**Microplastics act as an important protective umbrella for bacteria during water/wastewater disinfection**

Maocai Shen a, 1, Zhuotong Zeng b, 1, Long Li a, Biao Song a, Chengyun Zhou a, Guangming Zeng a,\*, Yaxin Zhang a,\*\*, Rong Xiao b,\*\*\*

a College of Environmental Science and Engineering, Hunan University and Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha, 410082, P.R. China;

b Department of Dermatology, Second Xiangya Hospital, Central South University, Changsha 410011, P.R. China.

\***Corresponding author:** E-mail: [**zgming@hnu.edu.cn**](mailto:zgming@hnu.edu.cn) (G. Zeng), Tel: +86-731-8-8822754 (o)

**\*\*Corresponding author:** E-mail:[**zhang\_yx@hnu.edu.cn**](mailto:zhang_yx@hnu.edu.cn)(Y. Zhang), Tel: +86-731-88822424

**\*\*\*Corresponding author:** E-mail: **xiaorong65@csu.edu.cn** (R. Xiao), Tel: +86-731-88822754

1 These authors contribute equally to this article.

**Abstract**

As an important contributor of environmental microplastics, wastewater treatment plants have attracted extensive attention. The negative effect of suspended particulate matter on disinfection effect has also been established. The main aims of this study were to understand the potential effects of granular polyethylene microplastic and fibrous polyamide microplastics on ultraviolet and chlorine disinfection in water/wastewater treatment. In the laboratory, microplastics were exposed to different disinfection methods, which represented the disinfection dose and disinfection conditions of real water and wastewater treatment. *Escherichia coli* (8099) were selected as the experimental object. Microplastic suspended solids were closely related to water turbidity and UV transmittance at 254 nm. The occurrence of microplastics significantly reduced the efficiency of ultraviolet and chlorine disinfection in water/wastewater treatment, and the efficiency decreased with the increase of microplastics concentration or the decrease of disinfectant dosage. When the concentration of microplastics reached a certain level (> 50 mg L-1), even if the exposure dose continued to increase, the disinfection efficiency could not be improved. Microplastics can reduce the concentration of disinfectants around them by reacting with disinfectants, thus protecting the microorganisms enriched on the surface of microplastics. Although the harsh conditions tested here are rarely met in a typical water treatment process, it represented a worse result. These results were intended to be used by water treatment plants to predict the effects of microplastics in the secondary effluent on UV and chlorine disinfection processes.

**Keywords:** Microplastics; Disinfection; Protective substrates; *Escherichia coli*; Water/wastewater treatment

**1. Introduction**

Microplastics, usually defined as particle < 5 mm in size ([Thompson et al., 2004](#_ENREF_22)), are now ubiquitous in the planet ([Wang et al., 2019](#_ENREF_24)). The accumulation of microplastics in water environment is an increasing concern all over the world, because freshwater and marine polymers pose a serious threat to human daily diet. Freshwater (surface water and underground water) is the main source of raw water for human consumption, agriculture, industries and energy production. Surface fresh water, including rivers, lakes and reservoirs, and groundwater are the main sources of drinking water. The occurrence of microplastics in raw water, tap water and bottle water was thoroughly investigated, which ranged from almost zero to several millions of particles per cubic meter ([Anderson et al., 2017](#_ENREF_1)).

Water environment is threatened by a variety of pollutants, which will increase the harmful substance in drinking water. Natural water is polluted by domestic sewage and industrial wastewater and contains various microorganisms, including pathogenic bacteria and viral pathogens. The quality of drinking water is closely related to human health, which may also be one of the ways for human body to be directly exposed to microplastics ([WHO, 2014](#_ENREF_26)). Biofilm in water/wastewater is the result of the growth of microorganisms in drinking water pipes and other surfaces, like microplastics. In the drinking water distribution system, biofilm can be separated from the pipe wall into the water, which represents the source of the amount of heterotrophic bacteria background found in all drinking water ([McCormick et al., 2014](#_ENREF_13)). Although most of the microorganisms found in biofilms are considered to be mainly nonpathogenic, some biofilms may include free-living microorganisms and opportunistic pathogens ([Roager and Sonnenschein, 2019](#_ENREF_15)). In addition, specific intestinal pathogens can hide in the biofilm, but generally do not propagate outside the host. The biological characteristics of organisms formed by biofilm also affect their adhesion to microplastic surfaces ([Rummel et al., 2017](#_ENREF_16)). Adaptive strategies include hydrophobicity of cell wall and repulsion/attraction interaction between biofilm forming organism surface and surrounding medium to promote adhesion ([Shen et al., 2021b](#_ENREF_18)). Moreover, environmental conditions, including high nutrient concentration (nitrogen and phosphorus), salinity, temperature, high ultraviolet radiation and oxygen content, also affect the formation of plastic and microplastic biofilm. Therefore, it is necessary to remove biofilm from drinking water before human consumption.

Water/wastewater treatment plays an important role in reducing the concentration of microplastics in the water. Although most of these microplastics can be removed during the wastewater treatment, some of them will escape into the environment. Since it is well known that biofilms are more resistant to disinfection than plankton, microplastic biofilms may allow wastewater bacteria (such as *Escherichia coli*) to bypass the disinfection of wastewater treatment plants, as these organisms are known to form biofilms on natural and man-made particles ([Shen et al., 2021a](#_ENREF_17)). This may explain why microorganisms are observed in microplastic biofilm far away from the wastewater discharge outlet. The research on fresh water microplastics biofilm mainly focused on the adsorption of organic compounds, the biodegradation potential of microplastics, and the enrichment of antibiotic resistance genes and pathogens. However, the public have not paid much attention to whether microplastic particles show different behaviors in the process of disinfection. Disinfection is an effective way to kill pathogenic microorganisms in water and prevent the spread of disease and usually the last step in the process of water treatment, so the microplastic that achieve this process are most likely to interact with microorganisms ([Shen et al., 2020](#_ENREF_20)). Ultraviolet (UV) disinfection has been widely used as a three-stage treatment method for drinking water disinfection. In fact, it is a robust and economically viable technology, which satisfactorily solves two important problems in chlorination: the formation of toxic by-products and the low removal rate of protozoa as *Cryptosporidium* ([Carré et al., 2018](#_ENREF_3)). Chlorine is the most widely used disinfectant in drinking water treatment plants around the world. Chlorine is a strong oxidant, which can damage cell membrane, release protein, RNA, DNA and other substances, and affect a variety of enzyme systems (mainly the sulfhydryl group of glucose phosphate dehydrogenase is oxidized and destroyed), thus killing bacteria ([Kelkar et al., 2019](#_ENREF_10)). However, the formation of biofilm on the surface of microplastics may reduce the efficiency of disinfection process ([Hou et al., 2021](#_ENREF_8)). Chlorination causes bacterial death by inhibiting the activity of their enzymes. The occurrence of micron suspended solids in water hinders the effect of chlorine on microorganisms, because they may be tapped by flocs or suspended particles. Therefore, microplastics with similar physical properties can act as protective substrates for bacteria, which can resist the disinfection process. UV radiation can destroy DNA and inactive pathogenic microorganisms, however, the existence of suspended particles protects microorganisms from ultraviolet rays radiation damage and disinfection. Therefore, microplastics may reduce the performance of ultraviolet disinfection process.

The subject of this research is to explore the potential impact of the presence of microplastics on disinfection water/wastewater treatment. To that aim, we have selected common disinfection methods of water (UV and chlorine disinfection) and *Escherichia coli* as the research objects, as well as evaluated the disinfection efficiency of these methods in the presence of microplastics. These parameters are of great significance of the determination of disinfection mode and size and real-time risk control in the process of water production. Moreover, this research can provide guidance for the needs and knowledge gaps in the field of water/wastewater treatment and microplastics.

**2. Materials and methods**

2.1 Materials

The model microplastics used in this study are composed of manufactured microplastic particles and fibers. The manufactured microplastic particles include granular polyethylene microplastic (PE) and fibrous polyamide microplastic (PA). The selected microplastic particle size of PE and PA was to be 10 μm and 50 μm, because this kind of microplastics can escape from the existing water/wastewater treatment process and occupy the largest proportion in the monitoring of the amount of microplastics in water/wastewater, and it is easy to be ignored. These microplastics were obtained from Aladdin Chemical Company (Shanghai, China). *Escherichia coli* (8099) were preserved in the laboratory. The eosin-methylene blue medium (EMB), sodium chloride (NaCl), hypochlorite water disinfectant (HClO), sodium thiosulfate (Na2S2O3), potassium dichromate (K2Cr2O7), sulfuric acid (H2SO4, 18.4 M), hydrogen chloride solution (12 M), and potassium iodide (KI) were of analytic grade and purchased from Aladdin Chemical Company (Shanghai, China). The water used in the experiments was ultrapure water extracted by Milli-Q mechanism.

2.2 Preparation of bacterial suspension

The freeze-dried bacteria were inoculated in nutrient broth medium, cultured at 37 ºC for 24 h, and then inoculated in nutrient agar medium (F1). After culture, single colony was inoculated again on the slope of nutrient agar medium at 37 ºC for 24 h (F2). The third generation culture (F3) was obtained after culture of F2. The coating was washed down by diluent, and the suitable concentration of viable count was measured to be 1× 109 – 3 × 109 CFU mL-1.

2.3 Reactor description

2.3.1 UV reactor

The 50 L UV reactor used in this study is a cylindrical reactor made of glass. The specific components of UV reactor are as follows (**Fig. S1**): (1) Overhead stirrer with a blade impeller. The mixing speed was set to 150 rpm. The mixing conditions were designed to ensure homogenization within 30 seconds without impacting bacteria; (2) Low pressure UV lamp of 6W with an electronic ballast; (3) UV intensity sensor. The applied UV flux was defined as the product of the average flux and exposure time for a given volume of water. The formula for calculation is as follows:

*F = Eavg × t*  (1)

where *F* is the applied flux (J m-2), *Eavg* is the average flux rate of UV lamp (w m-2), defined as the total radiation power incident on the infinite sphere from all directions, and t is the total contact time (s). It is very complex to determine the applied UV radiation doses in the reactor, because the dose is not uniform in the whole system ([Bolton and Linden, 2003](#_ENREF_2)). To determine the equivalent flux, biodosimetry was evaluated by collimated beam reactor experiments using microorganisms with calibrated UV inactivation responses. The inactivation observed by the UV disinfection system was compared with the calibrated UV inactivation reaction to establish the equivalence called reduction equivalent flux. This method of determining equivalent flux is considered to be one of the most accurate methods to evaluate the UV flux in the reactor ([Carré et al., 2018](#_ENREF_3)).

In this study, *Escherichia coli* (8099) were chosen for equivalent UV flux determination. Firstly, *Escherichia coli* (8099) were cultured in medium at 37 ºC for 2 – 4 hours to reach the logarithmic growth stage. The culture, about 1× 106 – 2 × 106 CFU mL-1, was diluted in 0.9% NaCl solution. After that, a certain volume of bacterial solution (30 mL) was exposed to 0 – 15 J cm-2 and a portable radiometer was used to measure the irradiance. This measurement experiment was repeated four times. By comparing the colony formation of *Escherichia coli* (8099) before and after UV irradiation, the inactivation rate was calculated. Bacterial colony count is achieved by culturing 1 μL sample in a ready-made plate containing a special medium for *Escherichia coli* (8099). Then, the colony forming units (CFU) per milliliter was counted after incubation at 37 ºC for 24 hours. The method for calculating the inactivation constant of *Escherichia coli* (8099) is as follows:

*log = – k × F*  (2)

where *N0* and *NUV* are the colony counts of *Escherichia coli* (8099) (CFU mL-1) before and after UV irradiation, respectively, and *k* is inactivation constant (cm2 J-1). The determination procedure is the same as the abovementioned method, and the inactivation after UV exposure is converted to equivalent flux by formula (2). In order to ensure the accuracy, the experiment was repeated four times.

2.3.2 Chlorine reactor

The 5 L chlorine reactor used in this study is a cylindrical reactor made of glass. The mixing speed was set to 150 rpm. The mixing conditions were designed to ensure homogenization within 30 seconds without impacting bacteria. Hypochlorous acid has a strong oxidation capacity, no charge and relatively low molecular weight. It is easier to penetrate the bacterial cell wall than other chlorine compounds, and irreversibly oxidizes the key components in the cells, and quickly kills pathogens. The water sample to be tested was transferred to the sample bottle after high temperature sterilization. The different concentrations of hypochlorite water disinfectant (HClO) were added and the contact reaction time was set as 30 min. Dechlorination was performed using the powder sodium thiosulfate immediately after the chlorination.

2.4 Experimental

2.4.1 UV disinfection tests

The type of microplastics was selected according to the abundance in wastewater ([Shen et al., 2020](#_ENREF_20)). The selected microplastics were washed by Milli-Q pure water, aired and weighted, and the concentration of was set up 0 – 100 mg L-1. After proper mixing in the UV reactor (microplastics + *Escherichia coli*), the simulated experiments were carried out at room temperature (25ºC ± 1ºC). After exposure to a given flux, 50 L of water sample was prepared with sterile pipette in the UV reactor, and stored in the dark and at 4ºC. Next, a certain volume of water sample of 0, 3, 5, 10, 20, and 30 min was cultured in a ready-made plate containing a special medium to calculate the bacterial colony count. The water samples of the control group which had been in the dark place were used instead of the reaction water samples in the positive control test. Quantification was performed after the dish was placed at 37ºC for 24 h. The inactivation was determined by comparing the number of microorganisms before and after exposure to a given concentration. The experiment was repeated three times.

2.4.2 Chlorine disinfection tests

By gradually increasing the chlorine concentration and contact reaction time, the simulated experiments of drinking water treatment and chlorination under two conditions were performed. The effective chlorine content of disinfectant was measured to be 1.8 mmol L-1. Microorganisms and treated microplastics were added to the reaction system. Then, chlorine disinfectant was added to the reaction system to achieve the required concentration while the pH of the solution was maintained within in 6 – 7 ([Kelkar et al., 2019](#_ENREF_10)). The reaction system was sealed with a lid and incubated ate the specific conditions. 100 mL mixture solution was sampled at 0, 3, 5, 10, 20, and 30 min, respectively. Then, dechlorination was performed using the powder sodium thiosulfate immediately after the chlorination to neutralize for 10 min, and the colony forming unit per milliliter was counted after incubation at 37ºC for 24 h. The positive control test was performed using sterile ultrapure water instead of disinfectants. All these measurements were performed in triplicate for experiment.

2.4.3 Neutralizer identification test

The test bacteria were *Escherichia coli* (8099), and the bacterial solution was diluted with BSA to be 1× 104 – 1 × 105 CFU mL-1. The reaction time of disinfection process was set as 10 min and the neutralization time was also 10 min. The neutralizer identification test was divided into four groups: a) (disinfectant + bacterial suspension) + sterile ultrapure water; b) (disinfectant + bacterial suspension) + neutralizer; c) (neutralizer + sterile ultrapure water) + bacterial suspension; and d) (neutralizer + disinfectant) + bacterial suspension. The positive control group was set as (dilute + sterile ultrapure water) + bacterial suspension, and the negative control group was set as (dilute + sterile ultrapure water) + neutralizer, respectively. The mixture was properly diluted and inoculated into four plates respectively. After culture at 37ºC for 24 h, the viable bacteria were counted, and the average values were obtained after 3 times of test.

2.5 Re-activation test

In order to explore whether microplastics can protect bacteria during water disinfection, the re-activation test was carried out. The 50 mL solution from each sampling point was neutralized first, and then filtered by 0.45 μm sterile membrane to separate solid and liquid. The filtrated solution and microplastics were added to the triple concentrated liquid nutrient growth medium, respectively, and cultured at 37ºC for 24 h. Sterile ultrapure water was used for the same culture after filtration. After culture, the mixed culture medium was taken and cultured again on the solid EMB medium. The viable bacteria were counted, and the average value was obtained after 3 times of test.

2.6 Quality assurance and quality control

To avoid the pollution, glassware was mainly used to instead of plastics and was sterilized by high temperature. The experiment was carried out in a clean and unmanned laboratory. The number of people in the laboratory was controlled and a cotton lab cloth was worn. Several blanks were prepared to detect possible air pollution. The microplastics (granular PE and fibrous PA) were a standard particle and may not exist in the laboratory environment.

2.7 Characterization of microplastics after disinfection

μ-Fourier transform infrared spectroscopy (μ-FTIR) was sued to identify the obtained microplastics from before and after water disinfection process to compare the changes of microplastics. Considering the wide application of plastic polymers and disinfection, it can be expected that traditional polymers are quite resistant to oxidation during disinfection. The microplastics obtained from each sampling were thoroughly washed with ultrapure water, dried and stored in a clean glass conical flask before further analysis. The spectral range was set to 4000 – 675 cm-1, and the spectral resolution was set to 6 cm-1. The number of scans was 32 times and the data interval was 0.482 cm-1. The spectra obtained were compared with those of reference microplastic spectral database.

**3. Results**

3.1 Determination of inactivation constant

The correlation function between the inactivation level of *Escherichia coli* and the ultraviolet flux in the reactor is shown in **Fig. 1**. Because *Escherichia coli* are very sensitive to UV radiation, the inactivation constant of UV flux was determined to be less than or equal to 16 mJ cm-2, which were consistent with [Bolton and Linden (2003](#_ENREF_2)) and [Carré et al. (2018](#_ENREF_3)). This inactivation constant was calculated by the slope value of linear regression between UV flux and inactivation data. In this study, the inactivation constant of *Escherichia coli* was 0.35 ± 0.03 cm2 mJ-1 (*R2* = 0.97), which was consistent with the data of various *Escherichia coli* found in the previous literature, and the inactivation constant was in range of 0.3 – 0.6 cm2 mJ-1 ([Hijnen et al., 2006](#_ENREF_7)). This value was used to determine the reduction equivalent fluences in the designed UV reactor. The culture diluted with dechlorinated household tap water was used to inactivate *Escherichia coli* in the UV reactor. The UVT254 values of four samples were measured to be 65.3%, 67.4%, 69.1% and 67.0%, respectively. The reduction equivalent fluences in the UV reactor should be 67 ± 2% of the equivalent radiation value of UVT254. The UV exposure time was set as 0 – 90 min and applied in the reactor corresponding to the reduction equivalent fluences in the range of 0 – 110 mJ cm-2.

3.2 Neutralizer identification test

The neutralizer identification effect of neutralization reagent on *Escherichia coli* was shown in **Table 1**. The results suggested that the neutralizer used in the process of chlorine disinfection can effectively neutralize the disinfectant and reduce its germicidal efficacy, and the neutralizer itself and the neutralizing product have no obvious antibacterial effect on *Escherichia coli*. When the effective chlorine content of disinfectant was 1.8 mmol L-1, there were no bacteria in the plate of *Escherichia coli* in the group b. When the concentration of effective chlorine was decreased to 0.9 mmol L-1, the colony count in the plate was 1.2 × 104 CFU mL-1. These findings confirmed that the test bacteria affected by disinfectant could not grow again after residual disinfectant was neutralized.

3.3 Effect of microplastics on ultraviolet disinfection process

**Fig. 2**, **Table S1** and **Table S2** showed the effect of microplastics (PE and PA) on inactivation of *Escherichia coli* during UV disinfection. The results demonstrated that UV radiation had significant killing effect on *Escherichia coli*, and the general trend was that the germicidal efficacy increased with the extension of irradiation time. The killing rate of E. coli in water sample can reach 99.24% after ultraviolet irradiation for 3 min without microplastics. When microplastics were added into the reaction system, the UV disinfection efficiency significantly decreased, and the decrease was more obvious with the increase of the concentration of microplastics. When the concentration of microplastics was increased to 50 mg L-1, the sterilization rate was measured to be 99.96% for PE and 99.43% for PA and the number of viable bacteria was 18 and 24 CFU mL-1, respectively. When the concentration of microplastics was increased to 100 mg L-1, the sterilization rate was 98.79% for PE and 98.76% for PA and the number of viable bacteria was 356 and 370 CFU mL-1, respectively. After the ultraviolet radiation reached a certain dose, even if the treatment time continued to increase, the bactericidal effect did not increase significantly. The current results showed that microplastics can significant affect the ultraviolet disinfection process. The agitation of the water sample can help the bacteria to distribute evenly, so as to get better ultraviolet radiation. The bacteria in the system can flow and be exposed to ultraviolet radiation to effectively improve the disinfection efficiency and eliminate the tailing phenomenon.

The sterilization principle of ultraviolet ray is that 280 nm ultraviolet (belonging to C-band 190 – 290 nm) destroys microbial genetic material. The sterilization effect of ultraviolet rays is affected by many factors, including irradiation time, microbial sensitivity, liquid turbidity exposure distance, and environmental temperature and humidity. There was a strong correlation between the inactivation of UV sensitive *Escherichia coli* and the presence of suspended microplastic particles. This increase in UV resistance associated with the presence of microplastic particles may be caused by two main factors: the scattering of ultraviolet light by particles and the binding of particles with bacteria ([Chahal et al., 2016](#_ENREF_4)). Particle size seems to determine the shading effect of microorganisms. A study done by [Madge and Jensen (2006](#_ENREF_12)) reported that the inactivation constant of fecal coliform associated with large particles ( > 20 μm) was significantly lower than that associated with small flocs (< 5 μm). [Frank et al. (2014](#_ENREF_6)) showed that when the turbidity increased from 1 NTU to 10 NTU, the UV doses loss increased by 28% due to UV refraction and scattering. With the increase of suspended particle, the penetration of ultraviolet light was reduced, and the resistance to ultraviolet disinfection was improved, which was consistent with the results of the experiment. When the turbidity of water increased, the ultraviolet penetration ability decreased, which led to the decrease of ultraviolet radiation dose reaching the surface of bacteria, thus reducing the inactivation of bacteria. From the **Fig. 2**, with the increase of the concentration of microplastics in the reaction system, the sterilization efficiency of UV decreased significantly. Compared with PE microplastics, PA microplastics had obvious interference on ultraviolet disinfection, that is, its disinfection efficiency was lower at the same concentration.

3.4 Effect of microplastics on chlorine disinfection process

The effect of PE microplastics on chlorine disinfection process was illustrated in **Fig. 3** and **Table S3**. The effective chlorine content in this study was set as 1.8, 0.9 and 0.45 mmol L-1, respectively. In the absence of microplastics, the ratio of action time to 100% bactericidal rate of *Escherichia coli* was 3 min for 1.8 mmol L-1, 20 min for 0.9 mmol L-1, and 30 min for 0.45 mmol L-1, respectively. With increase of the concentration of microplastics, the action time to reach 100% bactericidal rate of *Escherichia coli* was significantly prolonged under the same effective chlorine content. In the process of disinfection with high concentration of effective chlorine (1.8 mmol L-1), the sterilization efficiency can reach the corresponding standard under all the different microplastic concentrations. When the concentration of PE microplastic was 1 mg L-1 and the content of effective chlorine was 0.9 mmol L-1, the action time to reach 100% bactericidal rate of *Escherichia coli* took more than 30 min. With the increase of microplastic concentration, it would take longer time to reach the same sterilization rate. When the concentration of microplastics was 10 mg L-1 and 20 mg L-1, the sterilization time of 30 min could not meet the requirements of sterilization, and the concentration of *Escherichia coli* in the solution was measured to be 400 and 1640 CFU mL-1. When the content of effective chlorine was 0.45 mmol L-1 and the concentration of microplastic was less than 2 mg L-1, the sterilization efficiency could meet the requirements of sterilization. When the concentration of microplastics was 5, 10 and 20 mg L-1, the sterilization time of 30 min could also not meet the requirements of sterilization, and the concentration of *Escherichia coli* in the solution was 54, 1070 and 2100 CFU mL-1.

**Fig. 4** and **Table** **S4** showed the effect of PA microplastics on chlorine disinfection process. The interference of these two kinds of microplastics on chlorine disinfection process was similar. The ratio of action time to 100% bactericidal rate of *Escherichia coli* was 3 min for 1.8 mmol L-1, 20 min for 0.9 mmol L-1, and 30 min for 0.45 mmol L-1, respectively. When the effective chlorine content was mmol L-1, the sterilization efficiency can also reach the corresponding standard under all the different microplastic concentrations. The action time to reach 100% bactericidal rate of *Escherichia coli* took 30 min at 1 mg L-1 of PA microplastics and 0.9 mmol L-1 of effective chlorine. When the concentration of microplastics was 100 mg L-1, the sterilization rate after 30 min was 97.56%, which meant that at least 630 and 1440 CFU mL-1 was still present in the disinfected water. When the content of effective chlorine decreased to 0.45 mmol L-1, the sterilization time of 30 min only reached 99.97% for 5 mg L-1, 96.48% for 10 mg L-1, and 92.48% for 20 mg L-1, indicating that more than 10, 903 and 1920 CFU mL-1 of *Escherichia coli* in the solution remained. The results showed that the content of available chlorine should be increased or the disinfection time should be prolonged when the microplastics were contained in the water to be disinfected. In addition, it can be seen from **Fig. 3** and **Fig. 4** that the effect of PE microplastics on disinfection process was greater than that of PA at the same effective chlorine content and microplastic concentration, that is, the sterilization efficiency was lower under the same action time.

3.5 Re-activation test

After disinfection, microplastics used in the study were collected and added to concentrated liquid nutrient growth medium, respectively, and cultured at 37ºC for 24 h. **Table 2** showed the study on the reactivation of *Escherichia coli* carried on the surface of microplastics after UV disinfection. It can be clearly seen from **Table 2** that in the absence of microplastics, no bacteria were detected in the bacteria reactivation experiment at all time points after the sterilization rate of UV disinfection, which indicated that UV ray could easily kill *Escherichia coli* in the clean water. When the concentration of microplastics was 1 mg L-1 and the action time was 9 minutes, the sterilization efficiency had reached 100%. Problematically, whether it was PE group or PA group, *Escherichia coli* was detected in the reactivation medium. The detection also occurred in the 10 min and 15 min groups, but not in the 30 groups. The results showed that although the low concentration of microplastics interfered with the UV disinfection process, as long as the disinfection time was increased, that is, the amount of UV radiation, the purpose of complete disinfection could also be achieved. When the concentration of the microplastics increased to more than 50 mg L-1, the *Escherichia coli* on the surface of the microplastics could not be completely killed after 30 min exposure, which indicated that the presence of microplastics greatly interfered with the UV disinfection process. After the ultraviolet radiation reached a certain dose, even if the treatment time continued to increase, the bactericidal effect did not increase significantly. Compared with PE microplastics, the effect of PA microplastics on disinfection process seems to be greater, because under the conditions of 20 mg L-1 and 30 minutes of action time, *Escherichia coli* was not detected in PE group, but it was detected in PA group, which indicated that PA microplastics with larger particle size have stronger protective umbrella effect.

The results of the study on the reactivation of carried on the surface of microplastics after chlorine disinfection were shown in **Table 3** and **Table 4**. From the **Table 3**, when the concentration of PE microplastic was ≤ 20 mg L-1, the high content of effective chlorine (1.8 mmol L-1) could effectively kill *Escherichia coli* in water and prevent it from resurrecting again. Unfortunately, when PE concentration was ≥ 50 mg L-1, although the effective chlorine of 1.8 mmol L-1 can kill all the *Escherichia coli* in water, it cannot effectively kill *Escherichia coli* on the surface of PE microplastics due to the new growth of *Escherichia coli* in the medium. When the effective chlorine decreased to 0.9 mmol L-1, the reactivation of *Escherichia coli* became more obvious after disinfection. At the microplastic concentration of 20 mg L-1, the effective chlorine of 0.9 mmol L-1 was not enough to kill *Escherichia coli* on the surface of the microplastic. For 0.45 mmol L-1 of effective chlorine, the available chlorine concentration was also not enough to effectively kill *Escherichia* on the surface of microplastic, even after exposure for 30 minutes. The effect of PA microplastics on chlorine disinfection process was similar to that of PE microplastics, which can be seen from **Table 4**. These results showed that microplastics can protect the *Escherichia coli* enriched on its surface by some mechanism during the chlorine disinfection process. For example, the microplastic itself reacted with chlorine disinfectant, which made the concentration of chlorine disinfectant in the neighborhood of *Escherichia coli* too low to kill it.

3.6 Changes of microplastics before and after disinfection

**Fig. 5** showed the chemical changes of microplastics before and after UV and chlorine disinfection. In general, the chemical structure of PE microplastic particles did not change significantly during disinfection except for a new peak at 1743 cm-1 (**Fig. 5A**). The infrared characteristic peaks of PE (CH2, 1465 cm-1) moved at a higher chlorine dose, indicating that the peaks were compressed under strong chlorination ([Kelkar et al., 2019](#_ENREF_10)). In addition, no obvious change of PE microplastics was viewed when the chlorine concentration was low (0.45 mmol L-1). No obvious chemical changes of PA fibers were observed in the process of ultraviolet and chlorine disinfection (**Fig. 5B**), which demonstrated that the chlorine dosage used in water/wastewater treatment was not enough to cause chemical changes of PE and PA microplastics.

**4. Discussions**

The influence mechanism of microplastics on the disinfection process of water/wastewater treatment remained to be studied. It can be inferred from the results of this study and published related studies ([Enfrin et al., 2019](#_ENREF_5)) that microplastics play a protective umbrella role in the disinfection process and form a stable structure to interfere with the killing of *Escherichia coli* (**Fig. 6**). For UV disinfection, the existence of microplastics can not only block the penetration of UV rays in water, but also react with them. By measuring the changes of functional groups on the surface of microplastics before and after disinfection, it can be seen that in the process of disinfection, the microplastics reacted with UV and chlorine disinfectants ([Kelkar et al., 2019](#_ENREF_10)), which reduced the dosage of disinfectants. When UV or chlorine contact with free *Escherichia coli* in the water, it can be killed in a short time. In the presence of microplastics, microplastics can help *Escherichia coli* to resist extreme conditions. Interestingly, both PE and PA microplastics had similar interference on UV disinfection or chlorine disinfection. The purpose of water/wastewater disinfection is to kill most pathogenic microorganisms, including bacteria, viruses and protozoa, which are harmful to human health, and prevent the spread of diseases. Domestic sewage and surface freshwater contain a large number of bacteria, viruses, sporangia. After the traditional treatment process, only about 90% of coliform can be removed. In order to prevent the spread of diseases, the sewage must be disinfected after secondary biochemical treatment and discharged into the receiving water body or other purposes. On the premise of meeting the microbiological standards of drinking water quality, the risk of water-borne infectious diseases caused by drinking water should be minimized. However, unfortunately, microplastics can escape from existing water/wastewater treatment processes ([Sun et al., 2019](#_ENREF_21)). Several studies have found that the concentration of microplastics in the secondary effluent of sewage treatment plants varies from zero to hundreds ([Lares et al., 2018](#_ENREF_11)). It should be noted that the minimum particle size of microplastics in these studies is 10 μm, and the others smaller than this size are ignored. Evidence showed that with the decrease of particle size, the concentration of microplastics gradually increased ([Pivokonsky et al., 2018](#_ENREF_14)), indicating that the concentration of microplastics in effluent may be underestimated. Disinfection is usually the last step in the water/wastewater treatment process. The current study demonstrated that microplastics will carry the microorganisms protected by them into the receiving water or household tap water. The microplastics escaping from the sewage treatment plant can not only lead to the serious pollution of micro plastics, but also bring new micro plastics pollution. Consequently, it is necessary to improve the removal efficiency of microplastics by improving the existing water/wastewater treatment process.

*Escherichia coli* is a common model bacteria in environmental toxicology, and its advantage is that it is highly sensitive to a variety of chemicals. Additionally, *Escherichia coli* is a simple, fast and suitable bacterial model for this study, because it is easy to cultivate and process, and low maintenance cost. Through observation, interestingly, we found that when *Escherichia coli* came into contact with microplastics, it would like to migrate and enrich on the surface of microplastics (**Fig. 6**). When it is seriously threatened by external extreme conditions, it will choose to hide on the rough surface of micro plastic to avoid the threat. Actually, in the actual disinfection process, the existence of other contaminants, microorganisms and biofilms may also affect the disinfection effect. *Escherichia coli* is very sensitive to UV and chlorine disinfectants. Evidence showed that microplastics in the environment have biofilm ([Van Melkebeke et al., 2020](#_ENREF_23); [Wright et al., 2020](#_ENREF_27)), which can protect the microorganisms the harm of ultraviolet and chlorine ([McCormick et al., 2014](#_ENREF_13)). In addition, emerging pathogenic microorganisms, such as chlorine or UV resistant viruses and protozoa, antibiotic resistant bacteria and so on, greatly threaten the safety of drinking water supply. *Legionella*, *Mycobacterium*, *Pseudomonas aeruginosa* and other common chlorine resistant bacteria are also pathogenic bacteria, which may lead to the outbreak of water-borne infectious diseases when they enter the water. At the same time, some new chlorine resistant bacteria has been found, such as *Pseudomonas sludge* and *Sphingomonas* reported in recent years ([Jia et al., 2020](#_ENREF_9)). Some viruses, such as coxsackievirus in enterovirus, have been found to be resistant to chlorine ([Wati et al., 2019](#_ENREF_25)). Moreover, under the pressure of antibiotic selection, bacteria in water environment gradually develop drug resistance. The resistance genes can transfer vertically or horizontally, which makes the resistance spread continuously, and even lead to the emergence of multi drug-resistant bacteria, which poses a great threat to human health and ecological security. The existence of microplastics is just like adding wings to a tiger's wings to the spread and transfer of resistant genes ([Shen et al., 2019](#_ENREF_19)). The detection of antibiotic resistant bacteria and resistance genes in water supply systems or freshwater has become a new challenge to the existing water purification process. Therefore, there is a long way to go to explore the influence mechanism of microplastics on traditional disinfection process in order to improve the disinfection efficiency of water/wastewater treatment.

So far, the influence mechanism of microplastics on disinfection process is still unknown. This study provided new data and insights into the effect of microplastics on the disinfection process of water/wastewater treatment, but it also had some limitations. All experiments were conducted with reagent grade water. However, in the real environment, the microplastics rich in biofilm, bacteria with resistance genes and chlorine resistant bacteria may change, and the disinfection effect was observed by competitive reaction and chlorine quenching. Therefore, the effective doses of chlorine and ultraviolet that can be used to interact with microplastics in drinking water, especially wastewater, may be less than those used here. As a result, the results of the report were conservative and represent the worst case scenario. The selection of bacteria and microplastics was not necessarily representative, and other disinfectants used in the treatment plant may also have different effects on the disinfection process. In general, the presence of microplastics did have an impact on the disinfection process. It is urgent to study the potential impact mechanism of microplastics on the disinfection process, which is very important to assess the human and environmental health risks of microplastics.

**5. Conclusions**

The potential effects of PE and PA microplastics on UV and chlorine disinfection in water/wastewater treatment were studied. The current study showed that the presence of microplastics reduces the efficiency of UV and chlorine disinfection in water/wastewater treatment. When the concentration of microplastics reached a certain level (> 50 mg L-1), even if the exposure dose continued to increase, the disinfection effect could not be improved. The finding suggested that microplastics can reduce the concentration of disinfectant around it by reacting with disinfectants, which can protect the *Escherichia coli* enriched on the surface of microplastics. The new bond on the surface of the microplastics can prove that the microplastics participate in the reaction with disinfectant during the disinfection process. Although the harsh conditions tested here are rarely met in a typical water treatment process, it also represented a worse result. The presence of *E.coli* on the surface of the microplastics after the completion of the disinfection process was the main evidence that the microplastics interfered with the disinfection process, as observed in microplastics. In general, the data collected in this work can better assess the type and degree of risk posed by microplastics in the process of water/wastewater treatment and disinfection. These microplastics would carry the microorganisms protected by them into the receiving surface water.

**Acknowledgements**

The study is financially supported by the Program for the National Natural Science Foundation of China (82003363, 82073449, U20A20323, 51521006).

**Reference**

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., Rennie, M.D., 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. 225, 223-231. https://doi.org/10.1016/j.envpol.2017.02.072.

Bolton, J.R., Linden, K.G., 2003. Standardization of methods for fluence (uv dose) determination in bench-scale UV experiments. J. Environ. Eng. 129, 209-215. https://doi.org/10.1061/(ASCE)0733-9372(2003)129:3(209).

Boni, W., Parrish, K., Patil, S., Fahrenfeld, N.L., 2021. Total coliform and *Escherichia coliin* microplastic biofilms grown in wastewater and inactivation by peracetic acid. Water Environ. Res. 93, 334-342. https://doi.org/10.1002/wer.1434.

Carré, E., Pérot, J., Jauzein, V., Lopez-Ferber, M., 2018. Impact of suspended particles on UV disinfection of activated-sludge effluent with the aim of reclamation. J. Water Process Eng. 22, 87-93. https://doi.org/10.1016/j.jwpe.2018.01.016.

Chahal, C., van den Akker, B., Young, F., Franco, C., Blackbeard, J., Monis, P., 2016. Pathogen and particle associations in wastewater: significance and implications for treatment and disinfection processes. Adv. Appl. Microbiol. 97, 63-119. https://doi.org/10.1016/bs.aambs.2016.08.001.

Enfrin, M., Ludovic F. Dumee , Lee, J., 2019. Nano/microplastics in water and wastewater treatment processes - origin, impact and potential solutions. Water Res. 161, 621-638. https://doi.org/10.1016/j.watres.2019.06.049.

Frank, T.E., Scheie, M.L., Cachro, V., Munoz, A.S., 2014. The effect of increasing grain size in biosand water filters in combination with ultraviolet disinfection. J. Water Sanit. Hyg. De. 4, 206-213. https://doi.org/10.2166/washdev.2013.171.

Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. Water Res. 40, 3-22. https://doi.org/10.1016/j.watres.2005.10.030.

Hou, L., Kumar, D., Yoo, C.G., Gitsov, I., Majumder, E.L.W., 2021. Conversion and removal strategies for microplastics in wastewater treatment plants and landfills. Chem. Eng. J. 406, 126715. https://doi.org/10.1016/j.cej.2020.126715.

Jia, S., Jia, R., Zhang, K., Sun, S., Lu, N., Wang, M., Zhao, Q., 2020. Disinfection characteristics of Pseudomonas peli, a chlorine-resistant bacterium isolated from a water supply network. Environ. Res. 185, 109417. https://doi.org/10.1016/j.envres.2020.109417.

Kelkar, V.P., Rolsky, C., Pant, A., Green, M.D., Tongay, S., Halden, R.U., 2019. Chemical and physical changes of microplastics during sterilization by chlorination. Water Res. 163, 114871. https://doi.org/10.1016/j.watres.2019.114871.

Lares, M., Ncibi, M.C., Sillanpää, M., Sillanpää, M., 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Research 133, 236-246. https://doi.org/10.1016/j.watres.2018.01.049.

Madge, B.A., Jensen, J.N., 2006. Ultraviolet disinfection of fecal coliform in municipal wastewater: Effects of particle size. Water Environ. Res. 78, 294-304. https://doi.org/10.2175/106143005X94385

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48, 11863-11871. https://doi.org/10.1021/es503610r.

Pivokonsky, M., Cermakova, L., Novotna, K., Peer, P., Cajthaml, T., Janda, V., 2018. Occurrence of microplastics in raw and treated drinking water. Sci. Total Environ. 643, 1644-1651. https://doi.org/10.1016/j.scitotenv.2018.08.102.

Roager, L., Sonnenschein, E.C., 2019. Bacterial candidates for colonization and degradation of marine plastic debris. Environ. Sci. Technol. 53, 11636-11643. https://doi.org/10.1021/acs.est.9b02212.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. 4, 258-267. https://doi.org/10.1021/acs.estlett.7b00164.

Shen, M., Hu, T., Huang, W., Song, B., Zeng, G., Zhang, Y., 2021a. Removal of microplastics from wastewater with aluminosilicate filter media and their surfactant-modified products: Performance, mechanism and utilization. Chem. Eng. J. 421, 129918. https://doi.org/10.1016/j.cej.2021.129918.

Shen, M., Song, B., Zeng, G., Zhang, Y., Teng, F., Zhou, C., 2021b. Surfactant changes lead adsorption behaviors and mechanisms on microplastics. Chem. Eng. J. 405, 126989. https://doi.org/10.1016/j.cej.2020.126989.

Shen, M., Zhu, Y., Zhang, Y., Zeng, G., Wen, X., Yi, H., Ye, S., Ren, X., Song, B., 2019. Micro(nano)plastics: unignorable vectors for organisms. Mar. Pollut. Bull. 139, 328-331. https://doi.org/10.1016/j.marpolbul.2019.01.004.

Shen, M., Song, B., Zhu, Y., Zeng, G., Zhang, Y., Yang, Y., Wen, X., Chen, M., Yi, H., 2020. Removal of microplastics via drinking water treatment: current knowledge and future directions. Chemosphere 251, 126612. https://doi.org/10.1016/j.chemosphere.2020.126612.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.-J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21-37. https://doi.org/10.1016/j.watres.2018.12.050.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science 304, 838-838. https://science.sciencemag.org/content/304/5672/838.

Van Melkebeke, M., Janssen, C., De Meester, S., 2020. Characteristics and sinking behavior of typical microplastics including the potential effect of biofouling: implications for remediation. Environ. Sci. Technol. 54, 8668-8680. https://doi.org/10.1021/acs.est.9b07378.

Wang, J., Wang, M., Ru, S., Liu, X., 2019. High levels of microplastic pollution in the sediments and benthic organisms of the south Yellow Sea, China. Sci. Total Environ. 651, 1661-1669. https://doi.org/10.1016/j.scitotenv.2018.10.007

Wati, S., Robinson, B.S., Mieog, J., Blackbeard, J., Keegan, A.R., 2019. Chlorine inactivation of coxsackievirus B5 in recycled water destined for non-potable reuse. J. Water Health 17, 124-136. https://doi.org/10.2166/wh.2018.393.

WHO, 2014. Water safety in distribution systems. https://apps.who.int/iris/bitstream/handle/10665/204422/9789241548892\_eng.pdf.

Wright, R.J., Erni-Cassola, G., Zadjelovic, V., Latva, M., Christie-Oleza, J.A., 2020. Marine plastic debris: a new surface for microbial colonization. Environ. Sci. Technol. 54, 11657-11672. https://doi.org/10.1021/acs.est.0c02305.