Microbial diversity of water after ultraviolet disinfection in a rural water supply project with groundwater as a source

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ABSTRACT

Microorganism was an important part of water quality safety, which was related to the health of residents. To determine the microbial community structure of rural water supply projects using ultraviolet (UV) disinfection, the structures of the microbial communities were illustrated through 16S rRNA sequencing using the Illumina MiSeq platform. The results showed that, when the abundance of genus level was more than 1%, there were 52 genera. UV disinfection can reduce the biodiversity, the number of species and the abundance of species, but the effect was not so good, and microorganisms further breed in the pipe network. The order of biodiversity was Fe^{2+} and Mn^{2+} exceeding standard project > Fe^{2+} and Mn^{2+} containing project > Fe^{2+} and Mn^{2+} free project. The similarity of microbial population between the source water, the produces factory water and the terminal tap water after UV disinfection was low. The influence order of environmental factors on water samples was COD > WT > pH > DO > TN > Mn^{2+} > Fe^{2+}. After UV disinfection, the microbial community of produces factory water and the terminal tap water samples were positively correlated with WT, pH, Fe^{2+} and Mn^{2+}.

Keywords: Microbial diversity; UV disinfection; 16S rRNA genes; Redundancy analysis (RDA); Groundwater source; Fe^{2+}; Mn^{2+}

1. Introduction

Disinfection was an important part of the water treatment process. It can effectively prevent the spread of pathogenic microorganisms in water [1], prevent and control the occurrence of infection, and reduce the harm to the human body [2]. In WHO guidelines for drinking water quality [3], it ranked the importance of microbiological problems and disinfection in the top two. In rural areas of Heilongjiang Province, there were 19,020 water supply projects with groundwater as the water source, covering a population of 12.83 million, of which more than 90% of the projects are disinfected by UV, due to the large quantity of water supply projects, the small scale of water supply and low level of operation and management, UV disinfection was the main disinfection method. Therefore, it was very necessary to clarify the microbial community structure of water supply project, improve the evaluation method of UV disinfection effect, and optimize the disinfection process.

A large number of studies have been carried out on the evaluation of UV disinfection effect of water supply projects. The WHO drinking water quality guidelines put forward 28 items of drinking water microbial safety evaluation criteria. China had made mandatory provisions on the evaluation indexes and limits of drinking water disinfection in Standards for drinking water quality (GB5749-2006) [4].

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At present, the evaluation indexes of UV disinfection efficacy were mainly concentrated on 4 indicators, including total coliform, heat resistant coliform, *Escherichia coli* and colony count [5]. The water supply project with groundwater as the source lacks the bio-safety study of UV disinfection effluent. The phenomenon of Fe^{2+} and Mn^{2+} exceeding the standard was common in underground water sources in Heilongjiang Province [6]. Therefore, combined with the water quality situation in Heilongjiang Province, it was very important to study the bio-safety characteristics of UV technology in drinking water disinfection process. The Fe^{2+} and Mn^{2+} free project, Fe^{2+} and Mn^{2+} containing project and Fe^{2+} and Mn^{2+} exceeding standard project were taken as the research objects. The physical and chemical indexes of water quality were tested. High throughput sequencing technology was used to analyze microbial community structure, biodiversity, sample correlation and the relationship between microbial community and environmental factors.

In this study, our aim was (1) to analyze the microbial community structure, biodiversity and sample correlation of rural water supply projects with groundwater as water source and UV disinfection, so as to provide data support for the evaluation of UV disinfection effect. (2) To explore the relationship between biological community and water quality factors, so as to provide technical guidance for the optimal design of UV disinfection process and the operation management of disinfection equipment.

2. Materials and methods

2.1. Material

The water samples were collected from the rural water supply project in Heilongjiang Province, Northern China. Water samples D1, D2 and D3 were from rural water supply project of Houshan Village, Xiangyangchuan Town, Fujin County, Jiamusi City. D1 was the source water, D2 was the produces factory water after UV disinfection, and D3 was the terminal tap water. Water samples D4, D5 and D6 were from rural water supply project of Tuanzishan Village, Mengjiaxiang Town, Hulan District, Harbin City. D4 was the source water, D5 was the produces factory water after UV disinfection, and D6 was the terminal tap water. D7, D8 and D9 were from rural water supply project of Jubao Village, Xingfu Town, Beilin District, Suihua City. D7 was the source water, D8 was the produces factory water after manganese sand filtration and UV disinfection, and D9 was the terminal tap water.

2.2. Analysis methods

2.2.1. Analysis method of physical and chemical indexes of water quality

A total of nine water samples were collected at the rural water supply project using an automatic sampler (W2BC-9600) in July 2021, and the sampling interval was 24 h. Three replicates were taken at each sampling point, and 10 L groundwater was taken in each. And the physiochemical properties were tested.

For groundwater detection, the water temperature was measured by mercury thermometer, chemical oxygen demand (COD) was measured by a COD detector (5B-1, Lianhua Com., China), total nitrogen (TN) and total phosphorus (TP) were determined by total phosphorus and total nitrogen analyzer (HACH NPW-160H, Hach, American), Fe^{2+} and Mn^{2+} were determined by atomic absorption spectrophotometer (A3AFG, Beijing General Instruments Co., Ltd., China).

2.2.2. Analysis method of microbial community structure

Water DNA was extracted from 10 L fresh water samples using the E.Z.N.A. Soil DNA Kit (Omega, USA), following the manufacturer's instructions, and the purity and quantity of the DNA were determined using a Qubit 2.0 (life, USA). The amplicon PCR forward primer (CCTACGGGNGGCWGCAG) and amplicon PCR reverse primer (GACTACHVGGGTATCTAATCC) was used to amplify the V3-V4 hyper variable region of the16S rRNA genes in bacteria [7]. Illumina MiSeq pe300 (Illumina, USA) was the sequencing platform, and the main sequencing work was carried out in Shanghai Meiji Biological Co., Ltd., China [8,9].

2.2.3. Data analysis

The biological Alfa diversity of Shannon, Chao1, ACE and Simpson were analyzed by MOTHUR software [10], and the Venn diagram and sample correlation thermal map were also plotted by MOTHUR software. The community structure at the genus level was plotted by Circos software and R software respectively. The thermal map of the distance between samples was draw by gplots of R. The relationship between bacterial community and environmental factors was analyzed by Redundancy Analysis using CANOCO 4.5 software.

3. Results

3.1. Analysis of water quality characteristics

The groundwater quality data obtained from the nine sampling sites was statistically analyzed, and the results are shown in Table 1.

Table 1 shows that the water samples D1, D2, D3, D4, D5, D6, D8 and D9 met the limit requirements of the standard for drinking water quality GB5749-2006. D1, D2 and D3 came from the same water supply project. The source water D1 didn't contain Fe^{2+} and Mn^{2+}. After UV disinfection, the produces factory water D2 and the terminal tap water D3 were qualified. D4, D5 and D6 came from the same water supply project. The source water D4 contains Fe^{2+} and Mn^{2+} but does not exceed the standard. After UV disinfection, the quality of the produces factory water D5 and the terminal tap water D6 were qualified. D7, D8 and D9 came from the same water supply project. The concentration of Fe^{2+} and Mn^{2+} in the source water D7 was 1.45 and 0.64 mg/L, respectively, which exceeded the limits of 0.3 and 0.1 mg/L, and other indicators met the requirements of the standard. After manganese sand filtration and UV disinfection, in the produces factory water D8 and the terminal tap water D9, Fe^{2+} and Mn^{2+} were 0.13 and 0.12 mg/L, respectively, which met the requirements of the standard.

Table 1
Water quality characteristics of rural water supply project

No.	T (°C)	pH	DO (mg/L)	COD (mg/L)	TN (mg/L)	TP (mg/L)	Fe^{2+} (mg/L)	Mn^{2+} (mg/L)
D1	5.1	6.68	7.9	1.69	1.7	L	L	L
D2	5.3	6.67	7.8	1.71	1.7	L	L	L
D3	5.3	6.69	8.1	1.7	1.7	L	L	L
D4	5.2	6.58	7.8	1.45	2.2	L	0.23	0.07
D5	5.3	6.57	7.7	1.47	2.3	L	0.21	0.07
D6	5.4	6.59	7.8	1.48	2.3	L	0.22	0.07
D7	5.1	7.73	7.8	1.47	2.2	L	1.45	0.64
D8	5.9	7.39	7.8	1.02	0.4	L	0.13	0.12
D9	6.9	7.48	8.1	1.01	0.4	L	0.13	0.12

Note: L means the detection result was lower than the detection limit.

3.2. Analysis of Alpha diversity

According to OTU cluster analysis results, OTU microbial diversity index (Shannon, Chao1, ACE and Simpson index) was analyzed. The results showed that UV disinfection can reduce the biodiversity of the produces factory water. The order of biodiversity was Fe^{2+} and Mn^{2+} exceeding standard project > Fe^{2+} and Mn^{2+} containing project > Fe^{2+} and Mn^{2+} free project. Biodiversity has increased in terminal tap water. The four indicators of D1 were 3.00, 607.6, 618.38 and 0.49 respectively. D2 were 4.46, 721.21, 727.91 and 0.69. D3 were 3.61, 786.99, 765.88 and 0.56. D4 were 4.48, 765.62, 759.72 and 0.68. D5 were 3.19, 628.01, 644.62 and 0.51. D6 were 4.04, 754.32, 730.65 and 0.62. D7 were 4.18, 813.27, 787.45 and 0.64. D8 were 4.46, 829.25, 833.57 and 0.68. D9 were 4.38, 855.5, 855.09 and 0.06. Based on Shannon information, the order of microbial diversity was D4 > D8 = D2 > D9 > D7 > D6 > D3 > D5 > D1. Based on Chao1 and Ace information, the order of microbial diversity was D9 > D8 > D7 > D3 > D4 > D6 > D2 > D5 > D1. Based on Simpson information, the order of microbial diversity was D9 > D1 > D5 > D3 > D6 > D7 > D2 > D8 = D4. Fe^{2+} and Mn^{2+} in water were the basic elements of microbial life process, which have a great impact on microbial community structure [11,12]. In the same water supply project, the biodiversity of terminal tap water was higher than that of produces factory water and source water. This result was similar to other previous study [13,14].

3.3. Analysis of Venn

Venn diagram was an effective method to represent the number of unique and common OTUs in multiple samples [15]. The number of overlapping parts represents the number of OTU shared by multiple groups. The number of non-overlapping parts represents the number of OTU specific to the corresponding packets, and the results are shown in Fig. 1.

Fig. 1 shows that UV disinfection can reduce the number of species, but the effect was not so good, and microorganisms further breed in the pipe network. In Fe^{2+} and Mn^{2+} free project, the total OTU number was 824 and the intersection OTU number was 383, accounting for 46.5% of the total OTU number. D1, D2, D3 OTU number was 487, 662,

1,328 separately, after UV disinfection, the number of OTU did not decrease, and the number of OTU in water samples at the terminal tap water increased. In Fe^{2+} and Mn^{2+} containing project, the total OTU number was 1,049, and the intersection OTU number was 299, accounting for 28.5% of the total OTU number. D4, D5, D6 OTU number was 720, 509, 660 separately, after UV disinfection, the number of OTU number decreased, and the OTU number of the terminal tap water sample increased. In Fe^{2+} and Mn^{2+} exceeding standard project, the total OTU number was 930, and the intersection OTU number was 488, accounting for 52.5% of the total OTU number. D7, D8, D9 OTU number was 670, 723, 746 separately, after UV disinfection the produces factory water and terminal tap water samples all increased. The biodiversity of water samples containing Fe^{2+} and Mn^{2+} was significantly higher than that of water samples without Fe^{2+} and Mn^{2+}. The number of OTU number shared by 9 water samples was 196.

3.4. Microbial community composition

The distribution of microbial communities with abundance greater than 1% is shown in Fig. 2.

Fig. 2 shows that when the abundance of genus level was more than 1%, there were 52 genera. UV disinfection can't reduce the abundance of species very well, and microorganisms further breed in the pipe network. In the actual water supply project, the total coliform bacteria were didn't detected in the three water supply projects. Although the effluent and the terminal water after UV disinfection in line with Standards for drinking water quality (GB5749-2006), the UV disinfection effect was not good. The genus diversity of water source was positively correlated with the concentration of Fe^{2+} and Mn^{2+}. The order of water source species was D7 with Fe^{2+} and Mn^{2+} exceeding the standard (17) > D4 with Fe^{2+} and Mn^{2+} but not exceeding the standard (15) > D1 without Fe^{2+} and Mn^{2+} (12).

In the water supply project without Fe^{2+} and Mn^{2+}, after UV disinfection in the produces factory water sample the abundance of *Sphingobium*, *Hydrogenophaga*, *Bacillariophyta*, *Chlorophyta* and *Thiothrix* decreased, the abundance of *Massilia*, *unclassified_Hyphomicrobiaceae*, *Bradyrhizobium*, *unclassified_Alphaproteobacteria*, *unclassified_Comamonadaceae*, *unclassified_Betaproteobacteria*, *unclassified_Bacteria*,
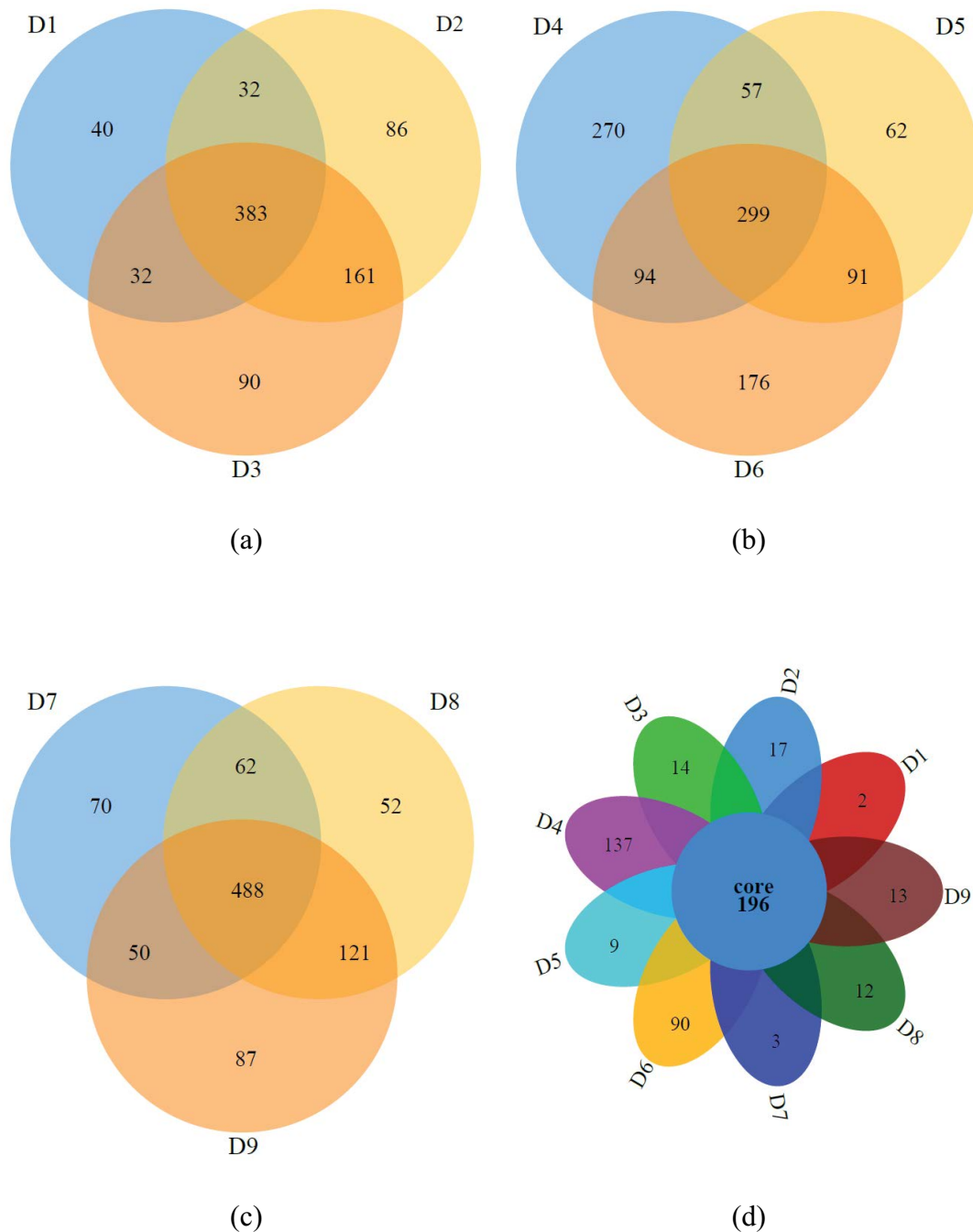
Fig. 1. Venn diagram of water samples distribution. (a) Distribution of qualified water samples, (b) distribution of water samples containing Fe^{2+} and Mn^{2+}, (c) distribution of water samples with excessive Fe^{2+} and Mn^{2+}, and (d) distribution of all water samples.

Brevundimonas, Clostridium_sensu_stricto, Nitrospira, Staphylococcus, Sediminibacterium increased. In the terminal tap water sample the abundance of *Sphingobium, Bacillariophyta, Thiothrix, unclassified_Hyphomicrobiaceae, unclassified_Bacteria, Nitrospira* increased. In the water supply project with Fe^{2+} and Mn^{2+} but not exceeding the standard, after UV disinfection in the produces factory water sample the abundance of *Sphingobium, Hydrogenophaga, Chlorophyta, Novosphingobium, Sphingomonas* decreased, the abundance of *Bacillariophyta, Pseudomonas, Massilia*

increased. In the terminal tap water sample the abundance of *Sphingobium, Hydrogenophaga, Bacillariophyta, Pseudomonas, Massilia, Chlorophyta, Novosphingobium, Sphingomonas, Haliscomenobacter, unclassified_Hyphomicrobiaceae, Bradyrhizobium* increased. In the water supply project without Fe^{2+} and Mn^{2+}, after manganese sand filtration and UV disinfection, in the produces factory water sample the abundance of *Sphingomonas, unclassified_Sphingomonadaceae, Rhodoferax, unclassified_Rhodocyclaceae, Sediminibacterium* decreased. The abundance of *Novosphingobium, unclassified_Rhizobiales,*
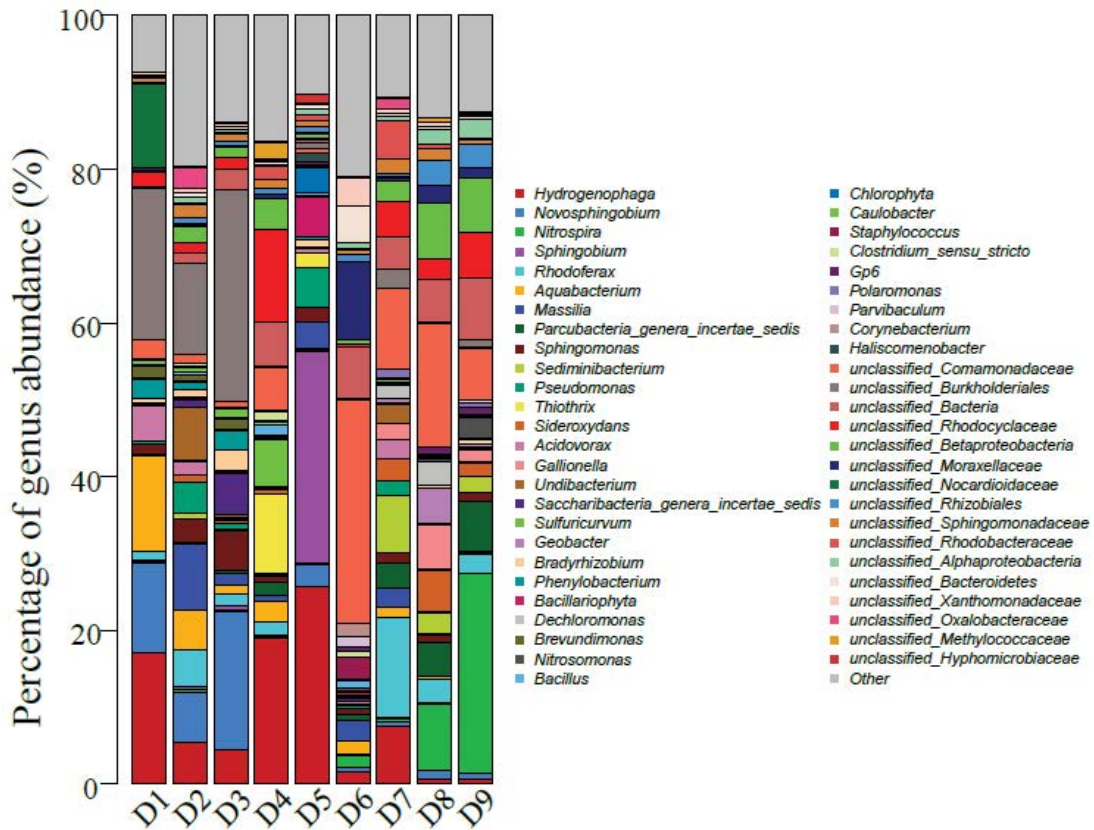
Fig. 2. Microbial community structure at genus level (abundance greater than 1%).

unclassified_Alphaproteobacteria, unclassified_Comamonadaceae, unclassified_Betaproteobacteria, unclassified_Bacteria, unclassified_Moraxellaceae, Nitrospira, Geobacter, Sideroxydans, Parcubacteria_genera_incertae_sedis, Gallionella, Dechloromonas increased. In the terminal tap water sample the abundance of unclassified_Rhizobiales, unclassified_Alphaproteobacteria, unclassified_Bacteria, Nitrospira, Parcubacteria_genera_incertae_sedis increased.

UV disinfection could effectively reduce the abundance of potential pathogenic bacteria Hydrogenophaga and unclassified_rhodocyclaceae, Geobacter and dechloromonas. However, potential pathogenic microorganisms such as unclassified_Rhizobiales, Parcubacteria_genera_incertae_sedis and Gallionella further breed in the pipe network. The results showed that, although UV disinfection could cause some damage to some organisms, some microorganisms could repair themselves under appropriate conditions [16,17], so as to restore their activity and lead to the deterioration of water quality.

3.5. Correlation analysis of water samples

The color blocks represent the distance between samples. The darker the color, the closer the distance between samples was. The sample correlation heat map is shown in Fig. 3.

Fig. 3 shows that at genus level, the similarity of microbial population between the source water, the produces factory water and the terminal tap water after UV disinfection
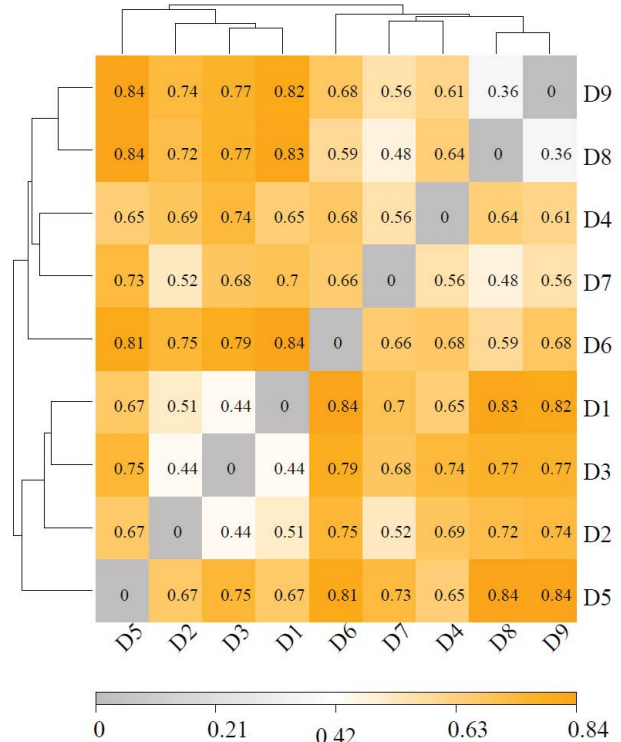

Fig. 3. Water sample correlation thermogram.

was low. The order of distance between water sample D1 and other samples was D6 (0.84) > D8 (0.83) > D9 (0.82) > D7 (0.70) > D5 (0.67) > D4 (0.65) > D2 (0.51) > D3 (0.44), the order of distance between water sample D2 and other samples was D6 (0.75) > D9 (0.74) > D8 (0.72) > D4 (0.69) > D5(0.67) > D7 (0.57) > D1 (0.51) > D3 (0.44), the order of distance between water sample D3 and other samples was D6 (0.79) > D8 (0.77) = D9 (0.77) > D5 (0.75) > D4 (0.74) > D7 (0.68) > D2 (0.44) = D1 (0.44), the order of distance between water sample D4 and other samples was D3 (0.74) > D2 (0.69) > D6 (0.68) > D5 (0.65) = D1 (0.65) > D8 (0.64) > D6 (0.61) > D7 (0.56), the order of distance between water sample D5 and other samples was D8 (0.84) = D9 (0.84) > D6 (0.81) > D3 (0.75) > D7(0.73) > D2 (0.67) > D1 (0.67) > D4 (0.65), the order of distance between water sample D6 and other samples was D1 (0.84) > D5 (0.81) > D3 (0.79) > D2 (0.75) > D4 (0.68) > D9 (0.66) > D7 (0.66) > D8 (0.59), the order of distance between water sample D7 and other samples was D5 (0.73) > D1 (0.77) > D3 (0.68) > D6 (0.66) > D4 (0.56) = D9 (0.56) > D2 (0.52) > D8 (0.48), the order of distance between water sample D8 and other samples was D5 (0.84) > D1 (0.83) > D3 (0.77) > D2 (0.72) > D4 (0.64) > D6 (0.59) > D7 (0.48) > D9 (0.36), the order of distance between water sample D9 and other samples was D5 (0.84) > D1 (0.82) > D3 (0.77) > D2 (0.74) > D6 (0.68) > D4 (0.61) > D7 (0.56) > D8 (0.36). The low similarity among water samples may be due to the different operation effects of UV disinfection equipment [18], and the groundwater was in a low-temperature and poor nutrition, which was not conducive to the growth and reproduction of microorganisms [19,20].

3.6. Correlation analysis of microorganism and environmental factors

At the level of 54.40% of the total contribution rate of x-axis and y-axis, the relationship between microbial community and water quality factors is shown in Fig. 4.

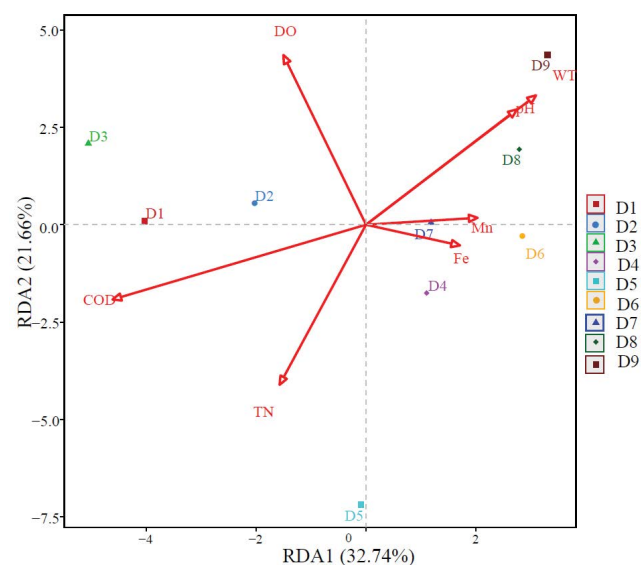Fig. 4 shows that influence order of environmental factors on water samples was COD > WT > pH > DO > TN >

Fig. 4. Redundancy Analysis at the generic level.

Mn^{2+} > Fe^{2+}. COD has the highest effect on water samples, it may be that the trace of biodegradable organic matter in water causes the regeneration of bacteria in the water supply network [21]. It leads to the change of drinking water quality and secondary pollution of pipe network. After UV disinfection, the produces factory water and the terminal tap water have high correlation with WT, pH, Fe^{2+} and Mn^{2+}, Among the 52 bacterial genera detected, 36 are related to Fe^{2+} and Mn^{2+} metabolism, it may be that Fe^{2+} and Mn^{2+} participate in the metabolism of iron and manganese oxidizing bacteria, thus maintaining the stability of microbial system [22]. D1, D2 and D3 were positively correlated with COD. D1, D2, D3 and D9 were positively correlated with Do. D1, D2, D3, D4 and D5 were positively correlated with TN. D4, D6, D7, D8 and D9 were positively correlated with pH, WT and Mn^{2+}. D4, D5, D6, D7, D8 and D9 were positively correlated with Fe^{2+}. UV disinfection has no residual disinfection effect, and can't guarantee the biological stability of water supply network. Many scholars have carried out a lot of research on this problem, pointing out that the combined use of several disinfection technologies can expand the coverage of microbial control [23,24], reduce the use of chemical agents, thereby reducing the generation of disinfection by-products and maximizing the safety of water supply.

4. Conclusions

Microbiological safety was the basis for ensuring the safety of drinking water, and also the basis for early warning in water treatment processes. The Fe^{2+} and Mn^{2+} free project, Fe^{2+} and Mn^{2+} containing project and Fe^{2+} and Mn^{2+} exceeding standard project were taken as the research objects. The microbial diversity of water after UV disinfection in rural water supply project with groundwater as water source were analyzed. The results showed that, when the abundance of genus level was more than 1%, there were 52 genera. UV disinfection could reduce the biodiversity, the number of species and the abundance of species, but the effect was not so good, and microorganisms further breed in the pipe network. The order of biodiversity was Fe^{2+} and Mn^{2+} exceeding standard project > Fe^{2+} and Mn^{2+} containing project > Fe^{2+} and Mn^{2+} free project. The similarity of microbial population between the source water, the produces factory water and the terminal tap water after UV disinfection was low. The influence order of environmental factors on water samples was COD > WT > pH > DO > TN > Mn^{2+} > Fe^{2+}. After UV disinfection, the microbial community of produces factory water and the terminal tap water samples were positively correlated with WT, pH, Fe^{2+} and Mn^{2+}.

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