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Removal efficiency and mechanisms of antibiotic resistance genes in secondary effluent by combined process of coagulation–sedimentation–ultrafiltration

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ABSTRACT

Antibiotic resistance genes (ARGs) is an emerging environmental contaminant that has been frequently detected in the secondary effluent of wastewater treatment plants, becoming a potential threat to human health and ecological security. In this study, Pre-treatment of coagulants of polyaluminum chloride (PAC) and polyferric sulfate (PFS) was used to investigate the removal effect of different ARGs using a coagulation–sedimentation–ultrafiltration (UF) combined process. The removal mechanisms of ARGs were also discussed. The main results were shown as follows: the combined process was 2–3 orders of magnitude higher than that of direct UF for the removal of tetracycline resistance genes (*tetA*, *tetC*, *tetG*, and *tetX*) and sulfonamide resistance genes (*sulI* and *sulII*), and the removal effect of PAC was better than PFS. The content of free ARGs in secondary effluent was higher than that of cellular ARGs and coagulation–sedimentation had a significant removal for cellular ARGs, and some free ARGs could be also removed. The removal of dissolved organic carbon (DOC) in the two combined processes was better than direct UF, and the concentration of ARGs had a significant positive correlation with DOC concentrations, indicating that the removal of DOC contributed to the reduction of ARGs. In comparison with PFS, PAC had larger floc size and smaller fractal dimension during the coagulation process, so PAC floc was easy for sedimentation and looser, and the hydrolysate of PAC was more complicated, therefore having a better effect on the removal of ARGs. The above results show that coagulation–sedimentation–UF is an effective technology for removing ARGs in secondary effluent.

Keywords: Antibiotic resistance genes; Coagulation; Sedimentation; Dissolved organic carbon; Floc size; Fractal dimension

1. Introduction

The occurrence of antibiotic resistance bacteria (ARB) in aquatic environments, due to an increase in drug resistance, is a global public health issue [1]. Antibiotic resistance genes (ARGs), such as tetracyclines, sulfonamides and β-lactams, have also been found in a variety of environments, such as lakes, rivers, soils and wastewater treatment plants [2]. Although the urban sewage treatment plant had certain removal of ARB and ARGs, the secondary effluent still contained higher concentrations of ARB and ARGs, which poses a threat to ecosystems [3], and the increase of ARB and ARG concentrations in water environments is an important health issue. Investigations into the detection and reduction of ARGs in urban sewage treatment plants have become a key area of research. Current studies have shown that sewage treatment systems with a high biological density and rich bacterial structure could effectively control pollutants

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such as aerobic organic matter, NH_3–N and TN, however, the removal effects on ARB and ARGs were still relatively limited [4]. Treatment processes such as activated sludge, the use of oxidation ditches and biological turntables only resulted in a magnitude of removal for ARGs of 1 to 3; in contrast, membrane biological reactors reached a magnitude of the order of 3 to 4 [5]. Membrane filtration, therefore, plays an important role in the removal of ARB and ARGs. As most sewage treatment systems are ineffective in removing ARGs, it is important to examine possible health and ecological risks associated with ARGs during wastewater reclamation processes.

Recent investigations have focused on the removal of ARGs in the secondary effluent of municipal wastewater treatment plants using advanced treatment processes such as oxidation, coagulation–sedimentation and membrane technology. Sharma et al. [6] confirmed that using Cl_2, O_3, and Fenton reagents as disinfectants could inactivate ARB and ARGs, and the order of magnitude of removal of ARGs was 1.65–2.2, 0.6–1.2, and 2.42–3.38, respectively. As a low dose of ultraviolet light could not effectively reduce the junction transfer rate of DNA, the removal effect of ARGs was not significant. Results for the coagulation treatment process indicated that this process could remove colloidal contaminants and ARGs by the mechanisms of charge neutralization, bridging and sweeping. The treatment of secondary effluent from wastewater treatment plants using $FeCl_3$ and polyferric chloride was shown by Li et al. [7] to have an order of magnitude of 0.5–3.1 for the removal of ARGs by coagulation; the removal of dissolved organic carbon (DOC), NH_3–N, and TP promoted the reduction of ARGs. Recently, treatment technologies using membranes to remove emerging pollutants have increased in focus. Studies have shown that microfiltration has no significant effect on the removal of ARGs, and ultrafiltration (UF) reduces ARGs by a magnitude of the order of 0.6–1.5 [8]. As the molecular weight of ARGs ranges from 100 to 9,000,000 Dal [9,10], and ARGs consist of flexible molecules with long and thin characteristics, they are easily squeezed and penetrate the ultrafiltration membrane when their size is greater than the membrane pore size. ARGs are not easily intercepted by the membrane when their size is smaller than the membrane pore size [11].

Single treatment processes only result in a low removal rate of ARGs, thus current studies focus on identifying an efficient treatment process. In this study, the coagulants of polyaluminum chloride (PAC) and polyferric sulfate (PFS) were used for pretreatment before being combined with ultrafiltration to remove ARGs in secondary effluent to select a coagulant with better removal effects of ARGs, and to explore the effect of hydrolysis products on the removal of antibiotic resistance genes. The removal effects and mechanisms of ARGs using this combined process are analyzed.

2. Materials and methods

2.1. Experimental reagents and materials

Experimental reagents: Polyaluminium chloride [$w(Al_2O_3)$ was 29%, basicity was 40%]. Polyferric sulfate [w(Fe) was 18.5%, basicity was 11%].

Experimental materials: Polyvinylidene fluoride (PVDF) flat-sheet membrane was used with a molecular weight cut-off of 100 K Dal. This material was customized according to the effective filtration area of the ultrafiltration bowl and stored in a 4°C constant temperature refrigerator. Water used in our experiment was taken from the secondary sedimentation tank of a sewage treatment plant in Beijing (Turbidity: 1.35 NTU, pH: 7.2, TP: 1.73 mg L^{-1}, NH_4^+: 1.85 mg L^{-1}, DOC: 18.95 mg L^{-1}, UV_{254}: 0.13 cm^{-1}). DNase I degrading enzyme was purchased from Beijing Solarbio Technology Co., Ltd., (Beijing). Its appearance is white powdered and molecular weight is about 30 K Dal. It was stored at –20°C constant temperature.

2.2. Experimental setup and instruments

In this experiment, after the addition of the appropriate amount of coagulants (PAC and PFS), water samples were stirred at 200 rpm for 2 min, stirred at 40 rpm for 30 min, and then left to stand for 1 h. The supernatant was then filtered through the ultrafiltration membrane. The filtration experiment was performed in a dead-end mode. In order to ensure that the water sample in the ultrafiltration bowl was filtered, nitrogen was used to maintain a constant filtration pressure of 0.10 MPa. The filtered liquid was collected in a beaker. The schematic diagram of the filtration system is shown in Fig. 1. Filter membranes that retained ARGs were stored at low temperatures (–20°C) for subsequent determination.

Experimental instruments: The process of coagulation was performed using a coagulation test agitator (ZR4-6, ZhongRun Water Industry Technology Development Co., Ltd., China). The determination of DOC was undertaken using a total organic carbon analyzer (TOC-VCPH, SHIMADZU, Japan). The fractal dimensions of the floc were determined using laser diffraction (Mastersize 2000, Malvern, UK). The content and purity of DNA were detected using an ultra-micro spectrophotometer (NanoDrop8000, Thermo, U.S.A.).

2.3. Identification experiment of the existence forms of resistance genes in water samples

In the experiment, DNase I degrading enzyme was used to degrade the free DNA fragment in the raw water and the treated water sample, thereby removing the free resistance genes in water, and then using the membrane to intercept the cellular resistance genes in a water sample, followed by qualitative and quantitative detection by qPCR. When the content of cellular resistance genes was obtained, the content of free resistance genes is equal to the total amount of resistance genes minus the content of cellular resistance genes. The experimental operation method was as follows: 1 mg of lysin granules were added to 500 ml of a water sample, and the mixture was shaken at 25°C for 2 h, then heated in water at 80°C for 10 min. After the water sample was cooled, it was filtered through the 0.22 μm membrane, then the membrane was stored in a refrigerator at –20°C and the cellular resistance genes were detected as soon as possible.

2.4. Extraction and quantification of ARGs

The total amount of resistance genes in the water sample was enriched by filtration using a 1,000 Da PVDF

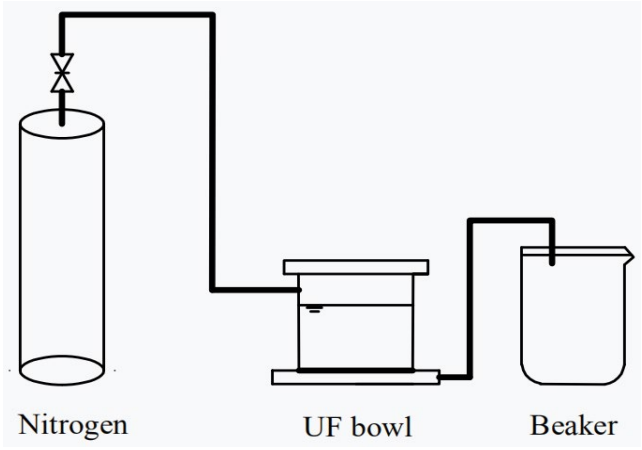

Fig. 1. Schematic diagram of the filtration system.

membrane, and the filtered membrane was collected. The above two membranes were chopped for DNA extraction. In this experiment, the DNA was extracted using the Fast DNA Spin Kit for soil kit, and the DNA was extracted according to the instruction manual. The extracted DNA samples were stored at –20°C until subsequent quantification experiments.

The tetracycline resistance genes (*tetA*, *tetC*, *tetG*, *tetX*), sulfonamide resistance genes (*sulI*, *sulII*), class I integron gene *intI1* and 16S rDNA in water samples were quantified using a real-time fluorescence quantitative polymerase chain reaction (qPCR). First, the extracted DNA was amplified by PCR, PCR products were analyzed using gel electrophoresis, and the presence of the target genes was confirmed by the TAE buffer. PCR products containing the target genes were purified and ligated to the PMD19-T vector before being subjected to plasmid sequencing. The method used to calculate the copy number (Eq. 1) was used to prepare a standard sample and then make quantifications of *tetA*, *tetC*, *tetG*, *tetX*, *sulI*, *sulII*, *intI1*, and *16S rDNA*. 20 μl of the qPCR reaction system is shown in Table 1. The reaction conditions are shown in Table 2. The PCR and qPCR primers are shown in Table 3.

$$\text{Copy Number} = \frac{C \times 6.02 \times 1{,}014}{\left[\left(2{,}692 + L\right) \times 660\right]} \quad (1)$$

where Copy Number is the copy number of a target gene (copies μL^{-1}); C is the mass concentrations of the product (ng μL^{-1}); 2,692 is the length of the vector, and L is the length of the target gene fragment (bp).

2.5. Fractal dimension

During the process of determination of fractal dimensions, the microbeam passed through the sample resulting in small particles scattering at high angles and large particles scattering at low angles. The light scattering of the sample was detected using a light-sensitive array detector at different angles, ranging from 0.01° to 40.6°. In a light scattering study, the scattered intensity I is a function of the magnitude of the scattering wave vector Q. The calculation method of the scattering wave vector Q was [12]:

$$Q = \frac{4\pi n \sin(\theta/2)}{\lambda} \quad (2)$$

where n is the refractive index of the suspended matter; θ is the scattered angle (degree); and λ is the wavelength of the light in the vacuum (nm).

For independent floc, the scattered intensity I, the scattering wave vector Q and the fractal dimension D_f had the following relationship [13]:

$$I \propto Q^{-Df} \quad (3)$$

Therefore, on a log–log plot, the negative of the slope was the fractal dimension, reflecting the compactness of the floc. The larger the fractal dimension, the denser is the structure of the floc; a lower fractal dimension indicates a looser floc structure [14].

Table 1
qPCR reaction system

Contents	Volume (μL)	Concentration
SybrGreen qPCR Master Mix	10.0	2×
F Primer	0.8	10 μM
R Primer	0.8	10 μM
DNA Template (5-fold dilution)	5.0	–
ddH_2O	3.4	–
Final volume	20.0	–

Table 2
Reaction time and temperature

Types	Initial state	Each cycle (40 in total)		
		Dissolve	Annealing	Extend
Time	2 min	10 s	5 s	15 s
Temperature	95°C	95°C	55°C	72°C (collection)

3. Results and discussion

3.1. Removal effect of coagulation–sedimentation–UF on ARGs

The removal effects of direct UF, coagulation (PAC) sedimentation–UF and coagulation (PFS) sedimentation–UF on the ARGs in the secondary effluent are shown in Fig. 2 and Table 4. The dosages of PAC and PFS were 0.85 mmol/L (calculated as aluminum) and 0.40 mmol/L (calculated as iron), respectively. Both dosages were determined according to the previous experiment. Concentrations of *tetA*, *tetC*, *tetG*, *tetX*, *sulI*, *sulII*, *intI1* and 16S rDNA in the secondary effluent were $10^{4.93}$–$10^{5.00}$, $10^{4.79}$–$10^{4.86}$, $10^{5.12}$–$10^{5.18}$, $10^{6.63}$–$10^{6.69}$, $10^{6.57}$–$10^{6.64}$, $10^{5.83}$–$10^{5.92}$, $10^{7.15}$–$10^{7.26}$ and $10^{8.34}$–$10^{8.49}$ copies mL^{-1}, respectively. Concentrations of sulfonamide resistance genes were recorded to be higher than that of tetracycline resistance

Table 3
Primers used in PCR and qPCR

Target genes	Primer sequence		Annealing temperature/°C	Amplification length/bp
tetA	*tetA-F*	GCTACATCCTGCTTGCCTTC	60	210
	tetA-R	CATAGATCGCCGTGAAGAGG		
tetC	*tetC-F*	CTTGAGAGCCTTCAACCCAG	68	418
	tetC-R	ATGGTCGTCATCTACCTGCC		
tetG	*tetG-F*	GCAGAGCAGGTCGCTGG	65	134
	tetG-R	CCYGCAAGAGAAGCCAGAAG		
tetX	*tetX-F*	AGCCTTACCAATGGGTGTAAA	55	278
	tetX-R	TTCTTACCTTGGACATCCCG		
sulI	*sulI-F*	CGCACCGGAAACATCGCTGCAC	57	158
	sulI-R	TGAAGTTCCGCCGCAAGGCTCG		
sulII	*sulII-F*	CTCCGATGGAGGCCGGTAT	60	190
	sulII-R	GGGAATGCCATCTGCCTTGA		
intI1	*intI1-F*	CCTCCCGCACGATGATC	55	280
	intI1-R	TCCACGCATCGTCAGGC		
16S rDNA	BACT1369R	CGGTGAATACGTTCYCGG	55	143
	PROK1492R	GGWTACCTTGTTACGACTT		

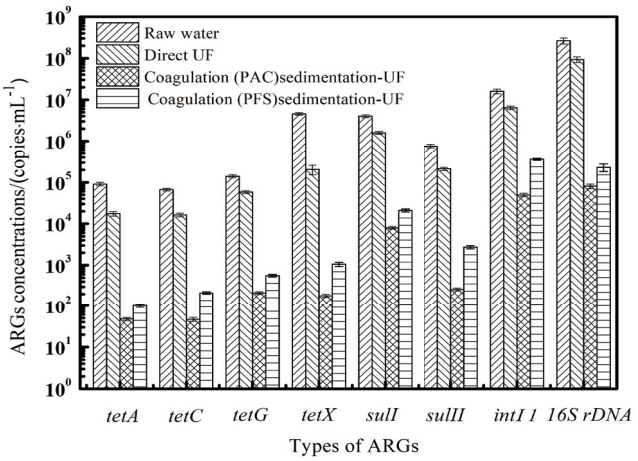

Fig. 2. Removal effect of different treatment processes on ARGs in secondary effluent.

genes, possibly due to the fact that *sulI* and *sulII* were the genes encoding dihydropterin synthase in gram-negative bacteria [15]. These genes were easily transferred horizontally with class I integrons and drug-resistance plasmids, respectively [16].

Results for the removal amount of ARGs by direct UF was low, with a removal order of magnitude of 0.38–1.35 (Table 4). The removal of ARGs by the combined process was 2–3 orders of magnitude higher than that of direct UF when PAC and PFS were added. Among the six resistance genes, the combined processes of coagulation (PAC/PFS) sedimentation–UF had the highest removal of *tetX*, with the removal of 4.40 and 3.63 orders of magnitude, respectively; *sulI* removal was the lowest (2.70 and 2.29 orders of magnitude, respectively). The removal effect of PAC was better than that of PFS, a finding that may be due to the different floc morphology formed in the process of coagulation–sedimentation pretreatment and the degree of different membrane fouling. The removal of ARGs was undertaken by the membrane which acted as a sieve in the process of direct UF. When coagulation–sedimentation was combined with UF, the combined effects of charge neutralization, bridging, sweeping and membrane interception further reduced the concentrations of ARGs.

3.2. Removal of cellular and free ARGs from raw water by coagulation–sedimentation

The removal effects of coagulation–sedimentation on cellular and free ARGs are shown in Fig. 3. By using the DNase I degrading enzyme to degrade the secondary effluent before the experiment, we found that ARG concentrations declined by 50%–80% compared with the raw water. This result indicated that the content of cellular ARGs in raw water was less than that of free ARGs, thus the reduction of free ARGs was crucial. When the water sample was degraded with DNase I after coagulation–sedimentation pretreatment, the concentrations of most ARGs decreased by more than 90%, indicating that free ARGs were dominant in the effluent. The majority of cellular ARGs were completely removed by coagulation–sedimentation pretreatment, thus their content was minimal. The average concentrations of the free resistance genes *tetA*, *tetC*, *tetG*, *tetX*, *sulI*, *sulII*, *intI1* and *16S rDNA* in the raw water were 1.42×10^6, 4.3×10^4, 1.01×10^4, 2.3×10^7, 8.51×10^5, 1.28×10^7, 1.5×10^7 and 1.02×10^9 copies mL^{-1}, respectively (Fig. 3). After PAC pretreatment, concentrations were 7.6×10^3, 2.2×10^3, 1.1×10^4, 5.6×10^6, 7.57×10^5, 8.0×10^5, 1.2×10^7 and 6.97×10^7 copies mL^{-1}, respectively. After PFS pretreatment, concentrations were 2.2×10^4, 5.5×10^3, 3.5×10^3, 3.3×10^5,

Table 4
Removal of ARGs by different treatment processes (–log)

	tetA	*tetC*	*tetG*	*tetX*	*sulI*	*sulII*	*intI1*	*16S rDNA*
Direct UF	0.72	0.61	0.38	1.35	0.40	0.55	0.40	0.45
Coagulation (PAC)–sedimentation–UF	3.28	3.16	2.81	4.40	2.70	3.46	2.51	3.52
Coagulation (PFS)–sedimentation–UF	2.96	2.49	2.39	3.63	2.29	2.44	1.84	2.87

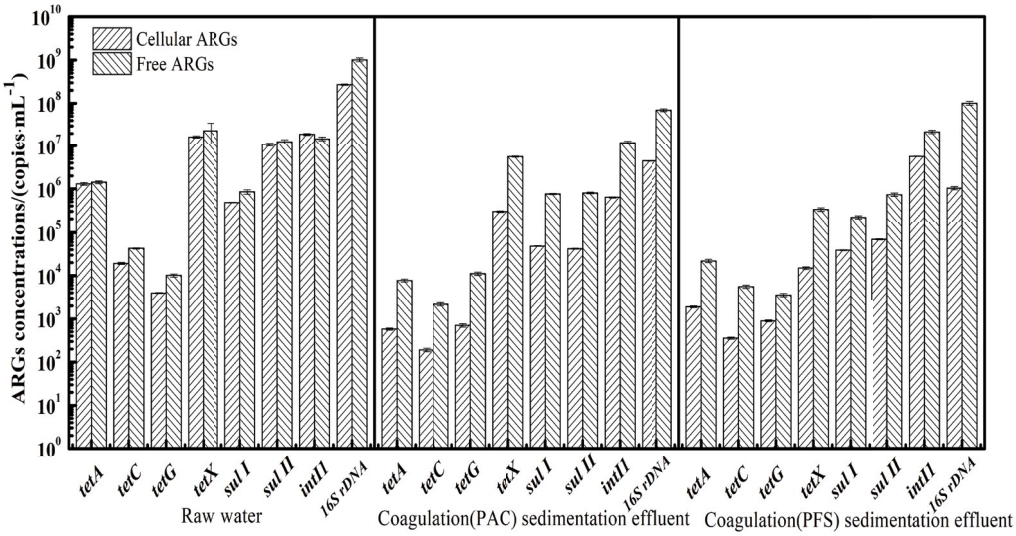

Fig. 3. Removal of cellular and free ARGs by coagulation–sedimentation.

2.15×10^5, 7.81×10^5, 2.17×10^7 and 1.0×10^8 copies mL^{-1}, respectively. It can be seen that the concentrations of free ARGs after coagulation–sedimentation pretreatment were much lower than those of free ARGs in raw water, indicating that coagulation–sedimentation pretreatment could effectively remove free ARGs. Coagulation–sedimentation removed ARGs with the combined effects of adsorption and sweeping towards free ARGs and gene hosts (such as bacteria). Free ARGs that was not adsorbed by floc after sedimentation could be intercepted by the UF membrane, thus ARG concentrations were further reduced in the effluent. Therefore, coagulation–sedimentation was a more effective pretreatment process for removing cellular and free ARGs.

3.3. Effect of organic matter removal on the reduction of ARGs

Breazeal et al. [17] examining the correlation between the removal of ARGs and TOC, identified a strong correlation between ARGs and protein, and a higher correlation coefficient between ARGs and the total concentrations of proteoglycans. These findings confirm that a reduction of ARGs was promoted by the removal of organic matter. In our investigation, therefore, the removal effect of different treatment processes on DOC in raw water was examined (Fig. 4). The correlation between DOC concentrations and ARGs concentrations is shown in Fig. 5.

Initial DOC concentrations in the raw water were 18.95 mg L^{-1}. After direct UF, coagulation (PAC) sedimentation–UF and coagulation (PFS) sedimentation–UF treatment, DOC concentrations declined to 15.43, 11.98 and 12.15 mg L^{-1}, respectively (Fig. 4). Removal rates of DOC by the three processes were 18.6%, 36.8%, and 35.9%, respectively, indicating that DOC removal effects by coagulation–sedimentation–UF combined processes were better than direct UF. The removal effect of PAC was slightly better than PFS, mainly due to the different floc morphology of the two coagulants formed in water and the different effects of adsorption of organic matter. DOC concentrations of the membrane influent of the two combined processes (Fig. 4) were 13.5 and 14.1 mg L^{-1}, respectively. These results were significantly lower than that of direct UF effluent, indicating that coagulation–sedimentation pre-treatment plays an important role in the removal of DOC. The removal of DOC by direct UF mainly depended on the mechanical sieving of the membrane. However, the larger pore size of the membrane had a limited effect on the removal of small and medium molecular organics, resulting in a low removal rate of DOC. After PAC and PFS were added into the secondary effluent, they hydrolyzed to form positively charged floc or hydroxide precipitates, resulting in the pollutants being destabilized and flocculating under adsorption-charge neutralization and sweeping. Most of the colloidal organic matter was removed after sedimentation, and the finely-divided floc in the supernatant was further intercepted by the membrane, resulting in the coagulation–sedimentation–UF combined process having better effects on DOC removal.

A significant positive correlation ($P < 0.05$) was recorded between the resistance genes (*tetA*, *tetC*, *tetG*, *tetX*, *sulI* and

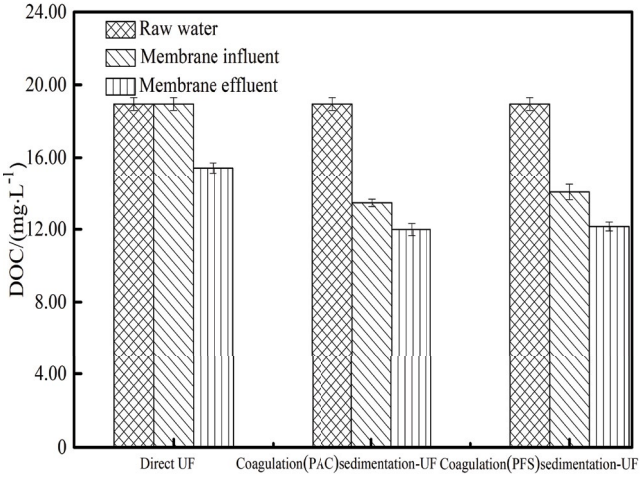

Fig. 4. Removal of DOC in raw water by different treatment processes.

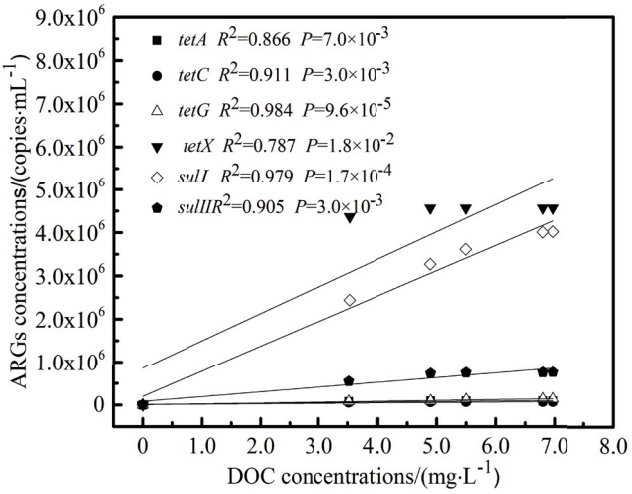

Fig. 5. Correlation between ARGs and DOC.

sulII) and DOC (R^2 values of 0.866, 0.911, 0.984, 0.787, 0.979, and 0.905, respectively; Fig. 5), indicating a high correlation between ARG concentrations and DOC concentrations. Cai et al. and Nguyen et al. [18,19] highlighted that ARGs could interact with various organic or inorganic particles, including different soil particle colloids, different types of clay and natural organic matter, and that they could be adsorbed onto colloids to protect them from the degradation of nuclease. Lorenz et al. [20] found that DNA adsorption increased with higher ionic strength and a lower pH. When PAC and PFS coagulants were added to the samples, hydrolyzed Al^{3+} and Fe^{3+} increased the ionic strength in the aqueous solution, and pH recorded a slight decrease during the process of forming the polymers of aluminum and iron, thus aiding adsorption of ARGs on colloidal organic matter. ARGs adsorbed on large particle colloids were removed by floc sedimentation; ARGs adsorbed on small particle colloids were not easily precipitated and they were removed by membrane interception. Therefore, the removal of DOC in the secondary effluent promotes the reduction of ARGs.

3.4. Effect of floc size and fractal dimension on ARG removal

Floc size had a significant influence on the coagulation effect. Peter et al. [21] showed that the removal of organic pollutants was closely related to the floc size: a larger floc size makes it easier for the floc to precipitate. Therefore, the removal rate of organic matter is higher, and the reduction of ARGs is positively correlated with the removal of organic matter, thus the change of floc size indirectly affects the reduction of ARGs. The coagulation process included mixing and flocculation, and the coagulant was added to the wastewater to destabilize the colloidal particles via charge neutralization. The destabilized particles were then flocculated to form micro-flocs, and large flocs were formed using the bridging effect. In our experiment, floc size was measured at 2 and 20 min in the coagulation process (Fig. 6), representing the phases of mixing and flocculation, respectively.

Floc size results over time (Fig. 6) showed that the volume percentage of PAC floc with a size of 4 μm was greatest when

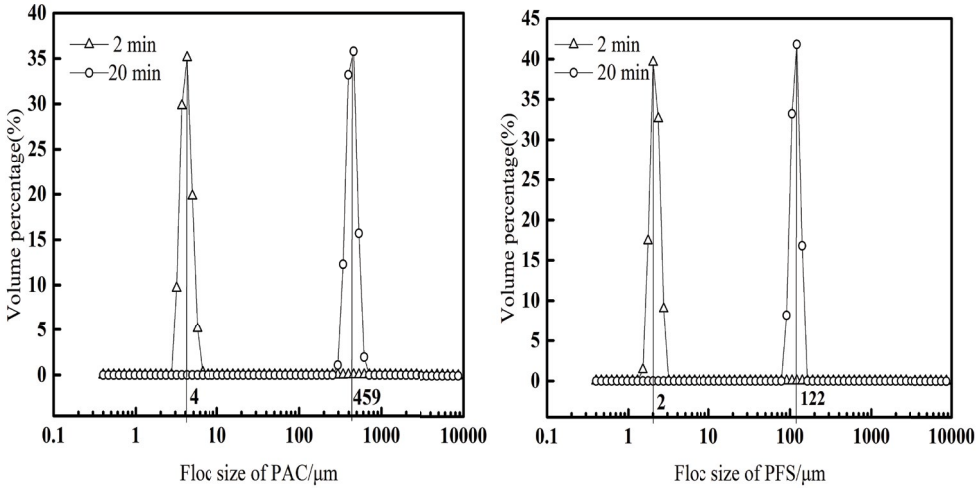

Fig. 6. Floc size of PAC/PFS at different coagulation times.

the solution had been mixed for 2 min, and the volume percentage of floc with a size of 2 μm was the greatest for PFS. When coagulation continued for 20 min, the volume percentage of PAC floc with a size of 459 μm was the greatest, while the volume percentage of floc with a size of 122 μm was the greatest for PFS. PAC floc size was larger than PFS during the coagulation process. Although the micro-floc had a large specific surface area, it was not easily precipitated, therefore the removal effect on the organic matter was not significant. Floc size increased during the later stage of coagulation, and it was easily adsorbed and swept by intracellular and extracellular ARGs attached to organic matter. After flocculation had been rapidly completed precipitated, the removal rate of ARGs improved.

During coagulation, the fractal dimensions of the floc also affected the removal of organic matter. Li et al. [22] recorded that the logarithm of floc size was inversely proportional to the logarithm of fractal dimension, and the fractal dimension of floc formed by charge neutralization was greater than the fractal dimension of floc formed by bridging. The fractal dimensions of PAC floc (2.76 and 2.58) and PFS floc (2.86 and 2.73) were recorded at 5 and 20 min, respectively (Fig. 7). Our results indicated that the fractal dimensions of the floc in the mixed state were higher than that of the flocculated state, and the fractal dimensions of PFS floc were higher than that of the PAC floc. PAC floc was therefore, looser than the PFS floc, confirming that the main effect of coagulant on pollutants in the mixed stage was charge neutralization, floc was smaller and denser, and the fractal dimensions were larger. In the stage of flocculation, the pollutants were mainly removed by bridging.

3.5. Effect of zeta potential on the reduction of ARGs

The changes in floc potential at different coagulation times are shown in Table 5. In general, ARB and ARGs are negatively charged in the effluent, and the initial potential of the secondary effluent was about –20 mV. Results in Table 5 indicate that the potential was about 7.52–8.95 mV and 2.55–3.56 mV after PAC and PFS were added, respectively, indicating that the absolute value of the potential decreased after the addition of the coagulant, the colloids were unstable and easily destabilized. After the addition of PAC, the surface potential of the floc was greater than PFS, which might be caused by the different floc components of the two coagulants. For PAC, a hydrated aluminum ion $Al(H_2O)_6^{3+}$ was formed after the addition of water; under further hydrolysis, H_2O in the ion hydration layer was replaced by OH^- to form various mononuclear hydroxy hydrolyzates, including $Al(OH)^{2+}$ and $Al(OH)_2^+$. Due to the condensation of the mononuclear hydroxy hydrolysates, a series of polynuclear hydroxy hydrolysates were formed, including $Al_2(OH)_2^{4+}$, $Al_6(OH)_{15}^{3+}$, $Al_7(OH)_{17}^{4+}$, $Al_8(OH)_{20}^{4+}$ and $Al_{13}(OH)_{34}^{5+}$ [23]. Under the effect of charge neutralization, the formed hydrolysates destabilized ARB and ARGs. Results by Xu et al. [24] indicated that Al_{13} had a stable structure, therefore being the best flocculating component. The surface of this structure also had a large amount of positive charge, thereby exerting a strong neutralization charge. Al_{30}, being composed of two Al_{13} molecules and four Al monomers, was another polycation formed during the hydrolyzed process of PAC. It is believed that Al_{30} molecules exhibit a good bridging effect during coagulation, therefore promoting the coagulation effect [25]. As the hydrolyzed process progressed, the floc potential decreased by 5 min. This result indicates that $Al(OH)_3$ sedimentation may occur, and the effect of sweeping could result in a rapid increase in the coagulation process, thereby enhancing the removal effect of ARB and ARGs.

For PFS, the stable iron polymer dominated (about 70%) after it was added to the water, and the unstable Fe^{3+} which could be hydrolyzed only accounted for a small portion; a small part of ionized Fe^{3+} underwent processes such as hydrolysis, complexation, polymerization, and sedimentation. The mononuclear hydroxy complexes produced by Fe^{3+} hydrolysis processes included $Fe(OH)^{2+}$, $Fe(OH)_2^+$,

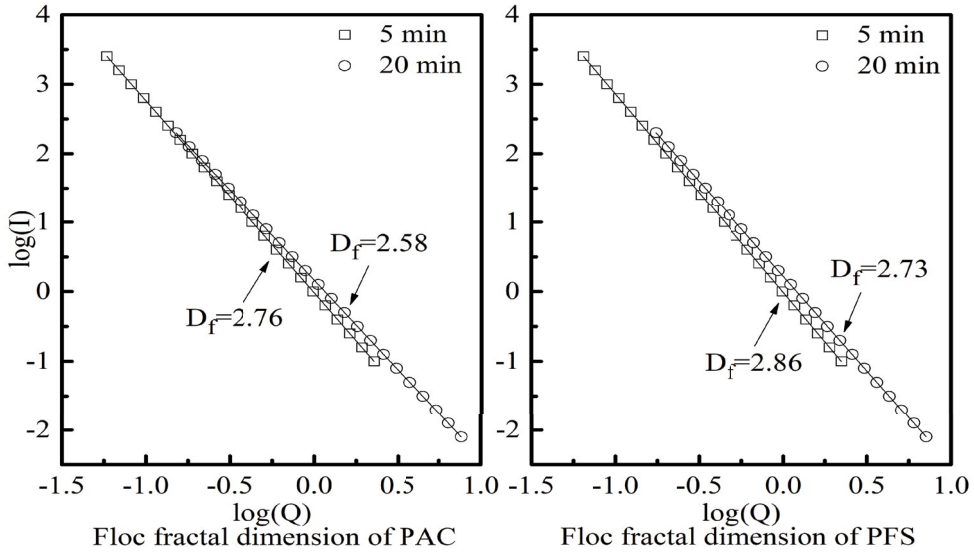

Fig. 7. Fractal dimensions of PAC/PFS floc at different coagulation times.

Table 5
Changes of floc potential at different coagulation times (mV)

Time	2 min	5 min	15 min	25 min
PAC	8.53	8.32	8.95	7.52
PFS	3.56	3.22	2.91	2.55

$Fe(OH)_3$ and $Fe(OH)_4^-$, and the polynuclear hydroxy complex was $Fe_2(OH)_2^{4+}$. When the coagulant PFS was added to the secondary effluent, the pH of secondary effluent was neutral, and the main hydrolyzate was $Fe(OH)_3$. The removal of ARGs by PFS was due to (i) the charge neutralization of positively charged mononuclear and polynuclear hydroxy complexes produced by the hydrolysis of Fe^{3+}; and (ii) adsorption and sweeping of $Fe(OH)_3$. Since the stable iron polymer was dominant in the solution, it could not be further hydrolyzed or converted into iron hydroxide precipitate. The main reason, therefore, for the removal of ARGs was the adsorption and sweeping effect of stable iron polymers on organic matter and colloidal particles, thus removing ARGs synergistically.

4. Conclusions

- The removal of resistance genes (*tetA*, *tetC*, *tetG*, *tetX*, *sulI*, and *sulII*) by coagulation (PAC/PFS)-sedimentation–UF combined processes were 2–3 orders of magnitude higher than that of direct UF. The removal effect of PAC was also recorded to be better than the removal effect of PFS. The two coagulants could remove more than 90% of cellular ARGs in secondary effluent by coagulation–sedimentation, and a part of free ARGs could be also removed.
- The removal rate of DOC by direct UF was 18.6%, and the removal rate of coagulation (PAC/PFS)-sedimentation–UF combined processes were 36.8% and 35.9%, respectively, indicating that the pre-treatment of coagulation–sedimentation played an important role in the removal of DOC. A significant positive correlation was also observed between the concentration of ARGs and DOC, indicating that the removal of DOC could promote the reduction of ARGs.
- The floc size of PAC was larger than PES, so PAC floc was easy to precipitate. The measurement of the fractal dimension showed that the removal effect of coagulants for resistance genes was charge neutralization and bridging. The hydrolysate formed by PAC during the coagulation process was more complicated than PES, so the removal rate of ARGs with PAC was higher.
- The results of this study indicated that coagulation–sedimentation–UF was an effective process for removing ARGs in secondary effluent, and the types of coagulants had a great effect on the removal of ARGs, so more experiments need to be done to determine the best coagulants. Sewage treatment plants should strengthen the removal of organic matters, thereby indirectly removing ARGs. The process of coagulation–sedimentation can be used to remove the cellular ARGs in the secondary effluent, and then use other processes to remove the free ARGs. In addition, coagulation–sedimentation as a pretreatment for UF can alleviate membrane fouling to a certain extent, prolong the operation time of UF and reduce the operating cost.

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