Synergism of Low-Pressure UV and Chlorine for Water Disinfection

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Introduction

The primary purpose of drinking water disinfection is to prevent the transmission of waterborne pathogens through water supplies. Chemical disinfectants like chloramines, chlorine dioxide, chlorine, and ozone are being used at present for the inactivation of micro-organisms despite the potential formation of harmful disinfection by-products (DBP) and the inability to inactivate some micro-organisms such as Cryptosporidium parvum and Bacillus subtilis spores (Driedger *et al.*, 2000, Clevenger *et al.*, 2007). UV disinfection has been increasingly adopted in water and wastewater treatment but one of its major drawbacks is that it is not able to provide residual disinfection. As a residual disinfectant is often required for water distribution systems, the use of UV as primary disinfectant followed by chemical dosing has been reported.

Sequential application of UV and chemical disinfection is being implemented with UV as a primary disinfectant followed by chlorine as a residual disinfectant and it has shown that there is a synergetic inactivation of MS2 bacteriophages and Bacillus subtilis spores; the reason behind this is that the portion of the target inactivation level caused by a strong primary disinfectant helps to facilitate the inactivation by chlorine at a secondary stage (Cho *et al.*, 2011). Nevertheless, an approach balancing the enhanced disinfection efficacy while minimizing the unwanted DBP formation would be necessary to make this approach of practical use.

The aim of this research is to determine optimal doses and the sequence of UV and chlorine to achieve adequate disinfection while minimizing DBP formation in drinking water disinfection. The study investigated the disinfection efficacy of UV and Cl_2 alone and the combination UV/Cl_2. The disinfection efficacy will be assessed for spore (*B. subtilis*) and virus (MS2 phage) inactivation.

Materials and Methods

Materials

UV disinfection experiments were performed with a collimated beam apparatus with a low-pressure (28W) UV lamp (Berson UV, The Netherlands) and the UV intensity was measured with a radiometer (ILT1700, USA) with an optical detector (SED240/QNDS2/W) targeting UV light at a wavelength of 254 nm. The UVA_{254} of water samples containing the target microorganism in phosphate buffer saline (PBS) was calculated from the UVT measured using a UV spectrophotometer (P254C, UV photometer). The true UV irradiance and consequently the exposure times were calculated according to the protocol designed by Bolton and Linden (2003). All inactivation experiments were performed by using Delft tap water. All glassware was cleaned with demineralized-water and sterilized by autoclaving at 121°C for 20 minutes. Tap water was spiked with *B. subtilis* spore and MS2 phage suspensions. The methods that were followed for the enumeration of *B. subtilis* spores and MS2 phages were the method specified by USEPA (2006) and the ISO 10705-1 (1995), respectively. Chlorination experiments were performed using sodium hypochlorite. All experiments were carried out at ambient temperature (22 ± 1°C) and pH (8 ± 0.25).

Experimental Methodology

Single step chlorine inactivation

Before spiking into tap water, the stock solution of *B. subtilis* spores was treated by heat at 80°C for 10 minutes in a water bath to inactivate any vegetative cells. Subsequently, the stock solution was sonicated at 42 kHz for 10 minutes and 10°C to ensure that there was no cell aggregation. After sonication, 1 mL of spore suspension was transferred into 100 mL of 0.001 M phosphate buffer saline (PBS) to obtain 100 times dilution. The spore

suspension was further diluted to obtain a concentration of the spore suspension of about 1.0 x 10^6 CFU/mL. A 100 mL brown amber glass bottle was used as a batch reactor for the chlorine inactivation experiments. About 45 and 90 mL of the spiked tap water were transferred in a 100 mL reactor. From the chlorine stock solution 1.5, 2.0, 3.0, 4.0, 5.6, 11.7 & 20.6 mg/L were added into the reactor and sterile tap water was added to make up 100 mL of reactor volume. The initial concentration of spores was determined by using a spread plate count technique from the blank sample (without chlorine) after incubation of the plates at 37°C for 24 hours. The inactivation experiments were conducted with contact time in the range of 1 minute to 3 hours. Samples for determination of chlorine residual, bacterial viability assessment, DOC, pH, electrical conductivity (EC), temperature and UVA_{254} were drawn from the reactor after every 10 or 20 minutes up to a maximum of 3 hours.

Single step UV inactivation

Prior performing the UV inactivation experiments, the Petri Factor was determined by scanning with a UV detector sensor at every 5 mm in both Y and X directions (- & +) on the surface of the Petri dish prior each experiment. The Petri factor was obtained by the average of the irradiance at each point divided by the central irradiance (Bolton and Linden 2003); the values obtained were always above the recommended threshold of 0.9. A 57 x 35 mm sterile Petri dish filled with 35 mL of sterile Delft tap water containing *B. subtilis* spore suspension or 25 mL of sterile Delft tap water containing MS2 phage suspension. Samples for viability assessment were withdrawn from the reactor at various exposure times and immediately plated in Petri dishes containing Columbia agar or tryptone yeast extract glucose agar (TYGA) and finally incubated at 37°C for 24 hours.

Sequential UV and chlorine inactivation

The experiments for the sequential inactivation of *B. subtilis* spore were initially conducted by applying free chlorine as a primary disinfectant and UV light as a secondary disinfectant. Subsequently the order of disinfectants was reversed. The experiments were carried out to achieve an overall target of 1 log inactivation of *B. subtilis* spores. Both primary and secondary disinfection were set to achieve at least 0.3 log inactivation of *B. subtilis* spores. The sequential experiments were carried out by fixing the chlorine dose to 11 mg Cl_2/L and varying the UV dose to 7 and 10 mJ/cm² respectively. In the case of the Cl_2-UV sequence, 11 mg Cl_2/L was injected in a batch reactor of 100 mL containing 45 mL of sterile tap water plus *B. subtilis* spores (1 x 10^6 CFU/mL). Sterile tap water was used to fill up the reactor volume to 100 mL. After this target log inactivation was achieved the sample solution was quenched immediately with excess sodium thiosulphate (0.001 M) and subsequently, a 35 mL sample was exposed in duplicate to UV as secondary disinfectant. For the UV-Cl_2 sequence, a *B. subtilis* spore solution was exposed to UV light as a primary disinfectant in duplicate at a UV dose of 7 and 10 mJ/cm² to achieve 0.3 and 0.6 log inactivation respectively. Immediately after UV inactivation a sample was drawn for secondary inactivation with free chlorine disinfectant and the same procedure as for primary inactivation was followed.

Results

Synergy in the removal of B. subtilis spores

The results of the inactivation of Bacillus subtilis spores with free chlorine (NaOCl) indicate that the strain used (12.01.31, GAP Lab, Canada) is very resistant to chemical disinfection by chlorine (NaOCl), as a high free chlorine dose and an extended contact time are required for a significant log inactivation. Figure 1 shows that with a chlorine dose of 5.6 mg Cl_2/L no more than

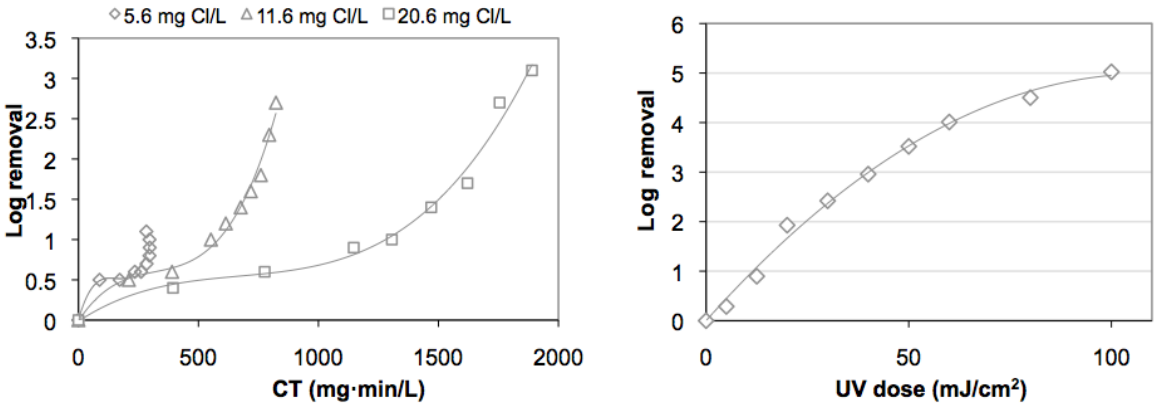

Figure 1. Inactivation of *B. subtilis* during single step application by free chlorine (left) and during the single step application of UV (right).

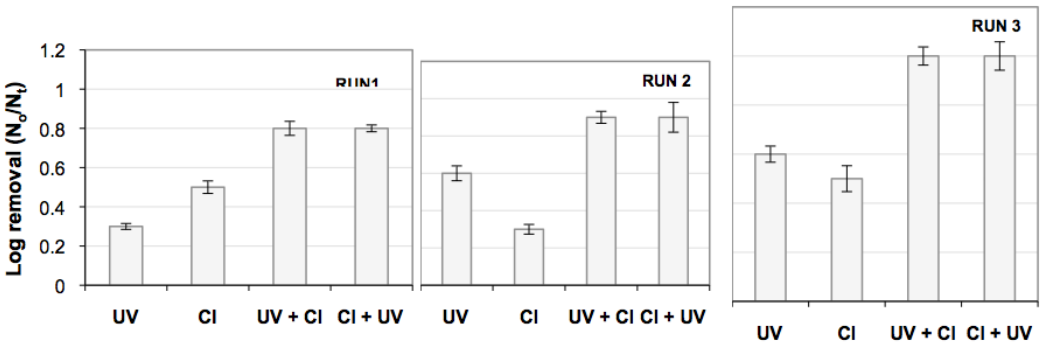

Figure 2. Inactivation of *B. subtilis* spore during sequential application of free chlorine and UV.

1-log inactivation of *B. subtilis* could be achieved. The log inactivation is not linear but it is fitting well to the polynomial function. To achieve a removal higher than 1-log, chlorine doses higher than 5.6 mg Cl_2/L and up to two hours contact time had to be applied for inactivation of *B. subtilis* spores. With a dose of 11.6 mg Cl_2/L dose and 2 hours contact time 2.7-log inactivation could be achieved while 100% inactivation could be achieved with a dose of 20.6 mg Cl_2/L and three hours contact time. About 3-log removal was achieved with 20.6 mg Cl/L after about 2 hours contact time.

Figure 2 shows the results of sequential application of UV and free chlorine in both forward and reverse order for three experimental runs, where the inactivation target was 0.8, 0.9 and 1 Log respectively. In these experiments the overall inactivation target was predetermined to be 1-log inactivation of *B. subtilis* spores. The three experiments were performed applying a UV dose of 7 mJ/cm² for the first run and 10 mJ/cm² for the following runs; the CT values applied were 396, 206, 396 mg Cl_2 min/L, respectively.

Synergy in the removal of MS2 phages

The experiments to test the inactivation of MS2 phages by free chlorine were performed with doses of 1 mg Cl_2/L, 0.5 mg Cl_2/L and 0.3 mg Cl_2/L. Table I shows the decrease in viability of MS2 phage versus CT during the single step free chlorine disinfection. The MS2 phages were very susceptible to chlorination and were effectively inactivated by free chlorine at a temperature of 22 ± 1o C and a pH of 7.9 ± 0.3. The UV dose response curve for MS2 phages is presented in Figure 3.

The sequential inactivation of UV and free chlorine was performed with UV doses of 11 mJ/cm² and 23 mJ/cm² and an initial free chlorine concentration of 0.3 mg Cl_2/L. Figure 4 shows that the inactivation efficiency of chlorine on MS2 phages is high compared to UV disinfection and the effect of pre-UV irradiation on secondary disinfection by chlorine could not be established since the application of free chlorine alone already inactivated 100% of the MS2 phages after 1 minute contact time.

Conclusions

The results showed that free chlorine is ineffective for the inactivation of *B. subtilis* spores as very high chlorine dosages of 5.6 mg Cl_2/L and long contact times of 2 hours are required to achieve at least 1-log removal. UV light is very effective for inactivation of *B. subtilis* spores with an optimum UV dose of 40 mJ/cm². At higher UV dosages tailing is observed. No synergistic effect in the combination of UV and free chlorine during the inactivation of *B. subtilis* spores was observed.

MS2 phages are very sensitive to chlorination as complete removal was already obtained at a CT value as low as 0.19 mg min/L. MS2 phages are more resistant to UV light disinfection than to chlorination. No synergistic

Table I. CT value and log inactivation for MS2 phage disinfection by free chlorine.

	0.3 mg Cl_2/L			0.5 mg Cl_2/L			1.0 mg Cl_2/L		
Contact time (min)	Residual chlorine (mg Cl_2/L)	CT (mg·min/L)	Log removal (%)	Residual chlorine (mg/L)	CT (mg·min /L)	Log removal (%)	Residual chlorine (mg/L)	CT (mg·min /L)	Log removal (%)
0	0.30	0.00	0.00	0.46	0.00	0.00	0.85	0.00	0.00
1	0.19	0.19	100	0.45	0.45	100	0.46	0.46	100
2	0.13	0.13	100	-	-	-	-	-	-
3	0.12	0.12	100	0.44	1.32	100	0.45	1.35	100
4	0.10	0.10	100	-	-	-	-	-	-
5	0.09	0.09	100	0.43	2.15	100	0.43	2.15	100
6	0.08	0.08	100	-	-	-	-	-	-
60	-	-	-	0.02	1.2	100	0.07	4.2	100

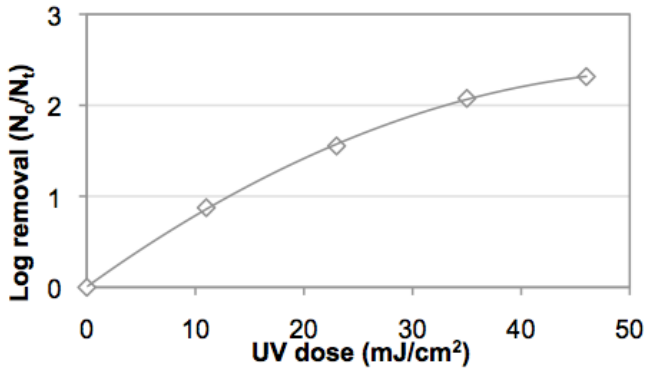

Figure 3. UV dose-response curve for MS2 phages.

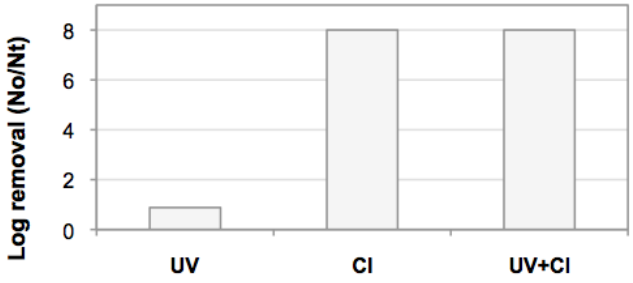

Figure 4. Inactivation of MS2 phages during sequential application of free chlorine and UV.

effects were observed when UV light was applied as a primary disinfectant followed by free chlorine as a secondary disinfectant.

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Investigation of UV-TiO_2 Photocatalysis and its Mechanism on Bacillus Subtilis Spores Inactivation

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Abstract

The inactivation levels of *Bacillus subtilis* spores for various disinfection processes (UV, TiO_2 and UV-TiO_2) were compared. The results showed that compared with UV treatment alone, the inactivation effect increased significantly with the addition of TiO_2. Increases in the irradiance or TiO_2 concentration both contributed to the increasing inactivation effect. Malondialdehyde (MDA) was used as an index to determine the extent of the lipid peroxidation. The MDA concentration surged with the simultaneous process. At the same time the cell membrane was totally damaged and cellular contents were completely lysed by UV-TiO_2 treatment.

Keywords: UV; TiO_2; *Bacillus subtilis*; spore; disinfection; lipid peroxidation

Introduction

Ultraviolet (UV) technology has shown its high efficiency, operational convenience and low yield of DBPs formation in drinking water and wastewater disinfection [1]. UV treatment is found to be effective with some chlorine-resistant microorganisms, such as *Cryptosporidium* and *Giardia* [2]. However, due to the limitations of physical disinfection processes such as UV, other chemicals are required in order to maintain the capability of continuous disinfection and to improve the inactivation level [3, 4].