A pulsed light system for the disinfection of flow through water in the presence of inorganic contaminants

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ABSTRACT

The use of ultraviolet (UV) light for water disinfection has become increasingly popular due to on-going issues with drinking water and public health. Pulsed UV light has proved to be an effective form of inactivating a range of pathogens including parasite species. However, there are limited data available on the use of pulsed UV light for the disinfection of flowing water in the absence or presence of inorganic contaminants commonly found in water sources. Here, we report on the inactivation of test species including *Bacillus* endospores following pulsed UV treatment as a flow through system. Significant levels of inactivation were obtained for both retention times tested. The presence of inorganic contaminants iron and/or manganese did affect the rate of disinfection, predominantly resulting in an increase in the levels of inactivation at certain UV doses. The findings of this study suggest that pulsed UV light may provide a method of water disinfection as it successfully inactivated bacterial cells and bacterial endospores in the absence and presence of inorganic contaminants.

Key words | *Bacillus*, endospores, flow through water, inactivation, pulsed UV

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INTRODUCTION

The incidence of water-related disease outbreaks resulting from the presence of pathogenic organisms has been well documented. The disinfection of public water supplies with chlorine has been instrumental in reducing the prevalence of disease; however, the resistance of parasitic organisms and microbial endospores to chlorination has led to the need for alternative disinfection methods. Furthermore, the formation of disinfection by products from chlorination raises concerns for public safety. The use of ultraviolet (UV) light for the disinfection of water has proved beneficial in terms of microbial inactivation and a reduction in water-associated disease outbreaks. The method of UV disinfection is due to the interference of UV energy with the genetic material of the microbial species, leading to the formation of DNA abducts (thymine dimers) and strand breaks that result in an inhibition of replication. The inability of the pathogen to reproduce itself (as indicated by bacterial growth in cultures or pathogen infectivity assays) is an indication of UV effectiveness. The higher the UV dose applied, the greater the nucleic acid damage, resulting in a lower percentage of cells that survive UV irradiation (Oguma *et al.* 2001). The UV systems which are currently applied for drinking water disinfection processes are monochromatic low pressure UV at a wavelength of 254 nm and medium pressure UV, which emits a wider range of wavelengths including UV-A, -B, -C and visible light. Although an effective means of inactivating pathogens, limitations of these lamps do exist including microbial repair mechanisms, depth of penetration and the presence of contaminants in the treatment medium. Alternative forms of UV delivery such as pulsed UV light have been developed which aim to overcome these issues. Pulsed UV systems operate by storing electrical energy in a capacitor and releasing it into the treatment chamber in very short duration pulses. These pulses contain a broader range of wavelengths and an increased penetration capacity than standard UV approaches. The use of pulsed UV light as a bench scale system has highlighted its effectiveness for the inactivation of bacteria, endospores, protozoans and viral test species (Garvey *et al.* 2010, 2014). However, there is limited information on the use of pulsed UV light for the disinfection of water as a flow through

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system, or in the presence of inorganic contaminants. Contaminants such as manganese and iron occur naturally in many groundwater, surface water and soil sources. Furthermore, human activities are responsible for much contamination of water in some areas leading to varying concentrations depending on location (WHO 2011). Manganese has uses in many industrial applications such as the production of iron, steel, aluminium and batteries, increasing its presence in waterways. Iron has many uses such as the construction of automobiles among many other industrial areas. Manganese II (Mn^{2+}) has been found at concentrations of up to 9.6 mg/L in groundwater while iron II is commonly found at a concentration of 0.3 mg/L in drinking water (WHO 1996). Due to the presence of these inorganic contaminants in water from natural and manmade sources, the aim of this study was to determine the efficacy of a pulsed light system for the disinfection of flowing water in the absence and presence of the inorganic contaminants.

METHODS

Pulsed UV system

For the studies described a pulsed power source (PUV-1, Samtech Ltd, Glasgow) was used to power a low pressure (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type NL4006 series constructed from a clear UV transparent quartz tube) that produced a high-intensity diverging beam of polychromatic pulsed light. This delivery system kills microorganisms by using ultra-short duration pulses of an intense broadband emission spectrum that is rich in the UV-C germicidal wavelength. Pulsed light is produced by storing electricity in a capacitor over relatively long times and releasing it as a short duration pulse using sophisticated pulse compression techniques. The emitted pulse has a broadband emission spectrum extending from the UV to the infrared region with a rich UV content (200–1,100 nm). The light source has an automatic frequency control function that allows it to operate at 1 pulse/s that was used throughout this study. The lamp was placed over the sterile treatment chamber giving a volume depth of 4.5 cm. In this study, standard treatments involved treating predetermined numbers of each test species to lamp discharge energy of 16.2 J directly under the light source. The UV dose was adjusted by increasing or decreasing the frequency of the pulsing.

Preparation of *Bacillus* species endospores

Bacillus endospores were cultivated as per the method of Periago *et al.* (2006) with some modifications. Endospores were prepared on petri dishes containing plate count agar (Cruinn Diagnostics, Dublin, Ireland) which was supplemented with 3 mg/L of manganese sulphate (Sigma-Aldrich Ltd, Arklow, Wicklow, Ireland). The agar surface was inoculated with 400 μL of a 24-h culture of either *Bacillus megaterium* (ATCC 14581) or *Bacillus cereus* (ATCC 11778) grown in nutrient broth at 37 °C. Plates were then incubated at 30 °C for 4 days to allow for bacterial sporulation due to nutrient depletion. *Bacillus* endospores were then collected by flooding the agar plate with sterile phosphate-buffered saline (PBS) (pH 7) and rubbing the surface with a sterile spatula. Samples were transferred to sterile bottles for storage. Spore samples were then heated to 90 °C for 25 min to kill any vegetative cells present. A control bottle was used to ensure the water inside each bottle reached 90 °C. After heating, endospores were washed twice by centrifugation at 10,000 rpm for 10 min and re-suspended in sterile PBS (Sigma-Aldrich Ltd, Arklow, Wicklow, Ireland). The purity of spore suspensions was checked using malachite green spore staining and phase contrast microscopy. The concentration of endospores in the final suspension was assessed by serial dilution of the stock culture followed by incubation on plate count agar at 37 °C for 24 h.

Pulsed UV inactivation of flowing test species

Flow through inactivation of test species was conducted on vegetative *Escherichia coli* (ATCC 25922), *B. megaterium* and *B. cereus* cells and on *Bacillus* bacterial endospores. Twelve-hour cultures (ca. 1×10^8 colony-forming units (cfu)/mL) of microbial species grown in nutrient broth at 37 °C or pre-cultured endospores (ca. 1×10^8 cfu/mL) prepared in sterile PBS were suspended in a 5 L sterile duran. The bacterial suspension was then pumped from the duran via a peristaltic pump (Watson Marlow Bredel pump, Wilmington, USA) to the sterile treatment chamber with a volume depth of 4.5 cm at set flow rates. The flow rates chosen for this

study, 200 and 375 rpm, gave a retention time (RT) of 60 and 120 seconds, respectively. Liquid was pulsed at a rate of 1 pulse per second (pps) and treated samples were collected aseptically at the exit of the treatment chamber.

Effect of the presence of inorganic matter in the treatment liquid

To determine the effect of inorganic contaminants on the inactivation rates of test species, pulsed UV studies were conducted in the presence of Mn^{2+} (Sigma–Aldrich Ltd, Arklow, Wicklow, Ireland) and iron (Sigma–Aldrich Ltd, Arklow, Wicklow, Ireland) and also in the presence of both. Concentrations of both were chosen to represent levels of contaminants commonly found in surface waters (0.3 mg/L iron, 10 mg/L manganese). Five-litre volumes of sterile PBS containing the set concentrations of contaminants were prepared by adding Mn^{2+} powder (w/v) and/or iron powder (w/v) to the liquid which was then inoculated with bacterial test species and treated as previously described.

Statistical analysis

All experiments were performed in triplicate with three plate replicates for each experimental data point (allowing for a total of nine replicates for each data point). The log reduction was calculated as the $\log_{10}$ of the ratio of the concentration (cfu/mL) of the non-treated (N0) and PUV treated (N) samples [$\log_{10}$ (N0/N)]. Linear regression analysis was used to determine the rate of inactivation for each test species under the regime of PUV treatments applied. Student's *t*-tests (MINITAB software release 16; Minitab Inc., State College, PA) were used to compare bacterial inactivation to PUV treatment in the absence and presence of inorganic contaminants and also to compare strain sensitivity to pulsed UV inactivation.

RESULTS

A decrease in cell and endospore viability was achieved with pulsed light when treated as a flow through system. However, a greater level of inactivation occurred at a lower flow rate, i.e. greater RT for all test species. A dose of 10.8 μJ/cm^2 resulted in a 4.30, 2.65 and 4.29 $\log_{10}$ inactivation of *E. coli, B. cereus* and *B. megaterium*, respectively, with an RT of 120 seconds (Figure 1 and Table 1). However, with a decrease in the RT (increase in flow rate) the inactivation rate also decreased significantly ($p < 0.05$) resulting in a 3.24, 2.44 and 2.2 $\log_{10}$ inactivation of *E. coli, B. cereus* and *B. megaterium* vegetative cells, respectively (Figure 2 and Table 1) with an RT of 60 seconds. A UV dose of 10.8 μJ/cm^2 is equivalent to 100 seconds of pulsing

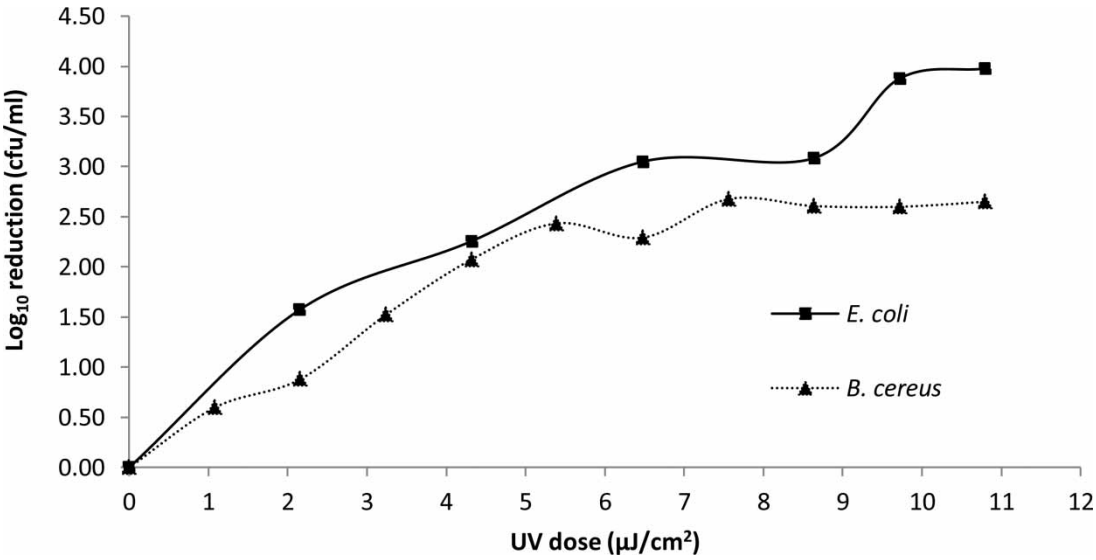

Figure 1 | $\log_{10}$ reduction of vegetative test species via a flow through pulsed UV system at an RT of 120 seconds and a flow rate of 24 L/h (±SD).

Table 1 | Pulsed light inactivation of *Bacillus megaterium* vegetative cells in the absence (O) and presence of organic contaminants manganese, iron and a mixture of both (±SD) at discharge energy of 16.2 J and a rate of 1 pps

Treatment time (seconds)	UV dose μJ/cm²	24 L/h – RT 120 seconds				30 L/h – RT 60 seconds			
		O	Manganese[a]	Iron[b]	M/I[c]	O	Manganese	Iron	M/I
10	1.08	1.2 (± 0.1)A	0.55 (± 0.3)B	1.05 (± 0.01)C	1.96 (± 0.2)D	1.06 (± 0.1)C	0.37 (± 0.4)B	1.66 (± 0.1)D	0.36 (± 0.2)B
20	2.15	1.19 (± 0.2)A	1.13 (± 0.2)A	2.26 (± 0.2)F	3.04 (± 0.04)G	1.64 (± 0.01)C	1.22 (± 0.1)A	1.54 (± 0.1)D	1.44 (± 0.08)C
30	3.24	1.21 (± 0.3)A	1.27 (± 0.02)A	2.46 (± 0.1)F	3.04 (± 0.2)G	1.31 (± 0.08)A	1.79 (± 0.2)D	1.66 (± 0.2)D	1.44 (± 0.1)C
40	4.32	1.26 (± 0.08)A	2.05 (± 0.3)E	2.35 (± 0.1)F	3.93 (± 0.1)H	1.26 (± 0.2)A	1.22 (± 0.1)A	2.25 (± 0.2)E	1.20 (± 0.3)A
50	5.39	1.35 (± 0.01)C	1.41 (± 0.1)C	2.35 (± 0.3)F	2.41 (± 0.09)F	1.96 (± 0.1)D	1.88 (± 0.01)D	2.14 (± 0.09)E	1.65 (± 0.2)C
60	6.48	2.11 (± 0.1)E	1.36 (± 0.01)C	1.99 (± 0.02)D	4.23 (± 0.3)I	2.36 (± 0.1)F	2.22 (± 0.2)E	1.82 (± 0.04)D	1.52 (± 0.07)C
80	8.64	2.99 (± 0.2)G	1.45 (± 0.08)C	2.42 (± 0.05)F	4.04 (± 0.1)I	2.37 (± 0.1)F	1.69 (± 0.3)D	1.49 (± 0.2)C	1.88 (± 0.09)D
100	10.8	4.29 (± 0.2)I	1.36 (± 0.1)C	2.56 (± 0.4)F	3.38 (± 0.3)H	2.20 (± 0.2)F	2.0 (± 0.2)E	1.38 (± 0.3)C	2.03 (± 0.2)E
120	12.96	4.34 (± 0.1)I	1.90 (± 0.2)D	3.46 (± 0.3)H	4.26 (± 0.01)I	2.60 (± 0.4)G	1.63 (± 0.1)C	2.45 (± 0.1)F	2.0 (± 0.1)E

A, B, C, D, E, F, G, H and I signify significant difference in sensitivity to pulsed UV treatment.
[a]Manganese concentration 10 mg/L.
[b]Iron concentration 0.3 mg/L.
[c]Manganese and iron.

(100 pulses) at a pulse rate of 1 pps. Although a significant level of inactivation was achieved for all test species, it must be noted that with an RT of 60 seconds the rate of inactivation decreased after a UV dose of 6.48 μJ/cm^2 (Table 1), equivalent to a treatment time of 60 seconds (60 pulses). The order of decreasing sensitivity to UV pulses for treated vegetative cells was *E. coli, B. megaterium* and *B. cereus*.

A similar trend was observed for bacterial endospores with a 1.52 and 1.45 $\log_{10}$ inactivation of *B. megaterium* endospores with 6.46 μJ/cm^2 (60 seconds treatment time) at an RT of 120 and 60 seconds, respectively. A maximal ca. 2 $\log_{10}$ (Table 2) inactivation was achieved with up to 32.40 μJ/cm^2 at an RT of 120 seconds (flow rate of 24 L/h), whereas, a maximal 1.4 $\log_{10}$ inactivation of *B. megaterium* endospores was achieved at an RT of 60 seconds (flow rate 30 L/h) with 29.16 μJ/cm^2. Endospores of the pathogenic *B. cereus* proved more UV sensitive than *B. megaterium* with a 1.39 and 1.49 $\log_{10}$ inactivation with 8.64 μJ/cm^2 (Figure 3) for an RT of 120 and 60 seconds, respectively. A maximal 1.67 and 1.51 $\log_{10}$ inactivation of *B. cereus* endospores was achieved with 25.9 and 21.6 μJ/cm^2 for RTs of 120 and 60 seconds, respectively. It is worth noting that *B. megaterium* vegetative cells were found to be more sensitive to UV pulses than *B. cereus*. However, *B. megaterium* endospores were found to be more resistant than *B. cereus*. These findings correspond to the research of Sharifi-Yazdi & Darghahi (2006), who suggests that this variance in sensitivity may be related to the size of the bacterium.

The findings also show that the presence of inorganic contaminants had an effect on pulsed UV inactivation rates for both vegetative cells (Table 1) and *B. megaterium* endospores (Table 2). At treatment doses up to 5.39 μJ/cm^2 there was an increase in the level of inactivation in the presence of manganese and iron for vegetative cells. However, with an increase in UV dose up to 12.96 μJ/cm^2 this pattern did not continue and there was a significant ($p \leq 0.05$) decrease in inactivation at an RT of 120 seconds. However, with a mixture of both contaminants present there was consistently more inactivation at all UV doses. With a decreased RT to 60 seconds the presence of iron increased the levels of inactivation up to a UV dose of 6.48 μJ/cm^2 after which a reduced level of cell inactivation occurred. At doses exceeding 1.08 μJ/cm^2 there was similar or slightly decreased levels of inactivation in the presence of manganese or both contaminants (Table 1).

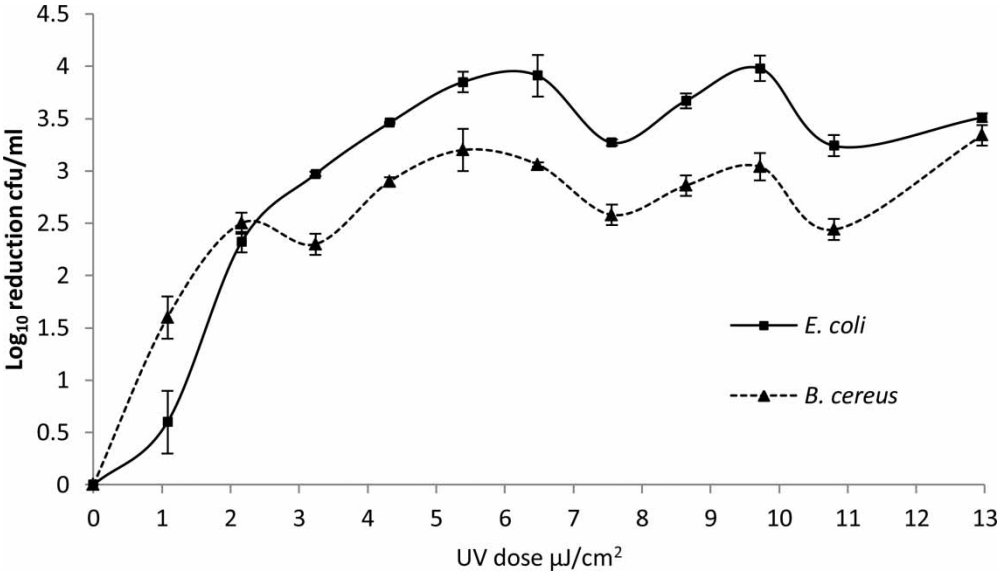

Figure 2 | Log_{10} reduction of vegetative test species via a flow through pulsed UV system at an RT of 60 seconds and a flow rate of 30 L/h (±SD).

For *B. megaterium* endospores the presence of manganese and iron resulted in a reduced level of inactivation at a 120-second RT at all treatment doses (Table 2); however, when treated in the presence of both contaminants there was a marked increase in the levels of inactivation. At an RT of 60 seconds (flow rate of 30 L/h) there was also a reduced inactivation rate up to a dose of 12.96 µJ/cm^2 after which significantly more inactivation of endospores occurred in the presence of manganese for each UV dose. The presence of iron led to a significant increase in disinfection occurring (Table 2) at UV doses exceeding 3.24 µJ/cm^2. In the presence of both contaminants the inactivation rates were more inconsistent with some doses providing a significant difference compared to others, e.g. UV doses of 3.34, 6.48, and 16.2 to 29.16 µJ/cm^2 led to an increase in activation while other doses were not significantly different.

DISCUSSION

Pulsed UV lamps are a relatively new technology that has had limited application to water treatment but has been used for the sterilisation of food, surfaces and pharmaceutical packaging (Bohrerova *et al.* 2008). Furthermore, research has shown that pulsed UV induces cellular damage to the organisms that cannot be undone by the cells repair mechanisms, meaning that, once inactivated, pathogens do not regain their ability to be infective. Such cellular damage is believed to include disruption to cell membranes and essential proteins following exposure to pulsed light. Indeed, studies conducted by Takeshita *et al.* (2003) to determine why pulsed UV results in the inactivation of species, which have resistance to standard UV, found that the high peak power of pulsed UV resulted in cellular damage not found with conventional UV approaches and attributed this to the higher levels of inactivation obtained for test species (Takeshita *et al.* 2003). Such bacterial potency suggests that pulsed UV may provide an excellent means of water disinfection. Studies described herein show that pulsed UV light provides significant bacterial and endospore inactivation when used to treat flowing water in the absence and presence of inorganic contaminants. Although the RT and flow rate did affect the rate of disinfection, significant levels of bacterial planktonic and endospore reduction were still achieved. Previous findings of this research group showed that *B. megaterium* endospores showed similar sensitivity to that of *Cryptosporidium parvum* when treated with pulsed UV light, suggesting that *B. megaterium* may be used as a surrogate organism for *C. parvum* disinfection studies (Garvey *et al.* 2012).

The presence of inorganic contaminants iron or manganese did affect the rate of disinfection, predominantly

Table 2 | Pulsed light inactivation of *Bacillus megaterium* endospores in the absence (O) and presence of organic contaminants manganese, iron and a mixture of both (±SD) at discharge energy of 16.2 J and a rate of 1 pps

Treatment time (seconds)	UV Dose μJ/cm^2	24 L/h – RT 120 seconds				30 L/h – RT 60 seconds			
		O	Manganese[a]	Iron[b]	M/I[c]	O	Manganese	Iron	M/I
30	3.24	0.25 (± 0.02)A	0 (± 0.4)B	0.31 (± 0.1)A	1.96 (± 0.2)	0.42 (± 0.08)C	0.22 (± 0.1)A	0.5 (± 0.2)C	0.2 (± 0.3)A
60	6.48	1.52 (± 0.1)G	0.5 (± 0.3)C	0.73 (± 0.2)D	3.04 (± 0.01)	1.45 (± 0.2)G	0.23 (± 0.02)A	0.82 (± 0.1)D	0.73 (± 0.1)D
90	9.78	1.95 (± 0.1)H	0.88 (± 0.2)D	0.93 (± 0.2)D	3.04 (± 0.01)	0.64 (± 0.4)D	0.29 (± 0.08)A	0.96 (± 0.1)D	0.73 (± 0.1)D
120	12.96	1.48 (± 0.2)G	0.95 (± 0.01)D	1.04 (± 0.01)E	3.93 (± 0.3)	0.79 (± 0.1)D	0.76 (± 0.1)D	0.62 (± 0.04)C	0.91 (± 0.02)D
150	16.20	1.73 (± 0.01)H	1.03 (± 0.01)E	1.31 (± 0.3)F	2.41 (± 0.2)I	0.82 (0.2)D	1.22 (± 0.1)E	1.08 (± 0.4)E	1.0 (± 0.1)E
180	19.44	1.61 (± 0.2)H	1.06 (± 0.07)E	1.14 (± 0.2)E	4.26 (± 0.2)J	0.57 (0.1)C	1.1 (± 0.2)E	1.16 (± 0.3)E	0.87 (± 0.2)D
210	22.68	1.61 (± 0.3)H	1.16 (± 0.1)E	1.07 (± 0.08)E	4.04 (± 0.1)J	0.54 (0.07)C	0.76 (± 0.04)D	1.52 (± 0.1)G	0.93 (± 0.1)D
240	25.92	1.54 (± 0.2)G	1.34 (± 0.2)F	1.06 (± 0.3)E	4.04 (± 0.1)J	0.84 (0.3)D	0.91 (± 0.01)D	1.66 (± 0.2)G	1.1 (± 0.08)E
270	29.16	1.67 (± 0.1)H	1.38 (± 0.08)F	0.87 (± 0.04)D	3.93 (± 0.2)J	1.4 (0.01)F	0.76 (± 0.3)D	1.79 (± 0.2)G	1.26 (± 0.08)F
300	32.40	1.52 (± 0.1)G	1.20 (± 0.2)F	0.96 (± 0.01)D	3.38 (± 0.03)K	1.31 (0.2)F	0.65 (± 0.01)C	2.49 (± 0.2)I	1.33 (± 0.1)F

A, B, C, D, E, F, G, H, I, J and K signify significant difference in sensitivity to pulsed UV treatment.
[a]Manganese concentration 10 mg/L.
[b]Iron concentration 0.3 mg/L.
[c]Manganese and iron.

resulting in an increase in the levels of inactivation of vegetative cells up 5.39 μJ/cm^2 at an RT of 120 seconds. The presence of Mn^{2+} is known to induce sporulation of *Bacillus* species (Vasantha & Freese 1979); therefore, it is possible that the reduced level of inactivation obtained after 12.96 μJ/cm^2 may be due to sporulation of the vegetative cells. At a reduced treatment time (60-second RT) the levels of inactivation remained similar in the absence and presence of Mn^{2+} or a mix of Mn^{2+} and iron, suggesting that this RT was too short to allow for sporulation to occur.

For *B. megaterium* endospores a reduced level of inactivation was obtained for iron and manganese at an RT of 120 seconds, suggesting that contaminants had a shielding effect on the endospores. However, a marked increase in inactivation for a mix of contaminants occurred. Interestingly, this was not consistent with the increased flow rate/reduced RT, suggesting that the variance in RTs or exposure time of endospores to the inorganic contaminants also impacts on UV disinfection. Further studies are warranted to determine the precise effect contaminants such as iron or manganese have on pathogens present in water sources. To fully elucidate their impact on UV disinfection rates, a clearer understanding of the reaction occurring between bacterial cell structures and inorganic contaminants is required.

CONCLUSION

Pulsed UV light successfully inactivated all test species when used as a flow through system at the flow rates or RTs studied. Significant levels of endospore inactivation were also achieved for both *B. megaterium* and *B. cereus*. The presence of inorganic contaminants iron and/or manganese did affect the rates of inactivation for *B. megaterium*. *B. megaterium* vegetative cells proved more UV sensitive than *B. cereus*; however, its endospores proved more resistant. From this study it can be concluded that lower flow rates equivalent to a longer RT under the UV lamp achieve higher levels of microbial inactivation. This technology may find application in the disinfection of water sourced from a high industrial area or water areas high in inorganic contaminants as it has proved effective for the inactivation of test species in the presence of inorganic contaminants.

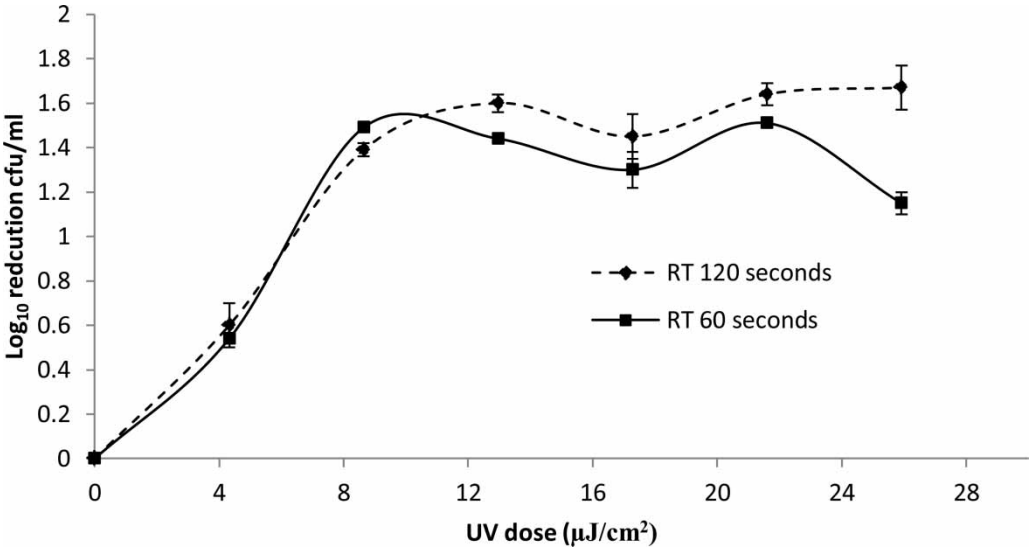

Figure 3 | Log_{10} reduction of *Bacillus cereus* endospores via a flow through pulsed UV system at an RT of 120 seconds and a flow rate of 24 L/h, and at a 60-second RT and a flow rate of 30 L/h (±SD).

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