Comparison of indicator bacteria inactivation by the ultraviolet and the ultraviolet/hydrogen peroxide disinfection processes in humic waters

Arzu Teksoy, Ufuk Alkan, Sevil Çalışkan Eleren, Burcu Şengül Topaç, Fatma Olcay Topaç Şağban and Hüseyin Savaş Başkaya

ABSTRACT

The aim of the present study was to evaluate responses of potential indicator bacteria (i.e. *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis*) to the ultraviolet (UV) radiation and the UV/hydrogen peroxide (H_2O_2) disinfection processes of surface waters with different qualities in terms of humic content. The UV and the UV/H_2O_2 processes were applied to waters containing various concentrations of fulvic acid in order to inactivate *E. coli*, *P. aeruginosa* and *B. subtilis* spores. Three fulvic acid (0, 2 and 6 mg l^{-1}) and four H_2O_2 (0, 10, 25 and 50 mg l^{-1}) concentrations were used. Results showed that the *k* values of *E. coli*, *P. aeruginosa* and *B. subtilis* spores varied between 2.22 and 4.00, 1.73 and 3.58, and 1.40 and 1.86, respectively, in all test conditions. The sensitivity of the test organisms followed a decreasing order of *E. coli* > *P. aeruginosa* > *B. subtilis.* Results of the study indicated that the blocking effect of fulvic acid for the UV light was diminished by using H_2O_2 in combination with the UV radiation. Findings of the present study strongly suggested that the UV/H_2O_2 process was significantly effective on the inactivation of *E. coli* and *P. aeruginosa* in humic waters, whereas it induced little or no apparent contribution to the disinfection efficiency of *B. subtilis* spores.

Key words | *B. subtilis* spore, *E. coli*, fulvic acid, hydrogen peroxide, *P. aeruginosa*, UV radiation

Arzu Teksoy
Ufuk Alkan (corresponding author)
Sevil Çalışkan Eleren
Burcu Şengül Topaç
Fatma Olcay Topaç Şağban
Hüseyin Savaş Başkaya
Department of Environmental Engineering,
Uludağ University,
Nilüfer,
Bursa,
Turkey
E-mail: *alkan@uludag.edu.tr*;
ufukalkan@yahoo.com

INTRODUCTION

Removing and inactivating microbial pathogens in drinking water is essential to protect the public from outbreaks of waterborne diseases. Chemical disinfectants such as chloramines, chlorine dioxide and especially chlorine are commonly used for drinking water disinfection because of their low cost, ease of handling, and their ability to provide disinfectant residual. However, the formation of carcinogenic or mutagenic chloro-organic by-products during chemical disinfection is more of a problem when surface waters containing natural organic matter are used as the drinking water source (Cairns 1994). In pursuit of alternatives to chemical disinfection in drinking water treatment, there has been increasing interest in the use of UV light because of its excellent biocidal properties without the formation of toxic disinfection by-products (DBPs) (Zimmer & Slawson 2002; Quek & Hu 2008). UV radiation has several advantages with the added benefit of being cost-comparable and environmentally friendly (Savoye *et al.* 2001; Madrid 2004). The extremely short contact times (ranging from seconds to a few minutes) and no by-product formation have also contributed to its rising popularity as an alternative disinfection process (USEPA 1999).

The germicidal effects of UV irradiation are due to the DNA absorption of the UV light, causing crosslinkage between neighbouring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Wang *et al.* 2005). These crosslinkages cause distortion of the DNA molecule, resulting in malfunctions in the replication of the cell, potentially leading to cell death in unicellular organisms (Zion *et al.* 2006). The efficiency and reliability of UV disinfection is

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greatly dependent on water quality parameters such as UV transmittance and organic matter content (Koivunen & Heinonen-Tanski 2005; Alkan *et al.* 2007a). Humic substances such as humic and fulvic acids are known to absorb UV light, and the UV transmittance decreases with increasing concentrations of humic compounds in natural waters (Corin *et al.* 1998; Lee *et al.* 2009). The absorption is mainly due to conjugated unsaturated bonds and to the presence of free electron pairs on heteroatoms (USEPA 2006). Photoactivated organic matter and other UV absorbing compounds may have a major role in the protection and rapid recovery of bacteria from UV and other disinfectant stresses (Kaiser & Herndl 1997; Lyons *et al.* 1998; Gu 2004; Alkan *et al.* 2007a).

Humic substances are normally considered to be recalcitrant towards microbial degradation. They generally constitute the major fraction of dissolved organic matter in surface water and may account for up to 90% of the total dissolved organic carbon (DOC) content (Corin *et al.* 1998); fulvic acid may constitute up to 80% of this humic substance fraction (Bolto *et al.* 2004). Aqueous DOC concentrations are highly variable; in surface water, values range from 1 to 60 mg l^{-1} DOC, with typical values from 2 to 10 mg l^{-1} (Beckett 1990).

Recently, advanced oxidation processes (AOPs) have been suggested for the control of DBPs and microorganisms in drinking water (Wang *et al.* 2000; Koivunen & Heinonen-Tanski 2005). In particular, the UV/H_2O_2 process has received attention because of its ability to break down pollutants and inactivate microorganisms. The bactericidal effect of hydrogen peroxide has been reported (Pedahzur *et al.* 1997; Wang *et al.* 2001; Kruithof *et al.* 2002; Labas *et al.* 2008). However, hydrogen peroxide has not been widely used as a sole disinfectant for water and wastewater treatment, mainly because of its slow disinfection action and low efficiency (Koivunen & Heinonen-Tanski 2005). On the other hand, application of UV in combination with hydrogen peroxide has been shown to enhance the disinfection efficiency, and synergistic lethal effects have been reported against both spores and vegetative cells by generation of reactive hydroxyl radical ($OH^{\bullet}$) (Bayliss & Waites 1980; Mamane *et al.* 2007; Alkan *et al.* 2007b).

Hydroxyl radicals are considered as the most reactive oxidizing agents in water treatment and this highly reactive species in waters react with a range of organic compounds and the surface molecules of microorganisms (Gu 2004). The cell wall is the primary site of hydroxyl radical attack. The combination of cell membrane damage, and further oxidative attack of internal cellular components, ultimately results in cell death (Hasset & Cohen 1989; Blake *et al.* 1999; Dunlop *et al.* 2002).

The drinking water industry uses bacterial indicators in order to assess the efficiency of processes. *Escherichia coli* is extensively used as a treatment efficiency indicator and, if not detected, the drinking water is regarded as free from faecal contamination (Dunlop *et al.* 2002). Similarly, *Pseudomonas aeruginosa* is known as an opportunistic pathogenic bacterium and as an indicator bacterium of drinking water treatment (Tosa *et al.* 2003). Commonly used test organisms for UV biodosimetry studies are bacterial spores, usually spores of *Bacillus subtilis*, because of their high degree of UV resistance, reproducible inactivation response, and ease of use (Nicholson & Galeano 2003). Resistance to disinfection can vary depending on the species of microorganism; even on the strains of the same species (Quek & Hu 2008). In general, it is known that microorganisms that form spores are more resistant to disinfection (Ibanez 2007).

It appears that an investigation regarding the behaviour of different indicator bacteria to the UV and the UV/H_2O_2 disinfection processes in natural waters with humic compounds will provide a significant contribution to the area of advanced disinfection processes of drinking water. Different water quality conditions may induce different inactivation behaviours and it is important to predict responses of indicator microorganisms to the disinfection processes in varying water quality conditions. The main objective of the present study was to evaluate responses of potential indicator bacteria, namely, *E. coli*, *P. aeruginosa* and *B. subtilis* spores, to the UV and the UV/H_2O_2 disinfection process in waters with different quality in terms of humic content.

METHODS

Preparation of microorganism cultures

Cultures of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 15542) and *B. subtilis* (ATCC 6633) were purchased from the American Type Culture Collection. *Escherichia coli* was cultured in tryptic soy broth (Oxoid) for 16 h at 37 ± 0.5 °C

in an orbital incubator. An aliquot of the 18 h culture was transferred to sterile tryptic soy broth (Oxoid). The bacterial growth was controlled during the incubation period by measuring the optical densities (OD_{595}). The *P. aeruginosa* (ATCC 15542) culture was inoculated into the nutrient broth (Oxoid) using a sterilized loop and was allowed to grow for 24 h at 37 °C in an incubator. The same procedure used for *E. coli* was applied in order to determine the exponential phase of *P. aeruginosa*. A culture of *B. subtilis* was inoculated to tryptic soy broth (Oxoid) and incubated at 35 °C for 24 h in order to prepare a late log phase suspension of vegetative cells of *B. subtilis*. A 3 mL aliquot of the diluted suspension was poured on top of R2A (Oxoid) medium to form a thin layer. The vegetative bacteria were allowed to incubate at 35 °C for 7 days until 95% of the microorganisms had sporulated. After harvesting of spores, the suspension was filtered using a 10-mm glass fibre filter in order to remove spore clumps (Barbeau *et al.* 1997). Cultures of *E. coli* and *P. aeruginosa* were transferred onto slanted nutrient agar (Oxoid) for maintenance. *Bacillus subtilis* spores were maintained on R2A agar plates. Isolates were stored at 4 °C and subcultures to fresh media were made monthly.

Preparation of fulvic acid solution

Fulvic acid was extracted from soil samples that were collected from the catchment area of Doğancı Dam in Bursa City. Soil samples were air-dried in laboratory conditions. After sieving, the soil samples were ground and 50 g was transferred to a plastic bottle to which 200 mL of 0.1 N NaOH was also added and the mixture was constantly shaken for 1 h. At the end of the shaking period, samples were kept at room temperature for 18 h. It was further shaken for an additional 1 h and centrifuged at 20,540 g for 30 min. The supernatant was passed through a folded filter (Schleicher & Schuell, 595 1/2) and the pH of the filtrate was adjusted to 1.0 by adding concentrated H_2SO_4. Acidification caused the separation of the fulvic acid and humic acid fraction following 30 min incubation in the oven at 80 °C and coagulation at room temperature for 18 h. Fulvic acid is soluble in acid medium, whereas humic acid is insoluble. Therefore, humic acid precipitated during the above treatment. The supernatant was separated by centrifugation at 20,540 *g* for 30 min and it was used as fulvic acid stock solution (Baskaya 1975). DOC concentration of the solution was measured by a TOC-V_{CPH} 5000A Shimadzu TOC analyzer.

Enumeration of microorganisms

Escherichia coli was enumerated by membrane filtration method using mFC agar as described in Standard Methods 9222 D (*Standard Methods* (APHA/AWWA/WEF 1998)). Plates were incubated at 44.5 °C ± 0.2 for 24 h, after which time blue colonies were counted. Then some of the typical colonies were inoculated into tubes of EC broth (Oxoid) and incubated for 24 h at 44.5 °C ± 0.2. Gas production and turbidity in tubes confirmed *E. coli* occurrence (*Standard Methods* (APHA/AWWA/WEF 1998)). *Pseudomonas aeruginosa* was determined with the membrane filtration method according to Standard Methods 9113E (*Standard Methods* (APHA/AWWA/WEF 1998)). After filtration, the membrane filter was placed onto M-PA agar and then plates were incubated at 41.5 °C ± 0.5 for 72 h. Typical *P. aeruginosa* colonies with light outer rims and brownish to greenish-black centre were streaked on a milk agar medium and incubated at 35 ± 1.0 °C for 24 h for confirmation. *Pseudomonas aeruginosa* hydrolyses casein and produces a yellowish to green diffusible pigment. Enumeration of viable spores of *B. subtilis* was done using a previously described modified plate filtration method (Barbeau *et al.* 2005). The sample was filtered through a 0.45 μm, 47 mm diameter, gridded filter. The filters were placed on 47 mm Petri dishes with pads soaked with 1.75-ml tryptic soy broth (Oxoid) containing 1% tetrazolium trichloride. The tetrazolium trichloride dyed the spores red as they germinated and ultimately formed red pin-head (slightly larger) colonies, aiding in the counting of microorganisms. The plates were then wrapped in water/airtight plastic bags and placed in a 75 °C water bath for 15 min, after which they were placed in a 37 °C incubator for 24 h and then counted. All samples were plated within 16 h of the experiments. Results were recorded as CFU per mL (Barbeau *et al.* 1997).

Experimental procedure

UV radiation experiments were carried out in a glass cylinder reactor that had an inner diameter of 10 cm and a

height of 40 cm. The temperature of the reactor was controlled at 20 °C by thermal cycler (Thermo, England). UV irradiation was performed by using a low pressure mercury vapour lamp (Lightech), producing predominantly 254 nm wavelength UV radiation. The lamp was installed in a quartz sleeve and placed at the centre of the reactor. The light intensity of the lamp was 40 μW cm^{-2}. A schematic diagram of the UV reactor radiation experimental set-up is shown in Figure 1. Before each exposure, the UV_{254} lamp was turned on for at least 10 min to ensure a uniform lamp output and to sterilize the glass cylinder. Two litres of bottled water was placed into a sterile container. After the addition of specific amounts of fulvic acid to the 2 L bottled water, pH was set to 7.5. Then bacteria were inoculated in the required numbers (i.e. approximately 10^6 CFU100 ml^{-1}) and mixed thoroughly with a magnetic bar. This bottled water containing specific amounts of fulvic acid (0, 2, 6 mg l^{-1}) and bacteria was added to the UV reactor, instantly. Following this, the required concentrations of hydrogen peroxide (0, 5, 10, 25 and 50 mg l^{-1}) were immediately added to the UV reactor containing water with fulvic acid and bacteria. The UV reactor was mixed with a magnetic bar and was irradiated for various times in order to achieve required UV doses. Then, subsamples were collected for the analyses of UV_{254} absorbance, *E. coli*, *P. aeruginosa* and *Bacillus subtilis* spores and pH. Disinfection experiments were carried out separately for *E. coli*, *P. aeruginosa* and *B. subtilis* spores. Each set of experiments was repeated twice and each dilution was plated in triplicate. Blank experiments without UV and H_2O_2 were carried out in order to determine inactivation of bacteria in dark conditions. Experiments with only H_2O_2 were also carried out in order to determine inactivation of bacteria in the presence of H_2O_2. The results of these experiments showed that no significant bacterial inactivation occurred.

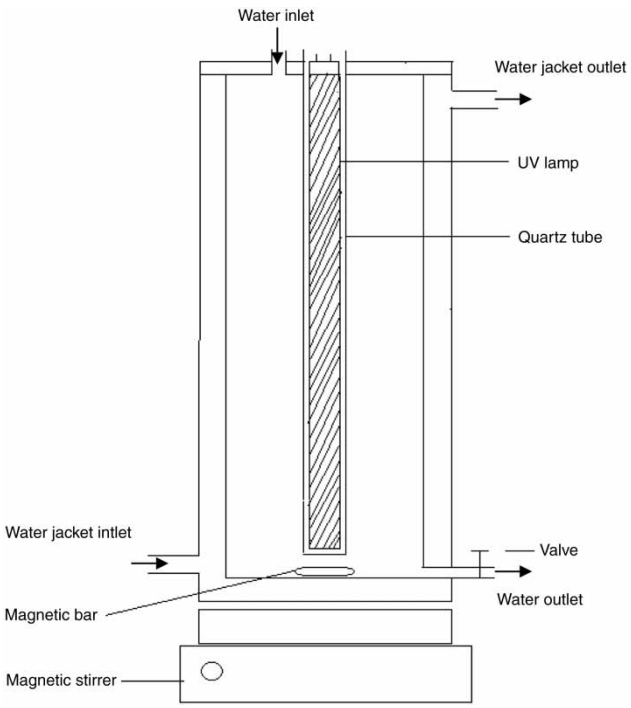

Figure 1 | Schematic diagram of the ultraviolet (UV) reactor.

Chemical and physical analyses

Dissolved organic carbon concentrations were determined by a Shimadzu TOC-V_{CPH} 5000A total carbon analyser (Shimadzu, Japan). UV absorbance and OD were measured at 254 and 595 nm, respectively, by a Jenway UV/Vis spectrophotometer (Barloword Sci. Ltd, UK). Samples were prefiltered with 0.45 μm Millipore filters before UV absorbance and DOC analyses. pH was measured by a Metrohm 704 pH-meter.

RESULTS

Log reductions of *E. coli*, *P. aeruginosa* and *B. subtilis* spores during the UV and the UV/H_2O_2 disinfection for all water types with 0, 2 and 6 mg l^{-1} fulvic acid are presented in Figures 2–4, respectively. The time spent for approximately 3 log reduction was selected as a baseline in order to evaluate the efficiency of different disinfection conditions. Figure 2 illustrates the log reductions of test organisms for increasing concentration of H_2O_2 in non-humic waters. It is clearly seen from the figure that *E. coli* inactivation was more rapid in any disinfection conditions compared with *P. aeruginosa* and *B. subtilis*; 3 log *E. coli* reduction was achieved within 40 s to 58 s for 0–50 mg l^{-1} H_2O_2 concentration. *Pseudomonas aeruginosa* required about 53 s to 73 s for 3 log reduction, whereas *B. subtilis* required 91 to 100 s (Figure 2).

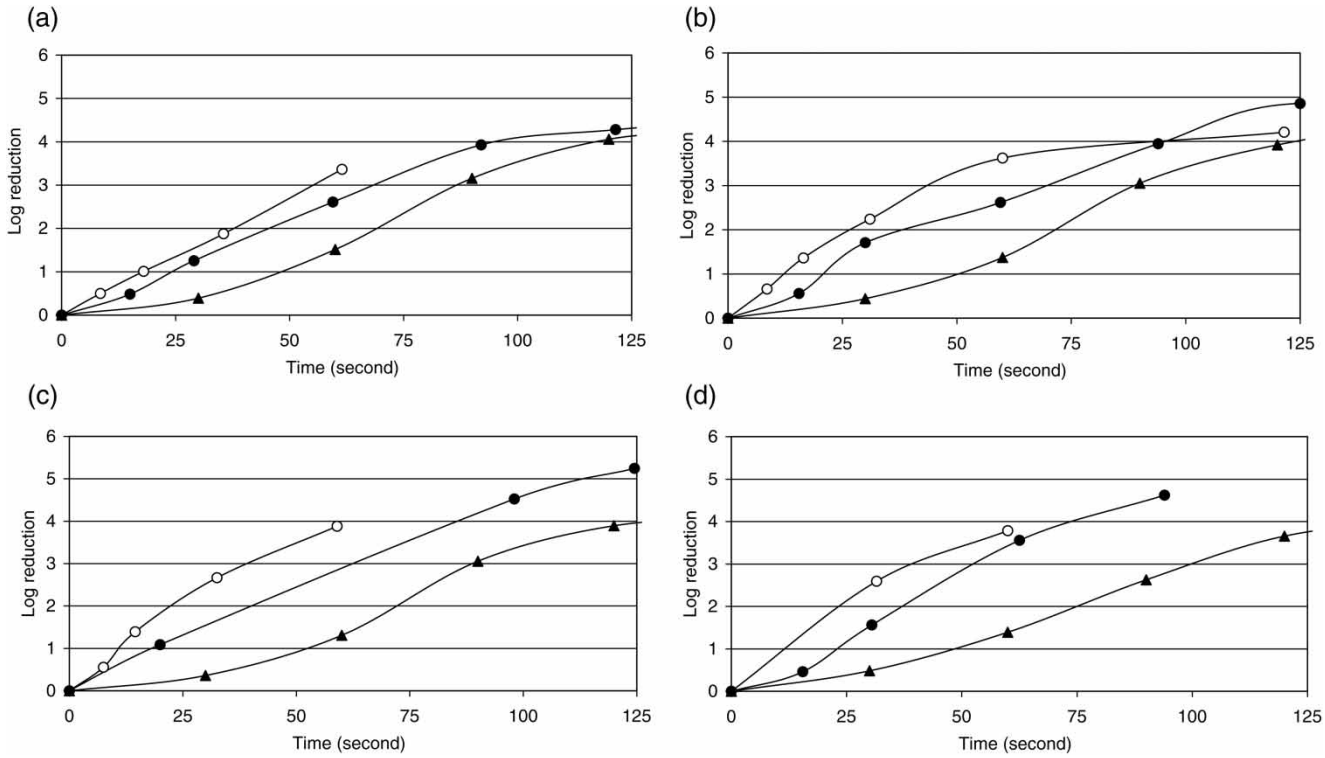

Figure 2 | Bacterial reduction by the UV and the UV/H_2O_2 processes in waters containing 0 mg l^{-1} fulvic acid. (a) $H_2O_2 = 0$ mg l^{-1}, (b) $H_2O_2 = 10$ mg l^{-1}, (c) $H_2O_2 = 25$ mg l^{-1}, and (d) $H_2O_2 = 50$ mg l^{-1}; key: –○– *Escherichia coli*, –●– *Pseudomonas aeruginosa*, –▲– *Bacillus subtilis*.

Figure 3 displays the effect of H_2O_2 concentration increase in water samples with 2 mg l^{-1} FA (fulvic acid). The time required for 3 log *E. coli* reduction decreased from 55 to 33 s when H_2O_2 concentration was elevated to 50 mg l^{-1} whereas time required for *P. aeruginosa* was reduced from 84 to 32 s. The use of 50 mg l^{-1} H_2O_2 appeared to reduce required time by 40 and 62% for *E. coli* and *P. aeruginosa*, respectively. On the other hand, the same dose of H_2O_2 induced no significant effect on the disinfection efficiency of *B. subtilis* spores (Figure 3).

Figure 4 shows the log reductions of test organisms in water samples with 6 mg l^{-1} FA. As can be seen from the figure, the required contact times of *E. coli* and *P. aeruginosa* increased significantly compared with the non-humic water. However, addition of 50 mg l^{-1} H_2O_2 during UV radiation in this highly humic water (i.e. 6 mg l^{-1} FA) again appeared to reduce required contact times from 88 s to 54 s for *E. coli* and from 92 to 60 s for *P. aeruginosa*. Figure 4 also indicates that increasing concentration of H_2O_2 slightly reduced the inactivation time of *B. subtilis* spores in highly humic water.

The effect of FA on the required contact times showed variations during different UV/H_2O_2 process combinations. The contact time required for *E. coli* increased by 35–60% with increasing FA concentrations (0 to 6 mg l^{-1}). The corresponding increases in contact times for *P. aeruginosa and B. subtilis* spores were 6–26% and 3–25%, respectively.

Figure 5 compares the inactivation coefficients (k) that were calculated by using the modified model of Chick-Watson (Hassen *et al.* 2000). The figure confirms that increasing FA concentration caused the decline of inactivation coefficients of all test organisms. It was calculated that the k value of *E. coli* in water that contained high concentration of FA decreased by 31% during the UV application when compared with non-humic water. The k values of *P. aeruginosa* and *B. subtilis* spores also showed similar declines (25%) during the UV application (Figure 5(a)).

Increasing concentrations of H_2O_2 increased the inactivation efficiency of *E. coli* and *P. aeruginosa* in non-humic water by 6–24% and by 4–28%, respectively, whereas, the increasing H_2O_2 concentrations appeared to reduce

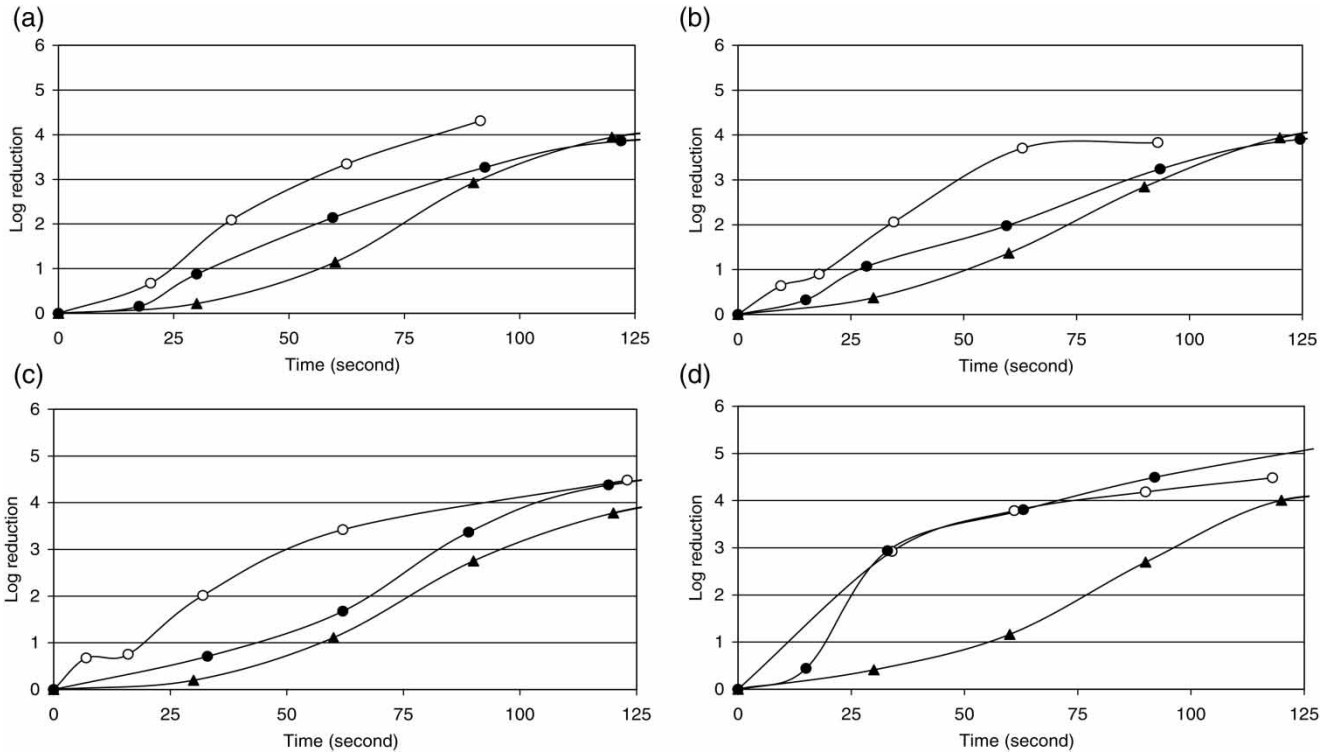

Figure 3 | Bacterial reduction by the UV and the UV/ H_2O_2 processes in waters containing 2 mg l^{-1} fulvic acid. (a) $H_2O_2 = 0$ mg l^{-1}, (b) $H_2O_2 = 10$ mg l^{-1}, (c) $H_2O_2 = 25$ mg l^{-1}, and (d) $H_2O_2 = 50$ mg l^{-1}; key: –○– *Escherichia coli*, –●– *Pseudomonas aeruginosa*, –▲– *Bacillus subtilis*.

inactivation efficiency of *B. subtilis* spores in non-humic water. The *k* value was reduced by 4–11% between 0 and 50 mg l^{-1} H_2O_2 (Figure 5).

As known, water transparency is an important factor affecting the efficiencies of the UV and the UV/H_2O_2 processes. The effect of water transparency is usually estimated by determining the UV absorbance at 254 nm. Figure 6 illustrates the effect of increasing H_2O_2 and FA concentrations on the UV_{254} absorbance of water. It is clear from the figure that addition of both fulvic acid and H_2O_2 to the water samples increased the UV absorbance. Figure 6 also indicates lower absorbance values for the disinfected water samples implying the partial removal of UV-absorbing FA and/or H_2O_2 during UV exposure.

Based on the *k* values presented in Figure 5, it can be stated that, in general, the use of H_2O_2 increased the disinfection efficiency of *E. coli* and *P. aeruginosa* in both moderately and highly humic waters. The inactivation coefficient increased from 3.00 to 4.00 for *E. coli* and from 1.90 to 3.58 for *P. aeruginosa* in moderately humic waters (i.e. 2 mg l^{-1}) with the application of 50 mg l^{-1} H_2O_2. While low H_2O_2 concentration (10 mg l^{-1}) did not affect the *k* value of *E. coli* and *P. aeruginosa*, higher concentrations of H_2O_2 caused larger inactivation of both bacteria. Maximum differences in inactivation of *E. coli* and *P. aeruginosa* were 33 and 88%, respectively. However, as can be seen from Figure 5, *B. subtilis* spores exhibited different behaviour in response to the increasing H_2O_2 concentrations. The inactivation efficiency of *B. subtilis* spores in this type of water (i.e. 2 mg l^{-1}) appeared not to be affected by the elevation of H_2O_2.

Increasing concentration of H_2O_2 provided further inactivation of all test organisms in highly humic waters (i.e. 6 mg l^{-1}). The *k* value showed an increase from 2.22 to 2.52 for *E. coli*. Corresponding values of *P. aeruginosa* varied between 1.73 and 2.67. Dissimilar to non-humic and moderately humic water, the elevation of H_2O_2 concentration positively affected the inactivation of *B. subtilis* spores in highly humic water.

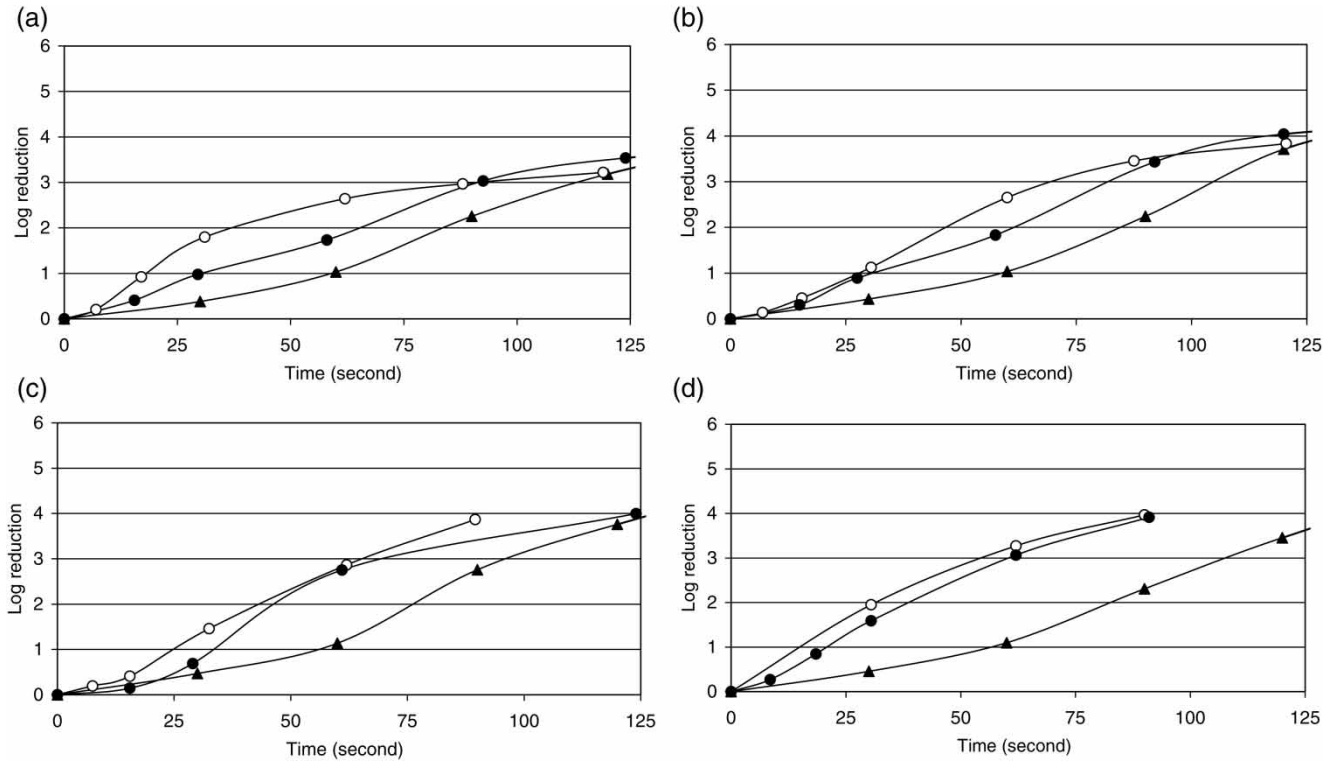

Figure 4 | Bacterial reduction by the UV and the UV/H_2O_2 processes in waters containing 6 mg l^{-1} fulvic acid. (a) $H_2O_2 = 0$ mg l^{-1}, (b) $H_2O_2 = 10$ mg l^{-1}, (c) $H_2O_2 = 25$ mg l^{-1}, and (d) $H_2O_2 = 50$ mg l^{-1}; key: –○– *Escherichia coli*, –●– *Pseudomonas aeruginosa*, –▲– *Bacillus subtilis*.

DISCUSSION

It is known that inactivation of microorganisms by disinfection processes exhibits variations in different environmental conditions. In this study, inactivation of different indicator microorganisms by the UV and the UV/H_2O_2 disinfection processes in humic waters were compared. Results indicated that *B. subtilis* spores were the most resistant organism while *E. coli* was the most sensitive bacteria to the UV and the UV/H_2O_2 processes in all conditions. This finding is in accordance with previous studies which show that the bacteria with spores are more resistant than vegetative bacteria to disinfection processes (Gardner & Shama 1998; Hijnen *et al.* 2006). Results of the present study apparently showed that *E. coli* and *P. aeruginosa* were affected by the increasing H_2O_2 concentration at different rates. It was found that the increases in the *k* values varied between 4 and 33% and 4 and 88% with the increasing concentration of H_2O_2 for *E. coli* and *P. aeruginosa*, respectively. This diversity within these species might be caused by the molecular and structural differences in their cell walls (Weiss & Fraser 1973; Cantwell *et al.* 2008) and outer structures such as capsule and pili. It is clear from Figure 5 that increasing concentration of H_2O_2 is less effective in inactivating *B. subtilis* spores than *E. coli* and *P. aeruginosa*. The resistance of *B. subtilis* spores is known to originate from the presence of an outer proteinaceous layer termed the spore coat. This coat is known to protect the spores from enzymes, mechanical disruption and some disinfectants (Riesenman & Nicholson 2000).

The variations in water quality significantly affect microbial inactivation. Reduction of UV transmittance depending on the humic content is one of the most important factors that influences the efficiency of the UV and the UV/H_2O_2 processes. Based on the results of the present study, it can be stated that increasing concentrations of fulvic acid caused decreasing inactivation rates of all test organisms in both the UV and the UV/H_2O_2 applications. Accordingly, the required contact times for 3 log reduction increased significantly in highly humic waters. It is known

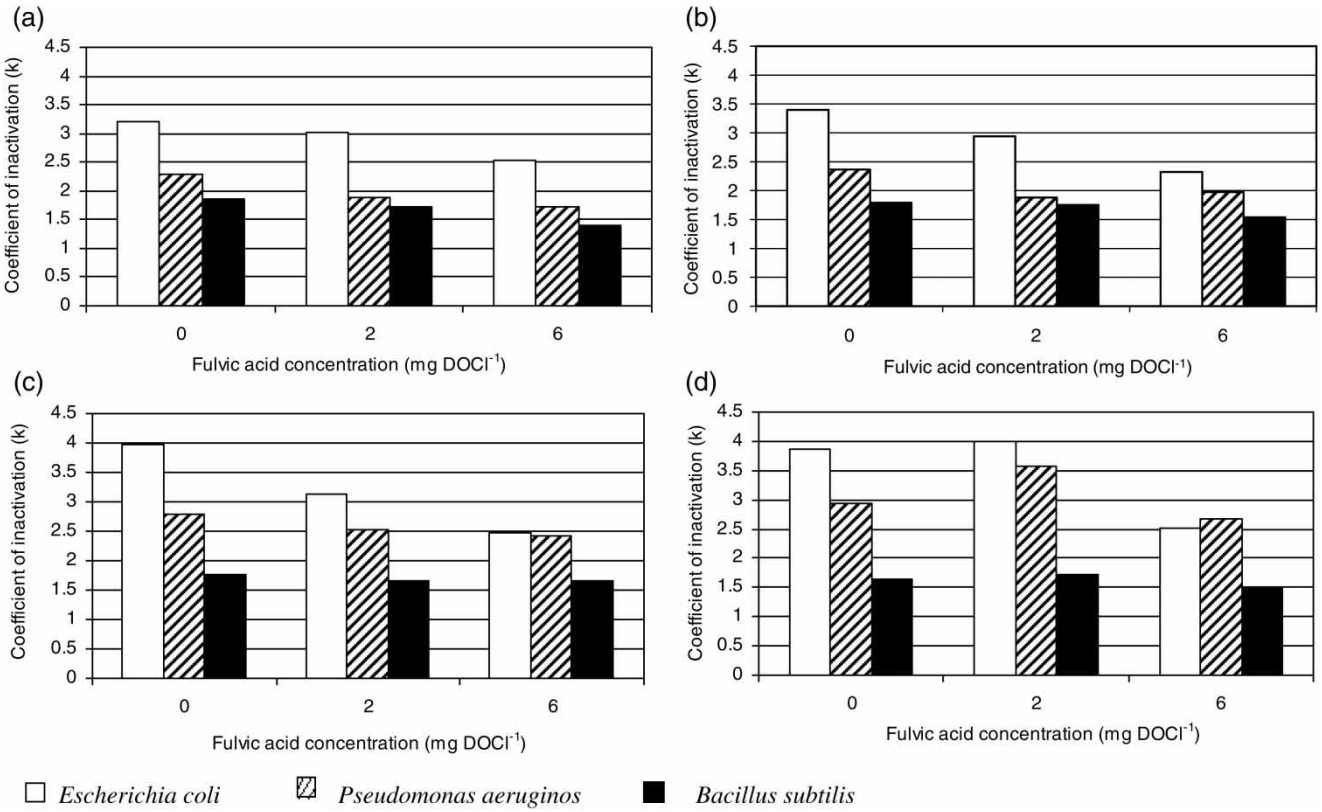

Figure 5 | Comparison of inactivation coefficients for *E. coli*, *P. aeruginosa* and *B. subtilis* spores in disinfected water containing various concentrations of fulvic acid. (a) $H_2O_2 = 0$ mg l^{-1}, (b) $H_2O_2 = 10$ mg l^{-1}, (c) $H_2O_2 = 25$ mg l^{-1}, and (d) $H_2O_2 = 50$ mg l^{-1}. DOC, dissolved organic carbon.

that humic substances are among the most highly UV-absorbing compounds present in natural waters (Corin *et al.* 1998). The harmful effect of UV radiation on bacteria diminishes in humic matter-containing waters owing to the absorption of UV light (Alkan *et al.* 2007a) and the humic-coating of bacterial cells (Cantwell *et al.* 2008). Cantwell *et al.* (2008) reported that humic matter in water negatively affected the inactivation efficiencies of both *E. coli* and *B. subtilis* cells. They also indicated that the magnitude of reduction in UV effectiveness increased with humic content, suggesting the level of protection is concentration dependent.

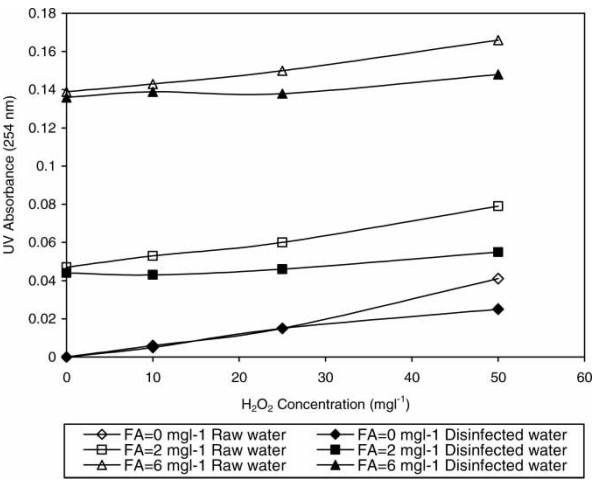

Figure 6 | UV absorbances of raw and disinfected waters containing various concentrations of fulvic acid (FA).

The results of the present study revealed that the test organisms were affected by the increase of FA to different extents. Calculated k values showed that fulvic acid was more effective in protecting *E. coli* from the UV and the UV/H_2O_2 processes compared with *P. aeruginosa* and *B. subtilis* spores. Although the inactivation rate of *E. coli* was higher in general than that of the other two microorganisms, the k value of *E. coli* was the most reduced by the increase in FA concentration. For the same FA concentration, differences between the k values of *E. coli* and

P. aeruginosa diminished with increasing H_2O_2 concentration. Closer k values were obtained for *E. coli* and *P. aeruginosa* for the 6 mg l^{-1} FA-containing waters, which confirmed the better protection of *E. coli* cells both from the UV and the UV/H_2O_2 processes. This protection appeared to occur against oxidative hydroxyl radicals produced by the UV/H_2O_2 process as well as the UV light and may be attributed to better humic-coating of *E. coli* cells. Oxidative action of hydroxyl radicals was thought to be primarily directed towards the potential humic-coating of *E. coli* cells and towards the cell wall of *P. aeruginosa*.

Bacterial cell wall surfaces contain numerous functional groups such as carboxyl, phosphate and phenolic, which results in a bacterial surface displaying hydrophilic or hydrophobic regions. On the other hand, humic matters have many different functional groups and may show affinity for surfaces. As a result, there is a good reason to suspect that humic molecules might interact with microbial surfaces (Cantwell *et al.* 2008) and this interaction may occur to different extents for different species.

The blocking effect of fulvic acid for the UV light was diminished by using H_2O_2 in combination with UV radiation. Significant increases were obtained in the inactivation rate of vegetative bacteria by using the combination of UV and H_2O_2. In comparison with UV radiation alone, the application of UV/H_2O_2 provided enhanced oxidative attack by the generation of highly reactive hydroxyl radicals. These radicals were thought to first attack microbial cell walls, membranes and enzymatic and transport systems. Thus, the effects of these mechanisms may have improved microbial reduction and caused synergistic benefit against the vegetative bacteria (Fantel 1996; Koivunen & Heinonen-Tanski 2005; Mamane *et al.* 2007).

Inactivation rates of *E. coli* and *P. aeruginosa* increased by the addition of H_2O_2 in all types of water tested. Yasar *et al.* (2007) reported that UV/H_2O_2 process was the most effective method for the disinfection of wastewater in comparison with UV, H_2O_2 or O_3 alone.

In contrast, the inactivation coefficient of *B. subtilis* spores was reduced by increasing H_2O_2 concentrations in non-humic water owing to an increase in UV absorbance. In the case of moderately humic water, no apparent variation or only a slight reduction was observed. Owing to the fact that the inner membrane exhibits extremely low permeability to hydrophilic and hydrophobic molecules, the spores show resistance to oxidizing agents such as OH. radicals (Setlow 2006). Therefore, transmission of UV light becomes more important in the disinfection of *B. subtilis* spores. However, the transmission of UV light to *B. subtilis* spores was expected to be blocked by the presence of H_2O_2 in both non-humic and moderately humic waters. In other words, increases in the concentration of H_2O_2 did not contribute to the inactivation of *B. subtilis* spores. The principal cause of the reduced inactivation performance in the presence of H_2O_2 appears to be the UV absorption characteristics of H_2O_2.

However, an increase in H_2O_2 concentration showed different effects on *B. subtilis* spores in highly humic waters compared with non-humic and moderately humic waters. k values of *B. subtilis* spores appeared to increase with increasing H_2O_2 concentration in waters with 6 mg l^{-1} FA. The presence of H_2O_2 in highly humic water is thought to break up FA molecules, which then results in a reduced UV absorbance (Moncayo-Lasso *et al.* 2009). In other words, the reducing effect of UV absorbance caused by FA on the inactivation coefficient of *B. subtilis* spores was counteracted by the oxidative effect of H_2O_2 on FA (i.e. reduction in UV absorbance). Light appears to be more detrimental for the spores of *B. subtilis* than oxidative agents produced by the UV/H_2O_2 process. The mechanism of inactivation was mainly dictated by the damaging effect of light on the genetic material rather than the damaging effect of oxidative agents on the outer membrane of the spores.

CONCLUSIONS

An overall evaluation of the present study showed that the k values of *E. coli*, *P. aeruginosa* and *B. subtilis* spores varied between 2.22 and 4.00, 1.73 and 3.58, and 1.40 and 1.86, respectively, in all test conditions. The sensitivity of test organisms to the UV/H_2O_2 process in humic waters followed a decreasing order of *E. coli* > *P. aeruginosa* > *B. subtilis*. Findings of the present study strongly suggest that the UV/H_2O_2 process was significantly effective on the inactivation of *E. coli* and *P. aeruginosa* in humic waters, whereas it induced little or no apparent contribution to the disinfection efficiency of *B. subtilis* spores. Results

also showed that *P. aeruginosa* was primarily affected by the increments of H_2O_2 concentration while the other two organisms were affected by the increments of the UV light. In addition, it is worth noting that the humic character of the water was found to be the major factor affecting the treatment efficiency of the UV/H_2O_2 process as well as the UV process.

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