Removal Ability of Antibiotic Resistant Bacteria (Arb) and Antibiotic Resistance Genes (Args) by Membrane Filtration Process

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Abstract. Recently, the environmental pollution caused by the extensive use of antibiotics is becoming more and more serious, and the existence of antibiotics may accelerate the prevalence and spread of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB), and thus endangering human health. The need for wastewater reuse is increasing due to the water scarcity, and membrane process including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) has been widely used in wastewater reclamation process. Consequently, understanding the removal efficiency of ARB and ARGs in membrane filtration process is of great significance. The main purpose of this study was to determine the removal efficiencies of ARB and ARGs by different membrane filtration processes (i.e., MF, UF, NF and RO), analyze the influencing factors, and summarize the removal ability of different membrane filtration processes. In addition, the removal ability of ARB and ARGs by membrane-based integrated processes was studied, and suggestion on future wastewater reclamation was also proposed.

1. Introduction
Recently, antibiotics have been widely used in the breeding industry and animal husbandry. Due to the incomplete metabolism of antibiotics by humans and livestock, they are frequently detected in water, soil and other environments. The environmental pollution caused by antibiotics is becoming more and more serious, which has become one of the current research hotspots in the world [1]. At the same time, the selective pressure caused by antibiotics may arouse the prevalence of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB), thus threatening human health. In the post-antibiotic era, it is assumed that 10 million people will die due to the failure of antibiotic
treatment each year, and the total loss of global GDP may reach US $10 billion [2]. Some new ARGs, such as ARGs NDM-1 and McR-1, have attracted great attention [3], which exhibited resistance to one of the latest classes of antibiotics, i.e. polymyxins [4]. NDM-1 is resistant to β-lactic acid drugs as well as many other antibiotics[5]. NDM-1 and McR-1 are abundant in the influent and biological treatment units of wastewater treatment plants (WWTPs), and significant amount of NDM-1 and McR-1 are detectable in the effluent of WWTPs [3].

Currently, as available water resources in the global range are greatly reduced, wastewater recycling has become one of the important measures for rational utilization of water resources [6]. China is a populous country with limited water resources, and its fresh water available per capita is only about a quarter of the world's per capita. With population growth and industrialization, water pollution is gradually aggravate [7]. Membrane separation is one of the most widely used technologies in wastewater recycling. The membrane filtration process can be classified into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) according to the molecular weight of membrane interception. MF can efficiently treat wastewater containing coatings and oil stains [8]. UF has been usually used in medication, food production, and ultra-pure water manufacturing [9]. During NF process, inorganic salt separation is not affected by electric potential and chemical gradient. Molecules with a diameter of about 1 nm can be effectively rejected by NF with actual pressure less than or equal to 1.5 MPa, resulting in the purification of wastewater. RO is widely used in urban wastewater reclaimation, and plays a vital role in wastewater treatment of chemical, metallurgical and papermaking industry [10]. With the increasing demand of wastewater reuse, the application of membrane separation will be more wide in wastewater treatment. As a result, investigating the removal ability of ARB and ARGs in different membrane separation processes is vital for the safety assessment of wastewater reclaimation and reuse.

The main purpose of this study is to determine the ability of different membrane filtration processes to remove ARGs and ARB, discuss the influencing factors to remove ARGs and ARB in the membrane filtration processes, and put forward the prospect of the development direction of wastewater reclaimation process towards the safety of wastewater reuse.

2. The occurrence of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) in wastewater treatment process and its potential threat

Generally, ARGs could be divided into six categories, i.e. tetracycline, sulfonamide and trimethoprim, quinolone, macrolide, β-lactam and multidrug resistance. Bacteria can obtain antibiotic resistance through two ways: one is that antibiotics below lethal dose induce new mutations in genes to develop resistance; The other is resistance acquired through vertical or horizontal gene transfer. Horizontal gene transfer was mediated by modified genetic elements (MGEs) like bacteriophages, integrons, transposons and plasmids [11]. Meanwhile, antibiotic resistance can also be obtained through uptake of extracellular ARGs (eARGs) [12]. When resistant microbial cells break down, the ARGs inside the cells is released into the environment. The extracellular antibiotic resistance genes (eARGs) dissociate or attach themselves to the surface of other particles in the environment, while the eARGs adsorbed on the surface of particles could persist for a long time. [13]
| ARB/ARGs type | Influent | Effluent | Removal efficiency | Treatment process | Ref |
|---------------|---------|----------|--------------------|-------------------|-----|
| Sulfamethoxazole resistant bacteria; Tetracycline resistant bacteria; total HPC | N.A. | Sulfamethoxazole resistant bacteria 3.09×10³ CFU/mL; Tetracycline resistant bacteria 1.05×10⁴ CFU/mL; total HPC 4.68×10⁴ CFU/mL | 2–3 logs | N.A. | [14] |
| tetO genes; tetW genes | tetO 5.13×10⁹ gene abundances/L; tetW 5.13×10⁹ gene abundances/L | tetO 9.12×10⁵ gene abundances/100 mL; tetW 5.13×10⁵ gene abundances/100 mL | 2–3 logs; tetO 3 logs | N.A. | [15] |
| sulI gene | 1.82×10⁸ gene abundances/100 mL (1.46±0.34) × 10⁵ to (1.78 ±0.51) × 10⁸ copies/mL | 1.05×10⁶ gene abundances/100 mL (2.08 ±0.16) × 10³ to (3.68 ±0.27) × 10⁶ copies/mL | 1-2 logs | N.A. | [16] |
| ARGs tet genes | N.A. | N.A. | 2–3 orders of magnitude | N.A. | [18] |
| sul genes | N.A. | N.A. | 1–2 orders of magnitude | N.A. | [19] |
| sulfonamide resistance genes | N.A. | (6.7±7.2)×10⁵ copies/mL | N.A. | conventional activated sludge process | [20] |
As summarized in Table 1, the removal efficiencies of ARB by the WWTPs with biological processes are relatively high, while ARGs could not be efficiently removed [23]. Generally, about 0.95-2.67 log removal of ARGs could be achieved by WWTPs [21]. Previous studies have found that even after the multi-stage treatment process of the wastewater treatment system, a variety of ARGs can still be detected in the effluent, which would cause resistance gene pollution to the receiving water bodies [24]. As wastewater treatment system contains a large number of microbial and nutritional elements, it is possible to transfer resistance genes from microbes (such as E. coli) to sewage or activated sludge[25]. Activated sludge and biofilm has been reported to be ideal environments for the transformation of ARGs from one host to another host through horizontal gene transfer [26,27,28]. Consequently, multiple resistance is easy to acquire in wastewater treatment system. In WWTPs,
ARGs may exist in each treatment unit, and various ARB may still persist after treatment processes. From previous studies, WWTPs have been regarded as hot spots of ARB and ARGs [24].

3. Removal efficiency of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) during membrane filtration process

3.1. Microfiltration process

Among all kinds of membrane technologies, MF is the most widely used and has the greatest economic value at present. Generally, MF with pore size larger than 0.1μm is mainly used for rejecting suspended particles with diameter of 0.1~10 μm, and widely applied in the removal of bacteria and suspended solid particles in water [29,30].

As the pore size of the MF membrane is generally smaller than the diameter of bacteria, the membrane bioreactor (MBR) using the MF membrane can efficiently remove ARB and iARGs by membrane rejection [31]. For example, Cheng et al. [32] found that polyvinylidene fluoride (PVDF) membrane (pore size: 0.3 μm) could eliminate three types of resistant microorganisms by orders of magnitude of 1.9-3.9. Wang et al. [33] reported that the average removal efficiencies of kanamycin (KAN) and ciprofloxacin (CIP), i.e., KAN-ARB and CIP-ARB were respective 93.9% and 87.1% by PVDF MF membranes (pore size less than 0.4 μm). However, Microbacterium Fluvii and Acinetobacter Junii were still detected after MF membrane with pore size at 0.2 μm. Regarding the removal efficiencies of iARGs by MF membrane, Lu et al. [34] reported that the abundances of tet (tetB, tetG, tetX), erm (ermF) and qnr (qnrS) genes decreased with 1–2 orders of magnitude after the MF process, however, the abundances of ermT, qnrA and qnrB remained nearly unchanged. Previous studies proved that eARGs could not be efficiently removed by single MF process. However, the removal efficiencies of eARGs in MBR with MF membrane were usually high, which could be attributed to the combined effects of biosorption, biodegradation and membrane separation. Zhang et al. reported that the concentration of 16SrDNA was reduced by 3 to 4 orders of magnitude [35], and the removal rate of eARG was nearly 100%[36] in a MBR process. Tsutsui et al. [37] found that the number of gene copy in the MBR effluents was only 1/5 to 1/500 of that in the supernatant of the mixed liquor. These previous studies demonstrate that the MBR process is suitable for the removal of eARGs through the combined effects of biodegradation, biological adsorption and membrane filtration [36]. However, due to the relatively low removal ability of antibiotics in one WWTPs, the relative abundance of eARGs was the highest in sludge. The biodegradation in this wastewater treatment process was inhibited by the residual antibiotics, and more eARGs tended to be adsorbed, leading to the enrichment of eARGs in MBR sludge [38,39].

In addition, it was found that the membrane material, the degree of membrane fouling and the substances in wastewater would influence the removal of ARGs and ARB in the MF process. For instance, Cheng et al. investigated three kinds of ARB removal by PVDF MF process, and found positive correlations between the removal of ARGs (log removal values) and the degree of membrane fouling [32]. Breazeal et al. [40] determined the removal of plasmids encoding bla TEM and vanA ARG by MF process, and found that ARG removal was correlated with the concentrations of protein, polysaccharide and organic colloid in wastewater. In addition, the occurrence of colloidal substances
might improve the removal efficiency of ARGs, and higher removal rate was observed by aluminum oxide membrane than polyvinyl fluoride membrane with the same pore size.

### 3.2. Ultrafiltration process

The diameter of UF membrane is usually 2 nm-0.1 μm with common molecular weight cut-off (MWCO) at 1, 10, 50 and 100 kDa, and its material mainly includes polysulphone, polyethersulfone, polysulphone permanently hydrophilic, polyamide thin film composite and regenerated cellulose RC, etc. The separation process of UF technology mainly relies on physical interception. Some pollutants are adsorbed on the surface of membrane, while some are trapped in the pores. UF technology can effectively remove microorganisms, particulate matter, algae, protoorganisms, bacteria, viruses, etc., and is widely used in many industries such as water treatment, food, refining, etc. [41,42,43]. Compared with MF process, UF process was more effective in ARB and ARGs removals. Schwermer et al. [44] found that complete removal of E. coli with antibiotics resistance from WWTP effluents was achieved during laboratory-scale UF process. The log removal value (LRV) of E. coli in raw wastewater was higher than 4.2 after UF treatment, resulting in less than 10 CFU/mL of E. coli. During single UF treatment (with MWCO of 100 kDa and the membrane material of regenerated cellulose RC), the removal rate of iARGs was over 90%, and the removal rate of eARGs was around 80% [45]. Krzeminski et al. [46] reported that the removal rate of plasmid DNA by lab-scale UF treatment could reach 99.15% (100 kDa, polysulphone) and more than 99.99% (1 kDa, polyamide thin-film composite). The LRVs of vanA and blaTEM by UF process were 4.2, 3.6 and 0.9 with MWCO at 1, 10 and 100 kDa respectively. With the application of UF membrane (0.1 μm), the concentration of ermB and tetO could be reduced from 5.59×10³ and 4.35×10⁴ copy/mL to those below the limit of detection.

Generally, UF could provide ARG removal of 1 to 4 LRV, and the removal efficiency of ARG in the UF process was mainly dependent on the water substances, membrane material, MWCO and DNA configuration [46]. Previous studies determined the ARGs removal from the secondary effluents of WWTP by UF processes with different MWCO (50 kDa and 100 kDa), and found that the removal efficiency was obviously higher with MWCO at 50 kDa, while the removal ability of ARGs by PES membrane was superior than that by PVDF membrane. PES membrane has a benzene ring structure on the surface, which can be used for molecular docking with DNA. Therefore, PES membrane has better removal ability than PVDF membrane. UF membranes with small MWCO have better removal efficiencies than those with large MWCO [41]. Decreasing pH value or increasing temperature will also improve the removal efficiency of ARGs by different UF-based integrated processes [47].

In practical application, ultrafiltration technology has a poor removal effect on humic acid, eARGs and other small molecular pollutants, as well as a relatively low removal efficiency on solute COD. Therefore, it is often necessary to combine ultrafiltration with other processes to reduce membrane fouling [48,49]. With the addition of coagulant polyaluminum chloride (PAC) at 0.85 mmol/L (as aluminium) and polyferric sulfate (PFS) at 0.50 mmol/L (as iron), the removal efficiency of three tetracycline resistance genes and two sulfonamide resistance genes by the integrated coagulation precipitation (PAC/PFS) – UF process was 83% higher than that of the single UF process, with 3-log of ARGs removed [47]. In the integrated nano iron (nFe)- and ultrasonic (US)-activated persulfate
(PMS) and ultrafiltration (MWCO at 100 kDa, PVDF) process, the three pre-oxidation treatments (PMS, nFe/PMS and US/PMS) could improve the ARGs removal in the secondary effluent. With the optimal dosages of PMS at 4 mM, nFe at 2 mM, and optimal frequency of US at 40 KHZ, the log removals of four ARGs including tetA, tetC, sul I and sul II were respective 0.66-2.06, 2.51-3.52 and 1.39-3.11 by the three integrated processes, with the best removal performance in nFe/PMS-UF process [48]. In the integrated ultrafiltration and biological powder activated carbon (BPAC) process which combined membrane filtration with biodegradation and adsorption, the removal efficiencies of tetA, tetC, tetX, sul I and 16 rRNA were all higher than 90%, and the removal efficiencies of tetG, sul II and intI 1 were 71.1%, 81.4% and 76.4% respectively. The integrated process could remove more than 90% of the iARGs and eARGs except for tetG [45].

3.3. Nanofiltration process
Generally, the MWCO of nanofiltration is between 200 and 1000 Da, and the material is mainly polyamide thin-film composite. Pore interception and electrostatic repulsion are the predominant mechanisms of pollutants removal in nanofiltration. The nanofiltration process is widely used for drinking water production from seawater, brackish water and wastewater [50,51,52,53]. Applied in the laboratory under controlled conditions, complete removal of free DNA was achievable during membrane filtration with nominal MWCO smaller than 1 kDa [53]. Slipko et al. ’s research showed that the removal efficiency of free DNA including different sizes of pure plasmids and linear fragments was higher than 99.8% by membranes with MWCO less than 5000 Da, and had a lower retention rate than neutral DNA due to repulsion, reduced adsorption and membrane fouling of free DNA [46]. The log removal value and removal rate of plasmid and total free DNA were respective 3.00 and 99.00% by membrane with MWCO less than 2500 Da. Latulippe and Zydney reported that due to the good elongational flesibility of linear free DNA, it could easily pass the membrane [54]. With application of nanofiltration, the average log removals of sul1, sul2, tetA, tetM, tetW and intI 1 during winter and summer were 5.29, 6.13, 8.12, 7.84, 6.62, 6.21 and 4.98 respectively. Nanofiltration could achieve 4.98-9.52 logs removal of the absolute abundance of ARGs. In addition, nearly unchanged relative abundance of sulfonamide resistant genes was observed, but 2.51, 3.47 and 0.88 log removal of tetW, tetM and tetA were achieved [55].

In the process of nanofiltration, free DNA configuration, the MWCO of membrane and charge on the membrane surface would affect the ARGs removal. Pawel et al. [53] found that the removal rate increased with the decrease of MWCO, which indicated that the removal effect was better with lower membrane pore size. The rejection of eARGs by nanofiltration membrane improved further (>99.99% removal) with a dense structure of membranes. Generally, the log removal of eARGs by nanofiltration membranes (MWCO 150-400 Da) was higher than 5.2 [55]. The salt retention mechanism of nanofiltration membrane was rather complex, and the charge of solute would have an obvious influence on it. With the same molecular size, the oppositely charged ion would pass the membrane more easily [51]. In addition, free DNA adsorbed and charge on the membrane surface were important in preventing free DNA penetration [46]. Previous studies reported that the Zeta potential of the membrane might influence the DNA interception [56]. With increased free DNA concentration in the solution, more free DNA molecules could be absorbed by the membrane surface with decreased
concentration of free DNA in the permeate [57]. Moreover, the different configurations of DNA would have an effect on the retention rate.

### 3.4. Reverse osmosis process

Reverse osmosis (RO) is often used in desalination, mainly for small molecule solute separation. The materials of RO membrane mainly include acetate fiber membrane, cross linked fully aromatic polyamid composite, interface composite membrane and thin film composite polyamide membrane on polyester support, etc. The RO operation generally requires a filtration pressure of 2-10 MPa, which can effectively remove amino acids and salt ions in water. Due to the high purity of the effluent, it is mostly used for drinking water supply [30,46]. Organic contaminants were mainly removed through hydrophobic interactions, electrostatic and size exclusion during RO filtration [58]. Generally, RO membrane is very effective in removing free ARGs. Krzeminski et al. [54] found that nearly all eARGs could be rejected by RO membrane with the effluent eARGs concentration below the limit of detection (LOD). The log removal values of eARGs by cross linked aromatic polyamide composite and thin film composite polyamide membrane on polyester support RO membranes (MWCO at 100-150 Da) were more than 5.0 and 6.6 respectively [46]. Similarly, Slipko et al. [54] found that the RO membrane was capable of free DNA removal, and the highest removal rate was achieved by polyamide-thin film composite RO membrane (MWCO at 200 Da), with 99.99% of eARGs could be removed, corresponding to a log removal value more than 4.00 of pure plasmid [54]. However, in the study of treatment of anaerobic digestion liquor from farm sludge with RO, it was found that although RO reduced the tetW, ermB and qnrS copies to a certain extent, ARG was still detected at medium concentration in the treated effluent [59]. Membrane fouling might be the reason for the difference in the treatment capacity of RO membranes, and greater ARG removal would be achieved by virgin membranes [60]. In previous study, the permeated concentration of supercoiled pure plasmid by polyamide-urea-thin film composite RO membrane (MWCO at 200 Da) was lower than the LOD, corresponding to a log removal value of more than 4.00 and removal efficiency of higher than 99.99%, while the removal efficiency by composite polyamide was significantly lower with a log removal value of 3.03), indicating that removal rate and retention rate were related to membrane material[54]. In Slipko et al.’s study, the charge of membrane would affect the retention of free DNA molecules, while the removal rate of free DNA by negatively charged membrane was relatively lower [54].

Different membrane processes has different removal ability of ARB and ARGs. Sum up above content to get Table 2.

| Antibiotic resistant bacteria | Microfiltration (MF) 0.1-0.4 μm | Ultrafiltration (UF) 2 nm - 0.1 μm | Nanofiltration (NF) < 2 nm | Reverse osmosis (RO) Dense membrane |
|-----------------------------|---------------------------------|-------------------------------|--------------------------|-----------------------------------|
| 1.9-3.9 log removal value   | >4 log removal value            | Complete                      | Complete                 | Complete                          |
4. Removal efficiency of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) during membrane-based integrated process

At present, the integrated processes based on membrane separation mainly include membrane bioreactor and combined pretreatment-membrane filtration process. In membrane bioreactors, pollutants including ARB and ARGs in water are removed through biodegradation, biological adsorption and membrane separation, which is often more efficient than the single membrane separation process. For example, the research conducted by Junya Zhang et al. [60] showed that compared with A2O, A2O-MBR could remove ARG more effectively. The removal of ARGs by MBR might be affected by the sludge retention time, and longer sludge retention time would probably favor the ARGs removal from water.

In the aspect of combined pretreatment-membrane filtration process, the commonly used pretreatment at present includes adsorption or coagulation. The results of integrated pre-coagulation and microfiltration process showed that ARB and eARGs were removed through solidification and combined effect of the following membrane separation process, resulting in efficient removal of ARGs from the effluent, e.g. more than 2.9 logs and 5.2 logs of total ARGs and eARGs were removed, respectively. More than 7.0 logs of eARG were removed by the combined pre-coagulation and microfiltration process. In contrary, only 3.2-3.3 and 0.9-1.0 logs of eARG removal were achieved in the single coagulation or microfiltration process. The excellent eARG removal could be attributed to that the positively charged Fe-coagulated flocs captured the eARGs and ARB with negative charge and was rejected during the subsequent microfiltration process [59]. Compared with single microfiltration process, the combined pre-coagulation and microfiltration process could not only significantly improve the removal effect of ARB and ARGs, but also greatly reduce membrane fouling[58]. Sun et al. [61] studied the fate of ARG during integrated powdered activated carbon (PAC) adsorption and ultrafiltration process. The treatment of wastewater by the integrated PAC-UF process could effectively reduce the concentrations of ARGs and intI 1. An average of 1.12-log, 1.48-log, 0.86-log and 1.56-log and a maximum 3.35-log, 3.18-log, 1.35-log and 3.10-log reductions of sulII, sulI, tetW and tetA were achieved by the filtration through 100 kDa membranes. The combined PAC-UF process could effectively reduce ARGs and intI 1 from the secondary effluents of WWTP, and the removal rate of ARGs was higher than that of the single UF process [61].
Fig.1 Shows the process flow diagram and advantages of some common membrane-based integrated processes.

5. Conclusions and perspective

In this study, the removal efficiencies of ARGs and ARB in different membrane filtration processes were explored, the influencing factors that affect the ARGs and ARB removal in the membrane filtration process were analyzed, and the removal ability of ARGs and ARB in different membrane filtration processes were summarized. Results showed that during MF process, the membrane material, degree of membrane fouling, colloidal substances in wastewater might affect the removal efficiency of ARGs and ARB. Generally, 2-4 logs removal of ARB and iARGs could be achieved in MF process, while single MF process could not effectively remove eARGs. The removal efficiency of ARGs and ARB in UF process mainly depended on the water matrix, DNA configuration and MWCO. The removal efficiency of ARB and ARGs in UF process was relatively obvious. In NF process, MWCO, surface charge of membrane and configuration of free DNA (linear and circular) might influence the removal efficiency of ARGs. The difference in removal efficiencies of ARGs by RO could be attributed to degree of membrane fouling. Generally, in NF and RO processes, the removal efficiencies of ARB and iARGs could reach nearly 100%, while the removal efficiencies of eARGs was more than 99.8% (i.e. more than 3 logs). Through investigation, it was found that the membrane-based integrated processes could effectively improve ARGs and ARB removals. Therefore, it is suggested that the application of membrane-based integrated process in water treatment could be widely promoted to improve the safety of effluent.

6. References

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