Microbial characterizations of water using tea polyphenols as a disinfectant for effluent treatment after the ultrafiltration process

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ABSTRACT

The microbial characteristics of water treated by tea polyphenols after the ultrafiltration process were studied based on the continuous disinfection performance of tea polyphenols. Metagenomics was carried out to investigate the microbial community distribution, functional characteristics, and variations after disinfection, to reveal the disinfection principle of tea polyphenols, and to provide the theoretical basis for using tea polyphenols as a disinfectant to better control the growth of microorganisms. The analysis of microbial diversity and community structure showed that the microbial diversity in water was significantly reduced after disinfection with tea polyphenols. The results of hierarchical clustering analysis showed that microbial metabolism, environmental information processing, and functional modules in genetic information processing were significantly inhibited after disinfection treatment using tea polyphenols. The tea polyphenols inhibit the growth and reproduction of microorganisms by selection on the microorganisms in the water, thus achieving the effect of bacterial inhibition.

Key words | metagenomics, microbial community, microbial function, tea polyphenols, ultrafiltration

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HIGHLIGHTS

- Tea polyphenols as disinfectants inhibit the regrowth of bacteria.
- Tea polyphenols disinfection significantly reduces the diversity of microorganisms in water.
- The gene function of many microorganisms in water is greatly changed after disinfection by tea polyphenols.
- Tea polyphenols disinfection exerts a greater inhibitory effect on the metabolism of various nucleotides and related processes of microorganisms.

INTRODUCTION

Some bacteria and viruses in natural water endanger human health. In the water supply system, disinfection of drinking

water is the last and most critical barrier to ensure the bio-security of drinking water; therefore, the safety of disinfection has received great attention both in China and abroad. With the innovation of purification processes in water plants, the ultrafiltration process is more and more widely used.

Tea polyphenols are a kind of high content and more kinds of polyphenols in tea (Dong *et al.* 2012). Numerous

studies have shown that tea polyphenols have good antibacterial effects and have certain inhibitions on almost all pathogenic microorganisms in nature, including Gram-negative and -positive bacteria, fungi, viruses, and the toxins they secrete. Antibacterial experiments have shown that tea polyphenols and their monomers have certain killing and inhibiting effects on oral pathogenic bacteria, food pathogenic bacteria, and a variety of skin pathogenic fungi, influenza viruses, and rotavirus in the human body, while inhibiting the growth of harmful intestinal bacteria and promoting the growth of beneficial intestinal bacteria. Many of the biological activities of tea polyphenols are reflected by its main ingredient, catechin, accounting for 65–80% of the total amount of tea polyphenols. Catechin has a phenol group or *o*-phenol group in its structure. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) are the two main types of tea polyphenols, with 36 and 24%, respectively (Huang *et al.* 2017), and are the main components that make tea polyphenols have antibacterial effects. The structures of EGCG and ECG are shown in Figure 1. As a new green and healthy disinfection method, tea polyphenols disinfection has become an important

Figure 1 │ Chemical structures of catechins.

means of drinking water disinfection due to its excellent continuous sterilization ability (Feng *et al.* 2016). The study on the disinfection effect of tea polyphenols by Feng *et al.* (2016) shows that tea polyphenols can be used as an auxiliary disinfectant in combination with ozone/ultraviolet/ultrafiltration processes to control the growth of microorganisms in the pipeline network and to avoid the generation of chlorine disinfection byproducts.

At present, initial progress has been made in the study of tea polyphenols for drinking water disinfection. However, the large-scale application of tea polyphenols still needs to solve a series of problems, including tea polyphenol extraction process, disinfection equipment, the normalization and standardization of tea polyphenol disinfectant production and application, and systematic economic evaluation, etc., which requires comprehensive research of relevant multidisciplinary. One of the possible applications of tea polyphenols is as an auxiliary disinfectant for ultrafiltration systems as technology develops and human beings demand a better quality of life. The microbial concentration in ultrafiltration-treated water is low, and the main function of adding the disinfectant after the ultrafiltration process is no longer to kill microorganisms, but to inhibit their growth, especially pathogenic microorganisms.

During the process of inhibiting microbial growth, the structure of microbial communities in water may undergo complex changes.

Metagenomics is the study of the genomes of all microorganisms in the environment. Therefore, this technology is used to investigate the variations in the community structure of microorganisms in water for better control of microbial growth (Chao *et al.* 2013). At the same time, the gene sequences can be further sequenced and translated into proteins for comparison with the KEGG database to obtain information on microbial genomes, chemical substances, and system functions. Then, according to the hierarchical cluster analysis, the variations of microbial functional unit modules and metabolic resistance mechanism can be explored from the perspective of macrobiological pathway information analysis.

Tea polyphenols were used as disinfectants to treat the effluent after the ultrafiltration process. The microbial characteristics of tea polyphenols after disinfection will be studied by metagenomics analysis, which will provide an
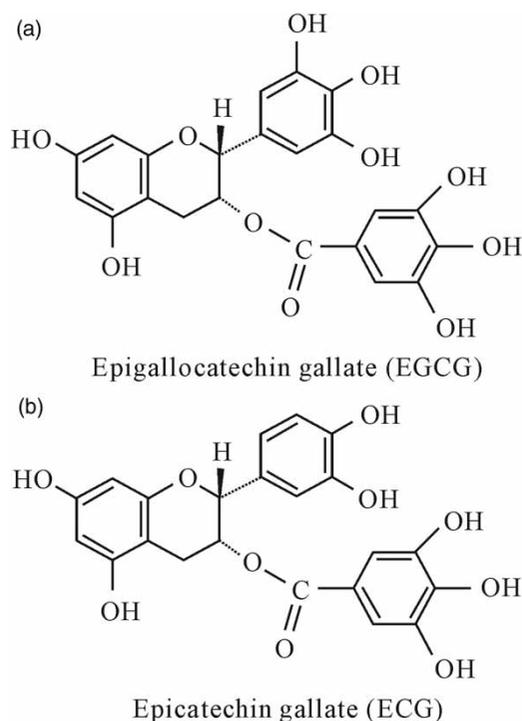
important theoretical basis for the promotion and application of green disinfectants and improve the microbial safety of drinking water.

MATERIALS AND METHODS

Materials and equipment

1. *Tea polyphenols*: Green tea extract, purity > AR98.0%, Anhui Red Star Pharmaceutical Co., Ltd, China.
2. *Raw water*: Water was taken from the outlet pipe of V-type filter in a typical water purification plant and filtered through a 0.01 μm (100 KDa) ultrafiltration membrane at a pressure of 0.10 MPa. The temperature of water was 11–20 °C, and the water quality characteristics are shown in Table 1.
3. Reagents and equipments for the determination of tea polyphenols.

   Analytical reagents: Potassium sodium tartrate, ferrous sulfate, disodium hydrogen phosphate, and potassium dihydrogen phosphate were purchased from Sinopharm Group Chemical Reagent Co., Ltd, China.

   Equipments: BSA124S-CW analytical balance (Sartorius, Germany); DR6000 ultraviolet spectrophotometer (HACH Instrument Co., Ltd, USA).

4. Reagents and equipments for the determination of total bacterial colonies.

   Reagents: Nutrient agar medium, sodium hydroxide, and hydrochloric acid were purchased from Sinopharm Group Chemical Reagents Co., Ltd, China.

   Equipments: Electric Furnace (Shanghai Chenlin Electric Furnace Co., Ltd, China); MLS-3781 L-PC Autoclave (Sanyo, Japan); TY10BCN-1360 Biological Clean Bench (Beijing Donglianhar Instrument Manufacturing Co., Ltd, China); SPX-250B-Z Biochemical Incubator (Shanghai Boxun Medical Biological Instruments Co., Ltd, China).

Dosage of tea polyphenols

Tea polyphenols were added to the raw water, and tea polyphenol solutions of different gradient concentrations were prepared from 0 to 10.0 mg/L in a unit of 2.5 mg/L. In accordance with *Standards for Drinking Water Quality* (GB 5749-2006) and *Standard Examination Methods for Drinking Water–Microbiological Parameters* (GB/T 5750.12-2006). Samples were taken in units of 6 h for different time gradients from 0 to 48 h of treatment, respectively, and the total number of bacteria was determined using the heterotrophic plate count (HPC) method. The test temperature was 10 °C, pH was 7.5–8.0, and DO was 8.26–9.41 mg/L.

Table 1 | Raw water quality

Parameter	Values	Instruments	Methods
Chroma (degree)	1–2	PFXi-995 High Precision Automatic Colorimeter	
Turbidity (NTU)	0.1	HACH-2100AN Turbidity Meter	
UV_{254} (1/cm)	0.011–0.015	DR6000 (HACH Instrument Corporation)	Direct reading
TOC (mg/L)	0.82–0.98	Shimadzu TOC-L Organic Carbon Analyzer	High temperature Catalytic Combustion
NO_3-N (mg/L)	0.6–0.9	DR6000 UV Spectrophotometers	Ultraviolet spectrophotometry
NH_4^+-N (mg/L)	0.05–0.06	DR6000 UV Spectrophotometers	Salicylic acid method
TP (mg/L)	0.64–0.73	DR6000 UV Spectrophotometers	Digestive-ascorbic acid method
Total number of bacteria (CFU/mL)	0–5		Plate counting
Total coliforms (CFU/100 mL)	0		Multi-tubular fermentation
Escherichia coli (CFU/100 mL)	0		
pH	7.5–7.8	FiveGo – Single Channel Portable pH Meter	

Metagenome sequencing

The samples were named with Raw for raw water and 5TP for 5 mg/L tea polyphenol disinfectant water sample. After cast 5 mg/L tea polyphenol for 48 h, the tea polyphenol disinfection water sample began to rich microorganisms, while raw water is the blank control water sample. About 100 L for each water sample was taken to intercept microorganisms by a 0.2 μm pore size membrane through a filtration device.

Total DNA was extracted using the UltraClean Soil DNA Kit. After passing the test for quality control, the genomic DNA was fragmented, and libraries were constructed and sequenced.

The whole metagenome sequencing was commissioned to Shanghai Sangon Bioengineering Co., Ltd, China.

RESULTS AND DISCUSSION

Determining the amount of tea polyphenols

Different dosages of tea polyphenols were added into ultrafiltration water to determine the total number of bacterial colonies in the water after contacting for different times. The effect on bacterial inhibition treating the effluent after ultrafiltration process using tea polyphenols was investigated and the results are shown in Figure 2.

As can be seen in Figure 2, the main function of tea polyphenols as a disinfectant is to inhibit bacterial regrowth. The addition of 2.5 mg/L tea polyphenols can significantly prolong the delayed growth of bacteria to 18–24 h. After 24 h, the bacteria grow rapidly and the total number of colonies was more than 100 CFU/mL after 36 h, exceeding the limit value mentioned in *Standards for Drinking Water Quality* (GB5749-2006).

The bacterial count was not significantly increased at 5.0, 7.5, and 10.0 mg/L of tea polyphenols, and bacterial regrowth was well inhibited within 0–48 h. The total bacterial count was lower than 10 CFU/mL at 5 mg/L of tea polyphenols. The total number of bacterial colonies in water was less than 10 CFU/mL at a dose of 5 mg/L of tea polyphenols after 48 h, and even lower at 7.5 and 10.0 mg/L. Therefore, tea polyphenols showed good
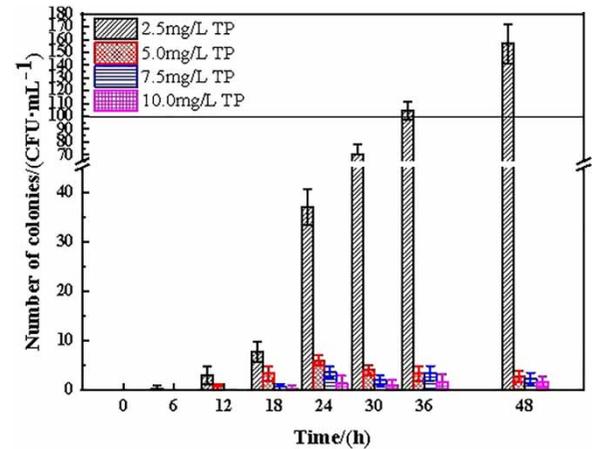

Figure 2 │ Inhibitory effect of tea polyphenols on bacterial growth.

performance of persistent inhibition at dosages of 5.0, 7.5, and 10.0 mg/L, respectively.

In order to further optimize the dosage of the disinfectant, the dosages of tea polyphenols were selected at 5.0, 7.5, and 10.0 mg/L, respectively. Correspondingly, the values of redox potential were measured at 0, 6, 12, 18, 24, 30, 36, 42, and 48 h, respectively. The results are shown in Figure 3.

The redox potential (ORP) is used to reflect the macroscopic redox properties exhibited by all substances in aqueous solutions (Wang 2014). A higher ORP shows stronger oxidation performance; a lower ORP shows stronger reduction performance (Wen 2017). A positive ORP indicates that the solution shows the oxidation ability, while a negative ORP indicates that the solution shows the
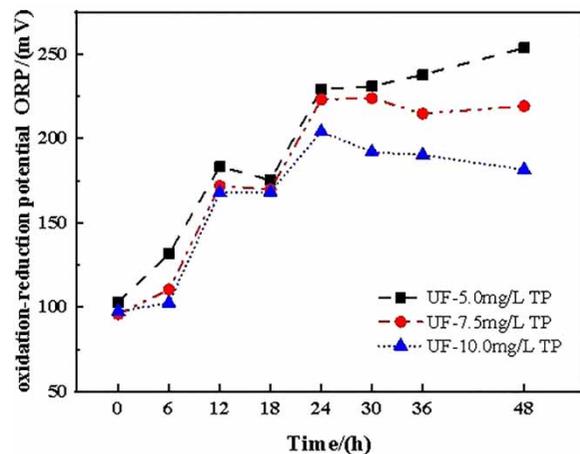

Figure 3 │ Changes of ORP during disinfection of tea polyphenols.

reduction ability (Chen *et al.* 2003). The ORP provides a comprehensive indication of the overall germicidal efficacy and effective oxidation capacity of the disinfectant in drinking water disinfection. As shown in Figure 3, the ORP value increased at all three doses of tea polyphenols from 0 to 24 h. After 24 h, the ORP showed a decreasing trend at doses of 7.5 and 10 mg/L. The redox potential of water sample (5 mg/L tea polyphenols) was always higher than other doses from 0 to 48 h, indicating that its oxidation ability was higher than that at 7.5 and 10.0 mg/L. Due to the presence of several hydroxyl groups, the oxidation ability of tea polyphenols is reduced with the variations of concentrations, which is consistent with the findings of Guo (2019) on the study of ozone–tea polyphenols combined disinfection.

To summarize the results of the above studies, the appropriate dosage of tea polyphenols reaching the highest disinfection efficiency was 5 mg/L. At this dosage of 5 mg/L, the microbiological characteristics of water samples were analyzed by metagenomics sequencing after contacting with tea polyphenols for 48 h.

Characteristics of microbial community structure

Microbial community diversity

Due to the different disinfection mechanisms of disinfectants, the effects on different bacterial populations are different, which affects the microbial diversity and community structure (Lin *et al.* 2013; Zhang & Liu 2019), as well as the safety of drinking water. Investigating the variations on the structure and function of microbial communities in water will not only help to reveal the principles of disinfection, but also contribute to better control of microbial growth (Gomez-Alvarez *et al.* 2012).

The index of community diversity includes Shannon index (Shannon 1948) and Simpson index (Simpson 1949). The Shannon index is used to estimate the level of community diversity, and the Simpson index is used to determine the diversity of species. More species indicate various individuals, and a higher index value shows better diversity.

Microbial community diversity statistics for Raw (raw water samples) and 5TP (tea polyphenols disinfected water samples) are shown in Table 2.

Table 2 | Genetic diversity statistics

Sample	Shannon	Simpson
Raw	11.13	1.0×10^{-4}
5TP	9.87	4.0×10^{-5}

Table 2 shows that both Shannon index and Simpson index of water samples (5TP) after tea polyphenols disinfection are lower than the values of raw water samples, indicating that the microbial diversity was significantly reduced by tea polyphenols disinfections, which is consistent with the results obtained by most disinfection methods such as chlorination, ozone, and UV disinfection (Zhang & Liu 2019).

Microbial community structure analysis

Analysis of metagenomic data shows that bacteria, archaea, viruses, and fungi can be detected in raw water after disinfection with tea polyphenols for 48 h. Most of the sequences were related to bacteria, accounting for more than 99% of the total number of annotated protein sequences; therefore, in-depth analysis on the community structure of bacteria should be conducted.

1. Characteristics of bacterial community distribution at the phylum level

At the phylum level, the dominant groups with more than 1% of relative abundance in Raw water samples were *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, accounting for 86.03, 4.73, and 3.99%, respectively, as shown in Table 3. *Proteobacteria* accounted for the highest proportion in raw water, due to the fact that *Proteobacteria* are more

Table 3 | Percentage of major species at the phylum level (%)

Flora species	Raw	5TP
Proteobacteria	86.03	96.63
Bacteroidetes	4.73	0.05
Actinobacteria	3.99	0.16
Nitrospirae	0.24	0.07
Others	5.01	3.09

Note: Raw stands for raw water; 5TP stands for 5 mg/L polyphenols in disinfected water samples.

adaptable to environmental conditions and are prone to regrowth after the ultrafiltration process. The diversity of '5TP' in tea polyphenols disinfected water samples decreased at the phylum level, and the predominant community was *Proteobacteria*, which increased from 86.03% of the raw water to 96.63% after disinfection. The proportions of *Bacteroidetes*, *Actinobacteria*, and *Nitrospirae* in the tea polyphenols disinfected water samples were significantly reduced, as shown in Table 3.

2. Characteristics of bacterial community distribution at the class level

The relative abundance of bacterial communities in raw water samples (Raw) and tea polyphenols disinfected water samples (5TP) at the class level is shown in Figure 4, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Flavobacteriia*, and *Actinobacteria* with relative abundance greater than 1% of raw water, accounting for 32.35, 28.32, 25.10, 4.54, and 3.99%, respectively. After disinfection with tea polyphenols, the proportion of the dominant group of bacteria increased to 49.74% for *Alphaproteobacteria* and 37.91% for *Betaproteobacteria*, while the proportion of *Gammaproteobacteria* decreased to 8.76%. The relative abundances of *Flavobacterium* and *Actinobacteria* were also reduced to 0.03 and 0.15%, respectively, indicating that tea polyphenols have a significant selective inhibitory effect on *Flavobacterium*, *Actinobacteria*, and *Gammaproteobacteria*.

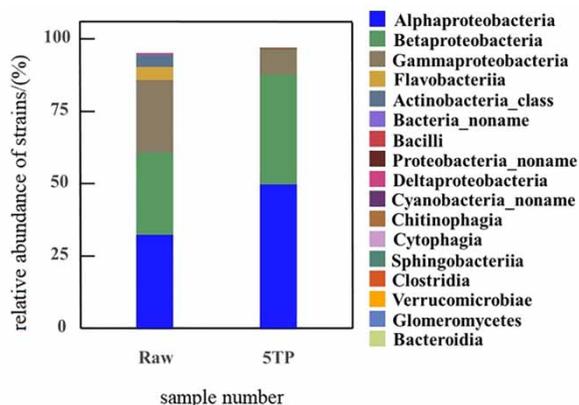3. Characteristics of bacterial community distribution at the genus level

Figure 4 │ Bacterial community structure at the class level. *Note*: The figure shows bacterial communities with relative abundances greater than 0.01%.

Bacteria whose relative abundance at the genus level in raw water samples greater than 1.00% were *Sphingobium* (19.60%), *Acinetobacter* (11.96%), *Pseudomonas* (10.53%), *Janthinobacterium* (5.69%), *Methylophilus* (5.07%), *Flavobacterium* (4.33%), and other 12 genera, which together constitute the basic community structure of raw water at the genus level, as shown in Figure 5.

The percentage of six major groups of bacteria at the genus level reduced significantly in the tea polyphenols disinfected water samples, compared with the raw water. Among them, *Acinetobacter* decreased from 11.96 to 0.06%, with the largest decrease of 11.90%. This genus has been proposed, but it is not confirmed whether organisms spread in drinking water (WHO 2003b, 2014), as listed in the World Health Organization Guidelines for *Drinking Water Quality*. Moreover, it is widespread in soil, water, and wastewater, which usually accounts for 1.0–5.5% of HPC in drinking water samples (WHO 2003a). *Mycobacterium* is resistant to conventional disinfectants, existing in natural aqueous environments, and some atypical *Mycobacterium* species are potentially pathogenic. *Bacillus* is also a potential bacterial pathogen listed in the World Health Organization Guidelines for *Drinking Water Quality*, and some bacteria of this genus are pathogenic to humans and animals (WHO 2003b; Magdolna *et al.* 2017). An important characteristic of *Bacillus* is that it can produce spores with special resistance to adverse conditions and thus to resist disinfection. The relative abundance of *Bacillus* was 0.15% in raw water and only 0.01% after tea polyphenols disinfection, whereas *Bacillus* can be easily detected in most drinking water supply systems by the HPC method (WHO 2003b). This shows that tea polyphenols can effectively control the growth and reproduction of opportunistic pathogens.

The main bacterial communities after tea polyphenols disinfection were *Sphingobium*, *Pseudomonas*, *Janthinobacterium*, *Methylophilus*, *Sphingomonas*, and *Novosphingobium*. The relative abundances were 38.84, 7.80, 18.52, 2.14, 3.42, and 1.59%, respectively. Among them, *Sphingobium* had the largest increase of 19.24%, followed by *Janthinobacterium* with an increase of 12.83%.
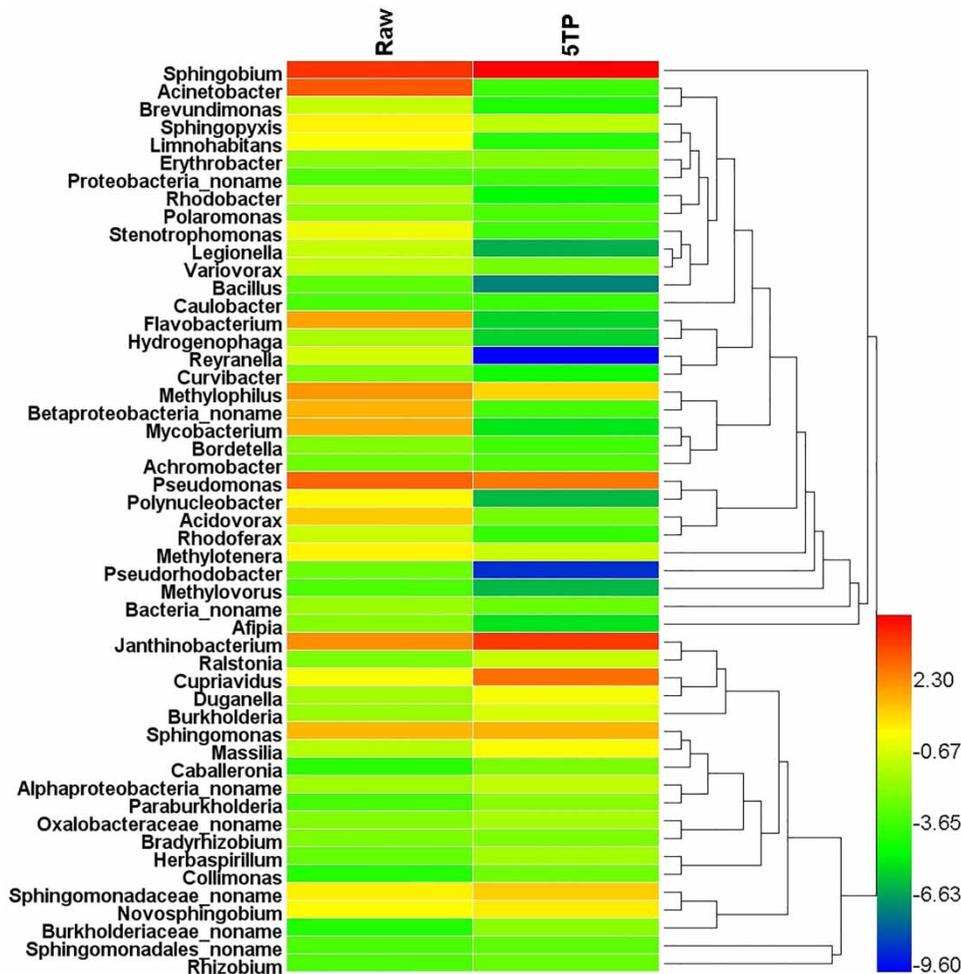
Figure 5 │ Heatmap of relative abundance at the genus level.

Functional clustering analysis of microbial systems

The effects of tea polyphenols on microbial diversity and community structure were investigated, and it was found that tea polyphenols disinfection played an important role in the reduction of microbial diversity and the variation of microbial community structure. The variation of microbial community structure may lead to changes in microbial community function. In this section, the gene sequences were translated into proteins and compared with the KEGG (Kyoto Encyclopedia of Genes and Genomes) database to obtain information on microbial genomes, chemicals, and system functions. Based on hierarchical clustering, the analysis of macrobiological pathway information gradually progressed to the analysis of microbial functional unit

modules and metabolic pathways, and genomics was used to study the genetic composition and community function of microorganisms in water.

The functional clustering of microbial systems in raw water samples (Raw) and tea polyphenols disinfected water samples (5TP) was obtained by annotating sequences of the translated proteins with the KEGG database, as shown in Figure 6.

At level 1, the microbial metabolic clustering of raw water samples (Raw) and tea polyphenols disinfected water samples (5TP) involved six major classes of KEGG biological pathway information (Shi & Huang 2006). They are cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems.
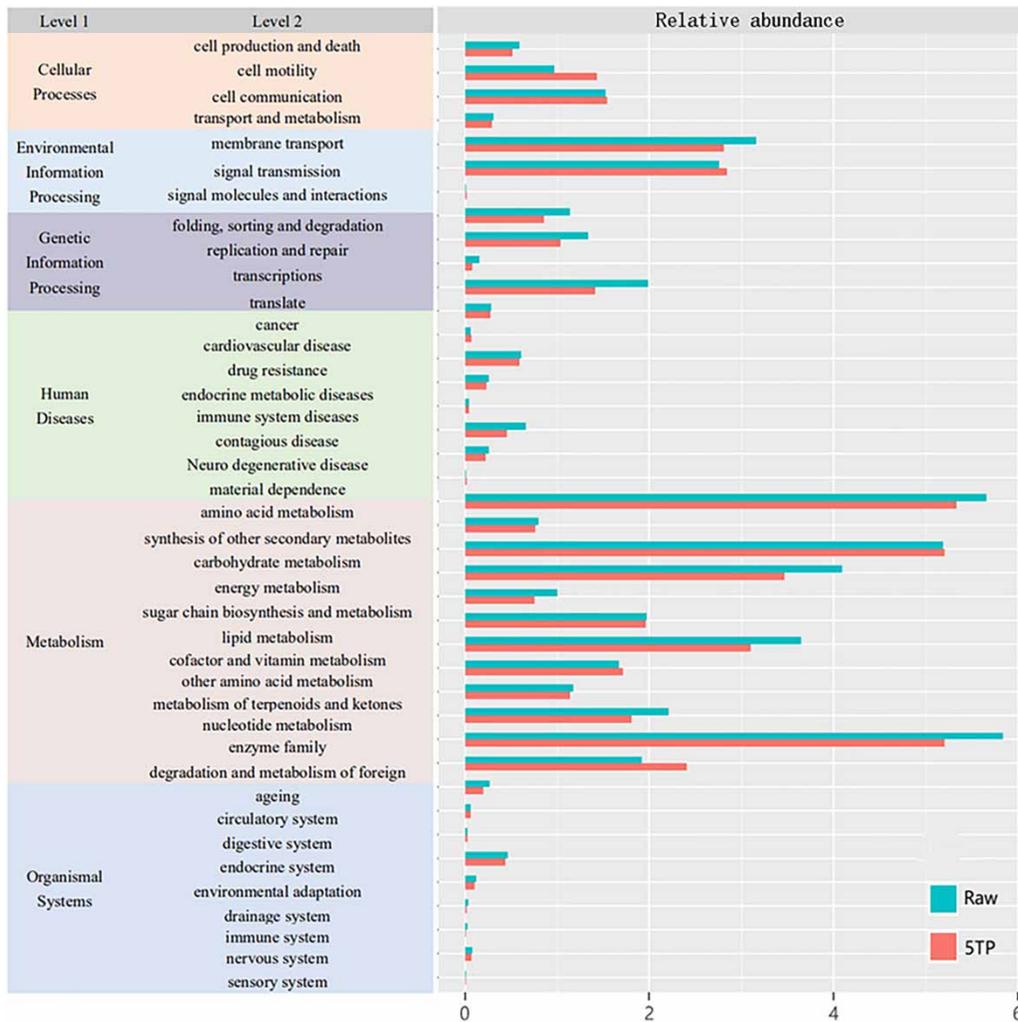
Figure 6 | KEGG statistics of functional genes.

According to the extent of the expression of gene function, the genes in the water samples can be divided into three major categories: metabolic modules showed a significant advantage in functional diversity and relative abundance; followed by the abundance of genes with corresponding functions in environmental information processing, genetic information processing, and cellular processes; the relative abundance of functional genes related to human disease and organism system was low. The abundance of microbial metabolic pathways varies significantly with different gene functions, and most of the microbial metabolic processes are essential for the maintenance of microbial growth.

At level 2, overview, amino acid metabolism, and carbohydrate metabolism in the metabolic function module are the largest taxa in the genome. The second and third largest taxa are energy metabolism, metabolism of cofactors and vitamins, nucleotide metabolism, lipid metabolism, as well as xenobiotics biodegradation and metabolism, followed by metabolism of other amino acids, metabolism of terpenoids and polyketides, glycan biosynthesis and metabolism, and biosynthesis of other secondary metabolites. Significant inhibitions on overview, energy metabolism, metabolism of cofactors and vitamins, amino acid metabolism, and glycan biosynthesis and metabolism were found after tea polyphenols disinfection. The metabolic processes were reduced by 10.83, 15.25, 14.94, 5.81, and 24.39%, respectively, indicating that tea polyphenols significantly inhibited microbial metabolism.

Clustering analysis of specific genes in different microbial populations with different gene sequences annotated with the KEGG database showed that tea polyphenols significantly reduced the performance of microbial membrane transport, translation and replication of genetic information, and the ability of microbes to process environmental and genetic information. Tea polyphenols can also reduce the relative abundance of functional genes for cell production and death, so as to slow down the process of cell cycle. These tea polyphenols can inhibit the growth and reproduction of microorganisms by selecting microorganisms in water and altering the characteristics of microbial communities, in order to achieve the disinfection effect. Tea polyphenols can also reduce the abundance of microorganisms with genetic functions related to human diseases by killing water-borne pathogens.

Microbial metabolic mechanism

Based on the clustering analysis of microbial metabolism, the results revealed that high functional diversity and relative abundance of functional modules for microbial metabolism were remarkable before and after tea polyphenols disinfection. The metabolic processes of most microorganisms are essential for the maintenance of microbial growth. The relative abundance of different gene function modules of microbial metabolism differed significantly before and after tea polyphenols disinfection, and the clustering of functional unit modules of microbial metabolism is shown in Figure 7.
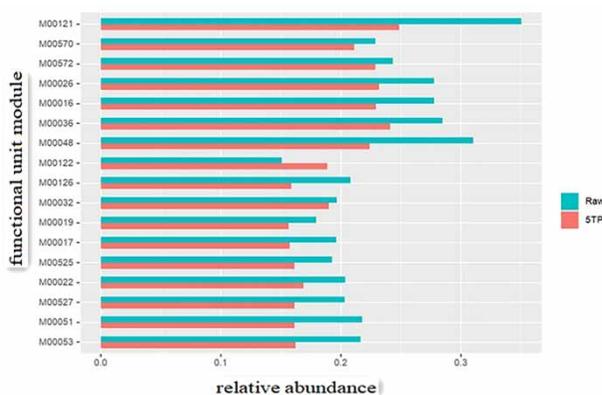

Figure 7 | Clustering statistics of metabolic function modules.

As shown in Figure 7, the relative abundance of modules related to the metabolism of various nucleotides and amino acids was significantly reduced in the clusters of tea polyphenols disinfection, especially the cofactor and vitamin biosynthesis, which had the greatest reduction in relative abundance. The relative abundance of modules associated with glutamate metabolism (M00121), purine metabolism (M00048), pyrimidine metabolism (M00053 and M00051), and lysine metabolism (M00527, M00525, M00032, and M00016) was significantly reduced in the system of tea polyphenols disinfection. Significant reductions of relative abundances were also found in the module of carbon fixation, including the reduced pentose phosphate cycle (M00165), the hydroxypropionate-hydroxybutylate cycle (M00375), the 3-hydroxypropionate bi-cycle (M00376), the reductive citrate cycle (M00173), and the dicarboxylate-hydroxybutyrate cycle (M00374). Tea polyphenols affect microbial metabolic mechanisms in two main ways.

1. Inhibition of the tricarboxylic acid (TCA) cycle

Glutamate plays an important role in living organism protein metabolism and is involved in a variety of important chemical reactions in microorganisms (Xiao *et al.* 2016). Glutamine in glutamate metabolism (M00121) is an important metabolic fuel that helps rapidly proliferating cells to meet the increasing demand for ATP, biosynthetic precursors and reducing agents, and itself promotes proliferative signaling (Sun *et al.* 2014). Glutamine and glutamate are involved in cellular energy metabolism through the TCA cycle; in addition, glutamate is critical for maintaining redox homeostasis and avoiding oxidative stress in cells through the production of glutathione (GSH). So, it was suggested that the use of tea polyphenols for water disinfection reduced the glutamate metabolism of microorganisms in water by inhibiting the TCA cycle, which was involved in cellular energy metabolism (M00121), resulting in an inhibited growth of microorganisms in water for disinfection.

2. Reducing the production of nucleic acid material

The disinfection by tea polyphenols produced a greater inhibition of purine metabolism (M00048) and pyrimidine metabolism (M00053 and M00051). Purines and pyrimidines are the most important components of nucleic acids.

The main physiological function of purines is involved in the formation of purine nucleotides, together with pyrimidine nucleotides, which form the basic structural unit of the nucleic acid molecule and play an important role in various physiological activities of cells (Zhang & Liu 2019). Microorganisms break down purine analogs to produce NH_3, CO_2, and organic acids (formic acid, acetic acid, and lactic acid). The biosynthetic pathway of purine nucleoside metabolites consists of two types: the *ab initio* synthesis pathway and the remedial synthesis pathway. In microorganisms, the biosynthetic pathway of purine nucleosides begins with ribose 5-phosphate pyrophosphate and glutamine, which react to produce inosinic acid (IMP), an important precursor for conversion to other purine nucleotides, hence the name IMP as the central metabolite of the purine biosynthetic pathway. In the remedial pathway, phosphoribo-syltransferases catalyze the formation of the corresponding purine nucleosides from PRPP and purine bases; in addition, some deaminases catalyze the conversion of different purines into each other. The purine biosynthetic pathway in microorganisms is long, GMP and AMP are precursors for the cellular synthesis of DNA and RNA, and purine nucleosides provide essential energy for cell growth and reproduction, so the purine biosynthetic pathway is tightly regulated by a variety of regulatory mechanisms.

Tea polyphenols affect the metabolic processes of microorganisms in the way of influencing the production of nucleic acids by inhibiting glutamate metabolism, purine metabolism, and pyrimidine metabolism processes.

CONCLUSIONS

1. The main role of tea polyphenols as disinfectants in the treatment of ultrafiltration effluent is the inhibition of bacterial regrowth. When the dosage of tea polyphenols was 2.5 mg/L, the total number of colonies in the water can be maintained without a significant increase in 24 h. During this period, the requirements of *Standards for Drinking Water Quality* can be met. When the dosages of 5.0, 7.5, and 10.0 mg/L were applied, respectively, the total number of colonies in the water can be controlled to meet the requirements of *Standards for Drinking Water Quality* for 48 h. Therefore, the dosage of 5.0 mg/L was recommended for the disinfection with tea polyphenols.

2. The microbial diversity of the water can be significantly reduced after tea polyphenols disinfection. Shannon and Simpson indices of the samples disinfected with polyphenols were lower than those of the raw water samples, which are consistent with the effects on microbial population of drinking water using most disinfection methods, such as chlorine disinfection, ozone disinfection, and UV disinfection. At the class level, the relative abundance of *Alphaproteobacteria* and *Betaproteobacteria* increased significantly, while *Gammaproteobacteria* decreased. The relative abundance analysis at the genus level indicated that tea polyphenols were effective against the bacterial pathogens *Acinetobacter*, *Mycobacterium*, and *Bacillus*, which are resistant to disinfection.

3. The relative abundance of gene sequences encoding the corresponding functions in the water samples after tea polyphenols disinfection was significantly changed compared with raw water samples. This indicated that the gene functions of various microorganisms in the water were greatly altered after tea polyphenols disinfection.

4. Clustering analysis of microbial-specific functional genes in different structures of communities showed that the metabolism of various nucleotides and related processes of microorganisms were greatly inhibited during the tea polyphenols disinfection process, and the relative abundance of various nucleotides and amino acid metabolism-related modules of microorganisms in the disinfected water was significantly reduced.

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CONFLICT OF INTEREST

We declare that we have no financial and personal conflicts of interest to this work.

AUTHOR CONTRIBUTIONS

Wei Tong is a Postgraduate candidate, majoring in Drinking Water Disinfection and Disinfection Byproducts.

Feng Cuimin is Professor, majoring in Theory and Technology of Water treatment.

DATA AVAILABILITY STATEMENT

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

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