Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1086693

57 (2016) 17537–17546
August

Taylor & Francis
Taylor & Francis Group

Considerable discrepancies among HPC, ATP, and FCM detection methods in evaluating the disinfection efficiency of Gram-positive and -negative bacterium by ultraviolet radiation and chlorination

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Received 20 January 2015; Accepted 14 August 2015

ABSTRACT

Up to now, the effects of ultraviolet (UV) and chlorine disinfection on microbes have been evaluated by heterotrophic plate counting (HPC). Recently, with the application of flow cytometry (FCM) in aquatic environment, it is used to analyze the membrane integrity, DNA damage and enzymatic activity of bacteria. The present work investigated the disinfection efficacy of UV-C and chlorine on *E. coli* (Gram negative) and *Bacillus subtilis* (Gram positive) and compared the detection methods for disinfection efficacy with HPC, Adenosine triphosphate (ATP), and FCM. The results show that there are considerable discrepancies among HPC, ATP, and FCM detection for *E. coli* and *B. subtilis* when disinfected by UV and chlorine. Specifically, the bacterium was sharply inactivated when evaluated by HPC, whereas the more gentle inactivation trend was observed when detected by ATP and FCM during both UV and chlorine disinfections for *E. coli*. In addition, for *B. subtilis* disinfection, the results between ATP and HPC detection were of little difference, especially with chlorine disinfection; thus, ATP measurement could be a replacement of HPC as it was much more fast and convenient. The results of FCM demonstrated that most of the bacteria undergo viable but non-culturable (VBNC) states which could not be detected by HPC but had the infection ability. Considering the risk of VBNC cells and the limitation of HPC, the following was suggested: when applied UV-C disinfection, HPC should combine ATP or FCM to evaluate the microbial viability, whereas FCM was a powerful tool to distinguish viable, but non-culturable cells (VBNC) applied for chlorine disinfection.

Keywords: Disinfection; Flow Cytometry; Adenosine triphosphate

1. Introduction

Chlorine has been the commonly used disinfectant in both drinking water and wastewater disinfections in the past decades, because it is cost effective, convenient, and safe compared to other disinfectants. But the efficacy of chlorine to microbes varied greatly. *E. coli* can be inactivated effectively at low chlorine dosage, whereas much higher dosage of chlorine is

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required to achieve the target goal with respect to *Cryptosporidium* [1]. The different effectiveness of chlorine to microbes may be related to the special structure of the spores [2]. Additionally, organic matter in water can react with chlorine resulting in the formation of disinfection byproducts (DBPs), i.e. when chlorine disinfection applied to wastewater effluent, high concentrations of eight N-nitrosamines are yielded [3]. DBPs have attained increasing attention due to its toxicity [4–8], alternative disinfectants have been used to avoid or control the formation of DBPs. Ultraviolet (UV) is very effective to *Cryptosporidium parvum oocysts* and *Giardia lamblia cysts* at normal dosage and can prevent pathogen infection [9–11], but the effect of UV disinfection is influenced by the water quality parameters to large extent and thus high UV dosage is needed to achieve target inactivation efficiency in wastewater disinfection [12]. The mechanism of UV disinfection is mainly due to the formation of thymine dimers, which ultimately results in reproductive cell death [13]. During UV disinfection, the formation of most DBPs is avoided due to no chemical reagent addition. So, UV disinfection especially low pressure UV radiation (253.7 nm) has been utilized in many water treatment plants to ensure the safety of microbes and the control of DBP formation. However, microbial re growth through photo-reactivated and dark-reactivated processes cannot be avoided without any residual disinfectants during water reservation and distribution [14]. Considering the disinfection efficiency, DBP formation, and the constancy of inactivated microbes, improvement should be made not only on disinfection process but also on the detection method to monitor the bacterium in time.

Traditionally, the death of bacteria is defined as it cannot grow on heterotrophic plate culture. In fact, as exposed to environment stress conditions, some bacterium have the ability to maintain low metabolic activity but are difficult to be cultured by HPC [15,16]. The above-mentioned bacterium are defined as viable but non-culturable cells (VBNC) or active but non-culturable cells (ABNC). During the disinfection process, bacterium experiences several steps: damage to enzymes activity, respiration cease, reduction of metabolic activity, change of membrane potential, and damage of membrane integrity [17]. Conventionally, HPC is used to detect the disinfection efficiency while the process is time consuming and laborious. The cells in intermediate state such as VBNC can hardly be detected by the culture-based method. In the last decades, flow cytometry (FCM) has been introduced to cell biology and microbiology, especially in single-cell analysis which can analyze cells rapidly and accurately [18]. FCM spreads widely and becomes a powerful tool for the detection of micro-organism in aquatic treatment.

FCM combined with advanced fluorescent dye technology can express detailed metabolic states of bacterium in aquatic environment and during water treatment process [19]. Until now, a range of fluorescent dyes can be obtained which target the cell components such as membrane integrity and potential, enzyme activity, and DNA [20,21]. With the FCM analysis, a better insight of the metabolic dynamic changes can be obtained during the cell injuries or disinfection process [22]. Bosshard et al. and Berney et al. have investigated the death process during solar disinfection with FCM and demonstrated the changes of ATP level, polarization, membrane integrity, membrane potential, and metabolic activity [21,23]. Schenk [20] reported that UV radiation also produces significant damage to the cytoplasmic membrane integrity and the cellular enzyme activity of *E. coli* and *S. cerevisiae* stained with FDA and PI. Usually, several fluorescent dyes are used to measure multiple parameters and give the relative information. In this work, SYBR Green I and propidium iodide (PI) were selected to measure the membrane integrity during disinfection process and compare with the results achieved by HPC.

ATP is often used as energy currency by almost all organisms. It serves as a mediator and energy carrier for energy transformation during cell metabolism. Therefore, ATP has been regarded as a potential indicator for viable biomass estimation [24]. Recently, ATP analysis has been developed and applied in assessing bacterium in granular activated carbon in water treatment [25], natural communities in aquatic environments like bacteria in biofilms [26] and biofilters in drinking water treatment plants [24,27,28]. ATP was a suitable indicator parameter to easily and rapidly assess biomass in drinking water distribution system as there was a linear relationship between log [ATP] and log (HPC-R2A/mL) [29] and quantitatively determine the total microbial activity in distributed drinking water [30]. Comparable assessment of ATP, FCM, and HPC in evaluation bacteria in water treatment and distribution system has been studied. The combination of ATP and FCM has been used to quantify the particle-associated bacteria, and revealed that 25 and 50 cells were found to be attached on a single particle which was contrary to the results of one single colony measured by HPC method [31]. Berney et al. [32] has detected the water treatment efficiency and compared them by FCM, ATP, and HPC and showed that: a combination of the FCM and ATP gives a more detailed insight into specific treatment processes than the use

of only one technique alone because only a minute fraction of intact cells detected by FCM was also cultivable, and conventional cultivation-based methods were unable to satisfactorily describe water treatment processes in all cases. It has been reported that both FCM and ATP were able to describe the microbiology accurately with the same trend in drinking water treatment and distribution systems and providing meaningful process data when combined with other parameters such as dissolved organic carbon analysis [33]. However, different results were obtained by ATP and FCM for the total cell counts (TCC) that ATP could accurately describe both suspended and particle-associated biomass, whereas files of TCC measurements needed a pretreatment process to load particles attached on samples [34]. Meanwhile, the correlation study of parameters describing biostability found no relationship among TCC, HPC, and ATP, and TCC was a valuable parameter to assess the drinking water biological quality and regrowth. TCC could directly and sensitively quantify biomass, detect small changes, and determine the subgroup of active HNA bacteria that were related to ATP [35]. Thus, it was also reported that a combination of methods were encouraged to use instead of any single method to assess the quantitative bacterial growth and community in water distribution system [36].

E. coli and *Bacillus subtilis* which are representative indicator micro-organisms of Gram-negative and Gram-positive strains are often used in various investigations. For Gram-negative bacterium, the cell wall is thin and the lipopolysaccharide layer of the exterior membrane is often tightly cross-linked by divalent cation, whereas for the Gram-positive bacteria, outside the cell membrane is the thick peptidoglycan layer in the cell wall. Due to the above-mentioned difference of the cell wall structure, the resistance to the physical or chemical disinfectants of the two kinds of strains varies consequently. In this work, the inactivation progresses of the two kinds of strains by UV and chlorine were investigated.

The disinfection efficiencies of UV and chlorine were detected by observing the bacteria that can grow into colony on HPC and the VBNC with FCM and Luminometer. The discrepancy among the different detection methods may give some suggestions to evaluate the disinfection efficiency when applying UV and chlorine disinfections. The goal of this study was to evaluate the inactivation efficiency of UV and chlorine on *E. coli* and *B. subtilis*, with several detection methods including HPC, ATP activity, and FCM, and compare the differences between the two bacteria when detected by various methods.

2. Material and methods

2.1. Preparation of bacterial suspension

E. coli and *B. subtilis* are chosen as the representative indicator micro-organisms of Gram-negative and Gram-positive strains separately.

Growth media and cultivation condition LB broth (10 mg/L NaCl, 10 mg/L tryptone, 5 mg/L yeast extract) was prepared with ultrapure water and sterilized at 121˚C, 20 min before cultivation. Bacteria were prepared for each individual batch experiment from the same stock culture stored at −80˚C by streaking the stock solution onto LB agar plate. For *E. coli* and the cells of *B. subtilis*, after 15–18 h of incubation at 37˚C, one colony was picked using loop inoculated into a 200-mL Erlenmeyer flask containing 100 mL of LB broth, and incubated at 37˚C on a rotary shaker at 200 rpm. To avoid the production of spores, after 10–12 h, with an optical density at 600 nm (OD600) is between 1.0 and 1.2 and the microbial growth reach stationary phase.

2.2. Sample preparation

Cells were harvested by centrifugation from batch culture (5,000 g 10 min), washed three times and suspended with sterilized 0.85% NaCl at pH 7.0. Bacterial suspension was diluted to 10^6–10^7 cells/mL with 5 mM phosphate buffer (pH 7). The concentration of free chlorine was determined by DPD/FAS titration.

2.3. Disinfection progress

For UV disinfection, water samples (15 mL) were placed in circular glass bowls (diameter 6 cm, water depth <2 cm) under the lamps at a distance of 30 cm at room temperature around 25˚C. The light wavelength emitted from UV lamps was 254 nm, and the light intensity was determined by UV detector and calculated according to Bolton [37]. As for chlorine disinfection, 15 mL of bacterial suspension was placed in 40-mL brown bottle to avoid the light effects and maintained at 25˚C. Certain NaClO was added to the suspension to achieve the target initial chorine concentration, mixed for 30 min and 10 μL of 0.1 M $Na_2S_2O_3$ was added to quench the reaction. Each experiment was replicated twice.

2.4. Flow cytometric measurement

Flow cytometric measurement was conducted using an Accuri C6 with excitation wavelength at 488 and 630 nm from an argon ion laser. With FCM detection, the following fluorescent dyes were used: SYBR

Green I with corresponding FL1 (green fluorescence) and PI (invitrogen) with corresponding FL3 (red fluorescence) and P3 was set as the gate to distinguish the cells intact or not. Samples were pretreated according to Berney et al. [32]. Specifically, the samples after treatment were diluted with bottled water Evian (filtered by 0.1-μm membrane) to the concentrations between 10^4–10^6 cells/mL, stained with SYBR Green I/PI and incubated in the dark at 30℃ for 10 min. The fluorescent dyes were prepared with dimethylsulfoxide (Sigma) (filtered with 0.1-μm membrane), and stored in −20℃. The final SYBR Green I concentration used was 10^4 times diluted from the original stock. The working concentration of PI was 30 μM.

2.5. ATP Measurement

ATP was measured by the BacTiter-Glo Microbial Cell Viability Assay and Luminometer. Both reagent and samples were incubated at 38℃ for at least 10 min (10–20 min), then added the samples to the reagent, and sustained at 38℃ for exactly 20 s, the luminescence was subsequently measured as an integral over 10 s, expressed as relative light units (RLU). The calibration curve of ATP and RLU were prepared by diluting pure ATP with filtered bottled Evian to concentration in the range of 0.01–10 nM. The RLU can convert into ATP according to the standard curve. To distinguish the total ATP and extracellular ATP, 0.1-μm filters were used to separate microbial and extracellular. ATP in the bacterial was calculated by subtracting extracellular ATP from total ATP, each experiment was replicated three times and standard deviations are shown in the figures.

2.6. HPC detection

After disinfection, an aliquot of suspension was withdrawn and diluted with sterilized 0.85% NaCl to achieve the cell concentration with 30–300 cells/mL, and mixed 0.1 or 1 mL with 10 mL of solid LB at 48℃. The plates were sustained in 37℃ for 18–24 h for further analysis, each experiment was replicated three times and standard deviations are shown in the figures.

3. Results and discussion

3.1. E. coli *and* B. subtilis *disinfection with UV*

The *E. coli* inactivation curve using UV disinfection is shown in Fig. 1. As shown in Fig. 1, *E. coli* was inactivated sharply by UV when detected by HPC, whereas the inactivation was very slow when analyzed by ATP content. Specifically, 4log inactivation of *E. coli* was achieved with a UV dosage of 20 mJ/cm^2, and maintained constant at about 4.5–5log inactivation with UV dosage increased to 80 mJ/cm^2. On the contrary, the ATP content changed slightly during the whole process, as no more than 0.5log inactivation was achieved with the UV dosage increased to 80 mJ/cm^2. When measured by FCM, compared to the bacteria without any treatment, the viable bacterial population demonstrated strong green fluorescence and weak red fluorescence, while a completely permeable bacterial population showed weak green fluorescence and strong red fluorescence. The change of cell permeability was reflected by the position fluorescence shift of the bacterium cluster. It was shown that UV disinfection had little effect on membrane integrity, as shown in Fig. 2. During the whole process, the position of bacteria cluster hardly changed and a small portion of bacteria moved to the outside of the gate which indicated the integrity of most of the bacterium (Fig. 2(b) and (c)). However, with the UV dose of 80 mJ/cm^2, the bacteria were hardly cultivated by HPC. This indicated that *E. coli* was sharply inactivated as detected by traditional method but slight damage of the cell membrane with UV disinfection. The phenomenon was correlated with the mechanism of UV disinfection that UV inactivated bacterium though the formation of pyrimidine dimer and inhibited DNA replication, which was contrary to the research of Schenk et al. who demonstrated that the membrane of *E. coli* was damaged during UV-C disinfection [20]. The above results combined with the mechanism of UV disinfection indicated that

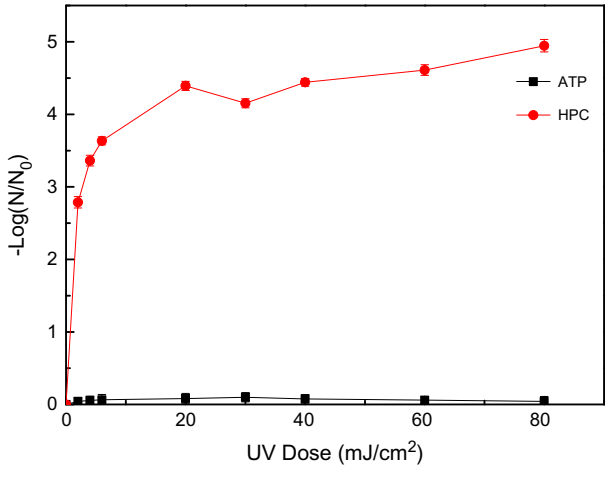

Fig. 1. Disinfection curve of *E. coli* by UV. Detected by HPC and ATP. Experimental conditions: initial concentration: 10^7 cells/mL, pH 7 with 5 mM phosphate buffer.

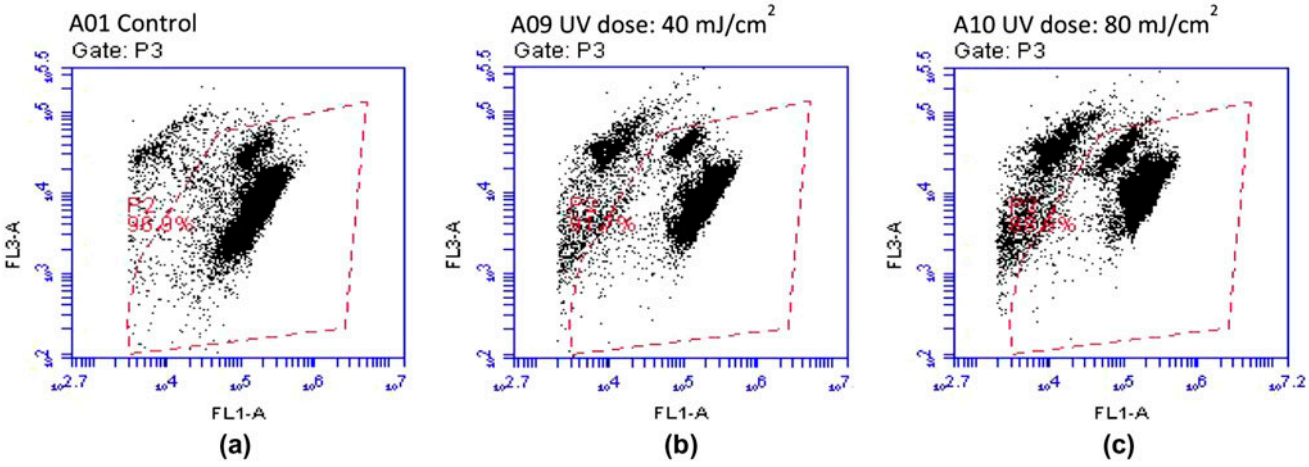

Fig. 2. FCM detection of the membrane integrity of *E. coli* during UV disinfection. (a) control; (b) 40 mJ/cm^2 treatment; (c) 80 mJ/cm^2 treatment.

HPC was more suitable than FCM and ATP detection during the progress.

Similar to *E. coli*, the cells of *B. subtilis* were sensitive to UV irradiation and were inactivated with a low dosage. The inactivation detected by HPC declined rapidly with low UV dosage, whereas ATP content depressed slower with the corresponding UV dosage, as shown in Fig. 3. Specifically, 3log inactivation of *B. subtilis* was achieved with 20 mJ/cm^2, whereas ATP content only decreased by 1.5log. With increased UV dosage, the inactivation of *B. subtilis* expressed the tailing phenomenon which fluctuated around 3–3.25log inactivation detected by HPC, whereas the ATP content continued to decrease to about 2.5log inactivation. The discrepancy between HPC and ATP detection along the whole process could be explained by the death progress during UV disinfection. The results of FCM detection demonstrated that the membrane of bacteria was hardly damaged during UV disinfection, as shown in Fig. 4.

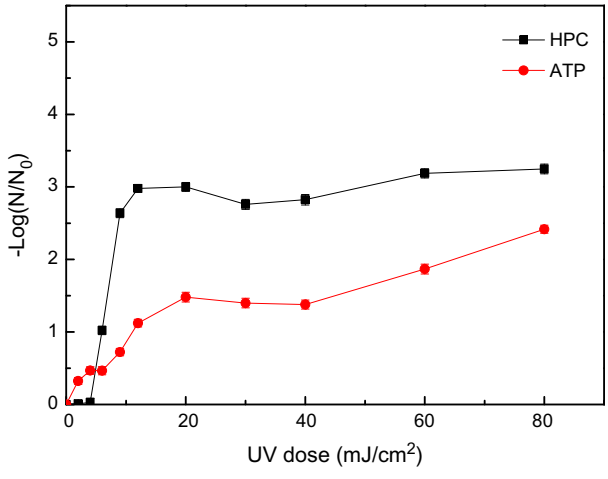

Fig. 3. Disinfection curve of *B. subtilis* by UV. The results were detected by HPC and ATP. Experiment conditions: initial concentration: 10^6–10^7 cells/L; pH 7 with 5 mM phosphate buffer.

As can be seen from Fig. 4, with the UV dosage increased from 0 to 80 mJ/cm^2, no significant change of the bacterial position in FCM was detected which indicated slight damage to the membrane during the process (Fig. 4(b) and (c)). Compared to the results of HPC detection, great discrepancy existed among them and both bacterium were inactivated only to small extent assessed by FCM. Specifically, ATP content in *E. coli* changed no more than 0.5log with UV dosage increased from 0 to 80 mJ/cm^2, which could be neglected compared to HPC detection with approximately 5log inactivation. For *B. subtilis*, about 2.5log inactivation was attained by ATP measurement while 3.25log inactivation was achieved by HPC detection with the UV dosage of 80 mJ/cm^2. The different trend of ATP content during UV disinfection was attributed to the different compositions and structures of the two cells which resulted in the different sensitive to UV disinfection.

3.2. E. coli *and* B. subtilis *disinfection with chlorine*

As for chlorine disinfection, as shown in Fig. 5, HPC detection indicated that *E. coli* was inactivated rapidly with low dosage of chlorine while ATP content changed with only small extent during the whole process. With the chlorine dosage of 0.12 mg/L,

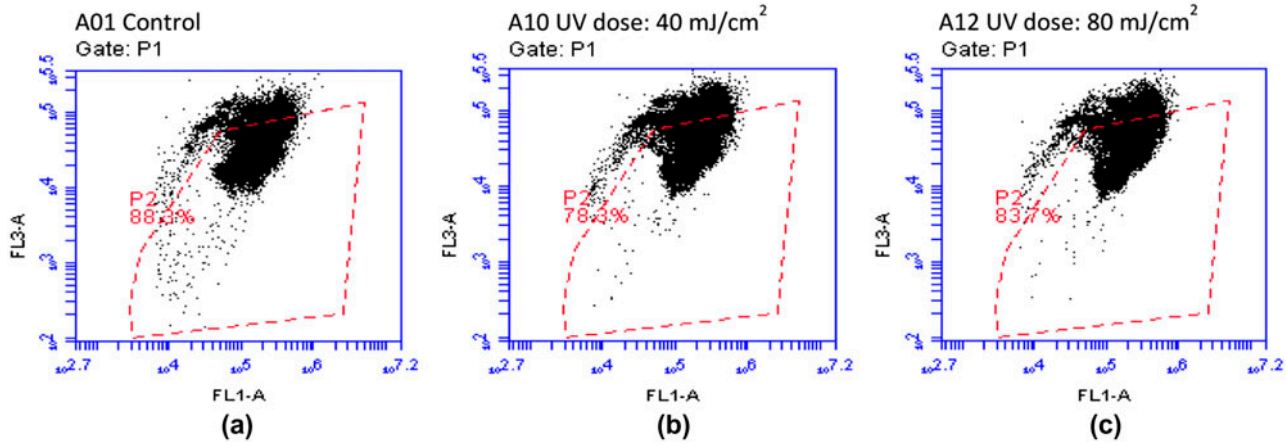

Fig. 4. FCM detection of membrane integrity of *B. subtilis* during UV disinfection. (a) control, (b) 40 mJ/cm^2 treatment, and (c) 80 mJ/cm^2 treatment.

contacting 30 min, 4log inactivation of *E. coli* were observed and maintained around 4–4.5 log with the increased chlorine dosage to 0.18 mg/L whereas when it was detected by ATP around 0–1log inactivation was observed. FCM detection implied that the membrane integrity changed more slowly than HPC cultivation with the chlorine increased from 0 to 0.2 mg/L, which indicated that most of the bacterium were VBNCs, but part of the bacterial membrane was completely damaged, as shown in Fig. 6. These cells could not grow into colony on nutrient culture but they sustained the bioactivity and could regrow in some conditions and have the infection ability. This was in accordance with the research of McDougald et al. [38], which pointed out that some bacteria were capable of sustaining metabolic activity but were uncultivable.

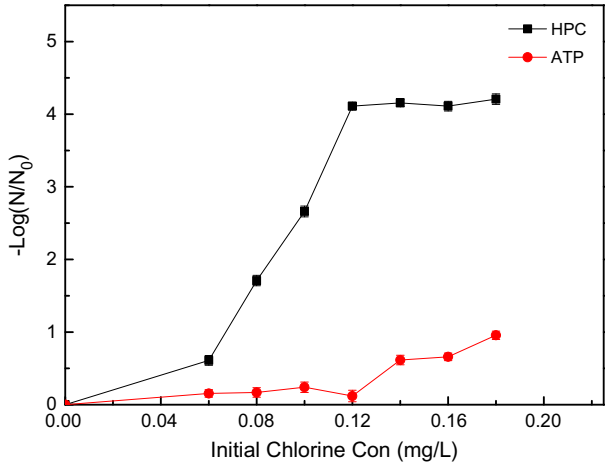

Fig. 5. Disinfection curve of *E. coli* by NaClO. The results were detected by HPC and ATP. Experiment conditions: initial concentration: 10^7 cells/L, contact time: 30 min, pH 7 with 5 mM phosphate buffer.

During chlorine disinfection, the cells of *B. subtilis* were effectively inactivated and detected by both HPC and ATP content, as shown in Fig. 7. With the initial chlorine dosage increased from 0 to 0.2 mg/L, contacting time 30 min, 3log inactivation of *B. subtilis* was observed steadily by HPC and 2.5log inactivation by ATP which indicated the discrepancy existed between HPC and ATP, but the difference was much smaller than *E. coli*. Besides, corresponding to chlorine dosage, *B. subtilis* was more resistant to chlorine disinfection than *E. coli*. FCM detection showed that most of the bacterium were VBNCs, but few of them were completely damaged, as shown in Fig. 8. The results of FCM were in accordance with HPC, while VBNCs were unable to grow into colony on HPC. The disparity among different detection methods was partly explained by the disinfection mechanism and the existence of VBNCs. Compared the Gram-positive and -negative strains, the sensitivity to UV and chlorine was affected by the composition and structure of the cell wall, while the mechanism of disinfection was of little difference to both of them. For *B. subtilis*, when applied to chlorine disinfection, ATP detection could be used to replace HPC, while the results of HPC and ATP detection were similar to each other and it was convenient and fast. When *E. coli* was disinfected by chlorine, great discrepancy existed between HPC and ATP which suggested that HPC should combine ATP to assess the bacterial viability and ensure the safety of microbes.

A summary of the mechanism of UV and chlorine disinfections and comparison of the different methods

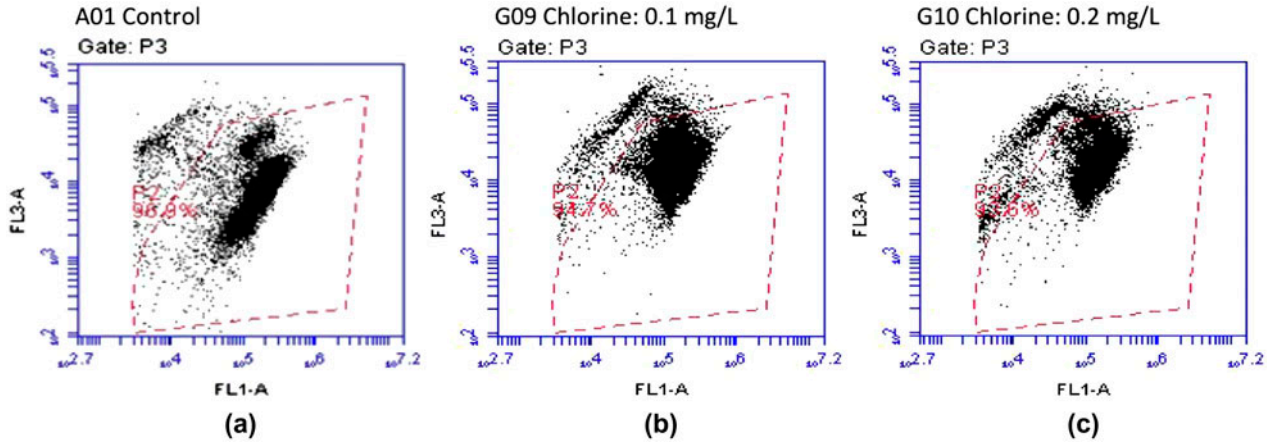

Fig. 6. FCM detection of the membrane integrity of *E. coli* during NaClO disinfection. (a) control, (b) 0.1 mg/L initial chlorine concentration treatment, and (c) 0.2 mg/L initial chlorine concentration treatment.

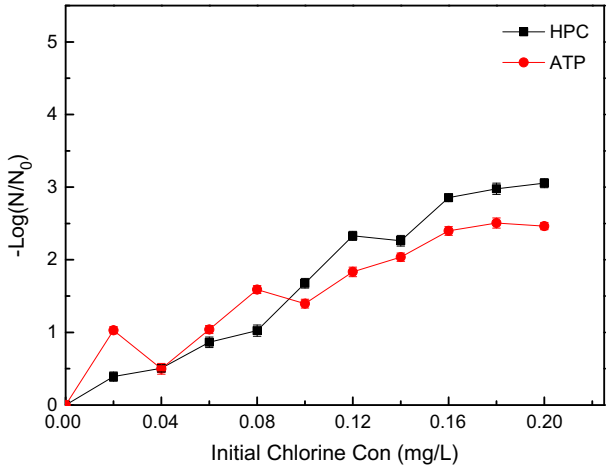

Fig. 7. Disinfection curve of *B. subtilis* by NaClO. The results were detected by HPC and ATP. Experiment conditions: 10^6–10^7 cells/L; contact time: 30 min; pH 7 with 5 mM phosphate.

for assessing of the efficiency of *E. coli* and *B. subtilis* disinfected by UV and chlorine are presented in the following:

The different disinfection results of various detection methods during UV and chlorine disinfections was ascribed to the mechanism of disinfection, the existence of the VBNC during the process to some extents, and the property of each detection technique. Specifically, nutrient culture was only applied to detect the culturable cells, whereas FCM could distinguish if the cell membrane is intact or not. So during UV disinfection, both of the Gram-positive and -negative bacteria, the cell membrane was only slightly damaged even at high dosages, the disinfection efficiency detected by FCM underestimated the actual effect and FCM was not suitable for evaluating the results of UV disinfection both for Gram-positive and -negative bacteria. On the other hand, to assess the microbial viability more accurately, more emphasis should be placed on the combination of ATP and HPC. For chlorine disinfection, *E. coli* and *B. subtilis* cells were easily inactivated at low dose detected by HPC. The main targets during chlorine disinfection are the membrane and the cell components inside the cells such as DNA, protein as well as enzymes. Comparing the results of FCM and HPC, the combination of them was recommended to assess the microbial viability and distinguish the bacterium in VBNC states (Table 1).

Comparing the results of *E. coli* and *B. subtilis*, which represent the Gram negative and Gram positive, respectively, some differences also existed between them. Generally speaking, both of them were very sensitive to UV, while *B. subtilis* was more resistant than *E. coli* to chlorine disinfection. To detect the disinfection efficiency of UV, HPC, FCM, and ATP were used, and great discrepancy existed among them. Rapid inactivation was observed by HPC, whereas FCM showed that cell membrane was seldom completely damaged. ATP measurement demonstrated that no more than 0.5log inactivation was achieved during the whole disinfection process for *E. coli*, whereas for *B. subtilis*, 1log disparity existed between HPC and ATP within the whole process. When applied to chlorine disinfection, about 4log inactivation discrepancy also existed between HPC and ATP detection for *E. coli*, whereas no more than 0.5log disparity was detected by HPC and ATP for *B. subtilis*. The especially different ATP results between *E. coli*

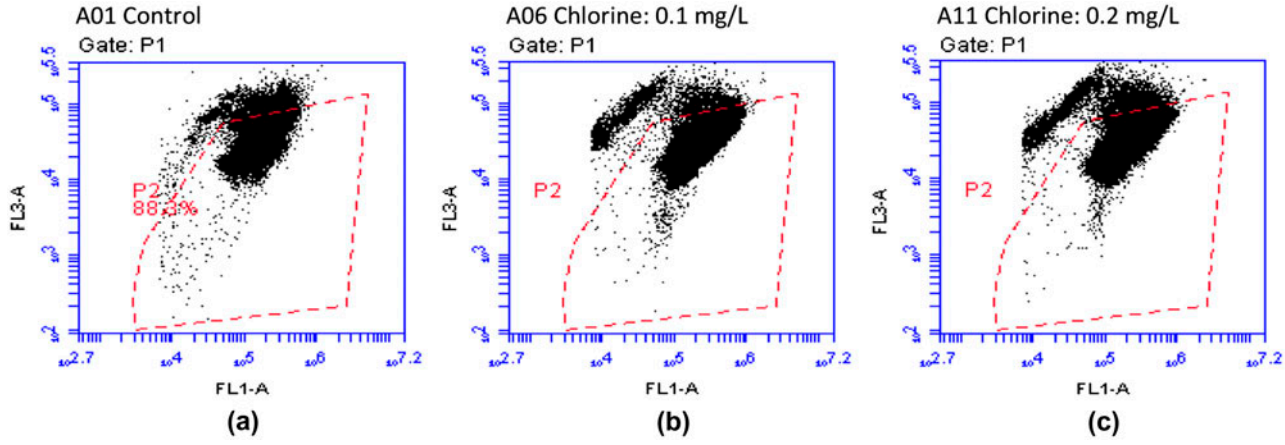

Fig. 8. FCM detection of membrane integrity of *B. subtilis* during chlorine disinfection. (a) control, (b) 0.1 mg/L initial chlorine concentration treatment, and (c) 0.2 mg/L initial chlorine concentration treatment.

Table 1
Summary of the mechanism of UV and chlorine disinfections and different methods for assessing of the efficiency of *E. coli* and *B. subtilis* disinfected by UV and chlorine

Detection methods			Disinfection mechanism	
	UV	Chlorine	UV	Chlorine
E. coli	HPC combined with ATP	HPC combined with ATP or with FCM	Via the formation of pyrimidine dimer (physical)	Via oxidation of membrane and inner cell (chemical)
B. subtilis	HPC combined with ATP	HPC or ATP or FCM combined with ATP		

and *B. subtilis* when applied to chlorine disinfection was attributed to the different composition and structure of the cell wall. As the cell wall of the former is thinner and easier to be penetrated, thus to achieve the same disinfection target, more chlorine dosage is required for *B. subtilis*. As a result of high chlorine concentration, the enzymes that was responsible for the metabolic was inhibited and ATP was hydrolyzed to maintain the cell viability which lead to the rapid decline of ATP. Overall, to assess the effects of UV disinfection, HPC was more suitable than FCM, and ATP measurement could assist in bacterial viability assessment for both *E. coli* and *B. subtilis*. For chlorine disinfection, ATP measurement was convenient and fast, and was a better method than HPC for cells of *B. subtilis* even for Gram-positive bacteria, whereas for *E. coli* or Gram-negative bacteria HPC should combined with ATP or FCM to detect the disinfection results accurately and ensure the safety of microbes.

As reported by McFeters et al. [39] and Hammes et al. [27], the disparity existed between HPC results and FCM detection during the different water treatment processes. Furthermore, there was no consistency between culturable and active bacteria. As the existence of VBNC, especially the infection ability of the pathogen bacteria [40], the microbial detection of water treatment should be improved to ensure the safety of human. The cells in the VBNC have the pathogenic genes/factors and may regrow in some circumstance upon restoration of normal environmental conditions [40]. During disinfection process, a proportion of the bacteria was converted into VBNCs cells and were ignored by HPC which would lead the deterioration of drinking water [39]. So the pathogenic bacteria in the water may regrow during the water transportation and pose a threat to human health.

4. Conclusion

This work demonstrates the limitation of HPC existed during UV and chlorine disinfections and indicates that the combination of ATP and FCM can

overcome the limitation of HPC detection and improve the measurement of disinfection efficiency to ensure the biosafety of the water and the biostability during distribution. In conclusion, the combination of HPC and ATP are recommended when the water is disinfected by UV, whereas for Gram-negative bacterium, HPC and ATP should be combined to assess the bacterial viability when the water is disinfected by chlorine and the ATP detection could be an alternative of HPC to assess the microbial activity for Gram-positive bacterium as it is fast, convenient, and has the similar trend to the HPC results. The work indicates that some improvement should be made to overcome the limitation of HPC detection to ensure the biosafety of the water and the biostability during distribution. The powerful tools of FCM and ATP for microbial analysis are better alternative in drinking water treatment and distribution process.

Acknowledgment

This work was supported by the National Science and Technology Pillar Program, China (No. 2012BAC05B02), the Funds for Creative Research Groups of China (51121062), the funds of the State Key Laboratory of Urban Water Resource and Environment (HIT, 2013TS04), the Natural Science Foundation of China (Grant No. 51308438).

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