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Investigation of the effect of microplastics on the UV inactivation of antibiotic-resistant bacteria in water

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ABSTRACT

This study investigated the effect of polyethylene and polyvinyl chloride microplastics on the UV fluence response curve for the inactivation of multidrug-resistant *E. coli* and enterococci in ultrapure water at pH 6.0 ± 0.1. In the absence of microplastics, the UV inactivation of the studied bacteria exhibited an initial resistance followed by a faster inactivation of free (dispersed) bacteria, while in the presence of microplastics, these 2 regimes were followed by an additional regime of slower or no inactivation related to microplastic-associated bacteria (i.e., bacteria aggregated with microplastics resulting in shielding bacteria from UV indicated by tailing at higher UV fluences). The magnitude of the negative effect of microplastics varied with different microplastics (type/particle size) and bacteria (Gram-negative and Gram-positive). Results showed that when the UV transmittance of the microplastic-containing water was not taken into account in calculating UV fluences, the effect of microplastics as protectors of bacteria was overestimated. A UV fluence-based double-exponential microbial inactivation model accounting for both free and microplastic-associated bacteria could describe well the disinfection data. The present study elucidated the effect of microplastics on the performance of UV disinfection, and the approach used herein to prove this concept may guide future research on the investigation of the possible effect of other particles including nanoplastics with different characteristics on the exposure response curve for the inactivation of various microorganisms by physical and chemical disinfection processes in different water and wastewater matrices.

1. Introduction

Millions of tons of plastics are produced annually to meet the needs of modern society, from which the majority ends up in landfills or the natural environment as plastic waste (Geyer et al., 2017). This has resulted in the presence of plastics of different sizes in various environmental compartments worldwide (Ateia et al., 2022; Koelmans et al., 2019). For example, plastic debris at the micro size (1-5000 μm (Lim, 2021)), called microplastics, have been detected globally in air (Gasperi et al., 2018), soil (Boots et al., 2019; Scheurer and Bigalke, 2018; Zhang and Liu, 2018), wastewater (Blair et al., 2019; Park et al., 2020), seawater (Collignon et al., 2012), drinking water and surface waters such as lakes and rivers (Koelmans et al., 2019; Li et al., 2018; Yonkos et al., 2014), and are considered as emerging contaminants (Amato-Lourenço et al., 2020; Blair et al., 2019; Browne et al., 2007). The presence of microplastics in various environmental matrices has initiated concerns related to their possible adverse effects on human health and the ecosystem (Campanale et al., 2020; de Souza Machado et al., 2018; Green et al., 2017; Prata, 2018).

The last decade, wastewater treatment plants (WWTPs) received great attention as significant sources for microplastics in the aquatic environment (Cheng et al., 2021; Liu et al., 2021; Murphy et al., 2016; Park et al., 2020; Ziajahromi et al., 2017). For this reason, there was an increasing interest in investigating the presence of microplastics in WWTPs. For example, microplastics of polyethelene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) have been detected in influents and effluents of WWTPs worldwide (~0.0-5.6 mg/L) (Cheng et al., 2021; Edo et al., 2020; Grbić et al., 2020; Park et al., 2020; Yang

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et al., 2019). This indicates clearly that WWTPs are a pathway for microplastics connecting the anthropogenic activity and the aquatic environment (Wei et al., 2021; Ziajahromi et al., 2017). The detection of microplastics in both influents and effluents of WWTPs suggests the presence of microplastics in all wastewater treatment stages and processes taking place in WWTPs. Similar observations were reported for drinking water treatment plants and processes (Sarkar et al., 2021; Shen et al., 2020; Wang et al., 2020). The current study dealt with the effect of microplastics on water treatment performance.

Recently, the effect of microplastics on biological wastewater treatment processes was studied (Liu et al., 2019; Wei et al., 2021, 2019; Zhang et al., 2020). It was reported that, in a short time of 2 h, microplastics such as polyester, PE and PVC do not affect significantly the activities of ammonium-oxidizing bacteria, nitrite-oxidizing bacteria, denitrifiers, and polyphosphate accumulating organisms, and hence it was concluded that microplastics do not affect the performance of biological wastewater treatment in terms of nitrogen and phosphorous removal efficiencies (Liu et al., 2019). On the other hand, in a longer time of 264 d, PVC microplastics were reported to negatively affect the anaerobic granural sludge treatment process in terms of chemical oxygen demand removal efficiency and methane production (Zhang et al., 2020). Furthermore, PE microplastics were shown to inhibit the aerobic and anaerobic digestion of waste activated sludge due to the reduction of key bacteria through oxidative stress and/or the release of toxic chemicals, and due to the induction of reactive oxygen species, respectively (Wei et al., 2021, 2019). Other water treatment processes such as membrane filtration are also negatively affected by microplastics because of fouling mechanisms (Enfrin et al., 2020, 2019; Li et al., 2021). The present study focused on the effect of microplastics on the process of disinfection.

It is known that particles may affect the efficiency of UV disinfection by absorbing, scattering and/or blocking UV light, resulting in lower amount of light available for disinfection, reduced available UV energy, and/or shielding microorganisms, respectively (Christensen and Linden, 2003; Qualls et al., 1983). For example, it was reported that suspended particles in wastewater can negatively affect the UV inactivation of microorganisms by increasing the UV absorbance of the water and by shielding microorganisms from UV light (Christensen and Linden, 2003; Emerick et al., 1999). To this end, the current study investigated if microplastics in water cause similar phenomena.

Limited work has been done so far on the effect of microplastics on water disinfection efficiency (Enfrin et al., 2019). To the best of the authors' knowledge, the only reported study on this topic evaluated the effect of 2 types of microplastics (i.e., granular polyethylene microplastic and fibrous polyamide microplastic) on the time-based inactivation of *E. coli* by ultraviolet (UV) irradiation and chlorine in water (Shen et al., 2021). In this study, the results were interpreted based on the exposure of bacteria to the disinfecting agent, i.e., for UV disinfection, the UV fluence (mJ/cm^2) which is the product of the average UV intensity (mW/cm^2) and exposure time (s), and can be corrected with the UV transmittance (UVT) of the water matrix. This is of utmost importance in differentiating between possible interaction of microplastics with UV at 254 nm (which can be taken into account by correcting the UV fluence by the UVT of the water) and interaction of microplastics with bacteria (e.g., protection of bacteria by microplastics). Moreover, the effect of other microplastics with different behavior in water (e.g., PVC or PE of different particle size) on the UV inactivation of other fecal indicator bacteria (e.g., Gram-positive bacteria such as enterococci) has not been investigated yet. Although microplastics were reported as hotspots of antibiotic resistance genes (Liu et al., 2021), their effect on the UV inactivation of antibiotic-resistant bacteria has not been studied so far. These are important to better understand the impact of different microplastics on the performance of UV disinfection and their possible contribution to the spread of antibiotic resistance in water, through lower inactivation of antibiotic-resistant bacteria.

The present study investigated the effect of 3 microplastics on the UV inactivation of multidrug-resistant *E. coli* and enterococci in ultrapure water at pH 6.0 ± 0.1 and room temperature (25 ± 1 °C). The objectives of the study were to: (i) evaluate the effect of the concentration of microplastics on the UV inactivation of multidrug-resistant *E. coli* and enterococci, (ii) investigate the effect of polyethylene 125 μm (deposited at the surface of water), polyethylene 40-48 μm (well mixed in water), and polyvinyl chloride ≤250 μm (deposited at the bottom of the petri dish) microplastics on the UV fluence response curve for the inactivation of multidrug-resistant *E. coli* and enterococci, (iii) explore and differentiate the effect of microplastics-UV and microplastics-bacteria interactions on UV disinfection performance, (iv) develop a UV fluence-based double-exponential microbial inactivation model to predict the inactivation of free bacteria and microplastic-associated bacteria, and (v) calculate UV fluence requirements for 1-5 log reductions of multidrug-resistant *E. coli* and enterococci in both the absence and presence of microplastics. This paper is a proof-of-concept study that may guide future research on the investigation of the possible effect of different particles including nanoplastics with different characteristics on the exposure response curve for the inactivation of other microbial targets by physical and/or chemical disinfection processes.

2. Materials and methods

2.1. Chemicals and reagents

Polyethylene microplastics (ultra-high molecular weight, surface-modified, powder, average particle size: 125 μm (PE1) and particle size: 40-48 μm (PE2)) were purchased from Sigma Aldrich (MO, USA). Polyvinyl chloride microplastics (unplasticised, powder, particle size: ≤250 μm (PVC)) were purchased from Goodfellow Cambridge Limited (England, UK). The selection of polyethylene (PE1 and PE2) and polyvinyl chlorine (PVC) plastics was based on their frequent detection in the environment (Alimi et al., 2018; Rochman et al., 2013). The particle size of PE1 and PVC microplastics (i.e., 125 μm and ≤250 μm respectively) is within the particle size range of the microplastics with the highest abundance in wastewater, i.e., 100-500 μm (Zhang et al., 2020). The lower particle size of PE2 microplastics (i.e., 40-48 μm) allowed for better mixing and hence the incorporation of the UVT of the PE2 microplastic-containing water in the calculation of UV fluence. Information about the microplastics used in the study is presented in Table 1. Details on other chemicals and reagents used in this study are given in Text S1.

2.2. Multidrug-resistant E. coli and enterococci

The *E. coli* and enterococci bacteria were isolated from raw wastewater collected at the inlet of a WWTP in Cyprus, through the inoculation of 0.1 mL of wastewater on Chromocult Coliform Agar (CCA) and Slanetz and Bartley Agar (SBA) respectively, which were spiked with the three antibiotics of interest, i.e., trimethoprim, ciprofloxacin and ampicillin, followed by incubation at 44 °C for 24 h for *E. coli* and 37 °C for 48 h for enterococci. Bacterial stocks of the cultivated multidrug-resistant bacteria to the aforementioned antibiotics were recultivated on Tryptic Soy Agar (TSA). Prior to UV disinfection experiments, ~4 recultivated colonies were selected and inoculated into sterile tubes containing 10 mL of tryptic soy broth (TSB), and then incubated overnight at 37 °C. The formed pellet was collected by centrifugation at 3500 RCF for 15 min and then resuspended in a phosphate-buffered saline (PBS) solution. The initial concentration of antibiotic-resistant bacteria spiked in water for disinfection experiments was in line with their concentration in wastewaters, i.e., ~10^4-10^5 CFU/100 mL (Balachandran et al., 2021; Pepper et al., 2018). Details regarding the selection of the concentrations of the antibiotics are given in Text S2.

Table 1
Information about the microplastics used in the study.

Microplastics	Abbreviation	Particle size (μm)	Density at 25 °C (g/mL)	Behavior in water	UV transmittance (UVT) at 254 nm (%)*
Polyethylene	PE1	125	0.94	deposited at the surface of water	98.5 ± 0.5
Polyethylene	PE2	40-48	0.94	well mixed in water	86.4 ± 2.7
Polyvinyl chloride	PVC	≤250	1.38	deposited at the bottom of the petri dish	99.5 ± 0.2

* The concentration of microplastics was 1.0 g/L; In the absence of microplastics: UVT(%) = 99.7 ± 0.3.

2.3. UV disinfection experiments in the presence of microplastics

A bench-scale collimated beam apparatus (Trojan Technologies, TrojanUV, London, Ontario, Canada) coupled with a low pressure-UV lamp emitting at 254 nm was used to study the UV fluence-based inactivation kinetics for multidrug-resistant bacteria, in the absence and in the presence of microplastics. The petri factor, which is the ratio of the average UV intensity over the area of the petri dish to the central UV intensity was determined as 0.91 indicating a well-designed collimated beam apparatus (Bolton and Linden, 2003). Experiments were conducted in Milli-Q water at pH 6.0 ± 0.1 and room temperature (25 ± 1 °C). To prove the concept of the effect of microplastics on the UV fluence response curve for the inactivation of bacteria, ultrapure water (Milli-Q) without buffer (pH 6.0) was used to rule out a possible effect of wastewater constituents and/or buffer ions on microplastics and/or on the inactivation of bacteria, and hence to ensure that the observed effect is only related to the interaction of microplastics with bacteria and/or microplastics with UV light. Microplastics (PE1, PE2 or PVC) were individually added in a glass petri dish of external diameter of 6.0 cm and height of 3.5 cm, followed by the addition of 59.95 mL water and the spiking of 0.05 mL of multidrug-resistant *E. coli* or enterococci solution. The concentration of microplastics was 0.25-1.0 g/L which is in agreement with the concentrations of microplastics used in reported studies on the interaction between microplastics and chemical pollutants in water (Atugoda et al., 2021; Chen et al., 2021; Elizalde-Velázquez et al., 2020; Liu et al., 2020). In addition to being in line with the aforementioned studies, these concentrations of microplastics were used to have a measurable difference in UVT between water and microplastic-containing water which is essential to prove the concept of the effect of microplastics on UV disinfection. The solution containing bacteria and microplastics was stirred using magnetic stirrer for 2 h, to allow a possible interaction between microplastics and bacteria. Then, a 10-mL sample was taken to measure the UVT of the solution at 254 nm and the initial concentration of multidrug-resistant *E. coli* or enterococci (i.e., before their exposure to UV). Prior to exposing the water to UV, the central UV intensity, at the same height with the surface of the water, was measured using a radiometer (the distance from the surface of the water to the UV lamp was 45 cm). To initiate a disinfection experiment, the 50-mL solution was exposed to UV by placing the petri dish on a magnetic stirrer beneath the collimated beam. Experiments were performed at several UV fluences, i.e., 0.0, 2.5, 5.0, 7.5, 8.5, 10.0, 12.5, 15.0, and 18.0 mJ/cm^2 for multidrug-resistant *E. coli* and 0.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 18.0, and 20.0 mJ/cm^2 for multidrug-resistant enterococci. The required exposure time (s) to achieve the targeted UV fluence (mJ/cm^2) was calculated by the UVT (%) of the water (with or without microplastics), the internal diameter of the petri dish (5.6 cm), the volume of the solution (50 mL), and the central UV intensity (mW/cm^2) (Bolton and Linden, 2003; Chowdhury et al., 2020; Venditto et al., 2022). Experiments were conducted in triplicate and the averages with standard deviations are presented. The 95% prediction interval of the UV fluence-based microbial inactivation kinetic model used in this study was calculated using SigmaPlot software.

2.4. Analytical methods

The cultivable multidrug-resistant bacteria were enumerated by the membrane filtration method (Novo and Manaia, 2010). Details on bacteria enumeration are given in Text S3. The UVT of water at 254 nm was measured using a UV/VIS spectrophotometer (Jasco, V-530) and the UV intensity at 254 nm was recorded by a UVC light meter (Lutron Electronic, UVC-254A). An EZDO PL-600 pH meter was used to measure the pH.

3. Results and discussion

3.1. Effect of the concentration of microplastics on UV disinfection performance

Initially, the effect of concentration of microplastics on the UV inactivation of multidrug-resistant *E. coli* and enterococci was assessed. Experiments were performed at different concentrations of PE1 microplastics (i.e., 0.25 g/L, 0.5 g/L and 1.0 g/L), at a UV fluence of 10.0 mJ/cm^2 and 15.0 mJ/cm^2 for multidrug-resistant *E. coli* and enterococci, respectively. The results in the presence of microplastics were compared with the results in the absence of microplastics (control or 0.0 g/L). Fig. 1 shows no effect of PE1 microplastics on the UV inactivation of the studied bacteria at concentrations of 0.25 g/L and 0.5 g/L, as the same reduction of bacteria in the presence and in the absence of microplastics was observed. This was consistent for both multidrug-resistant *E. coli* and enterococci (Figs. 1a and b). At the highest PE1 concentration used (1.0 g/L), the log reduction of multidrug-resistant *E. coli* decreased from ~5.5 to ~2.5. At 1.0 g/L PE1 microplastics, a lower disinfection performance was also observed for multidrug-resistant enterococci, i.e., the log reduction decreased from ~5.5 (without microplastics) to ~3.5 (with microplastics). These observations indicate a negative effect of microplastics on the UV disinfection performance which resulted in a 3-log and 2-log lower reduction of multidrug-resistant *E. coli* and enterococci, respectively, due to the presence of 1.0 g/L PE1 microplastics compared with their absence. It is worth noting that preliminary experiments showed no difference in the concentration of multidrug-resistant *E. coli* or enterococci before (without microplastics) and after the 2-h mixing with microplastics (Figs. S1a and b), indicating no reduction of bacteria due to microplastics alone (i.e., before their exposure to UV). This shows clearly that microplastics at 1.0 g/L affected negatively the performance of UV disinfection (Figs. 1 and S1). This observation is in line with the known negative effect of suspended solids on the efficiency of UV disinfection of secondary effluent wastewater (Azimi et al., 2012). It is also well documented in the literature that particles in water cause tailing phenomena at high UV fluences (Azimi et al., 2012). To examine if microplastics cause a similar tailing effect, experiments at different UV fluences were required.

3.2. Effect of UV fluence on UV disinfection performance in the presence of PE1 microplastics

To evaluate the effect of UV fluence on the inactivation of multidrug-resistant *E. coli* and enterococci, experiments were performed at different UV fluences, i.e., 0.0-18.0 mJ/cm^2 and 0.0-20.0 mJ/cm^2 respectively, in the absence and in the presence of PE1 microplastics. For the tests in the presence of microplastics, the concentration of PE1 microplastics was kept constant at 1.0 g/L (Fig. 1). The developed UV fluence response curves for the inactivation of multidrug-resistant *E. coli* and enterococci in the absence and in the presence of PE1 microplastics are presented in Figs. 2a and 3a, respectively.

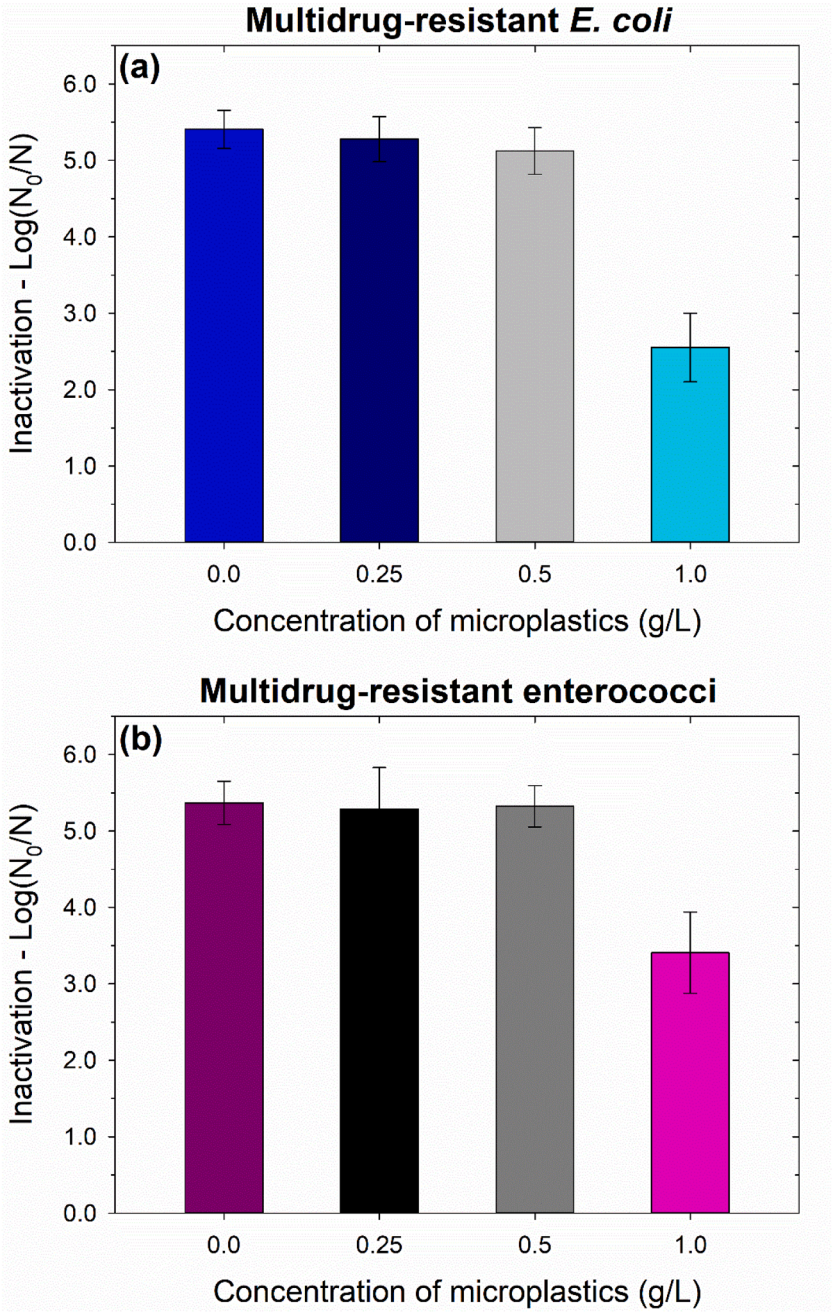

Fig. 1. Effect of the concentration of polyethylene 1 (PE1; 125 μm) microplastics on the UV inactivation of multidrug-resistant *E. coli* (a) and enterococci (b) in water. (Experimental conditions: [multidrug-resistant *E. coli*] = $7.35{\cdot}10^4$-$9.97{\cdot}10^5$ CFU/100 mL; [multidrug-resistant enterococci] = $4.10{\cdot}10^4$-$7.80{\cdot}10^5$ CFU/100 mL; UV fluence (multidrug-resistant *E. coli*) = 10.0 mJ/cm^2; UV fluence (multidrug-resistant enterococci) = 15.0 mJ/cm^2; [PE1] = 0.0-1.0 g/L; pH = 6.0 ± 0.1; T = 25 ± 1 °C).

Lower inactivation of multidrug-resistant *E. coli* was observed in the presence than the absence of PE1 microplastics. For example, at a UV fluence of 8.5 mJ/cm^2, the inactivation of multidrug-resistant *E. coli* decreased from ~3.5 log (without microplastics) to 2.0 log (with microplastics) (Fig. 2a). At the highest UV fluence applied in the absence of PE1 microplastics (10.0 mJ/cm^2), the difference in inactivation of multidrug-resistant *E. coli* was the highest, i.e., ~2.5 log and ~5.5 log reduction in the presence and in the absence of PE1 microplastics respectively. A lower inactivation was also seen in the case of multidrug-resistant enterococci. The log reduction of multidrug-resistant enterococci decreased from ~3.5 to 2.0 at a UV fluence of 12.5 mJ/cm^2, and from ~5.5 to 3.5 at UV fluence of 15.0 mJ/cm^2, in the absence and in the presence of PE1 microplastics, respectively. Results of experiments at higher UV fluences in the presence of PE1 microplastics showed tailing effect, i.e., a slower kinetic regime, which has not been observed in the absence of microplastics (Figs. 2a and 3a). This tailing was observed for both multidrug-resistant *E. coli* and enterococci.

The aforementioned negative effect of PE1 microplastics on the efficiency of the process of UV disinfection may be due to the interaction of microplastics with UV light (i.e., consumption of the UV irradiation by microplastics) and/or the interaction of microplastics with bacteria (i.e., bacteria aggregated with microplastics resulting in shielding bacteria from UV). It should be noted that the UVT of water in the absence and in the presence of PE1 microplastics was similar, i.e., 99.7% and 98.5% respectively (Table 1). The reason for this similarity in UVT is that PE1 microplastics have lower density than water (i.e., 0.94 g/mL versus 1.00 g/mL), causing PE1 microplastics to be deposited at the surface of the water in the cuvette and hence a similar measurement of UVT by the spectrophotometer was recorded. This means that the interaction of PE1 microplastics and UV light was not captured during the UVT measurement, and therefore it was not taken into account when calculating the UV fluence. Considering this, we could not differentiate if the observed

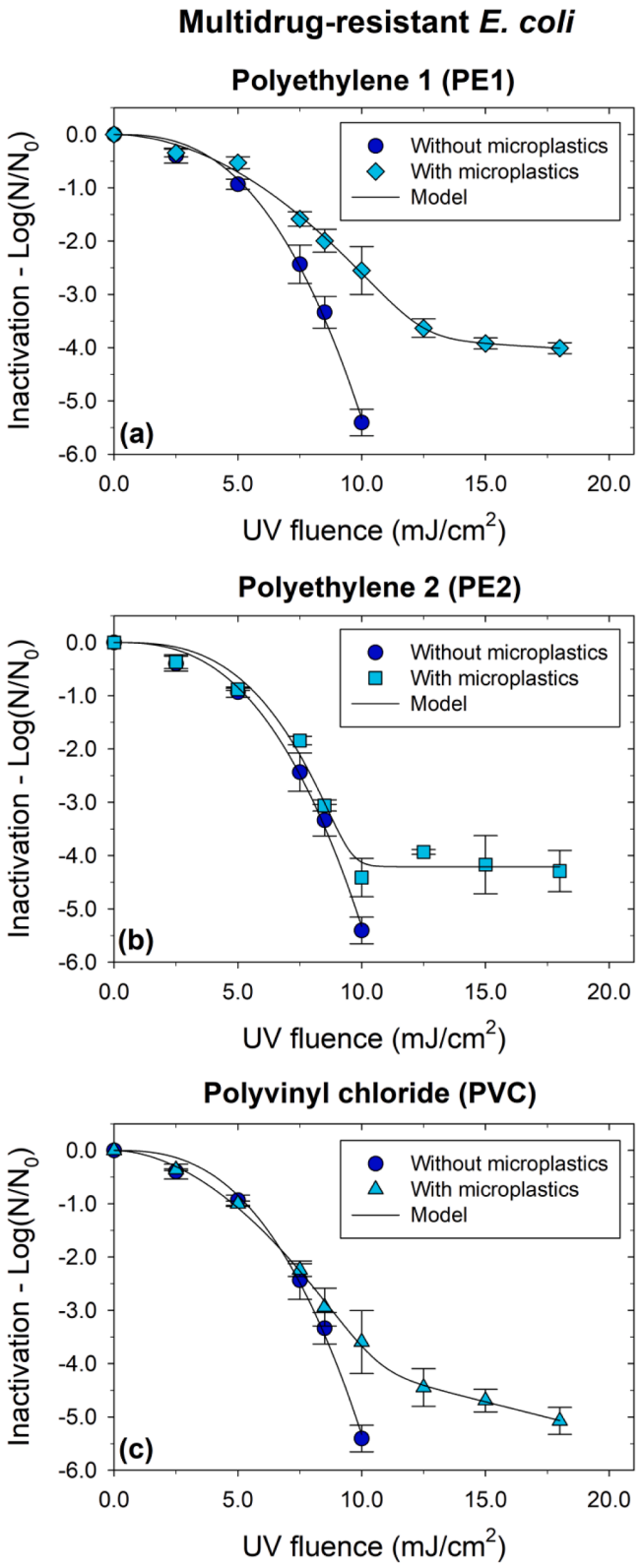

Fig. 2. Effect of polyethylene 1 (PE1; 125 μm) (a), polyethylene 2 (PE2; 40-48 μm) (b), and polyvinyl chloride (PVC; ≤250 μm) (c) microplastics on the UV fluence response curve for the inactivation of multidrug-resistant *E. coli* in water. (Experimental conditions: [multidrug-resistant *E. coli*] = $4.35 \cdot 10^4$-$9.97 \cdot 10^5$ CFU/100 mL; [PE1] = [PE2] = [PVC] = 1.0 g/L; pH = 6.0 ± 0.1; T = 25 ± 1 °C).

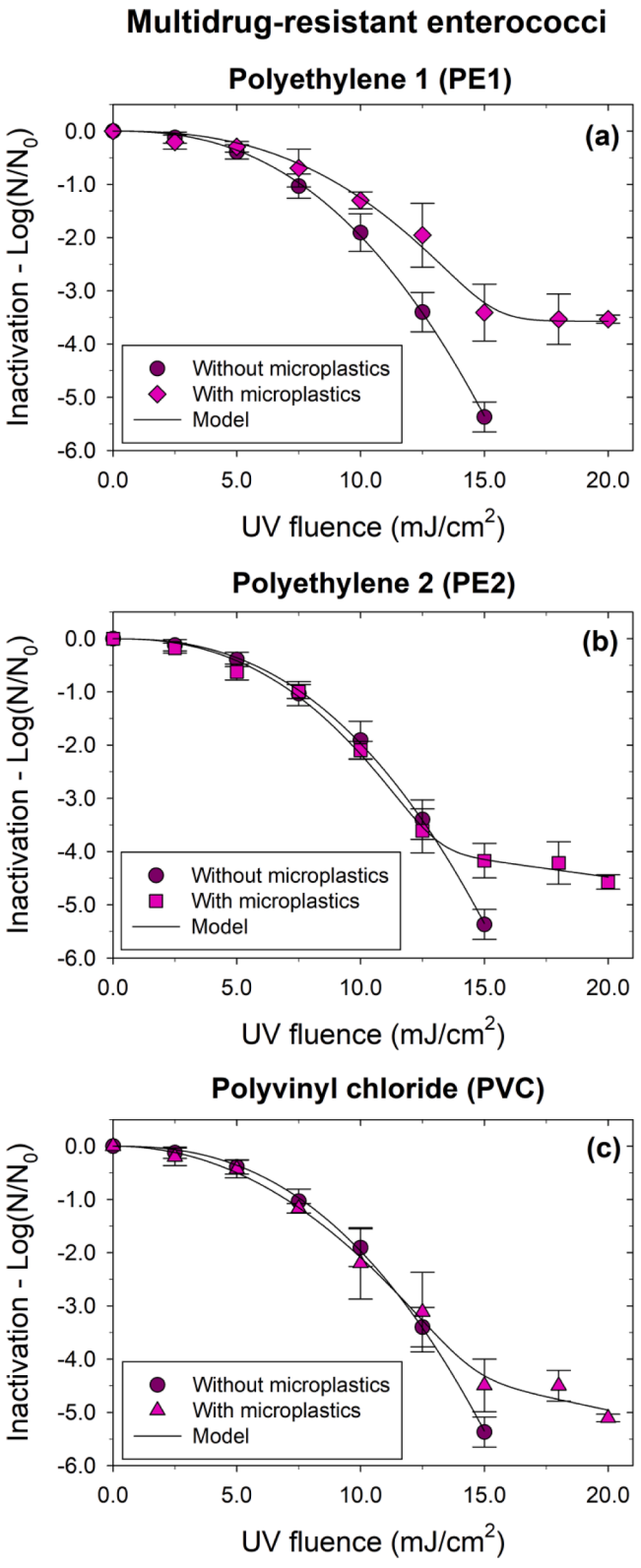

Fig. 3. Effect of polyethylene 1 (PE1; 125 μm) (a), polyethylene 2 (PE2; 40-48 μm) (b), and polyvinyl chloride (PVC; ≤250 μm) (c) microplastics on the UV fluence response curve for the inactivation of multidrug-resistant enterococci in water. (Experimental conditions: [multidrug-resistant enterococci] = $1.25 \cdot 10^4$-$9.80 \cdot 10^5$ CFU/100 mL; [PE1] = [PE2] = [PVC] = 1.0 g/L; pH = 6.0 ± 0.1; T = 25 ± 1 °C).

negative effect of PE1 microplastics on the inactivation of multidrug-resistant *E. coli* and enterococci was due to the interaction of PE1 microplastics with UV and/or with bacteria. It is known that the accurate measurement of UV fluence is important for the interpretation of results obtained by UV experiments (Almuhtaram et al., 2021). The presence of particles in water generally increases the UV absorbance of water and hence decreases the UVT, which is one of the factors affecting the UV fluence measurement (Christensen and Linden, 2003). However, the effect of microplastics on the inactivation of bacteria in the domain of UV fluence corrected by the UVT of the microplastic-containing water has not been investigated yet (Shen et al., 2021). This is essential to elucidate the role of microplastics in decreasing the UV inactivation of bacteria in water, and requires the use of a microplastic that allows for the incorporation of UVT in calculating the UV fluence.

3.3. Effect of PE2 microplastics on UV disinfection performance

To get insights into the reason for the observed negative effect of microplastics on the UV disinfection performance, an identical set of experiments was performed using a microplastic of lower particle size, i.e., PE2 (Table 1). Although the 2 polyethylene microplastics used in the study, PE1 and PE2, have the same density, the lower particle size of PE2 microplastics allowed for a good dispersion of the microplastics in water under mixing, and the interaction of PE2 microplastics and UV light at 254 nm could be captured by the UVT measurement and used for the calculation of UV fluence (i.e., UVT of 86.4% in the presence of PE2 microplastics and 99.7% in the absence of PE2 microplastics; Table 1). Figs. 2b and 3b show the UV fluence response curve for the inactivation of multidrug-resistant *E. coli* and enterococci, respectively, in the presence of PE2 microplastics compared with the absence of PE2 microplastics. A similar UV fluence-based inactivation of the studied bacteria without and with PE2 microplastics was observed at a UV fluence range of 2.5-8.5 mJ/cm^2 for multidrug-resistant *E. coli* and 2.5-12.5 mJ/cm^2 for multidrug-resistant enterococci. Interestingly, the log reduction decreased at the highest UV fluence, i.e., from ~5.5 to ~4.5 (multidrug-resistant *E. coli*) and from ~5.5 log to ~4.0 log (multidrug-resistant enterococci) in the absence and in the presence of PE2 microplastics, respectively. A tailing was observed for both bacteria at higher UV fluences (Figs. 2b and 3b).

Considering that, in the case of PE2 microplastics, the interaction of microplastics and UV was taken into account by UVT (Table 1), the findings suggest that the ~1-log lower inactivation of multidrug-resistant *E. coli* and the ~1.5-log lower inactivation of multidrug-resistant enterococci are due to a possible protection of bacteria by microplastics. The aforementioned observation of the negative effect of PE2 microplastics indicates that only a small fraction of the multidrug-resistant *E. coli* or enterococci was associated with microplastics, i.e., ~1 log for *E. coli* and ~1.5 log for enterococci. This is in agreement with the similar inactivation with and without PE2 microplastics at UV fluence < 10.0 mJ/cm^2 for multidrug-resistant *E. coli* and UV fluence < 15.0 mJ/cm^2 for multidrug-resistant enterococci, and the tailing effect at higher UV fluences, i.e., free bacteria were inactivated first followed by a slower or no inactivation of microplastic-associated bacteria. This is in line with reported studies on the inactivation of fecal indicator bacteria (e.g., *E. coli*, fecal coliforms and enterococci) by several chemical disinfecting agents such as peracetic acid, performic acid, and ferrate (VI), where the small fraction of bacteria (1-2 log) that was associated with particles, such as total suspended solids, in wastewaters (i.e., particle-associated bacteria) exhibited slower exposure-based inactivation than free (dispersed) bacteria, indicated by tailing at higher exposure values (Campo et al., 2020; Maffettone et al., 2020; Manoli et al., 2020, 2019). This was also reported for the UV disinfection of wastewater containing suspended solids (e.g., bioflocs) (Azimi et al., 2012). The results suggest a similar phenomenon in the case of microplastics, i.e., the multidrug-resistant *E. coli* and enterococci aggregated with microplastics (i.e., microplastic-associated multidrug-resistant *E. coli* or enterococci) resulting in shielding these bacteria from the UV irradiation leading to a slower inactivation than free bacteria.

It is worth noting that the differentiation between the two possible reasons for the negative effect of microplastics on UV disinfection can only be made in the domain of UV fluence corrected by the UVT of the water with or without microplastics. This is important because the water containing PE2 microplastics has a lower UVT than the water without PE2 microplastics (Table 1), and therefore, a longer exposure time is needed to deliver the same UV fluence in the presence than in the absence of PE2 microplastics. To further elucidate this, we performed experiments in the presence of PE2 microplastics where the UV fluence was calculated using the UVT of the water without PE2 microplastics ($UVT_{Control}$) instead of the UVT in the presence of PE2 microplastics (UVT_{PE2}), and we compared the results with the ones obtained by the experiment without microplastics (Control). Based on the hypothesis of 2 different reasons for the negative effect of microplastics on UV disinfection (i.e., microplastics-UV and microplastics-bacteria interactions), this experiment should have given different results, i.e., higher inactivation of bacteria at UVT_{PE2} than $UVT_{Control}$, since the first indicates the effect of PE2-bacteria interaction only and the later includes both the effects of PE2-UV and PE2-bacteria interactions. Results are shown in Fig. 4. When the $UVT_{Control}$ was used to calculate the UV fluence, the inactivation of multidrug-resistant *E. coli* decreased from ~5.5 log to ~3.5 log in the absence of PE2 microplastics (Control) and in the presence of PE2 microplastics ($UVT_{Control}$), respectively. This 2-log difference in the reduction of bacteria is two times higher than the difference in inactivation when the UVT_{PE2} was used to calculate the UV fluence (1-log lower reduction, from 5.5 log to 4.5 log) (Fig. 4a). This suggests that the PE2-bacteria interaction (i.e., protection of bacteria by PE2 microplastics) and the PE2-UV interaction (consumption of UV by PE2 microplastics) are responsible for 1-log lower inactivation of multidrug-resistant *E. coli* each (Fig. 4a). This observation is consistent with the results obtained for multidrug-resistant enterococci. At $UVT_{Control}$ with PE2 microplastics, a 2-log lower reduction than the absence of PE2 microplastics was observed, while the inactivation decreased by 1.5-log at UVT_{PE2} (Fig. 4b). To the best of the authors' knowledge, this is the first time that the effect of microplastics on disinfection was clarified in this manner, showing that microplastics may interact with bacteria in water and that these microplastic-associated bacteria have slower inactivation kinetics compared with the free bacteria. It was also clearly shown herein that no incorporation of the UVT in calculating the UV fluence may result in overestimation of the effect of microplastics as protectors of bacteria (Figs. 4a and b).

3.4. Effect of PVC microplastics on UV disinfection performance

To expand the study to another commonly found type of microplastic in water, similar experiments were performed using PVC. Figs. 2c and 3c show the UV fluence response curve for the inactivation of multidrug-resistant *E. coli* and enterococci, respectively, in the presence of PVC microplastics compared with their absence. In the case of multidrug-resistant *E. coli*, for up to a UV fluence of 8.5 mJ/cm^2, no effect of PVC microplastics was observed. Interestingly, at a higher UV fluence of 10.0 mJ/cm^2, the inactivation of multidrug-resistant *E. coli* decreased from ~5.5 log to ~3.5 log due to the presence of PVC microplastics (Fig. 2c). A similar trend was observed for the inactivation of multidrug-resistant enterococci, i.e., no effect of PVC microplastics for up to 12.5 mJ/cm^2, while at a UV fluence of 15.0 mJ/cm^2, the inactivation of multidrug-resistant enterococci decreased from ~5.5 log to 4.5 log without and with PVC microplastics, respectively (Fig. 3c). At higher UV fluences, a tailing was observed for both multidrug-resistant *E. coli* and enterococci.

It should be noted that PVC microplastics had different behavior than PE microplastics in water, i.e., PVC microplastics have higher density than water (i.e., 1.38 g/mL versus 1.00 g/mL) resulting in their deposition at the bottom of the petri dish. That being said, the observed effect

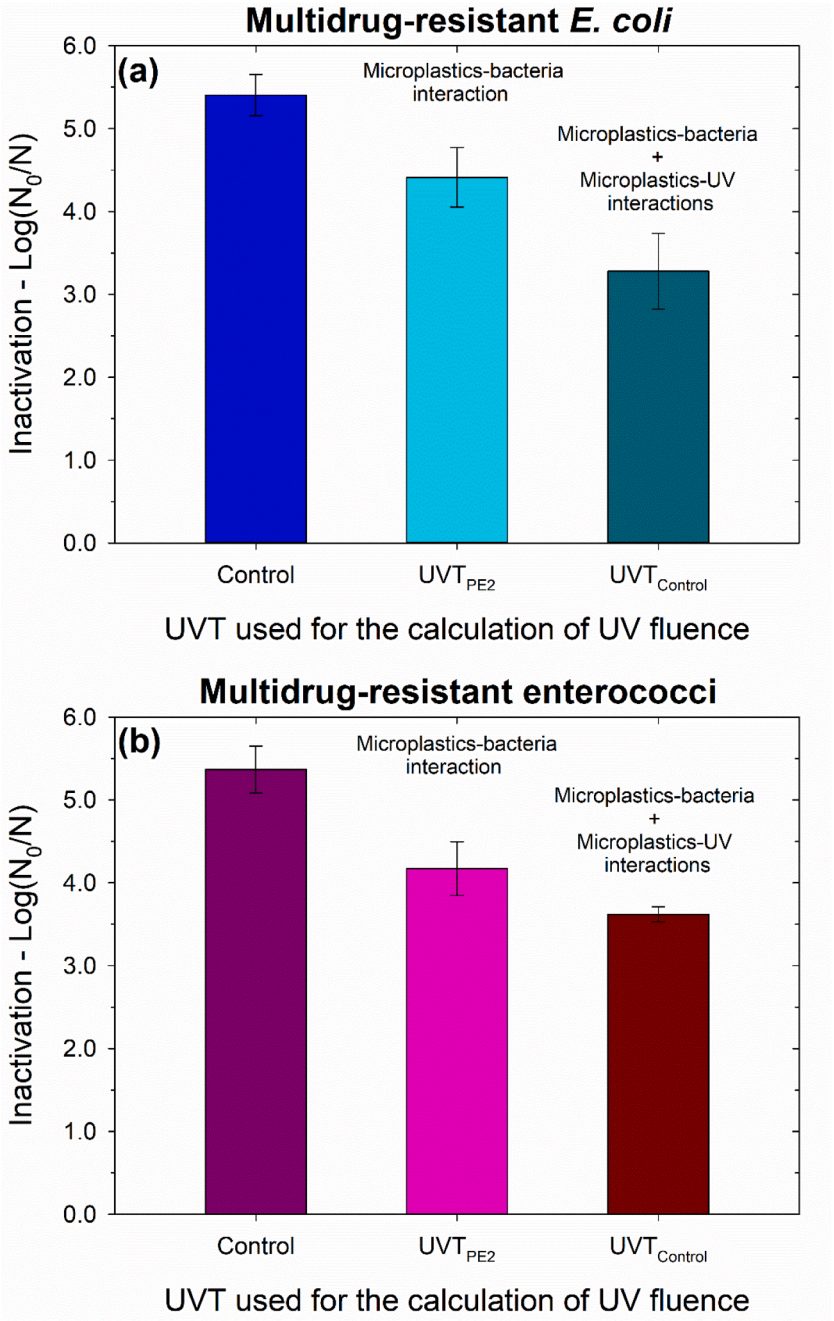

Fig. 4. UV inactivation of multidrug-resistant *E. coli* (a) and enterococci (b) in the absence of microplastics (Control), and in the presence of polyethylene 2 (PE2; 40-48 μm) in water when the UV transmittance (UVT) of the PE2 was taken into account in calculating the UV fluence (UVT_{PE2}) and when it was not ($UVT_{Control}$). (Experimental conditions: [multidrug-resistant *E. coli*] = $4.20{\cdot}10^4$-$9.97{\cdot}10^5$ CFU/100 mL; [multidrug-resistant enterococci] = $3.05{\cdot}10^4$-$6.14{\cdot}10^5$ CFU/100 mL; UV fluence (*E. coli*) = 10.0 mJ/cm^2; UV fluence (enterococci) = 15.0 mJ/cm^2; [PE2] = 1.0 g/L; $UVT_{Control}$ (%) = 99.7 ± 0.3; UVT_{PE2} (%) = 86.4 ± 2.7; pH = 6.0 ± 0.1; T = 25 ± 1°C).

of PVC microplastics on UV disinfection is due to a possible interaction of bacteria with PVC microplastics (or protection of bacteria by PVC microplastics), despite the fact that the measured UVT in the presence of PVC microplastics is similar to their absence (i.e., 99.5% and 99.7% respectively) (Table 1). Since the UV irradiation occurs from the top to the bottom using a collimated beam, the UV light may interact with the PVC microplastics only after it is transmitted through the water sample. Significantly, the PVC results (Figs. 2c and 3c) are consistent with the results obtained using PE2 microplastics (Figs. 2b and 3b), where a negative effect of microplastics on the UV disinfection was only observed at a UV fluence of 10.0 mJ/cm^2 for multidrug-resistant *E. coli* and 15.0 mJ/cm^2 for multidrug-resistant enterococci. The results of both PE2 and PVC microplastics suggest that a fraction of multidrug-resistant *E. coli* or enterococci was associated with microplastics, leading to their slower inactivation than free bacteria, which was indicated by a tailing effect. This is consistent with the fact that no difference in the inactivation of the studied bacteria with and without PVC or PE2 microplastics was observed at UV fluence ≤ 8.5 mJ/cm^2 for *E. coli* and UV fluence ≤ 12.5 mJ/cm^2 for enterococci, i.e., rapid inactivation of free bacteria followed by a slower or no inactivation of microplastic-associated bacteria (Figs. 2 and 3). The results are in line with the slower UV inactivation of particle-associated microorganisms than free microorganisms due to the presence, for example, of total suspended solids (>1.2 μm) in wastewater (Azimi et al., 2012; Christensen and Linden, 2003; Emerick et al., 2000; Hu et al., 2007). Interestingly, the magnitude of the effect of solids on the UV inactivation of bacteria varied with the size of the particles (Azimi et al., 2012). Taking this into account and considering that, in the UV fluence domain, the effect of microplastics on UV disinfection depends predominantly on the interaction of microplastics with bacteria (i.e., microplastic-associated bacteria), the different effect of microplastics observed herein (Figs. 2b, c, 3b and c) may be due to either their different type (i.e., polyethylene

versus polyvinyl chloride) or their different particle size (i.e., 40-48 μm versus ≤250 μm) or both.

3.5. Inactivation kinetic modeling in the absence and presence of microplastics

In the absence of microplastics, the UV inactivation of multidrug-resistant *E. coli* and enterococci, which were free (dispersed) bacteria in water, exhibited an initial resistance followed by a faster inactivation (Figs. 2 and 3). To describe both inactivation regimes, a UV fluence-based inactivation model that includes a parameter m describing initial resistance of bacteria to UV (shoulder effects; $m > 1$) was used (Eq. (1)) (Balachandran et al., 2021; Haas and Joffe, 1994):

$$\frac{N}{N_0} = e^{-k_F \cdot (\text{UV fluence})^m} \quad (1)$$

where N_0 and N are the concentrations of multidrug-resistant *E. coli* or enterococci (CFU/100 mL) initially and after exposure to UV respectively, UV fluence (also known as UV dose) is the exposure of multidrug-resistant *E. coli* or enterococci to UV (mJ/cm^2), and k_F is a UV fluence-based inactivation rate constant for free multidrug-resistant bacteria ((cm^2/mJ)^m).

In the presence of microplastics, the aforementioned inactivation regimes were followed by a tailing phase, i.e., a slower or no inactivation of the microplastic-associated bacteria. The biphasic behavior due to the different UV inactivation kinetics of free and microplastic-associated bacteria was described with a UV fluence-based double-exponential microbial inactivation model (Eq. (2)) (Manoli et al., 2019; Santoro et al., 2015):

$$\frac{N}{N_0} = \left((1-\beta)\cdot e^{-k_F \cdot (\text{UV fluence})^m}\right) + \left(\beta \cdot e^{-k_{MP} \cdot (\text{UV fluence})}\right) \quad (2)$$

where β is the fraction of the microplastic-associated bacteria and k_{MP} is a UV fluence-based inactivation rate constant for microplastic-associated multidrug-resistant bacteria (cm^2/mJ). The inactivation model parameters, k_F and m in the absence of microplastics (Eq. (1)) and β, k_F, m and k_{MP} in the presence of microplastics (Eq. (2)), were fitted simultaneously by Excel solver with the aim to minimize the difference between experimental and model-predicted inactivation data. The determined parameters are presented in Table 2.

Table 2
Inactivation kinetic parameters for multidrug-resistant *E. coli* and enterococci by UV in water, in the absence and presence of microplastics at pH 6.0 ± 0.1 and 25 ± 1 °C.

Microplastics*	β (10^{-2})	k_F (cm^2/mJ)^m	m	k_{MP} (cm^2/mJ)
Multidrug-resistant *E. coli*				
No microplastics	-	0.027 ± 0.004	2.657 ± 0.174	-
PE1**	0.031 ± 0.004	0.077 ± 0.032	1.895 ± 0.344	0.064 ± 0.011
PE2***	0.006 ± 0.001	0.014 ± 0.002	2.918 ± 0.746	0.000 ± 0.000
PVC****	0.109 ± 0.009	0.129 ± 0.030	1.838 ± 0.110	0.270 ± 0.053
Multidrug-resistant enterococci				
No microplastics	-	0.016 ± 0.002	2.462 ± 0.046	-
PE1	0.027 ± 0.006	0.009 ± 0.004	2.511 ± 0.426	<0.001
PE2	0.063 ± 0.003	0.020 ± 0.005	2.398 ± 0.414	0.147 ± 0.006
PVC	0.107 ± 0.041	0.041 ± 0.003	2.070 ± 0.284	0.229 ± 0.020

* The concentration of microplastics was 1.0 g/L
** Polyethylene (125 μm)
*** Polyethylene (40-48 μm)
**** Polyvinyl chloride (≤250 μm)

Interestingly, a similar initial resistance was observed in the absence and in the presence of different microplastics. For example, at a UV fluence of 5.0 mJ/cm^2, a log reduction of ≤ 1 was seen without and with microplastics for multidrug-resistant *E. coli*. This was consistent for all microplastics used in the study, i.e., PE1, PE2 and PVC microplastics. Significantly, the initial resistance of multidrug-resistant *E. coli* to UV that was observed herein for up to 5.0 mJ/cm^2 was in reasonable agreement with the initial resistance of *E. coli* to UV of up to ~4.0 mJ/cm^2 without microplastics reported earlier (Sun et al., 2016). In the case of multidrug-resistant enterococci, the initial resistance, which was similar for all microplastics and without microplastics, was up to 7.5 mJ/cm^2. The findings suggest that the studied microplastics did not affect the initial resistance of bacteria to UV. To the best of the author's knowledge, this is the first study to draw the abovementioned conclusion. This observation is in agreement with the inactivation model parameter m, which was determined as higher than 1 in both the absence and the presence of microplastics, indicating shoulder effects (Table 2). The phenomenon of initial resistance of bacteria to UV observed herein ($m > 1$) is in line with the initial resistance of bacteria to disinfecting agents in the absence of microplastics, e.g., *E. coli*, fecal coliforms and enterococci to peracetic acid (Campo et al., 2020; Maffettone et al., 2020), *E. coli*, fecal coliforms, enterococci, pseudomonas aeruginosa and total heterotrophs to ozone (Iakovides et al., 2021), enterococci to performic acid (Maffettone et al., 2020), and antibiotic-resistant *E. coli* and enterococci to peracetic acid (Balachandran et al., 2021; Campo et al., 2020). Phenomena of initial resistance were also reported for UV disinfection (without microplastics), e.g., *E. coli* and *Bacillus subtilis* spores (Hijnen et al., 2006; Sun et al., 2016). This is not always the case though, for example, no shoulder effects were observed for the inactivation of fecal coliforms by ferrate(VI) (Manoli et al., 2020), and murine norovirus did not exhibit initial resistance to UV, peracetic acid, ferrate(VI) and performic acid (Maffettone et al., 2020; Manoli et al., 2020).

The inactivation of microplastic-associated bacteria by UV in water varied with different microplastics and bacteria (Figs. 2 and 3). For example, the PVC microplastic-associated multidrug-resistant *E. coli* had faster inactivation than enterococci, i.e., k_{MP} of 0.270 cm^2/mJ and 0.229 cm^2/mJ, respectively. On the other hand, no UV inactivation of PE2 microplastic-associated *E. coli* was seen, while the observed inactivation of PE2 microplastic-associated enterococci was slower than PVC microplastic-associated enterococci (i.e., k_{MP} of 0.147 cm^2/mJ). These findings suggest that the magnitude of the effect of microplastics on UV disinfection depended on the characteristics of both microplastics (e.g., PE (40-48 μm) and PVC (≤250 μm)) and bacteria (Gram-negative (*E. coli*) and Gram-positive (enterococci) which have structural differences such as the presence versus the absence of an outer lipid membrane, respectively (Balachandran et al., 2021)).

The model used herein could describe well all 3 regimes of the UV inactivation of multidrug-resistant bacteria in the presence of microplastics Eq. (2)), i.e., initial resistance at lower UV fluences followed by a faster inactivation of free bacteria followed by tailing at higher UV fluences due to the slow inactivation of microplastic-associated bacteria (Figs. 2 and 3). Importantly, the microbial inactivation models (Eqs. (1) and ((2)) predicted well the inactivation of the studied bacteria in both the absence and presence of microplastics ($R^2 \geq 0.993$) (Fig. 5). The good prediction of the model allowed for the calculation of model-predicted UV fluences to achieve 1-5 log reductions of multidrug-resistant *E. coli* or enterococci in the absence and in the presence of PE1, PE2, or PVC microplastics. The results are shown in Table 3.

In the case of PE1 microplastics, where the UV fluence could not be corrected by the UVT, higher UV fluences were required compared to the absence of microplastics for 2-5 log reductions of multidrug-resistant *E. coli* and 1-5 log reductions for multidrug-resistant enterococci. For PE2 and PVC microplastics, where the UVT was incorporated in calculating UV fluence, higher UV fluence requirements than the absence of

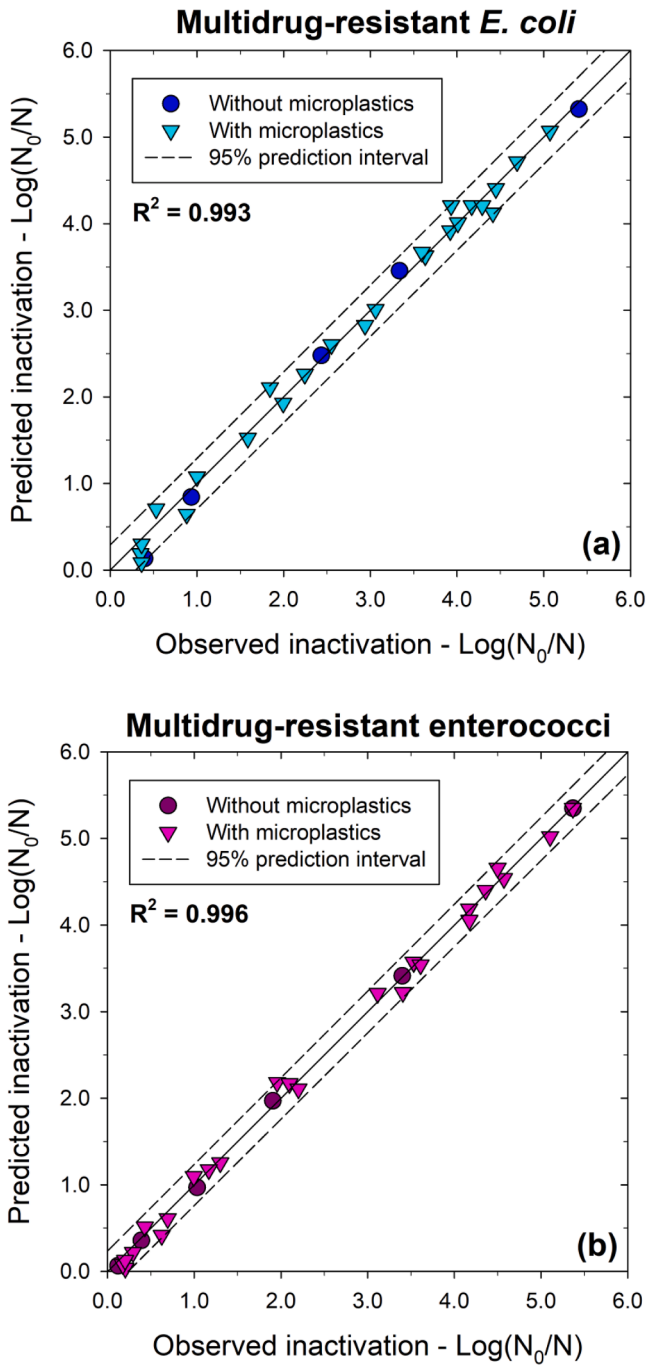

Fig. 5. Observed versus model-predicted UV inactivation of multidrug-resistant *E. coli* (a) and enterococci (b) in the absence and in the presence of microplastics in water.

microplastics were determined for 5-log reduction only, which is related to the slower inactivation of microplastic-associated bacteria than free bacteria. For example, to achieve a 5-log reduction, the UV fluence requirement increased from 9.8 mJ/cm^2 (without microplastics) to 17.5 mJ/cm^2 (with PVC microplastics) for multidrug-resistant *E. coli*, and from 14.5 mJ/cm^2 (without microplastics) to 20.5 mJ/cm^2 (with PVC microplastics) for multidrug-resistant enterococci (Table 3). In the presence of PE2 microplastics, a 5-log reduction of multidrug-resistant *E. coli* was not calculated due to tailing, i.e., no further reduction of bacteria was observed at a UV fluence > 10.0 mJ/cm^2 (Fig. 2b and Table 3). For a 5-log reduction of multidrug-resistant enterococci, ~2 times higher UV fluence was required in the presence of PE2

Table 3
Model-predicted UV fluence requirements for 1-, 2-, 3-, 4- and 5-log reductions of multidrug-resistant *E. coli* and enterococci in water, in the absence and in the presence of microplastics, at pH 6.0 ± 0.1 and 25 ± 1 °C.

Microplastics*	Reduction	UV fluence (mJ/cm^2)	
		Multidrug-resistant *E. coli*	Multidrug-resistant enterococci
No microplastics	1 log	5.5	7.5
	2 log	7.0	10.0
	3 log	8.0	12.0
	4 log	9.0	13.5
	5 log	9.8	14.5
PE1**	1 log	6.0	9.0
	2 log	9.0	12.0
	3 log	11.0	14.5
	4 log	17.5	NA
	5 log	53.5	NA
PE2***	1 log	6.0	7.5
	2 log	7.5	10.0
	3 log	8.5	11.5
	4 log	10.0	14.0
	5 log	NA*****	28.0
PVC****	1 log	5.0	7.0
	2 log	7.0	10.0
	3 log	9.0	12.0
	4 log	11.0	14.0
	5 log	17.5	20.5

* The concentration of microplastics was 1.0 g/L
** Polyethylene (125 μm)
*** Polyethylene (40-48 μm)
**** Polyvinyl chloride (≤250 μm)
***** NA: not-achieved due to tailing

microplastics than the absence of microplastics (Table 3). These results indicate that a certain UV fluence to achieve 5-log reduction of multidrug-resistant *E. coli* or enterococci in the absence of microplastics does not guarantee the same reduction of these bacteria in the presence of PE2 or PVC microplastics, due to aggregation of bacteria with microplastics resulting in shielding bacteria from UV.

4. Conclusions

The findings of this investigation allowed for the following conclusions:

- Microplastics negatively affected the UV inactivation kinetics of bacteria in water. This was indicated by an additional inactivation regime in the UV fluence response curve for the inactivation of both bacteria studied. The additional regime, which was observed after the initial resistance and fast inactivation of free bacteria, was related to the slow or no inactivation of microplastic-associated bacteria known as tailing effect. The magnitude of the negative effect of microplastics on the UV disinfection performance varied with different microplastics (PE2 (40-48 μm) and PVC (≤250 μm)) and bacteria (multidrug-resistant *E. coli* (Gram-negative) and enterococci (Gram-positive)), and was described well by a UV fluence-based double-exponential microbial inactivation model.
- It was shown that when the UVT of the microplastic-containing water was not taken into account in calculating UV fluence, the effect of microplastics as protectors of bacteria was overestimated. This is important considering that an accurate measurement of UVT of microplastic-containing water is not trivial due to the unique behavior of microplastics in water (i.e., deposited at the surface of water; well mixed in water; deposited at the bottom of the petri dish).
- The findings suggest that microplastics may contribute to the spread of antimicrobial resistance in the environment in terms of lower UV inactivation of antibiotic-resistant bacteria in the presence than in the absence of microplastics in water.

- This paper is a proof-of-concept study on the effect of microplastics on the efficiency of UV disinfection of water (i.e., microplastic-associated bacteria exhibited slower UV inactivation than free bacteria). The approach used herein to elucidate the effect of microplastics on the inactivation of bacteria may guide future research on the effect of other particles at the nano and micro size, including nanoplastics and other engineered nanoparticles with different characteristics that have been studied for water treatment applications, on the exposure response curve for the inactivation of various microorganisms by physical and chemical disinfecting agents in water/wastewater. It is worth noting that the effect of microplastics on the UV fluence response curve for the inactivation of bacteria should eventually be evaluated in real wastewater matrices (e.g., secondary effluent).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118906.

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