicles is another possible approach to achieve stabilization of bacteriophage structure and activity (Balcão et al., 2014).

An alternative solution is to use bacteriophage-based enzymatic (protein) antibiotics to alleviate membrane fouling. Compared to antibiotics and bacteriophages, such protein antibiotics display a few advantages, such as (1) protein antibiotics have their specificity for the pathogen without affecting normal flora; (2) protein antibiotics induce less chance of bacterial resistance compared to antibiotics and bacteriophages; (3) a small amount of protein antibiotics is sufficient to rapidly lyse a dense suspension of cells within minutes or even seconds, indicating high lysis activity and efficiency; (4) it is more practical and advisable to administer protein antibiotics compared to conventional antibiotics even at high doses (Loessner, 2005). Importantly, bacteriophage-based protein antibiotics have been proven to more efficiently remove biofilms compared to antibiotics or bacteriophages (Meng et al., 2011; Schmelcher et al., 2012). Meanwhile, a combination of protein antibiotics with conventional antibiotics tends to synergistically promote biofilm dispersal and inactivate the released cells (Djurkovic et al., 2005; Meng et al., 2011). It is also suggested to combine different enzymatic proteins with different substrate specificities to improve lysis kinetics

673 (Loeffler and Fischetti, 2003). In this regard, protein antibiotics may be promising and
674 feasible membrane biofouling control agents in an age of mounting antibiotic resistance.

675 As aforementioned, the encapsulation technique provides a possibility to integrate these
676 protein antibiotic particles into nanometer-sized vesicles so as to prohibit them from
677 deactivation by the immune system and dilution effects. Such vesicles are expected to be
678 integrated into the membrane matrix to perform as "antifouling membrane". In addition, such
679 bacteriophage-based enzymatic proteins could be considered as "cleaning chemicals" for
680 maintenance cleaning during membrane operation, especially at a low pressure dead-end
681 filtration mode (e.g., gravity-driven membrane filtration). Future studies should place a focus
682 on developments of novel protein antibiotics-based anti-fouling membranes and protein
683 antibiotics-based membrane cleaning protocols.

684 **5. Conclusions**

685 As indicators of human pathogenic waterborne viruses, bacteriophages perform crucial roles
686 in assessing membrane performance and integrity in membrane-based water and wastewater
687 treatment processes. Although the use of bacteriophages in the bench-scale and lab-scale
688 experiments has been well illustrated, detection accuracy, sensitivity, and practical feasibility
689 are major challenges for large-scale membrane-based water and wastewater treatment
690 processes. Many strategies have been proposed to overcome such limitations, for instances,
691 integrating bacteriophages with fluorescence dye/enzymatic probes and developing
692 bacteriophage-based protein particles.

693 On the other hand, bacteriophages display a potential role in alleviating membrane fouling.
694 However, maintaining bacteriophages or bacteriophage-encoded protein antibiotics in
695 membrane processes has faced some challenges. Recently developed controlled-release
696 encapsulation techniques are anticipated to explore opportunities to integrate protein

antibiotics with the membrane matrix. This could open a new perspective towards fabricating novel bacteriophage-associated anti-fouling membranes. In addition, bacteriophages-associated membrane cleaning protocols should potentially be developed as an alternative strategy to achieve sustainable membrane operation.

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