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RESEARCH ARTICLE

Changes of microbial composition during wastewater reclamation and distribution systems revealed by high-throughput sequencing analyses

Yiwen LIN[1], Dan LI (✉)[2], Siyu ZENG[1], Miao HE (✉)[1]

1 State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China
2 Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP^3), Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China

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Abstract This study employed 454-pyrosequencing to investigate microbial and pathogenic communities in two wastewater reclamation and distribution systems. A total of 11972 effective 16S rRNA sequences were acquired from these two reclamation systems, and then designated to relevant taxonomic ranks by using RDP classifier. The Chao index and Shannon diversity index showed that the diversities of microbial communities decreased along wastewater reclamation processes. *Proteobacteria* was the most dominant phylum in reclaimed water after disinfection, which accounted for 83% and 88% in two systems, respectively. Human opportunistic pathogens, including *Clostridium*, *Escherichia, Shigella*, *Pseudomonas* and *Mycobacterium*, were selected and enriched by disinfection processes. The total chlorine and nutrients (TOC, NH_3-N and NO_3-N) significantly affected the microbial and pathogenic communities during reclaimed water storage and distribution processes. Our results indicated that the disinfectant-resistant pathogens should be controlled in reclaimed water, since the increases in relative abundances of pathogenic bacteria after disinfection implicate the potential public health associated with reclaimed water.

Keywords wastewater reclamation systems, microbial community, pathogenic community, 454-pyrosequencing

1 Introduction

Wastewater reclamation is an important water resource for water-shortage zones worldwide [1,2]. The most important concern of wastewater reclamations is related to human public health and ecological safety [3,4]. Particularly, wastewater contains a variety of pathogens, which are mainly derived from faecal matter. Although pathogens can be efficiently removed or/and inactivated by multiple reclamation processes, part of them are still alive and reproduce during reclaimed water storage and distribution, and then cause some safety problems, such as biofilm formation, microbial-mediated corrosions, and even infectious diseases caused by pathogens [1,3,5]. Therefore, systematically investigation of microbial and pathogenic communities in wastewater reclamation and distribution systems is important, which will promote the efficiencies of wastewater reclamations and optimize the storage and distribution processes of reclaimed water.

A number of methods have been developed and applied to detect microorganisms in waters, such as culture-based assays, quantitative PCR (qPCR) and microarray. Those techniques mainly detect one or several microorganisms, and cannot describe all the features of the frequently existed pathogenic microorganisms in reclaimed water systems. The high-throughput 454-pyrosequencing[6], which applies shotgun sequencing to characterize the diversities and abundances of various microorganism at species levels, has been applied to investigate the microbial communities in varies environmental samples, including fresh groundwater [7], sea water [8] and wastewater [9]. However, these previous reports mainly focused on the microbial communities in conventional drinking water [10–12] and wastewater treatment systems [9]. These researches have evaluated the microbial communities along the wastewater treatment processes and provided important results for optimization and improvement of wastewater reclamation and distribution. However, the variability and stability of microbial and pathogenic communities in the whole wastewater reclamation and

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E-mails: lidanfudan@fudan.edu.cn, hemiao@tsinghua.edu.cn

distribution systems, which include wastewater treatment processes, disinfection, storage and distribution pipes, were not reported systematically.

The objective of this research is to investigate the microbial and pathogenic communities by using high-throughput 454-pyrosequencing in two full-scale wastewater reclamation plants with different treatment and disinfection processes, as well as distribution systems. The effects of reclaimed water quality and total chlorine on the diversities and abundances of microbial and pathogenic communities during storage and distribution processes were also analyzed. The results provide a comprehensive understanding of microbial communities and pathogenic microorganisms in reclamation and distribution systems, and would benefit the safety usage of reclaimed water.

2 Materials and methods

2.1 Water sampling, concentration and DNA extraction

In present study, reclaimed waters were sampled in December 2012 from two full-scale wastewater reclamation plants (WRP) (Q and W) in Beijing, China. Waters were sampled from Q WRP and distribution system, including: 1) influent (secondary effluent from wastewater treatment plant) (Q1); 2) after ultrafiltration treatment (Q2); 3) after ozonation and granular activated carbon filtration (Q3); 4) after chlorine disinfection (WRP effluent, Q4) and 5) at the end point of the distribution pipes (Q5). Samples collected from W WRP and distribution system included: 1) influent (secondary effluent from wastewater treatment plant) (W1); 2) after chlorination, coagulation and sand filtration (W2); 3) after UV and chlorination (WRP effluent, W3); and 4) at the end point of the distribution pipes (W4).

The water samples were collected in sterile plastic containers. All water samples were kept on ice and then delivered to the laboratory within 4 h. A volume of 2-L water sample was concentrated by filtering a 0.45-μm-pore-size cellulose nitrate filter (Shanghai Xingya Company, Shanghai, China). Then the filters were transited into a sterile 10-mL centrifuge tube, and 8 mL PBS was added to each tube and vortexed 10 min to release the bacteria into the eluant. After centrifuging at 13000 $r \cdot min^{-1}$ for 15 min at 4°C, the supernatant was removed and the sediment was transferred into a 2-mL tube, and then centrifuged at 13000 $r \cdot min^{-1}$ for 5 min, and then the DNA was extracted from the remaining pellet by FastDNA@ Spin Kit for Soil (MP Biomedicals, Catalog: 6560200, California, USA) [13]. From each sample, 50 μL of DNA was obtained and stored at –80°C until further analysis. Raw DNA was purified and measured the concentration of DNA by using Nanodrop-2000 spectrophotometer (Nanodrop Inc., Wilmington, USA). Besides, the total organic carbon (TOC) was quantified using a TOC analyzer (TOC-V CPH, Shimadzu, Japan). The total chlorine was measured by a colorimeter (46700, Hach, USA) using the *N*,*N*-diethyl-*p*-phenylendiamin (DPD) colorimetric method. The nutrient parameters of reclaimed water were also analyzed according to standard procedures and the results were shown in Table S1.

2.2 PCR amplification and pyrosequencing

The DNA of the samples was amplified by using a couple of primers targeting the hyper variable V1–V3 regions (about 500 bp) of bacterial 16S rRNA gene [10]. The forward primer was 27F: 5′-Fusion A-Barcode-CA linker-AGAGTTTGATCMTGGCTCAG-3′ and the reverse primers was 533R: 5′-Fusion B-TC linker-TTACCGCGGCTGCTGGCAC-3′ [10]. For the purpose of differentiating sequences in the mixed reactions, 10-bp barcodes were combined between the 454 adapters and the reverse primers. The PCR reaction was carried out in 50 μL mixtures, including 10 μL of 5 × FastPfu Buffer, 5μL of 2.5 $mmol \cdot L^{-1}$ dNTPs, 2 μL of 5 $\mu mol \cdot L^{-1}$ each of forward and reverse primers, 1 μL of FastPfu Polymerase, and 5 μL of DNA. The thermocycling profile includes 95°C for 2 min, then 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s and followed by 72°C for 10 min. The PCR products were confirmed by running on a 2% (*w*/*v*) agarose gel at a size about 500 bp, and then the PCR amplification products were purified with Wizard® DNA Clean-Up System (Promega code: A7280, Madison, USA).

The DNA mixtures containing samples and different barcodes were analyzed by a Roche 454 sequencer (Roche, Nutley, USA) at the Chinese National Human Genome Center [14].

2.3 Sequence analysis and phylogenetic assignment

After 454-pyrosequencing, scripts were written to filter and screen the sequences with the following principles: 1) verify the integrity of barcodes and remove sequences with even a single base bias; 2) eliminate sequences shorter than 150 bp; 3) exclude sequence with a quality value less than 20; 4) and assure sequences quality values of higher than 20 [9].

Valid sequences acquired from 454-pyrosequencing were assigned to taxonomic ranks with RDP classifier on the Website of Ribosomal Database Project (http://rdp.cme.msu.edu/classifier/classifier.jsp), and the bootstrap cutoff was set at 50% [9]. To study the α-diversity, a rarefaction analysis was performed at 97% sequences similarity for each sample. R (v 2.14.2 http://www.r-project.org/) was employed to compute the α-diversity indices (Chao1 richness estimator and Shannon diversity index), β-diversity index (Sørensen similarity index) and principal coordinate analysis (PCoA). Canonical correspondence analyses (CCA) were also conducted by

Canoco4.5 (Microcomputer Power, USA) to cluster the microbial communities of water samples. All graphs were generated by OriginPro 8.1 (OriginLab, MA, USA).

3 Results and discussion

3.1 Overall taxonomy of 454-pyrosequencing from two reclamation systems

In total, 32211 raw sequences were sequenced from 9 water samples collected from two reclamation systems by using high-throughput 454-pyrosequencing. After trimming, sorting, and quality control, 11972 effective sequences remained, accounting for about 37% of the total sequences. The quantity of Operation Taxonomy Units (OTUs) ranged from 96 to 512 in those samples (as shown in Fig. S1A). The Chao1 richness estimator and the Shannon diversity index, which were estimated at 3% (as shown in Figs.S1B and C), showed lesser richness and diversity in effluent than in influent of those two water reclamation plants (Q and W). The rarefaction curves (Fig. S2) for each sample (observed OTUs) also indicated that the effluent waters had less OTUs than the influent waters. To identify the potential pathogens in reclamation systems, reference sequences of human pathogenic bacteria (shown in Table S3) were listed according to previous reports [4,15,16].

3.2 Structures and diversities of microbial communities in two different reclamation systems

The similarity and the compositions of microbial communities between two different systems were quantified by using Sørensen similarity index (Table S2). The values of similarity indices were varied between these two different reclamation systems. The PCoA also exhibited similar results with the clustering analyses (Fig. 1). These samples from two reclamation systems (Q and W) were grouped into four clusters: 1) influent, after ultrafiltration treatment and after ozone treatment (Q1, Q2, Q3, and W1); 2) after chlorine treatment (W2); 3) after UV disinfection (W3 and W4) after storage and distribution systems (Q4, Q5 and W4). The differences of microbial communities in these waters samples were subsequently analyzed by using RDP Classifier.

As shown in Fig. 2(a), *Proteobacteria* were the predominant phylum, which accounted for about 69.0%, 59.6%, 41.7%, 83.5% and 76.2% in the samples of influent (Q1), after ultrafiltration (Q2), after ozone (Q3), effluent (Q4) and the end of distribution pipes (Q5), respectively. These results were consistent with previous researches [8,17,18] *Firmicutes* were the secondary dominant phylum in the ultrafiltration (Q2) and effluent (Q4) waters, which accounted for about 15.0 and 6.0% of the total microbial communities. However, the secondary phylum was

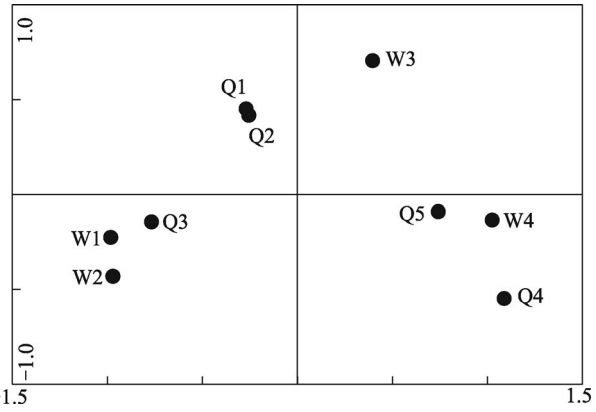

Fig. 1 Principal coordinates analysis (PCoA) analysis of 16S rRNA genes in reclaimed water samples collected from Q (Q1–Q5) and W (W1–W4) systems. Principal coordinates plots were generated using the presence of each OTU (at a distance level of 3%)

Actinobacteria (21.0%) in the ozone treated water samples (Q3), and was *Bacteroidete* (12.1%) in the end of pipe samples (Q5). The total phyla numbers were 19, 13, 16, 11 and 13 in the samples of Q1–Q5, respectively. These results suggested that the diversities of the microbial community in the samples after water reclamation treatments become lower than that in the influent samples at the phylum levels.

There were 6 phyla in total shared by all these five samples in Q system, which were *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*, and *candidate division TM7*. *Proteobacteria* and *Firmicutes* in Q system were further analyzed (Fig. 3). *Proteobacteria* subclasses were largely constituted of *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria*, which were consistent with previous studies [19]. *Betaproteobacteria* dominated in all treatment processes in plant Q. After ozone treatment, the relative abundance of the *Alphaproteobacteria* and *Gammaproteobacteria* increased from 4.0%, 6.0% to 26.0% and 16.9% of the total *Proteobacteria*, respectively. In contrast, the relative abundance of *Betaproteobacteria* decreased from 63.2% to 48.3% in *Proteobacteria* after ozone treatment. The microbial community structures of these four subdivisions of *Proteobacteriaon* were shown in Fig. 4, and significant divergences on composition among samples were observed. For *Firmicutes*, the class composition was homogenous (Fig. 3), and the majority orders were *Clostridia* and *Bacilli*. After ozone treatment, the percentages of *Clostridia* increased from 30.1% to 91.1% of the total *Firmicutes*.

The relative constituents of different phyla in four samples from W reclamation system were shown in Fig. 2(b). The samples, which were collected from influent, after chlorine treatment, in effluent, and at end of distribution pipes, were significantly different, in terms of either the most predominant phylum or the contents of

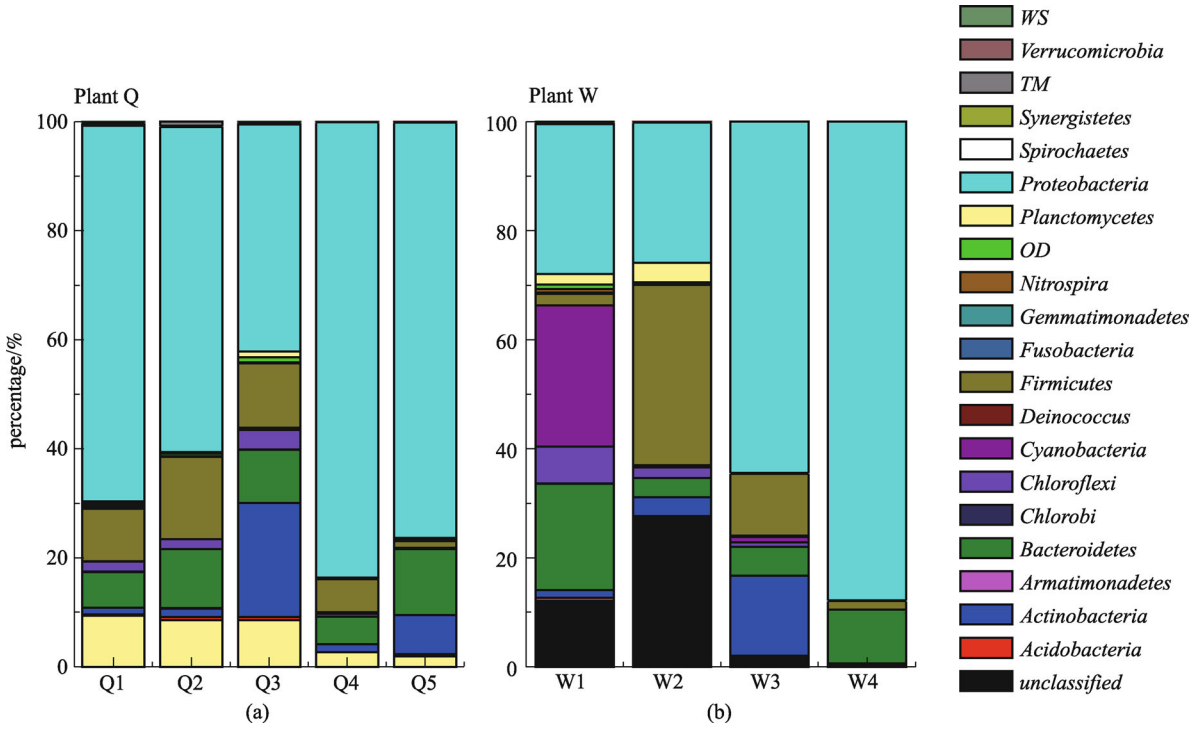

Fig. 2 Bacterial community structures in Q (Q1–Q5) and W (W1–W4) reclamation systems. The proportions of bacteria are presented by the percentage of certain bacteria in the total effective bacterial sequences in each water sample, which were classified according to the RDP Classifier at the confidence threshold of 50%

each phylum. In the influent sample (W1), the most predominant phylum was *Proteobacteria* (28%), followed by *Cyanobacteria* (26%) and *Bacteroidetes* (20%). However, in the W2 sample, which were treated with coagulation, sedimentation, chlorination (2.0 $mg \cdot mL^{-1}$) and sand filtration in sequence, the most predominant phylum was *Firmicutes* (33%), followed by *Proteobacteria* (26%). In the samples of effluent (W3) and the end of distribution pipes (W4), however, the microbial communities changed significantly, and the dominant phylum was *Proteobacteria* at percentages of 64% and 88%, respectively. The secondary phylum was *Actinobacteria* (15%) in the effluent sample (W3). However, in the W4 sample, the secondary phylum was *Bacteroidetes* (10%). The total phyla numbers in W1–W4 samples were 17, 14, 9 and 5, respectively, which suggested that the diversities of microbial community at the phylum level decreased after wastewater reclamation treatments and distribution.

There were five phyla in total shared by all these four samples in W system, which were *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Candidate division OD*.

For *Proteobacteria* and *Firmicutes*, further analysis was also made (Fig. 3). *Proteobacteria* subclasses were dominantly composed of *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria*, which were similar with the Q system. For the W1 (influent) and W4 (the end point of distribution pipe) samples, *Betaproteobacteria* was the dominance subclasses accounting for 50.6% and 71.6% of the total *Proteobacteria*, respectively. For the water samples collected from the effluent and distribution pipes (W3 and W4) in W system, which were treated with UV and chlorine, *Gammaproteobacteria* was the dominant subclasses accounting for 54.0% and 64.0%, respectively. The divergent composition of these four subdivisions of *Proteobacteria* at the order level was shown in Fig. 4. The predominant class composition of *Firmicutes* was *Clostridia*, however, after UV treatment, the relative abundance of *Bacilli* increased from 4.9% (W2) to 30.4% (W3) of the total *Firmicutes*.

Comparing the microbial community structures of these two different wastewater reclamation systems (Figs. 2–4), it was notable that the compositions of microbial communities after disinfection (Q3, W2 and W3) were significantly changed with those in other samples. This divergence might be explained by the different disinfections, including different types and dose of disinfectants. In Q plant, bacteria were removed mainly by ultrafiltration, and the concentrations of disinfection were relatively low, which were 2 $mg \cdot L^{-1}$ of ozone and 0.18 $mg \cdot L^{-1}$ total chlorine in the effluent. However, in W plant, bacteria were removed mainly by disinfection and the concentrations were much higher, which were 80 $mJ \cdot cm^{-2}$ UV and 1.21

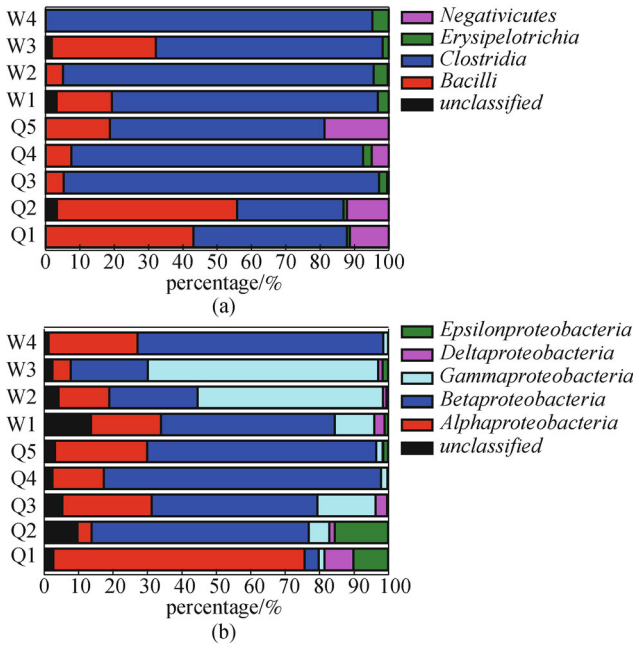

Fig. 3 (a) *Proteobacteria* and (b) *Firmicutes* compositions in reclaimed water samples collected from Q (Q1–Q5) and W (W1–W4) systems

$mg \cdot L^{-1}$ total chlorine in the effluents. As the total chlorine concentration in W plant was higher than that in Q plant, the bacteria that survived in W plant were more resistant than that in Q plant.

3.3 Diversities and abundances of pathogenic bacteria in different reclamation systems

Figure 5 shows the percentages of each pathogen genus in all of the 13 pathogenic genera listed in Table S3. It should be noted that bacteria were classified by genus level and not all the bacteria are pathogens. Among the genera of pathogens that occurred in the reclaimed water samples, including influents, treatment processes, effluents and end points of distribution pipes, there were significant differences between these two reclamation systems.

3.3.1 Pathogenic communities in Q reclamation system

Among the 13 genera detected in Q reclamation system (influent, after ultrafiltration, after ozone treatment, effluent and at end of distribution pipe), only *Clostridium* existed in the whole system (Fig. 5). The most dominant pathogenic genus in influent (Q1) and after ultrafiltration (Q2) samples was *Arcobacter*, which accounted for 63 and 81% of total identified pathogen populations, respectively (Table S4). However, *Clostridium* was the most abundant genus in the ozone treated (68%) and effluent (70%) samples, and the genus of *Mycobacterium* were responsible for 73% of pathogenic bacteria in the end of pipe sample. Besides, pathogen sequences accounted for 7.1%, 9.8%, 11.7%, 5.2% and 7.8% of total sequences for influent, after

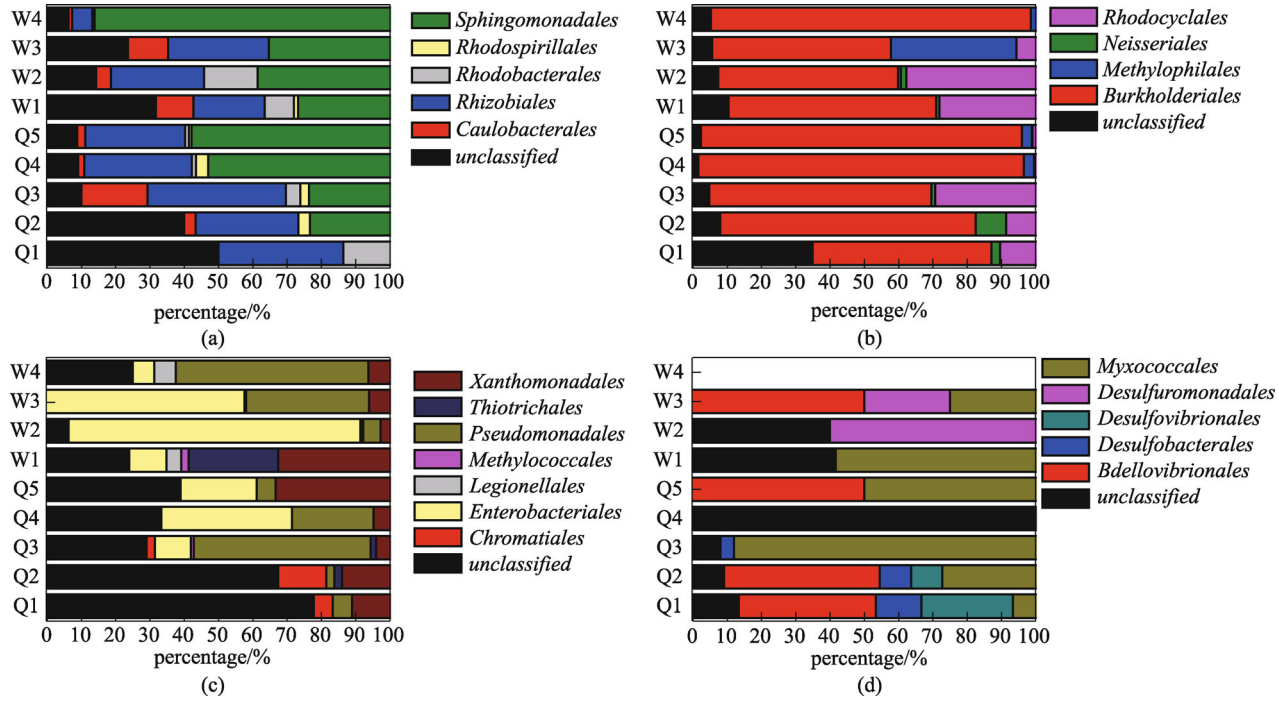

Fig. 4 (a) *Alphaproteobacteria*, (b) *Betaproteobacteria*, (c) *Gammaproteobacteria* and (d) *Deltaproteobacteria* compositions by order in reclaimed water samples

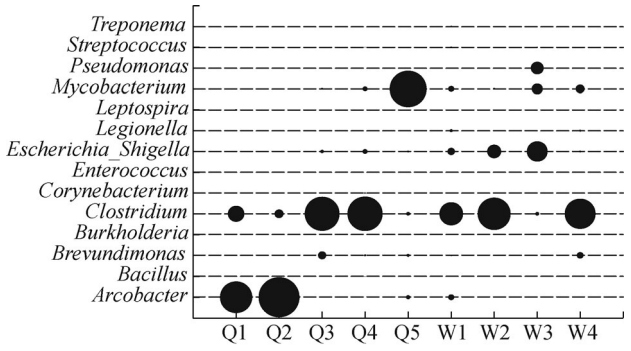

Fig. 5 Relative proportions of pathogenic bacterial genera in different reclaimed water samples. The reclaimed water samples were collected from Q (Q1–Q5) and W (W1–W4) systems. The circles in the figures are presented by the percentages of a pathogenic genus in total identified pathogen populations

ultrafiltration, after ozone, effluent and end point of distribution pipes samples, respectively (Table S4). The significant difference of genera diversities and abundances among the treatment processes indicated that pathogenic bacteria population changed during water reclamation.

3.3.2 Pathogenic communities in W reclamation system

In W reclamation system, the total number of pathogenic bacteria sequences in the influent water sample was less than that of Q reclamation system. The most abundant genus was *Clostridium* at percentages of 47.1%, 65.4% and 60.9% of total pathogenic bacteria for samples from influent (W1), after coagulation, sedimentation, chlorine treatment and sand filtration (W2) and at the end point of distribution pipes (W4), respectively. After UV disinfection in the effluent (W3), however, *Escherichia* and *Shigella* increased significantly from 28.3% to 41.2% of total identified pathogen populations. Additionally, *Mycobacterium* and *Pseudomonas* also increased significantly, which were at percentages of 21.1% and 25.9% of total identified pathogen populations in the effluent (W3) (Fig. 5 and Table S4). Unlike Q reclamation system, pathogenic bacteria sequences accounted for 2.3, 41.7, 59.9 and 1.7% of total sequences in the samples of W1–W4, respectively (SI Table S4). Those results indicated that disinfections selected the pathogenic communities significantly.

3.3.3 Bacterial resistance to disinfections

Arcobacter, which have the capacity to form and adhere to biofilms in various pipes therefore can colonize in water distribution systems, have been reported to cause several waterborne outbreaks [20]. *Arcobacter* was detected in the influent samples, however, was removed by reclamation treatments and disinfection (Fig. 5), which indicated that *Arcobacter* was susceptible to disinfections. Previous study also reported that this pathogen was susceptive to chlorine [21].

In recent decades, *Clostridium* spp. has rapidly reemerged as human and animal pathogens, which is a spore-forming bacterium [22]. The germinated spores are rather resistant to a variety of lethal factors such as chemicals, high hydrostatic pressure and disinfections [23]. The present results indicated that *Clostridium* was the most abundant genus in the ozone treated and effluent samples (Fig. 5). But we were not sure if these sequences were *Clostridium* of their spores due to the disinfection. After distribution, however, the aerobic environment resulted in a significant decrease of *Clostridium*spp [24]. The majority of *Mycobacterium* species are opportunistic pathogens that cause a variety of infections in humans [25]. The slow growth property indicates that *Mycobacterium* is less sensitive to conditions and agents (e.g., chlorine) [26]. The hydrophobic membrane of *Mycobacterium*, which reduces the transportation rate of hydrophilic compounds, affords resistance to antimicrobial agents [27]. In addition, *Mycobacterium* also has ability to utilize several kinds of hydrocarbons, such as chlorinated hydrocarbons [28]. Furthermore, an amoeba-associated lifestyle can also increase their resistance to disinfections [29]. *Mycobacteria* are poor competitors in eutrophic conditions, because they grow very slowly and cannot keep up with the reproduction of other bacteria. Only under dystrophic conditions (e.g., acid disinfectant and oligotrophic conditions), the *Mycobacterium* become excellent competitors and can become the dominant species. Our results also indicated that *Mycobacterium* were rare in the influent samples and after ultrafiltration sample in both Q and W plants (Q1, Q2 and W1). After disinfection treatments (ozone, chlorine or UV), however, the occurrences of *Mycobacterium* increased dramatically (Table S4).

Even in the end point samples (Q5 and W4), these two reclamation systems showed different results. As shown in Fig. 5 and Table S4, the occurrences of *Mycobacterium* increased significantly in Q system, whereas decreased in W system. This can be explained by the different storage periods of reclaimed water. The effluent of plant Q was immediate used for replenishment of a river and storage period was less than 2 h. However, the effluent of plant W was used for municipal miscellaneous with an unstable storage time and the storage periods ranged from several hours to several days. Those results indicated that the disinfection, storage and distribution system enriched *Mycobacterium* in reclaimed waters.

Shigella and *Escherichia coli* are agents of bacillary dysentery, a disease that remains a scourge of impoverished communities with little access to clean water [30]. Over the years, researchers have also shown that the percentages of antibiotic-resistant *Shigella* and *Escherichia coli* in waters may survive and grow when they were treated with low doses of UV light or chlorine [31,32]. It's

also reported that antibiotic-resistant *E. coli* was more resistant to chlorine than the antibiotic-sensitive one. For UV disinfection, however, the antibiotic-resistant *E. coli* showed unobvious resistance [32]. In our study, the results were consistent with these previous studies. The sequences and percentage of *Escherichia* and *Shigella* (Fig. 5 and Table S4) were increased after disinfections (ozone and chlorine). Those results indicated that *Escherichia* and *Shigella* were resistant to chlorine and can regrow during the storage and distribution processes. However, the UV treatment showed unobvious effect.

In summary, disinfections influence the microbial communities significantly. And they act as an allogenic factor to shape communities and to select a certain communities. Human pathogens, including *Clostridium*, *Escherichia, Shigella*, *Pseudomonas* and *Mycobacterium*, were selected and enriched during disinfection and distribution processes, implying that more attentions should be paid to the removal of disinfection-resistant pathogens.

3.4 Correlations of environmental factors and microbial communities during storage and distribution of reclaimed water

Canonical correspondence analysis (CCA) was employed to evaluate the correlations between environmental parameters and microbial communities structures (Fig. 6). Based on variance inflation factors (VIF) within 1000 Monte-Carlo permutations, eight significant environmental parameters, including chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), nitrate (NO_3-N), ammonia nitrogen (NH_3-N), total phosphorus (TP), pH, and total chlorine (Cl), were evaluated in the CCA biplot. The length of the arrows in the ordination plot shows the significance of the relationship of that environmental parameter to the compositions of microbial communities.

As shown in Fig. 6, it's observed that total chlorine (Cl), TOC, TN, NO_3-N and NH_3-N, significantly impacted the bacterial communities during reclaimed water storage and distribution systems (Fig. 6). These results were consistent with the results of Williams et al. [33], who also declared that chlorine was one of the most import factors that impacted the structures of bacterial communities in water. Comparable results have also been reported in a model laboratory drinking water distribution system (DWDS) [34]. Nutrients, including TN, NH_3-N, NO_3-N and TOC, also play vital roles in the stability and regrowth of bacteria in reclaimed water. Previous studies have also found that organic matter was an important factor for bacterial growth and stability [5]. However, the effect of TP on variance of microbial community seemed weakly (Fig. 6), which might be explained that reclaimed water typically contains sufficient phosphorus for microbial growth [35].

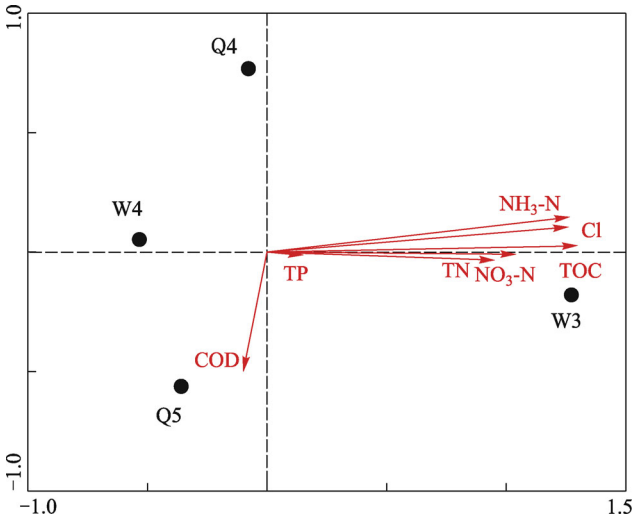

Fig. 6 Canonical correspondence analysis (CCA) of samples based on microbial communities in reclaimed water samples. Those samples include effluents (Q4 and W3) of reclaimed treatment plants and samples (Q5 and W4) collected at the end of distribution pipes from Q and W systems. The arrow shows the magnitude and direction of the parameters related with the structures of bacterial communities. Each solid-circle indicated a structure of bacterial community from a reclaimed water sample

3.5 Effects of reclamation systems on the diversities and abundances of pathogenic communities

Variances in microbial and pathogenic communities in these two different reclaimed water treatments and distribution systems (Q and W) were observed (Figs. 3–5). The compositions of microbial communities in these two systems were shown in Figs. 3 and 4. The combined results further revealed that prolonged disinfection acted as a selective pressure that altered the microbial community compositions in reclaimed water system. The abundances of pathogenic populations were shown to be disinfection-dependent. The results of present studies showed that *Aquabacterium*, *Hydrogenophaga* (*Betaproteobacteria*), *Arcobacter* (*Epsilonproteobacteria*), and *Bacteroides* (*Bacteroidetes*) were dominant in reclaimed water samples before disinfection, suggesting that those bacteria were sensitive to disinfection treatments [16]. In contrast, the percentages of *Acidovorax*, *Limnobacter* (*Betaproteobacteria*), *Clostridium* (*Firmicutes*), *Mycobacterium*, *Nocardioides* (*Actinobacteria*), *Brevundimonas* (*Alphaproteobacteria*), and *Escherichia*, *Shigella*, *Pseudomonas* (*Gammaproteobacteria*) were increased after disinfection, which agreed closely with previous findings [36,37]. These results showed that a variety of pathogenic bacteria could be selected and enriched by disinfection processes during wastewater reclamation. And the presence of those pathogenic bacteria would reduce the disinfection efficiencies. After distribution, however, the percentages of

pathogens decreased, however, they still pose microbial health risks during uses of reclaimed water.

4 Conclusions

The present results indicated that microbial and pathogenic communities varied along the wastewater reclamation and distribution processes. *Proteobacteria* dominated the microbial communities in the reclaimed water samples. And a number of pathogenic bacteria were also selected and enriched by disinfection processes. The total chlorine and nutrients (TOC, NH_3-N and NO_3-N) significantly impacted the bacterial and pathogenic communities during storage and distributions. Through the comparisons of the two different reclamation systems of nine water samples, disinfection, storage and distribution system had effects on the structures of microbial and pathogenic communities.

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