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Mitigation of Antimicrobial Resistance during Wastewater Treatment by Membrane Technologies

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ABSTRACT

The threat of antimicrobial resistance (AMR) to human health is predicted to become a significant infectious disease. Domestic sewage and wastewater treatment plants (WWTPs) are critical hotspots for controlling the spread of antibiotic resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) in the environment. A wastewater treatment system is not necessarily designed to mitigate AMR problems in wastewater. Furthermore, the presence of ARB and ARGs for a long time in WWTPs is reported as a reservoir of intracellular and extracellular ARG through horizontal gene transfer. Based on the studies, the additional membrane filtration with either microfiltration (MF) or ultrafiltration (UF) can reduce ARB and ARGs effectively through the separation mechanism. However, there are still inconclusive results when comparing ARG removal efficiencies between MBR and conventional processes. Further studies are required to clarify the effect of water qualities and membrane fouling conditions on ARG removal.

Keywords: Antimicrobial resistance (AMR), antimicrobial resistance gene (ARG), membrane bioreactor (MBR), wastewater treatment, reclaimed water

1.0 INTRODUCTION

Antimicrobial resistance (AMR) is a global threat to human health and become a major cause of death in the world [1]. The dissemination of AMR from anthropogenic activities causing adverse impact to human and animal health leading to the emergence of AMR [2]. In the context of 'One Health', which is the integrated approach to attain optimal health of human, animals, and ecosystems, it is essential to mitigate the spread and development of AMR in the environment. Wastewater is a major source of AMR, namely antibiotic resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) harboured by ARB [3-5]. Wastewater treatment plants (WWTPs) are an important barrier to controlling the dissemination of AMR from wastewater. However, conventional wastewater treatment processes are generally designed to remove suspended solids, organic matter and nutrients from wastewater and their operating conditions are not necessarily optimum to mitigate AMR in wastewater. Recently, the application of membrane technologies to water treatment is reportedly promising to

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reduce AMR in wastewater and reclaimed water. This review aims to provide up-to-date knowledge on the reduction of AMR by membrane incorporated wastewater treatment processes and perspectives for future research and development.

2.0 AMR IN WASTEWATER AND ITS REDUCTION

Anthropogenic pollution after the post-antibiotic era tends to be the threat of AMR in domestic wastewater. WWTPs are believed to be the key hotspots for AMR dissemination into aquatic environments [6, 7]. The presence of antibiotics inside the water environment is normally reported at low concentrations [8]. Still, it persisted there for a long time and accelerated the occurrence of ARB and ARGs in WWTPs [9]. The design of conventional WWTPs is focused mainly on removing organic matter and nutrients [10] but not for antibiotics, ABR and ARGs [11]. The abiotic conditions (pH, temperature) and biotic conditions (microorganisms, enzymes) of WWTPs affected the abundance of ARB and ARGs [12, 13]. The presence of 10^7 level of colony forming unit/100 ml of ARB in municipal wastewater [14, 15]. Alexander *et al*. [16] detected up to 10^{15} cell equivalents per day in a WWTP catchment area of 34,000 population. In WWTPs, microbes will provide and have ARGs via horizontal gene transfer (HGT) through mobile genetic elements such as integrons, transposons, bacteriophages, and plasmids. Cell-to-cell contact (conjugation) and phage infection (transduction) are also spreading intracellular ARGs (iARGs). Meanwhile, extracellular ARGs (eARGs) originating from cell auto-secretion, death, lysis, predation, and phage infection represents a significant proportion of the total genetic elements [17] and is assimilated by bacteria through transformation [18]. However, the profiles of iARGs and eARGs in WWTPs have not been thoroughly explored especially in different treatment stages.

Previous studies at WWTPs showed a significant decrease in the prevalence of ARGs and antibiotics during the treatment [19]. The relative abundance of most ARGs was found decreased after biological treatment in both high and low antibiotic consumption groups in seven European countries. Others reported an increased relative abundance of some ARGs in sludge and treatment effluent [20]. This observation may relate to the dynamics of the ARB bacterial host in wastewater treatment processes, and operational conditions, e.g., temperature, hydraulic and sludge retention times. Some researchers verified the evidence of sludge in WWTP as a pool for ARB and ARGs as they were detected in higher concentrations. In a conventional WWTP, wastewater is treated in biological (activated sludge) and physical (solid separation) steps [21]. The potency of conventional WWTPs on the removal of ARB is shown at various degrees, ranging from 1 to 2 log [15, 22, 23]. On the other hand, the appearance of ARGs and antibiotics was still detected in wastewater effluent in the surface water body [24]. In this context, additional treatments such as membrane filtration can be applied as advanced processes for mitigating AMR problems [25,26].

3.0 AMR MITIGATION BY MEMBRANE TECHNOLOGIES

A membrane bioreactor (MBR) process is being widely applied because of its capability to produce stable effluent qualities through membrane filtration of mixed liquor sludge. It has a more robust removal performance of AMR components than other conventional wastewater treatment processes [27, 28]. In general, the MBR process often achieves 1-5 log higher removal of ARB than other biological processes [29, 30]. A study reported up to 6 log reduction of coliform and *E. coli* in MBR [31]. Moreover, aerobic conditions in MBR also contributed to the mitigation of facultative ARB growth [32]. As sludge sorption and its separation from water are the main mechanism for ARB reduction during wastewater treatment [33], their removals in MBR could be enhanced due to higher sludge concentrations maintained in the MBR as well as more effective solid separation through membrane filtration. Wang *et al*. [34] reported that membrane filtration by pore size sieving is the key channel for the ARB removal in full-scale MBR. Meanwhile, the ARB reduction in conventional wastewater treatment process is mainly relies on solid-liquid separation performance i.e., sedimentation of sludge adsorbing ARB and ARGs [35]. Additionally, MBR showed 1-3 orders of magnitude higher removals of ARGs than other conventional biological processes [36]. The concentration of ARGs in MBR permeate reportedly reached at least 1 log less than that in conventional activated sludge effluent [29]. The aforementioned studies [29,36] showed that additional membrane filtration enhances the removals of abundant AMR components in MBR, rather than differences in efficiency for adsorption compared with the conventional processes. Overall, MBR can effectively remove ARGs in wastewater through both (i) adsorption on sludge and (ii) separation of sludge where ARGs are adsorbed such as effective size exclusion, hydrophobic adsorption, and electrostatic repulsion [37, 38]. Retaining of high biomass concentration in MBR is conducive to enhancing adsorption and thus promotion of ARGs removal [39]. The adsorption on sludge particles and rejection on the cake layer are major mechanisms for the removal of eARGs in the MBR [40]. Likewise, Zhu *et al*. [38] reported that dense fouling layers on membranes can enhance the removal of ARGs in MBR. The fouling layer on the membrane reduced the effective permeate pore size of the membrane for rejection of ARGs, although ARGs are smaller than the nominal micro-membrane pore size. Therefore, high biomass concentration and additional membrane filtration including cake layer filtration contributed to enhancing ARGs removal in MBR to compare the conventional processes. Moreover, fouled membranes caused surface characteristics to become more hydrophobic to enhance the removal of both the ARB and ARGs to adsorption [41]. Wastewater colloid components (protein and polysaccharides) and membrane removal of ARGs also found correlated significantly [42]. Genetic elements with negatively charged phosphate groups binds to soluble microbial products (SMP) and extracellular polymeric substances (EPS), which contain protein and polysaccharides in foulants with a negative charge in presence of divalent cations such as Ca^{2+} and Mg^{2+}. The removal mechanisms of AMR components during membrane filtration are illustrated in Figure 1.

To control the release of ARB and ARGs more effectively, the use of MBR with post-treatment using either tight ultrafiltration (UF) or nanofiltration (NF) membranes or strong chemical oxidation would be required. The differences in membrane types, i.e., microfiltration (MF), UF, NF as well as membrane materials are likely to impact the removal of ARGs on membrane interior and surface.

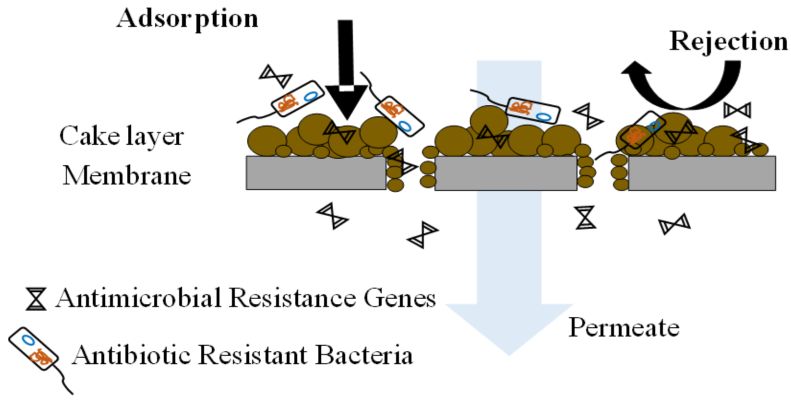

Figure 1 Removal mechanisms of AMR components during membrane filtration

Table 1 summarizes ARB and ARG removal performance of different membranes. Generally, higher degree of ARB could be achieved even using larger pore-size MF membranes. However, the capacities of MF and high molecular weight cut-off (MWCO) UF membranes are limited for ARGs removal. Applying tight UF or NF membrane filtration would be required to achieve high removal of ARGs [43]. Therefore, there is a high potential for tight UF or NF membranes to be an effective barrier for enhancing the removal of AMR components.

Table 1 Log reduction values (LRVs) of ARB and ARGs by different membranes

Membrane type/ material	Pore size or MWCO	Target ARB or ARGs	Log reduction values (LRVs)	References
MF/PVDF	0.3μm	*Escherichia coli* PI-7, *Klebsiella pneumoniae* L7, *E. coli* UPEC-RIY-4	5.4-6.5	[41]
MF/PVDF	0.22μm	*tet*A, *int*1, *sul*I, *sul*II	2.7-4.0	[44]
MF/PVDF	0.1μm	*sul*I, *sul*II, *tet*C, *tet*X, *ere*A, *int*1	0.6-5.6	[38]
UF/cellulose	100kDa	bla_{TEM}, *van*A,	0.9	[42]
	10kDa		3.6	
	1kDa		4.2	

4.0 CHALLENGES AND FUTURE PERSPECTIVES

Since ARBs could be effectively removed during membrane filtration through the size exclusion effect, challenges remain in the retention of ARGs during wastewater treatment. Integration of membrane filtration in secondary treatment processes such as MBRs relies on the use of larger pore size membranes such as MF or UF as they would allow achieving reasonable permeate flux during mixed liquor filtration. Meanwhile, their applications in tertiary treatment usually utilize denser membranes such as low MWCO UF, NF, or reverse osmosis (RO) membranes as the main focuses are to achieve efficient pollutant rejections so that the treated water could be purified sufficiently for their reuse purposes.

The extent of ARGs prevalence and retention in MBRs and their influencing factors are still relatively unclear. ARB population can be potentially promoted through HGT mechanism as the MBR are normally operated at higher biomass concentrations than those in other conventional processes. Nevertheless, long sludge age conditions maintained in the MBRs could also reduce ARB propagation [32]. For ARGs, higher aeration intensity usually employed in MBRs could also promote cell lysis and ARGs leakage from cells thus resulting in higher concentrations in the mixed liquor. But their removals in MBRs could be effective due to adsorption to sludge and membrane filtration effects. There are still contradictory reports on whether MBRs were more effective for ARG removals than other conventional processes or vice versa [36,45]. Li *et al.* [36] reported high ARG removals than parallel oxidation ditch and sequencing batch reactor process from membrane separation effect even though their abundance in activated sludge were distributed similarly in MBR and parallel processes. Moreover, the role of the membrane fouling layer in MBRs to help to mitigate ARG leakage has been reported [38]. On the other hand, Luo *et al.* [45] reported that high activated sludge concentrations and biofilm bacteria in the MBR process provide favourable conditions for HGT of ARGs. As good settling of activated sludge may lead to the transfer of most bacteria including ARGs to biological solids therefore higher sludge volume index in MBR process could not effectively prevent the leakage of eARGs. More studies are therefore needed to identify appropriate operating conditions of MBRs and their fouling control strategies for mitigating ARGs release.

In tertiary treatment, significant ARGs removal would require tight membranes and the presence of wastewater colloids enhanced their removals [46]. Moreover, membrane materials and their surface charges also play an important role in the prevention of ARG penetration. The use of MF with pre-coagulation is reported effective in ARG removal [44]. However, the effect of water qualities and membrane fouling conditions should be further studied to clarify their impact on ARG removals.

5.0 CONCLUSION

Membrane bioreactor (MBR) technologies have the potency to tackle AMR dissemination in an environmental ecosystem. The concentration of ARB and ARGs in wastewater treatment effluent is reportedly better in the MBR than in conventional treatment processes. Size exclusion, hydrophobic adsorption, electrostatic repulsion, etc., is one of the reasons for MBR performance to reduce ARGs prevalence. The exact process of MBR or other membrane-applied wastewater treatment processes to mitigate AMR problems is still unclear and needs further study.

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