Aggravated biofouling caused by chlorine disinfection in a pilot-scale reverse osmosis treatment system of municipal wastewater

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ABSTRACT

The reverse osmosis (RO) system is widely applied to produce reclaimed water for high-standard industrial use. Chlorine disinfection is the main biofouling control method in the RO systems for wastewater reclamation. However, researchers reported the adverse effects of chlorine disinfection which aggravated biofouling in laboratory-scale RO systems. In this study, four parallel 4-inch spiral wound RO membranes were used to study the effect of chlorine on biofouling in a pilot-scale RO system. The free chlorine dosages in four experimental groups were 0, 1, 2 and 5 mg/L, respectively. After continuous chlorination and dechlorination, the feed water entered the RO system. It was found that chlorine pretreatment caused a 1.9–36.7% increase in relative feed water pressure of the RO system, suggesting that chlorine aggravated the membrane fouling in the pilot-scale RO system. The microbial community structures of living bacteria in the feed water of the RO system were determined by the PMA (propidium monoazide)-PCR method and showed that the relative abundance of chlorine-resistant bacteria (CRB) was significantly increased after disinfection. Nine major genera which maintained higher relative abundance in experimental groups with high chlorine dosage were considered as possible key species causing membrane fouling, including *Pedobacter*, *Clostridium* and *Bradyrhizobium*.

Key words | biofouling, chlorine disinfection, chlorine-resistant bacteria (CRB), reverse osmosis, wastewater reclamation

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HIGHLIGHTS

- Revealed the effects of chlorine disinfection on fouling of a pilot-scale RO system.
- Chlorine disinfection aggravated the membrane fouling in the pilot RO system.
- Analyzed community structure of residual bacteria after disinfection in feed water.
- Identified key chlorine-resistant bacteria (CRB) causing RO membrane fouling.

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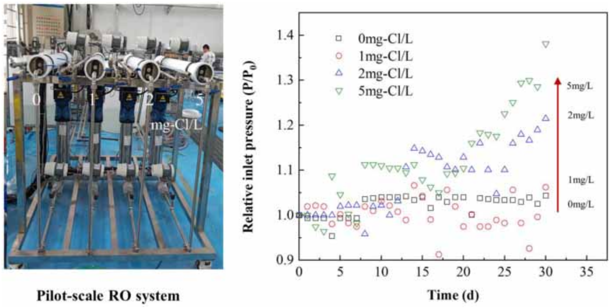
GRAPHICAL ABSTRACT



INTRODUCTION

Wastewater reclamation is an attractive approach to addressing water scarcity. The wastewater reclamation reverse osmosis (RO) system was widely applied to produce reclaimed water for municipal or industrial use in water-deficient and economically developed areas, such as Singapore and Beijing, the capital of China (Khan *et al.* 2015; Yen *et al.* 2017; Gu *et al.* 2019; Zhu & Dou 2019; Bai *et al.* 2020).

However, membrane fouling frequently occurred in the wastewater reclamation RO system, which leads to membrane pressure rise and energy consumption increase (Khedr 1998; Khan *et al.* 2015). The attachment and growth of microorganisms on the RO membrane, which was also called biofouling, was one of the main types of membrane fouling (Matin *et al.* 2011; Jiang *et al.* 2017).

Chlorine disinfection as pretreatment and adding biocides were the main strategies to control biofouling (Nguyen *et al.* 2012; Tan *et al.* 2017; Manalo *et al.* 2019). In recent years, some studies have reported the adverse effects of chlorine disinfection, which aggravated biofouling, on the seawater or municipal wastewater desalination RO systems (Khan *et al.* 2015; Wang *et al.* 2019a). The change of community structure and secretory products were considered to be the reasons for the change of RO biofouling. Researchers found that biofouling was more related to extracellular products (EPS) rather than bacterial cells and the fouling potential of EPS from different bacterial strains differed significantly (Ridgway *et al.* 1985; Gomez-Suarez *et al.* 2002; Chen & Ma 2004; Wang *et al.* 2019b). Wang *et al.* (2019a) reported that the abundance of chlorine-resistant bacteria (CRB) and EPS amount on the RO membrane surface significantly increased after chlorine disinfection, and it explained the adverse effects of chlorine disinfection.

However, the adverse effects of chlorine disinfection have only been reported in the laboratory-scale wastewater reclamation RO system with flat RO membranes (Wang *et al.* 2019a), and they have not been verified by pilot-scale experiments with spiral wound RO membranes. This study only analyzed the changes in the bacterial community structure in fouling layers after chlorine pretreatment but did not analyze the influence of chlorine on the community structure in feed water. The changes in community structures in feed water were the direct reason for the changes in community structures in fouling layers.

Therefore, the objectives of this study are to verify the adverse effects of chlorine disinfection to biofouling in the pilot-scale wastewater reclamation spiral wound RO system and reveal the influence of chlorine on the feed water community structure of the RO system.

MATERIALS AND METHODS

The pilot-scale RO system

Water source

Secondary effluent from a wastewater treatment plant in northern China is used as the water source of the pilot-scale

RO system. The treatment process of the wastewater treatment plant includes A^2O, a high-efficiency sedimentation tank and a deep-bed filter.

Process flow

The process flow of the pilot-scale RO system is shown in Figure 1. This system consisted of four parallel experimental groups, and each group consisted of three main parts: pretreatment tank, RO feed tank and RO membrane. For example, A1 pretreatment tank, B1 feed tank and B1 RO membrane constituted an experimental group. In addition, the pretreatment tanks and RO feed tanks were equipped with dosing tanks, respectively. In this experiment, four pretreatment water tanks used the same feed water, the water source mentioned in the section 'Water source' (the secondary effluent from a wastewater treatment plant). Meanwhile, four new 4-inch RO membranes (LP100, Vontron) were installed in the places of B1–B4 RO membranes. The cartridge filters were installed between the pretreatment tanks and RO feed tanks. The filter element size of these cartridge filters was 40-inch, the filter element material was polypropylene fiber, and the filtration accuracy was 1 μm.

Operation conditions and modes of the pilot-scale RO system are also shown in Figure 1. The four experimental groups adopted the same operation mode, but only the dosage of free chlorine (NaClO) and reductants ($NaHSO_3$) was different. Sodium hypochlorite was added to the pretreatment water tanks for disinfection. The volumes of the pretreatment tanks were 1 m^3, the feed water rates were 1 m^3/h, and the hydraulic retention time was 1 h. Excessive reductants $NaHSO_3$ were added into RO feed tanks for dechlorination. The mixed solution of NaClO and excess $NaHSO_3$ were used to adjust the conductivity and ions concentration of each experimental group equal. It can maintain a consistent amount of NaClO and $NaHSO_3$ dose to each of the feed water and exclude the effects of conductivity and ion concentration. The feed water rates of RO feed tanks were 1 m^3/h, and the hydraulic retention time was 1 h. The flow rates of permeate were kept constant at about 0.15 m^3/h by adjusting the feed water flow rates and pressures. The initial feed water flow rates of the RO system were set to 0.8 m^3/h and continuously increased with the aggravation of membrane fouling. The feed water flow rates were adjusted every

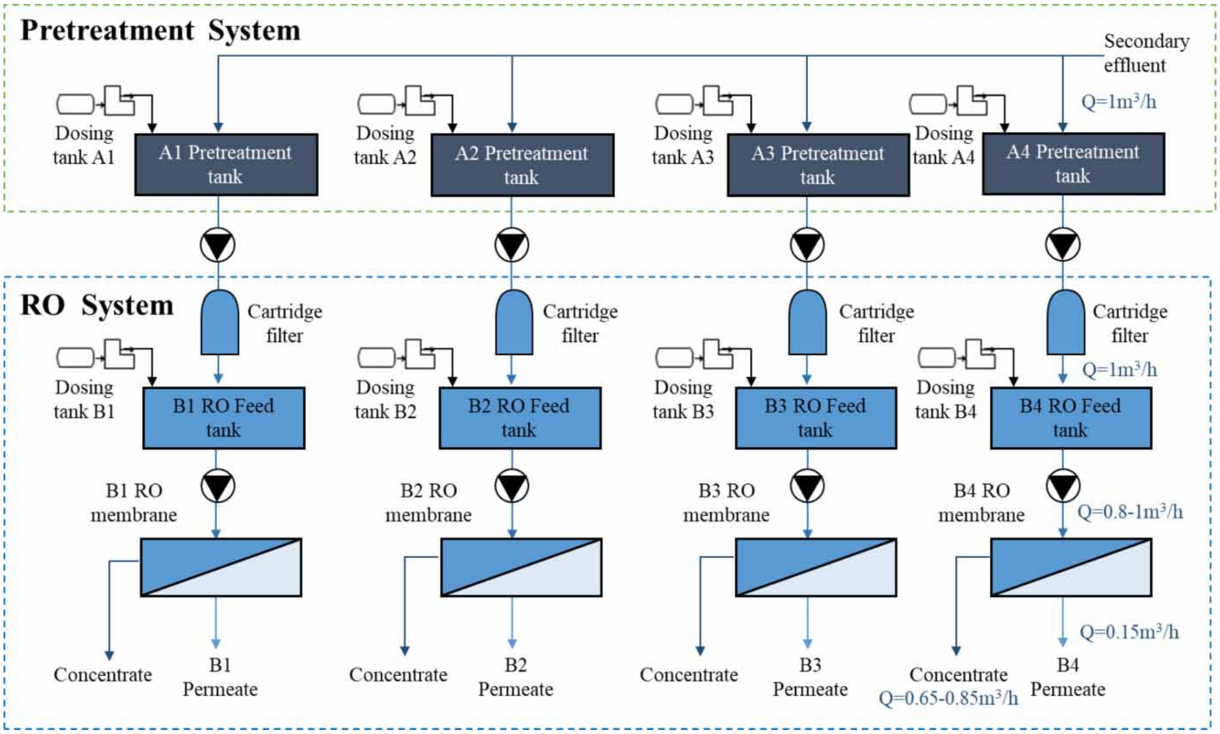


Figure 1 | Process flowchart of the pilot-scale wastewater reclamation RO system.

12 h. All concentrates of RO were discharged without backflow.

Dosing mode

The dosing mode of the pretreatment system is shown in Table 1. The sodium hypochlorite solution was continuously added to the pretreatment tanks from the dosing tanks. The free chlorine concentrations of A1–A4 pretreatment tanks were 0, 1, 2 and 5 mg/L, respectively.

The dosing mode of the RO system is shown in Table 2. The mixing solution of excess $NaHSO_3$ and sodium hypochlorite was continuously added to the RO feed tanks. Sodium hypochlorite was replenished to the B1–B3 feed tanks to make the total amount of sodium hypochlorite in each experimental group equal. The concentration of ions in each group could be comparable by adding sodium hypochlorite. Excess $NaHSO_3$ completely consumed free chlorine in the RO feed tanks to ensure that free chlorine did not damage the RO membranes.

Table 1 | Dosing mode of pretreatment tank

Dosing tank	**A1**	**A2**	**A3**	**A4**
Volume (L)	100	100	100	100
Flow rate (L/d)	100	100	100	100
Free chlorine (g/d)	0	24	48	120
Pretreatment tank	**A1**	**A2**	**A3**	**A4**
Free chlorine (mg/L)	0	1	2	5

Sodium hypochlorite solution was continuously pumped from the A1–A4 dosing tanks to pretreatment tanks.

Table 2 | Dosing mode of RO feed tanks

Dosing tank	**B1**	**B2**	**B3**	**B4**
Volume (L)	100	100	100	100
Flow rate (L/d)	100	100	100	100
Free chlorine (g/d)	120	48	24	0
$NaHSO_3$ (g/d)	600	600	600	600
Feed tank	**B1**	**B2**	**B3**	**B4**
Free chlorine (mg/L)	0	0	0	0

Excessive sodium bisulfite and sodium hypochlorite solution were continuously pumped from the B1–B4 dosing tanks to RO feed tanks to realize dechlorination and salt concentration supplement.

Operational data monitoring

Eleven kinds of operating parameters of the pilot-scale RO system were monitored online and uploaded to the computer in real-time. These operating parameters include: (1) flow rate, pressure, temperature, pH and conductivity of feed water; (2) flow rate, pressure, pH and conductivity of permeates and (3) flow rate, pressure, pH and conductivity of concentrates.

Water quality analysis

TOC (Total Organic Carbon) of secondary effluent (feed water of pretreatment tanks) was measured with a Sievers 5310C analyzer (GE, USA). The concentrations of inorganic elements in the secondary effluent water sample were measured with ICP-AES (Varian, USA) and ICP-MS (Perkin-Elmer, USA). An HPC (heterogeneous plate counties) method (APHA 2012) was used to determine the concentrations of bacteria in water samples of B1–B4 feed tanks.

Water quality parameters of secondary effluent (feed water of pretreatment tanks) are shown in Table 3. The concentration of inorganic ions in secondary effluent (feed water of pretreatment tanks) is shown in Table 4. The concentration of TOC in the water sample was 7.1 ± 0.7 mg/L. The number of bacteria represented by the HPC was $3.2 \pm 0.4 \times 10^4$ CFU/ml. The pH of the water sample was 7.20 ± 0.23. The turbidity of the water sample was lower than the detection limit of the turbidity meter (WZS-186, China). Studies have shown that TOC, pH, salt concentration and other water quality parameters will have an important impact on membrane fouling potential (Cai *et al.* 2019).

Microbial community structure analysis

The community structures of living bacteria of the water samples in B1–B4 feed tanks were determined by the PMA-PCR method. PMA (propidium monoazide) staining

Table 3 | Water quality of secondary effluent (feed water of pretreatment tanks)

TOC (mg/L)	**HPC (CFU/ml)**	**Conductivity (μs/cm)**	**pH**	**Turbidity (–)**
7.1 ± 0.7	$3.2 \pm 0.4 \times 10^4$	1,533.5 ± 114.4	7.20 ± 0.23	N.D. < 0.50

Table 4 | Concentration of inorganic ions in secondary effluent (feed water of pretreatment tanks)

TN mg/L	**NH_3-N** mg/L	**TP** mg/L	**Cl^-** mg/L	**SO_4^{2-}** mg/L	**PO_4^{3-}** mg/L
6.09 ± 0.33	0.11 ± 0.01	0.17 ± 0.02	313 ± 15	211 ± 11	0.19 ± 0.02
Fe mg/L	**Ca** mg/L	**Na** mg/L	**Mg** mg/L	**K** mg/L	**Zn** mg/L
15.9 ± 0.3	64.1 ± 5.3	266 ± 12	37.0 ± 1.0	20.2 ± 1.7	48.0 ± 4.2

can prevent the amplification of DNA of dead cells in PCR and avoid the interference of dead bacteria on the determination of community structures (Gensberger *et al.* 2014; Li *et al.* 2014).

The experimental method was based on the research of Pang *et al.* (2016) and Gensberger *et al.* (2014). The experimental steps are as follows. (1) Bacteria in 300 ml of the water samples were enriched on the clean 0.1 μm nylon membrane filter (47 mm, Whatman). (2) The ultrafiltration membrane was cut into pieces and placed in a 15 ml tube. Then, 3 ml of sterile phosphate buffer saline (PBS) solution was added to the tube. The tube was shaken for 60 s. The supernatant was separated into another tube. (3) Step (2) was repeated five times to obtain 15 ml of solution with enriched bacteria. (4) The solution with enriched bacteria dewatered by centrifugation (Beckman, USA) at 10,000 rpm for 20 min and that supernatant was discarded. The precipitate (cells) was suspended again with 1 ml of sterile PBS solution. (5) 5 μL of PMAxx dye (Biotium Inc., USA) was added to the solution with cells. After incubation at room temperature for 5 min in the dark, the water sample was irradiated with a PMA-Lite LED photolyzer (Biotium Inc., USA) for 15 min to fully cross-link PAMxx with DNA. (6) DNA was extracted and amplified by PCR. An Illumina high-throughput sequencing method was used to analyze amplified RNA.

RESULTS AND DISCUSSION

The operational conditions of the pilot-scale RO system under different chlorine dosages

The reduction of HPC represented the bacterial inactivation rate of chlorine on water samples in B1–B4 feed tanks, and the results are shown in Figure 2. The log inactivation rate in experimental groups with 1, 2 and 5 mg Cl_2/L were 0.69 ± 0.05, 1.09 ± 0.07, 2.28 ± 0.06 log. Chlorine reduced the concentrations of bacteria in feed water of the RO system significantly.

The operational characteristics of the pilot-scale RO system, namely feed water flow rate, permeate flow rate, feed water pH and salt rejection, are monitored every 12 h and is shown in Figure 3. During the 30-day operation of the pilot-scale RO system, the final feed water flow rates of these experimental groups with 0, 1, 2 and 5 mg Cl_2/L were 0.86, 0.86, 0.96 and 1.09 m^3/h, respectively, as shown in Figure 3(a). Under the low concentration of chlorine (0, 1 mg/L), the feed water flow rates remained unchanged in 30 days. However, the feed water flow rates increased significantly with the high concentration of chlorine (2, 5 mg/L) as pretreatment. The feed water flow rates provided additional pressure to overcome the resistance of the fouling layers.

The permeate flow rate of each experimental group fluctuated around 0.15 m^3/h, as shown in Figure 3(b). The pH of secondary effluent (feed water of pretreatment tanks) was 7.18 ± 0.08 during the 30-day operation. Simultaneously, the salt rejection rate of each group always remained above 98%, as shown in Figure 3(d).

Effect of chlorine disinfection on the membrane fouling of the pilot-scale RO system

The feed water pressures (P) of the RO membrane were normalized based on the initial feed water pressures (P_0). The normalized pressures are called the relative pressure of feed water (P/P_0). It was used to reflect the degree of RO membrane fouling. Higher relative pressure indicated higher resistance of the fouling layer and more serious membrane fouling. In this study, a significant rise of relative feed

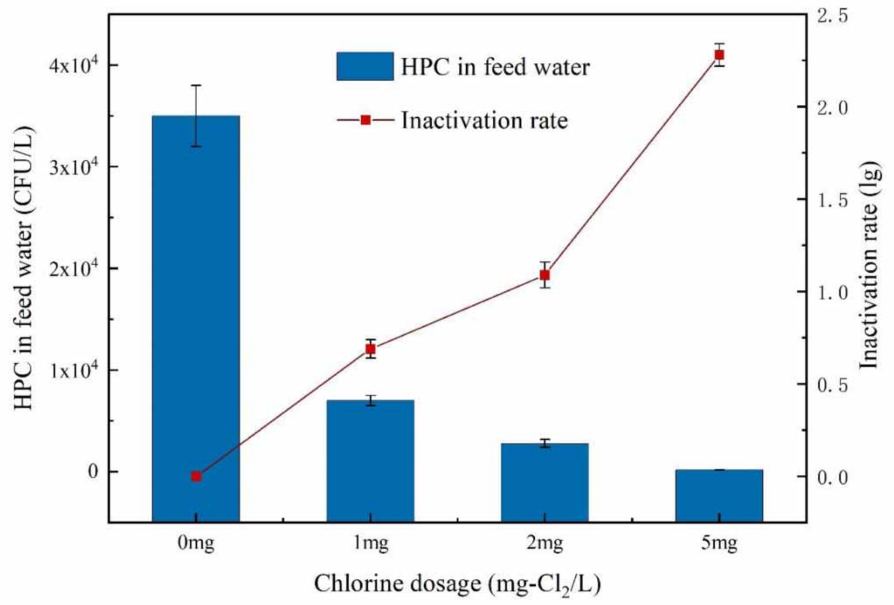


Figure 2 | Bacterial concentrations (CFU/L) and inactivation rates (log) of the water samples in B1–B4 feed tanks measured by the HPC method.

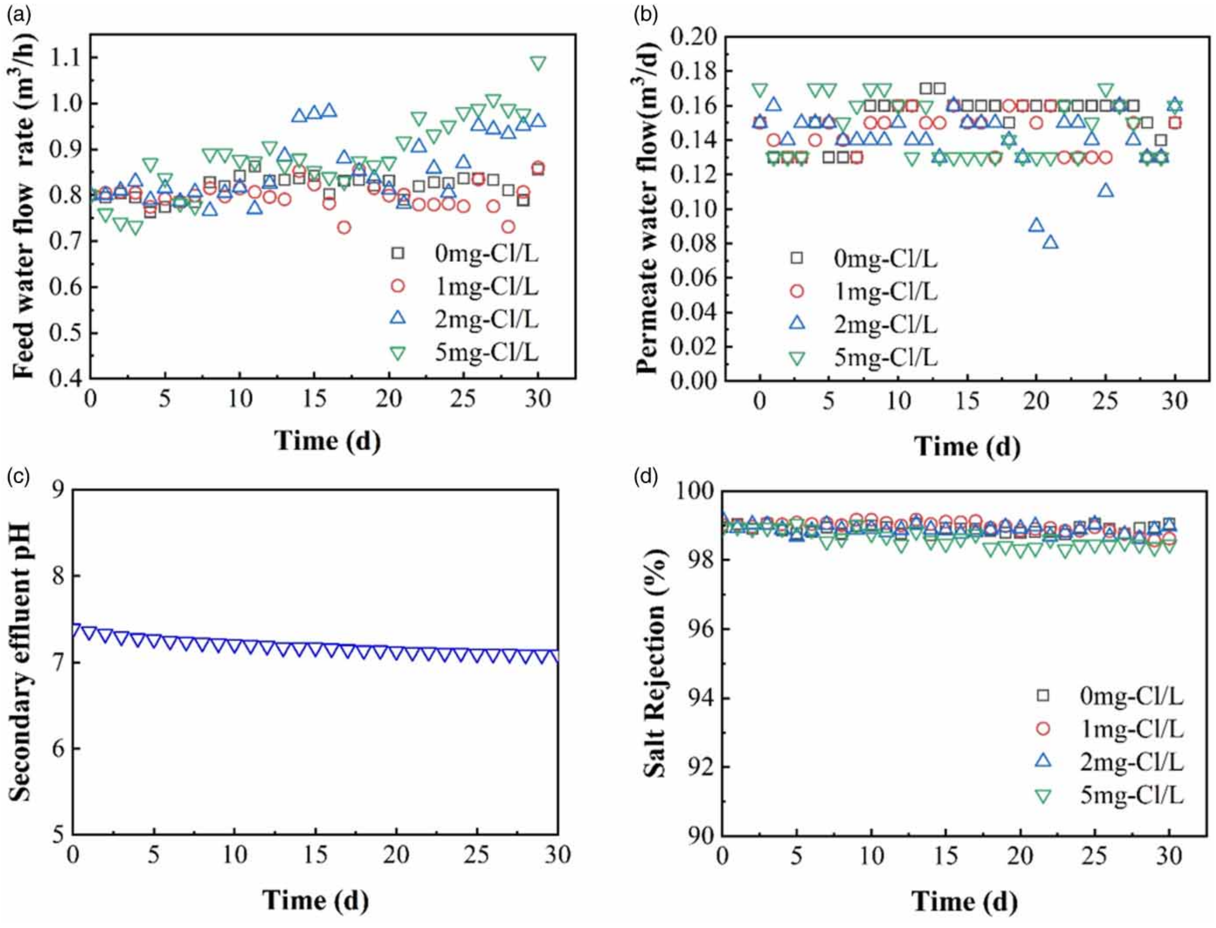


Figure 3 | Operational characteristics of the pilot-scale wastewater reclamation RO system. (a) Feed water flow rate, (b) permeate flow rate, (c) secondary effluent pH and (d) salt rejection.

water pressure after chlorine disinfection was observed. During the 30-day operation, the change of relative feed water pressure of each experimental group is shown in Figure 4. The initial feed water pressures (P_0) of the B1–B4 RO membrane were 867, 844, 856 and 872 kPa, respectively. The relative pressures of the water samples with 0, 1, 2 and 5 mg Cl_2/L as pretreatment finally reached 1.04, 1.06, 1.21 and 1.38, respectively.

Compared with the control group, the final relative feed water pressures of experimental groups with 1, 2 and 5 mg Cl_2/L increased by 1.9, 16.3 and 36.7%, respectively. Chlorine disinfection did not alleviate biofouling but aggravated it in this study.

These results were in accordance with the conclusion obtained by Wang *et al.* (2019a) in a laboratory-scale wastewater reclamation RO system. In addition, researchers also reported some cases, where biofouling could not be controlled by sodium hypochlorite in seawater desalination RO systems (Obaid & Ben Hamida 1998; Khan *et al.* 2015). Wang *et al.* (2019a) found that although chlorine disinfection reduced the number of bacteria in RO feed water, the number of bacteria on the membrane surface did not decrease, but the quality of organic matter on the membrane surface and the thickness of fouling layer increased instead. Since the abundance of CRB in the biofouling layer increased after disinfection, CRB was considered to be the key species leading to the aggravated fouling effect of chlorine (Wang *et al.* 2019a). Studies have proved that the EPS production of a strain was positively correlated with the chlorine resistance and the biofouling potential of RO membrane (Tsuneda *et al.* 2003; More *et al.* 2014; Wang *et al.* 2019b). Therefore, the remaining CRB after disinfection have stronger membrane fouling potential.

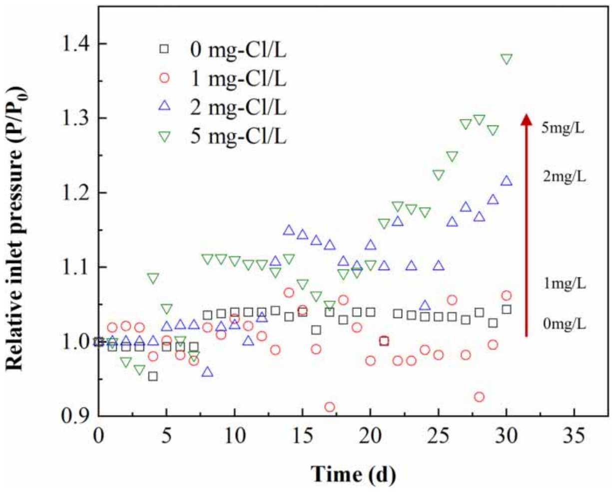


Figure 4 | Relative feed water pressure curves of the pilot-scale wastewater reclamation RO system under different chlorine dosages. The operation time lasted for 30 days.

Researchers reported that chemical cleaning with NaClO causes increases in eDNA and quorum sensing molecules N-acyl homoserine lactones (AHLs) secreted by pure bacteria in the regrowth stage, which eventually lead to the aggravation of biofouling of ultrafiltration membrane (Wang *et al.* 2020). After disinfection, the increase of eDNA and AHLs secretion may also lead to the increase of fouling of the RO membrane.

The aggravated fouling phenomenon of disinfection showed that the limited inactivation of bacteria might not effectively alleviate biofouling of the RO membrane. Because the size of bacteria (0.5–5 μm) is much larger than the pore size (0.1–0.7 nm) of RO membrane, bacteria could not directly block the pores. The process of RO biofouling can be described in three stages: adhesion, *in situ* regrowth and biofilm diffusion (Matin *et al.* 2011; Liu *et al.* 2020). Biofilm diffusion means that the bacteria in the mature biofilm fall off and enter the liquid phase, and then, the bacteria adhere, grow and form a new biofilm in other places (Matin *et al.* 2011). The effects of chlorine on the three stages of biofouling formation were not completely clear. So far, there is no research about the effects of chlorine on the adhesion ability of a single bacterium from a microscopic perspective. Wang *et al.* (2019a) studied the effects of chlorine on the regrowth of disinfection residual bacteria and found that chlorine increased the competitive advantage of CRB on the RO membranes. In addition, chlorine might promote the regrowth of bacteria by affecting the water quality. Liu *et al.* (2002) found that chlorine disinfection could improve the level of assimilable organic carbon (AOC) of the water samples. The rise of AOC reduced biological stability and promoted bacterial regrowth.

Effect of chlorine disinfection on the community structure of the feed water

The microbial community structures of the water samples in B1–B4 feed tanks were analyzed by 16S rRNA gene sequencing, and the results are shown in Figure 5.

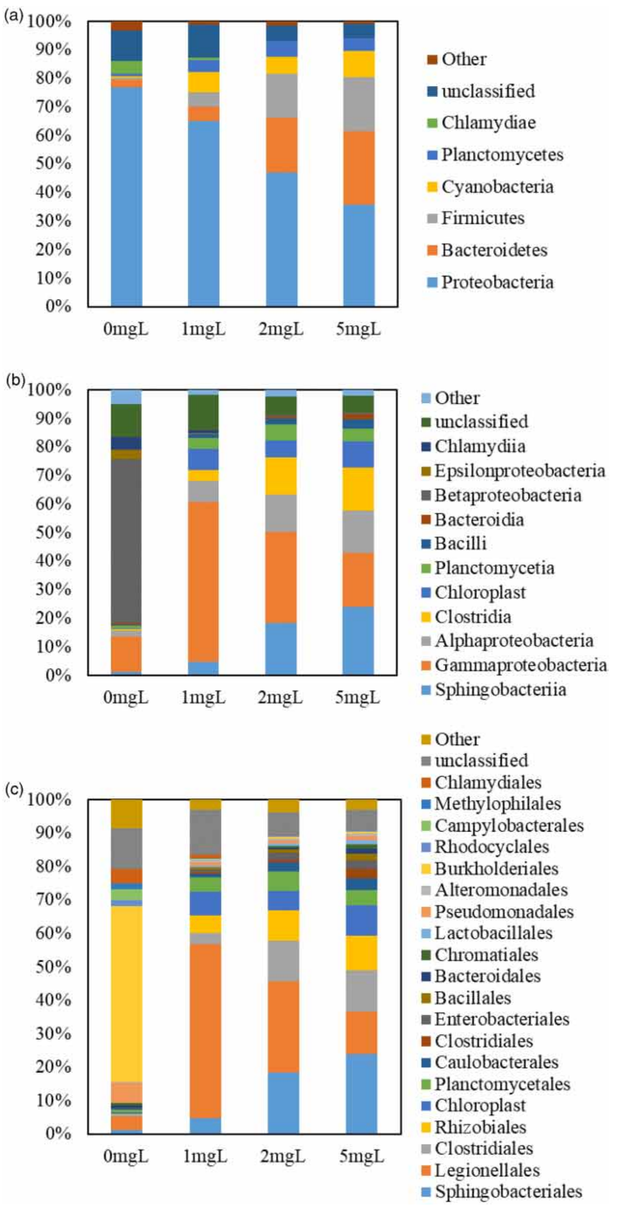


Figure 5 | Effect of chlorine disinfection on the community structure of the water samples in B1–B4 feed tanks at the (a) phylum level, (b) class level and (c) family level. 'Others' in the legend included bacteria whose abundances were lower than 1%.

On the phylum level, with the increase in chlorine concentration, the relative abundance of Bacteroidetes and Firmicutes increased and that of Proteobacteria decreased obviously. In the water samples with 0 and 5 mg Cl_2/L as pretreatment, the relative abundance of Bacteroidetes was 2.42 and 25.8%, that of Firmicutes was 0.77 and 18.9% and that of Proteobacteria was 76.9 and 35.6%, respectively.

On the class level, Betaproteobacteria, the dominant bacteria in the control group (with 0 mg Cl_2/L), almost completely inactivated after disinfection. Gammaproteobacteria, Sphingobacterium, Alphaproteobacteria and Clostridia became the dominant bacteria in the microbial community after disinfection. With the increase in chlorine concentration, the relative abundance of Gammaproteobacteria increased firstly and then decreased, while that of Sphingobacterium, Alphaproteobacteria and Clostridia increased continuously. The relative abundance of Gammaproteobacteria, Sphingobacterium, Alphaproteobacteria and Clostridia was 19.1, 23.9, 14.5 and 15.2%, respectively, at the experimental group with 5 mg Cl_2/L.

The community structure on the order level and the class level were compared. Betaproteobacteria mainly include Burkholderiales. Gammaproteobacteria mainly include Legionellales. Sphingobacteria mainly include Sphingobacteriales. Clostridia mainly include Clostridiales.

The differences of the water samples on the genus level were shown with the heat map (Figure 6). After chlorine disinfection, the relative abundance of some bacteria increased, showing different levels of chlorine resistance between different genera. The relative abundance of *Acidovorax*, *Acinetobacter*, *Arcobacter* and *Undibacterium* in the control group (0 mg Cl_2/L) was 47.5, 4.83, 2.15 and 2.12%, respectively. They were dominant bacteria in the control group. After disinfection, their relative abundance decreased significantly to less than 1%.

In this study, the aggravated fouling of chlorine disinfection only occurred when the chlorine concentrations in the pretreatment stage was relatively high (2, 5 mg Cl_2/L). Therefore, the dominant species in these two experimental groups with 2, 5 mg Cl_2/L might be more closely related to the aggravated fouling. On the genus level, nine major genera maintained higher relative abundance in experimental groups with 2, 5 mg Cl_2/L than the groups with 0, 1 mg Cl_2/L. The relative abundances of these major genera are shown in Figure 7. Among them, *Clostridium*, *Citrobacter* and *Bacillus* were reported as CRB in many other researches (Khan *et al.* 2016; Owoseni & Okoh 2017; Roy & Ghosh 2017; Luo *et al.* 2020). *Legionella* was also CRB and showed higher abundance in all three disinfection groups (with 1, 2 and 5 mg Cl_2/L)

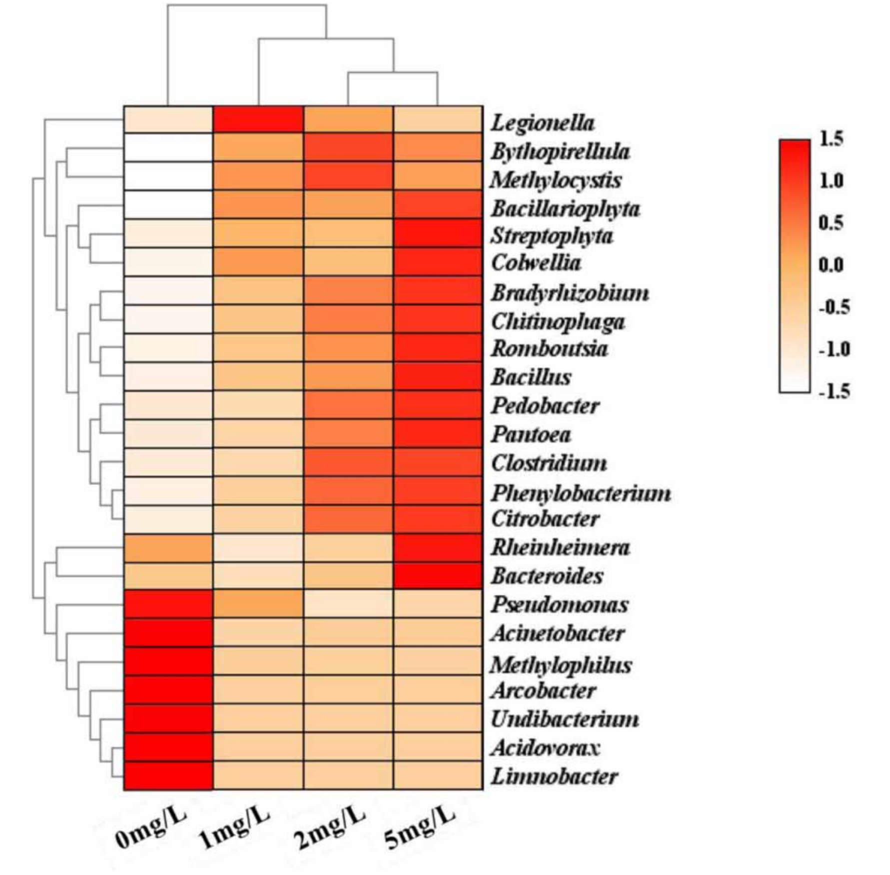


Figure 6 | Heat maps of microbial communities of the water samples in B1–B4 feed tanks on the genus level. Genera whose abundances were higher than 1% were shown and counted.

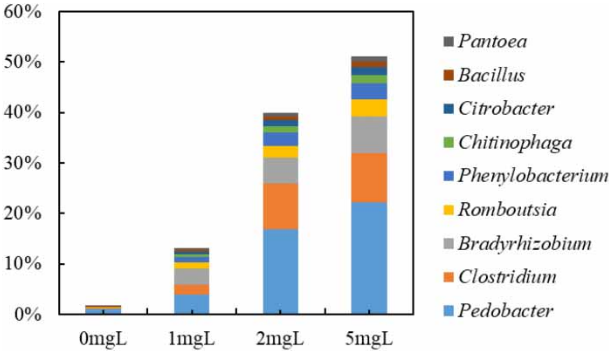


Figure 7 | Nine species with higher abundance under the high concentration of chlorine (2, 5 mg/L) than the low concentration of chlorine (0, 1 mg/L).

than the control group (with 0 mg Cl_2/L). Since *Legionella* was pathogenic bacteria, many studies focused on its chlorine resistance (Kuchta *et al.* 1983; Miyamoto *et al.* 2000). However, the relative abundance of *Legionella* in the experimental group with 1 mg Cl_2/L (53.2%) was higher than that with 2 and 5 mg Cl_2/L chlorine (27.9 and 13.0%). This trend suggested that *Legionella* might not be the cause of the aggravated biofouling after disinfection. These nine bacteria with abundance advantages in the experimental groups with 2 and 5 mg Cl_2/L were more likely to be the key species causing the increased fouling potential of RO.

It is noted that the abundances change of predominant CRB were not linear with increasing chlorine concentration. As mentioned previously, the low concentration of chlorine increased the relative abundance of *Legionella*, but the high concentration decreased it. In addition, some strong CRB did not dominate in the low concentration of chlorine. Therefore, the change of fouling potential caused by the change of the community structure was not linear, either. This might explain the sudden and significant increase in fouling potential at the experimental groups with 2, 5 mg Cl_2/L.

CONCLUSION

The pilot-scale RO system verified the conclusion of the laboratory-scale trial that chlorine disinfection in the wastewater reclamation RO system could aggravate RO membrane fouling. Chlorine pretreatment caused a 1.9, 16.3–36.7% increase in the relative feed water pressure of the RO system in this study. Analysis of the bacterial community structure in the feed water showed that the relative abundance of CRB was significantly increased after disinfection. The changing trend of dominant species with the increase in chlorine concentration was analyzed, and nine strains were considered as possible key species causing membrane fouling, including *Pedobacter*, *Clostridium*, *Bradyrhizobium* and *Romboutsia*.

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DATA AVAILABILITY STATEMENT

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

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