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Comparison of Fe(VI) (FeO_4^{2-}) and ozone in inactivating *Bacillus subtilis* spores

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ABSTRACT

The protozoan parasites such as *Cryptosporidium parvum* and *Giardia lamblia* have been recognized as a frequent cause of recent waterborne disease outbreaks because of their strong resistance against chlorine disinfection. In this study, ozone and Fe(VI) (i.e., FeO_4^{2-}) were compared in terms of inactivation efficiency for *Bacillus subtilis* spores which are commonly utilized as an indicator of protozoan pathogens. Both oxidants highly depended on water pH and temperature in the spore inactivation. Since redox potential of Fe(VI) is almost the same as that of ozone, spore inactivation efficiency of Fe(VI) was expected to be similar with that of ozone. However, it was found that ozone was definitely superior over Fe(VI): at pH 7 and 20 °C, ozone with the product of concentration × contact time ($\overline{CT}$) of 10 mg L^{-1} min inactivate the spores more than 99.9% within 10 min, while Fe(VI) with $\overline{CT}$ of 30 mg L^{-1} min could inactivate 90% spores. The large difference between ozone and Fe(VI) in spore inactivation was attributed mainly to Fe(III) produced from Fe(VI) decomposition at the spore coat layer which might coagulate spores and make it difficult for free Fe(VI) to attack live spores.

1. Introduction

Cysts of *Cryptosporidium parvum* and *Giardia lamblia* are known resistant to chlorine. Since they have caused a number of waterborne disease outbreaks worldwide, water utilities that employ chlorination as a disinfection process are evaluating alternative disinfectants such as ozone, chlorine dioxide, and UV irradiation.

The chemistry of irons with high oxidation states (i.e., Fe(VI), Fe(V) and Fe(IV)) in aqueous solution has been of interest because of their implication in numerous hydroxylation/oxidation reactions of environmental, industrial, and biological importance (Lee and Chen, 1991). Over the past decade or so, a number of papers have been published on the detailed reaction mechanisms of Fe(VI) ion (especially, FeO_4^{2-} oxidations). Most of them paid their attention to novel properties of the chemical such as high oxidizing power, reaction selectivity, stability as a salt, and production of non-toxic by-products (i.e., Fe(II) and Fe(III)). Under the acidic condition, the redox potential of Fe(VI) is 2.20 V and 0.57 V under basic condition (Wood, 1958). With its high oxidizing power, Fe(VI) can oxidize various organic and inorganic chemicals. For example, Rush et al. (1995) applied Fe(VI) to oxidize phenol. Sharma et al. (1997) oxidized sulfur-containing compounds like thiourea. Hydrogen sulfide can be oxidized to sulfate (de Luca et al., 1996), or it can be removed by forming precipitates with Fe(II) and Fe(III) produced from the Fe(VI) decomposition (Calvano et al., 1992).

The potential advantage of using Fe(VI) in water or wastewater treatment processes is the fact that it does not produce any toxicologically harmful by-products unlike other disinfectants such as chlorine (de Luca et al., 1996). Therefore, Fe(VI) is often cited as an effective alternative disinfectant for water and wastewater.

Fe(VI) also has been applied to inactivate microbes in water and wastewater. Gilbert et al. (1976) could inactivate 99.9% of *Escherichia coli* with 6 mg Fe L^{-1} in 7 min. Gilbert et al. (1976) and Sharma et al. (2005) investigated Fe(VI) as an alternative disinfectant and also found it was effective in inactivating *E. coli* and total coliforms. Recently, Jiang et al. (2007) reported that inactivation of *E. coli* by Fe(VI) requires less chemical dose and contact time (i.e., $\overline{CT}$; product of concentration and contact time) than that by sodium hypochlorite in achieving the same *E. coli* killing efficiency. In addition, the disinfection by Fe(VI) was less affected by the water pH in their study.

Kazama (1995) demonstrated that Fe(VI) in micromolar concentrations rapidly could inactivate *f2 Coliphage* at low concentrations and a survival ratio of the virus decreased rapidly within 10 min contact time. Later, it was proven that Fe(VI) could inhibit DNA polymerization irreversibly (Goldfield et al., 2000).

Recently, ozone has been increasingly applied in water treatment systems due to its strong biocidal oxidizing property and its ability to diffuse through the cell membrane (Driedger et al., 2000). Finch et al. (1993) observed that ozone could disinfect

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99.5% of *G. lamblia* within 2 min of contact time at initial ozone concentration of 0.79 mg L^{-1}. Hunt and Mariñas (1999) also reported effective inactivation of *E. coli* and *C. parvum* by ozone. If ozone is applied along with UV illumination, inactivation of spores can be further enhanced (Jung et al., 2008).

The oxidation involving ozone in water can occur mainly in two pathways: (1) one involves molecular ozone (direct reaction), and (2) the other involves ·OH radicals produced from ozone decomposition and from other ozone involving reactions (indirect reaction). A direct reaction with molecular ozone is known the predominant mechanism that inactivates microorganisms (Hunt and Mariñas, 1999), although the importance of ·OH radicals is emphasized in other studies (Bancroft et al., 1984; Jung et al., 2008). At higher pH, ozone decomposition rate is accelerated and more ·OH radicals can be created. Therefore, the water pH is frequently related to the role of ·OH radicals in the inactivation of microorganisms.

In this study, ozone and Fe(VI) were individually applied at different pH and water temperatures to inactivate *Bacillus subtilis* spores (ATCC 6633) and their results were compared. Since the direct measurement of *C. parvum* or *G. lamblia* was difficult, time-consuming, and expensive, *B. subtilis* spores were selected as an indicator (Driedger et al., 2001a,b). This spore is often accepted as a good surrogate for inactivation studies of inert protozoan cysts, especially for comparing or optimizing inactivation conditions (Rice et al., 1996).

Although Fe(VI) has been applied to disinfect various microorganisms as stated above, it has not been applied to inactivate spores in water. The inactivation mechanism of Fe(VI) for spore itself might be different from that for bacterial cells, since spores are often protected by thick outer layers unlike bacterial cells. If Fe(VI) is effective in spore inactivation, it can be more beneficial to utilities since it does not produce any harmful disinfection by-products but Fe(III) which acts as a coagulant.

2. Materials and methods

All solutions used in this study was prepared with deionized water purchased from Baker (New Jersey, USA), and all the experimental apparatuses were sterilized at 121 °C for 15 min before their use.

2.1. Spores preparation and growth conditions

A freeze-dried pellet of *B. subtilis* (ATCC 6633) was purchased from American Type Culture Collection (Manassas, Virginia, USA). Upon receipt, the pellet was rehydrated aseptically into saline solution. Columbia agar (contents: 23 g L^{-1} peptone, 1 g L^{-1} starch, and 5 g L^{-1} NaCl; Oxoid CM331, England) was then inoculated using a sterile inoculating loop dipped in the saline solution suspension. The inoculated agars were incubated for 24 h at 37 °C and subsequently stored at 4 °C until used. A stock suspension of spores was produced by first scrapping bacterial cells from the agars into a sterile 50 mL centrifuge tube containing 20 mL of nutrient broth medium, then by applying vortex. These *B. subtilis* suspensions were allowed to grow and sporulate in Sporulation medium (contents: 8.9 g L^{-1} nutrient broth, 0.28 g L^{-1} $MgSO_4{\cdot}H_2O$, 1.11 g L^{-1} KCl, and 0.0031 g L^{-1} $FeSO_4{\cdot}7H_2O$; Difco, USA) for 6–7 d at 37 °C.

The resulting spore suspension was cleaned by centrifuging for 15 min, at 10 °C, and at 4500*g*, and by decanting. This cleaning step was repeated three times. After the cleaning step, the spores were resuspended in 30 mL of 1 mM phosphate buffer solution (pH 7). In order to inactivate any remaining vegetative *B. subtilis* cells, the spore suspension was heated at 80 °C for 20 min using a thermostatically controllable water bath (Rice et al., 1996). The initial spore concentration in this study was maintained at 10^5–10^7 CFU mL^{-1} in plate count agar (contents: 5 g L^{-1} casein peptone, 2.5 g L^{-1} yeast extract, 1 g L^{-1} glucose, and 9 g L^{-1} agar; Oxoid 0463, England).

2.2. Viability assessment

Viability was determined by the membrane filtration method (Barbeau et al., 1999), where nitrocellulose filters (Millipore, USA) with a diameter of 47 mm and a nominal pore size of 0.45 μm were used to filter spores. A series of dilutions were made by transferring 1 mL subsample from the 5 mL sample aliquot to a 10 mL dilution test tube containing 9 mL 1 mM phosphate buffer solution (pH 7.0).

After filtration, the a membrane filter was placed in a petri-dish containing 10 mL plate count agar medium, and then incubated at 37 °C for 24 h. Finally, each CFU was counted.

2.3. Inactivation of B. subtilis spores by Fe(VI)

The Fe(VI) stock solution was prepared by dissolving 9 mg Fe L^{-1} K_2FeO_4 which was prepared by modifying the method proposed by Thompson et al. (1951) into borate buffered solution (pH 9) and its concentration over inactivation time was determined by a UV/Vis spectrophotometer (SPEKOL 1200, Germany). A molar absorptivity of 1150 M^{-1} cm^{-1} at 510 nm was used to calculate the Fe(VI) concentration (Rush and Bielski, 1986).

Once an experiment started, approximately 5×10^5–5×10^7 spores mL^{-1} were injected into the reactor of a 1 L glass Erlenmeyer flask (Fig. 1a). Sample aliquots of 5 mL were subsequently taken with an auto-pipette at various times corresponding to predetermined $\overline{C}T$ values. 10 μL $Na_2S_2O_3$ of 1.67 g L^{-1} was immediately injected into the sample to quench the Fe(VI) residual.

Three sets of experiments were performed. In the first set of experiments, solution pH was adjusted to 6, 7, or 8 while maintaining solution temperature at 20 ± 1 °C. In the second, the water temperature was varied (i.e., 5, 20 and 30 °C) while maintaining pH at 7.0. In the third, higher Fe(VI) dose (i.e., 20 mg Fe L^{-1}) was applied to inactivate the spores at pH 7.0 and 15 °C. In total, nine samples were collected at different time periods (i.e., up to 480 min) in each experiment. The viability was determined for each of the nine samples by the membrane filtration method.

2.4. Inactivation of B. subtilis spore by ozone

Ozonation experiments were performed in a glass reactor with a working volume of 1 L (Fig. 1b). Ozone gas was produced by an ozone generator fed with 99.9% dry oxygen (Triogen, Ozonia, Switzerland), and was bubbled into the reactor with a diffuser. The reactor was stirred to provide additional mixing and allowed to reach steady state for 20–30 min. The dissolved ozone concentration was measured by using a UV/Vis spectrophotometer (SPEKOL 1200, Germany). A molar absorptivity of 2960 M^{-1} cm^{-1} at 260 nm was used to calculate the ozone concentration (Elovitz et al., 2000).

In the beginning of each experiment, approximately 5×10^5–5×10^7 spores mL^{-1} were injected into the reactor. Then, 5 mL sample aliquots were taken with an auto-pipette at various times corresponding to the product of the predetermined concentration and contact time values ($\overline{C}T$). As the case of the experiments with Fe(VI), $Na_2S_2O_3$ solution was immediately added into the sample to quench the ozone residual.

Two sets of experiments were carried out. The first set of experiments was conducted by varying solution pH (i.e., pH 5–9) while maintaining ozone concentration at 1 mg L^{-1} and temperature at 20 ± 1 °C to evaluate the effect of pH on the ozonation of *B. subtilis* spores. In the second set of experiments, the water temperature

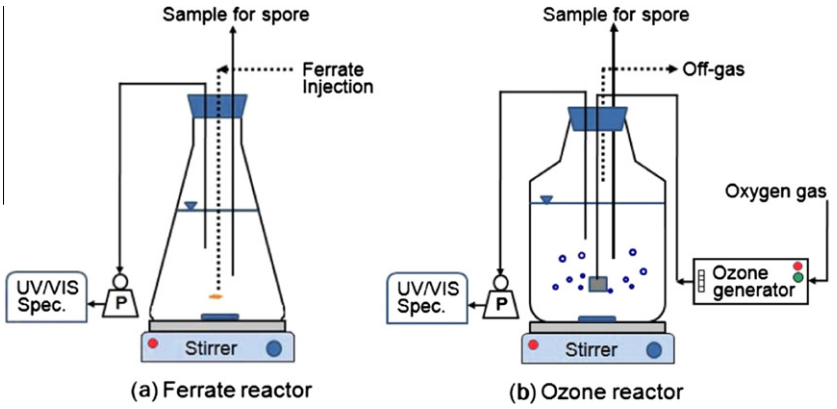

Fig. 1. Experimental set up for *B. subtilis* spore inactivation by: (a) Fe(VI), and (b) ozone.

was varied (i.e., 5, 20 and 30 °C) while maintaining ozone concentration at 1 mg L^{-1} and at pH 7.

2.5. Bacillus subtilis spore inactivation curves

Bacillus subtilis spore inactivations by Fe(VI) and ozone were compared by drawing spore inactivation curves with the following Delayed Chick–Watson model Eq. (1) (Rennecker et al., 1999).

$$\frac{N}{N_0} = \begin{cases} 1 & if \quad \overline{CT} \leqslant \overline{CT}_{lag} \\ e^{-k(\overline{CT}-\overline{CT}_{lag})} & if \quad \overline{CT} > \overline{CT}_{lag} \end{cases} \quad (1)$$

where k is the post-shoulder second-order inactivation rate constant [L mg^{-1} min^{-1}], and $\overline{CT}$ is the product of the dissolved disinfectant concentration [mg L^{-1}] and contact time [min]. N and N_0 are the numbers of live spores [CFU mL^{-1}] with and without disinfection treatment. $\overline{CT}_{lag}$ is the $\overline{CT}$ value during the lag phase.

Data from *B. subtilis* inactivation using ozone or Fe(VI) were fitted to Eq. (1) with the following two-step approach. First, the data with $N/N_0 < 0.5$ was fitted with the portion of Eq. (1) for $\overline{CT} > \overline{CT}_{lag}$ and obtained values for k and $\overline{CT}_{lag}$. A lag phase was then obtained by extending a horizontal line from $\overline{CT} = 0$ to $\overline{CT} = \overline{CT}_{lag}$.

Because ozone concentration could be maintained at a level (i.e., 1 mg L^{-1}) by bubbling ozone gas in the reactor, the model could be readily applied to the spore inactivation by ozone. However, Fe(VI) concentration decreased with time and could not be maintained at a certain level during spore inactivation experiments. Therefore, $\overline{CT}$ for each data point within each experiment was determined with the product of the integrated average Fe(VI) concentration over time and the batch contact time. Measurement of Fe(VI) decomposition revealed the decomposition kinetics followed the first-order rate, i.e., $C = C_0 e^{-k_d t}$, where C is Fe(VI) concentration at time t, and C_0 is the initial Fe(VI) concentration. The decomposition rate constant, k_d, can be readily evaluated. Once the k_d was determined, $\overline{CT}$ for each data during a spore inactivation experiment was obtained from Eq. (2) (Ruffell et al., 2000; Larson and Mariñas, 2003), and Eq. (1) could be applied to spore inactivation by Fe(VI).

$$\overline{CT} = \frac{C_0}{k_d}(1 - e^{-k_d t}) \quad (2)$$

3. Results and discussion

3.1. Effect of pH and temperature on B. subtilis inactivation by Fe(VI)

Initially, *B. subtilis* spore inactivation by Fe(VI) was tried with the initial Fe(VI) concentration of 1–5 mg Fe L^{-1}. However, any significant reduction in live spore number was not observed (data not shown). Noticeable spore inactivation could be observed when the initial Fe(VI) dose was increased to 9 mg Fe L^{-1}. At this concentration, therefore, *B. subtilis* inactivation was evaluated at three different pH, i.e., 6, 7 and 8 (Fig. 2a). $\overline{CT}$ values for the experiment set were calculated using Eq. (2); k_d values for Fe(VI) at pH 6, 7 and 8 were estimated 0.65, 0.36, and 0.023 min^{-1}. In general, the inactivation of *B. subtilis* by Fe(VI) was very pH dependent, although it was not greatly effective. The inactivation rate constant obtained at pH 6 was 8 times higher than the one obtained at pH 8; $k = 0.08$ and 0.01 L mg^{-1} min^{-1} for pH 6 and 8, respectively. In water, three different Fe(VI) species, i.e., H_2FeO_4, $HFeO_4^-$, and

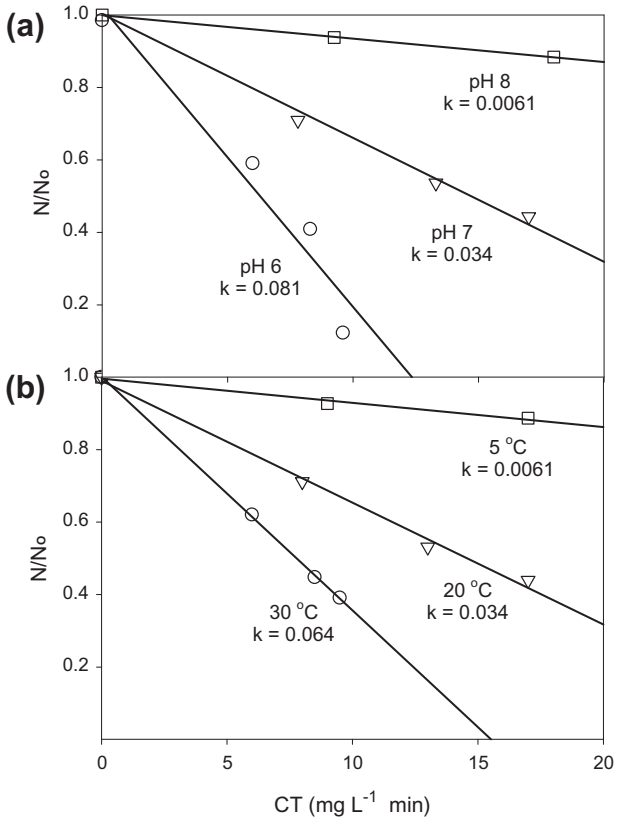

Fig. 2. Inactivation of *B. subtilis* spores using Fe(VI) at: (a) various pH (initial FeO_4^{2-} conc. of 9 mg Fe L^{-1}; Temp. of 20 °C; N_0 of 5×10^6–10^7 CFU mL^{-1}), and (b) various temperatures (initial FeO_4^{2-} conc. of 90 mg Fe L^{-1} and pH 7; N_0 of 5×10^6–10^7 CFU mL^{-1}).

FeO_4^{2-} can exist depending on water pH (Eq. (3)). Protonated Fe(VI) species (H_2FeO_4, and $HFeO_4^-$) are unstable and very reactive, while unprotonated Fe(VI) is not very reactive but relatively stable (Sharma et al., 2000); namely, the oxidation power is the following order: $H_2FeO_4 > HFeO_4^- > FeO_4^{2-}$. By the same token, the protonated Fe(VI)s can show higher biocidal effect than unprotonated one (Rush et al., 1996), as shown in this study.

$$\begin{aligned} H_2FeO_4 &\overset{K_{a1}}{\leftrightarrow} H^+ + HFeO_4^- \quad pK_{a1} = 3.50 \\ HFeO_4^- &\overset{K_{a2}}{\leftrightarrow} H^+ + FeO_4^{2-} \quad pK_{a2} = 7.23 \end{aligned} \tag{3}$$

Fig. 2b shows dependence of spore inactivation by Fe(VI) on water temperature. *B. subtilis* inactivation rate of Fe(VI) was significantly affected by water temperature (Fig. 2b). Dependence of the inactivation rate constants on water temperatures is often quantified with the Arrhenius equation. The activation energy (E_a; unit: J mol^{-1}) and the collision frequency (A; unit: L mg^{-1} min^{-1}) in Arrhenius equation for Fe(VI) were evaluated by fitting the equation to the inactivation rate constants of Fe(VI) at different temperatures (R^2 = 0.986). In fact, very high dependence of Fe(VI) on temperature could be found; E_a and A were calculated 6.7×10^4 J mol^{-1} and 2.5×10^{10} L mg^{-1} min^{-1}, respectively.

3.2. Effect of pH and temperature on B. subtilis spore inactivation by ozone

B. subtilis spore inactivation by ozone at different pH was evaluated by carrying out ozonation experiments (Fig. 3a). Measured spore inactivation efficiency (i.e., N/N_0) along with fitted line drawn with Eq. (1) was plotted for each pH as a function of $\overline{C}T$. For all pH, a lag phase could be observed at lower $\overline{C}T$ values, followed by the logarithmic inactivation phase.

Unlike the studies performed by Driedger et al. (2001b) and Craik et al. (2002) where insignificant effects of pH on the spore inactivation by ozone were observed within the range of pH 6–8, *B. subtilis* spore inactivation rate increased as the water pH increased in this study (Fig. 3a); about 100% higher inactivation rate at pH 9 (k = 1.36 L mg^{-1} min^{-1}) than at pH 5 (k = 0.67 L mg^{-1} min^{-1}).

The difference between the results from previous studies and ours can be attributed to the difference in the pH range evaluated in the studies. Although it needs further research, more ·OH of higher redox potential (E^0 = 2.80 V: Bielski, 1990; Jung et al., 2008) could be formed through ozone decomposition at higher pH to contribute to the spore inactivation. It also should be noted that at pH 9 much more ozone must have been supplied to maintain the residual ozone concentration at 1 mg L^{-1}, resulting in higher spore inactivation rate. Regarding the length of lag phases, only slight difference could be observed at different pH, as the case with Craik et al. (2002).

The effect of water temperature on *B. subtilis* inactivation by ozone was more apparent than that of pH; the rate constant was proportionally related with temperature (Fig. 3b). The spore inactivation rate of ozone at 30 °C ($k_{30°C}$) was 1.37 L mg^{-1} min^{-1}, while the one at 5 °C ($k_{5°C}$) was 0.45 L mg^{-1} min^{-1}. $k_{30°C}$ was about three times faster than $k_{5°C}$, indicating higher dependence of ozone on temperature.

As the case of Fe(VI), spore inactivation rate constants of ozone obtained at different temperatures were fitted with R^2 = 0.996. The high R^2 value proves the spore inactivation by ozone is temperature-dependant. From the data fit, E_a was calculated 3.1×10^4 J mol^{-1} which was similar to that obtained from Larson and Mariñas (2003), i.e., 4.6×10^4 J mol^{-1}. However, A, the collision frequency, was significantly lower. A obtained in our study was 3.2×10^5 L mg^{-1} min^{-1}, while the one from Larson and Mariñas (2003) was 4.0×10^8 L mg^{-1} min^{-1}. From these parameters, inactivation of *B. subtilis* by ozone is much less temperature dependent than that by Fe(VI).

$\overline{C}T_{lag}$ also showed apparent dependence on the water temperature. About three times longer $\overline{C}T_{lag}$ could be observed at 5 °C ($\overline{C}T_{lag}$ = 3.0 mg L^{-1} min) than at 30 °C ($\overline{C}T_{lag}$ = 0.9 mg L^{-1} min). Similar result was observed by the others (Driedger et al., 2001b; Larson and Mariñas, 2003). The shorter $\overline{C}T_{lag}$ at higher temperatures might be related with the collision frequency, A of the Arrhenius equation; A is proportional to water temperature (Stone and Morgan, 1990). Namely, the probability of ozone molecules to collide and react with spores would be higher at higher temperatures than the one at lower temperatures, resulting in a shorter $\overline{C}T_{lag}$. Nonetheless, ozonation could achieve 99% spore inactivation if enough $\overline{C}T$ would be provided even at lower temperatures.

3.3. Comparison of ozone and Fe(VI) in B. subtilis spore inactivation

First, disinfectant residuality of ozone and Fe(VI) was compared by calculating decomposition rate constants, k_ds at pH 6 and 8. k_d of ozone was 0.077 min^{-1} which is much lower than that of Fe(VI) at pH 6 (i.e., 0.65 min^{-1}), meaning ozone can persist longer than Fe(VI). On the other hand, k_d of Fe(VI) (0.023 min^{-1}) was much lower than that of ozone (0.35 min^{-1}) at pH 8. Nonetheless, the *B. subtilis* inactivation by Fe(VI) was not comparable with that by ozone.

Fig. 4 shows *B. subtilis* spore inactivation by Fe(VI) (Fig. 4a) and by ozone (Fig. 4b) at pH 7 and 20 °C. From the figure, it is so apparent that the magnitude of spore inactivation by Fe(VI) is much lower than that by ozone, although Fe(VI) has higher oxidation potential than ozone under neutral to acidic condition. If the y axis

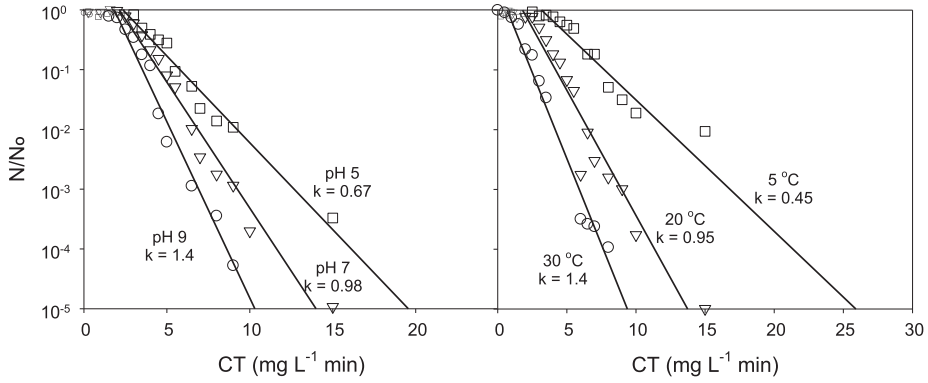

Fig. 3. Inactivation of *B. subtilis* spores using ozone: (a) at various pH (O_3 conc. of 1 mg L^{-1}; Temp. of 20 ± 1 °C; 5×10^5–10^6 CFU mL^{-1}) and (b) at various temperatures (O_3 conc. of 1 mg L^{-1}; pH 7; 10^5–5×10^5 CFU mL^{-1}).

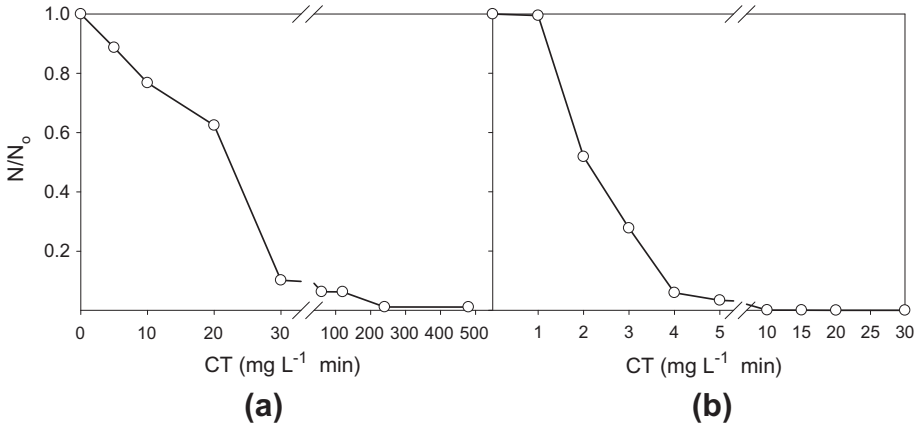

Fig. 4. Inactivation of *B. subtilis* spores using: (a) FeO_4^{2-} and (b) O_3 at 20 °C and pH 7.

of Fig. 4a is redrawn in logarithmic scale as Fig. 4b, the reduction in viable spores in Fig. 4a can be seen insignificant; only at $\overline{CT}$ of more than 30 mg L^{-1} min, about 90% reduction could be observed. However, almost complete inactivation could be achieved only with $\overline{CT}$ value of less than 10 mg L^{-1} min in ozonation.

Although it should be researched further in the future, the poorer *B. subtilis* spore inactivation by Fe(VI) was attributed to the spore structure and the property of Fe(VI), i.e., Fe(III) production upon decomposition, resulting in aggregation of spores.

In fact, *B. subtilis* spores are known to be very resistant to most oxidizing agents. With its multiple outer layers (Fig. 5), especially the coat layer mainly made of the complex of peptidoglycans and more than 25 different proteins the spore is protected from the exogenous toxic oxidants (Young and Setlow, 2004; Setlow, 2006). Especially, hydrophilic molecules such as oxidants in an ionized form can be easily blocked by the coat layer (Nicholson et al., 2000). Therefore, it was hypothesized that Fe(VI) added in the reactor was consumed and reduced to Fe(III) by the coat layer. Fe(III) is a commonly used coagulation agent in water or wastewater industry. The produced Fe(III) might aggregate spores to protect them from the attack from the remaining free Fe(VI). In fact, the formation of reddish colored aggregates could be observed after water samples with *B. subtilis* spores were treated with Fe(VI) in our study. The reddish color is common in Fe(III) oxides.

When the water sample with aggregates was filtered using a glass fiber filter (nominal pore size of 2.7 μm: GF/D from Whatman, USA), no viable spore could be counted. The result might indicate that some viable spores coagulated with inactivated ones via Fe(III) to form larger particles than filter pore size; the size of
B. subtilis is known about 0.9 μm in length and 0.2 μm in diameter (Fig. 5).

It is also known that the penetration of ozone into the center of a spore can be limited to some degree (Young and Setlow, 2004). Ozone is consumed by the spores reactive barrier (Khadre and Yousef, 2001), i.e., the coat layer resulting in the failure in complete spore inactivation. However, once proteins and other constituents of the coat layer are completely oxidized, inner spore cores are exposed to ozone. It was, therefore, hypothesized that continuous supply of ozone into the reactor eventually decoated the spores that were subsequently inactivated. Unlike the case of Fe(VI), ozone did not result in the spore aggregation that would otherwise prevent other live spores from being exposed to ozone. Due to the direct exposure to ozone molecules, the free decoated spores could not survive.

4. Summary and conclusion

Inactivations of *B. subtilis* spores by ozone and Fe(VI) were evaluated. In general, both oxidants showed high dependence both on water pH and temperature in inactivating the spores; especially Fe(VI) was much more dependent on the water pH probably due

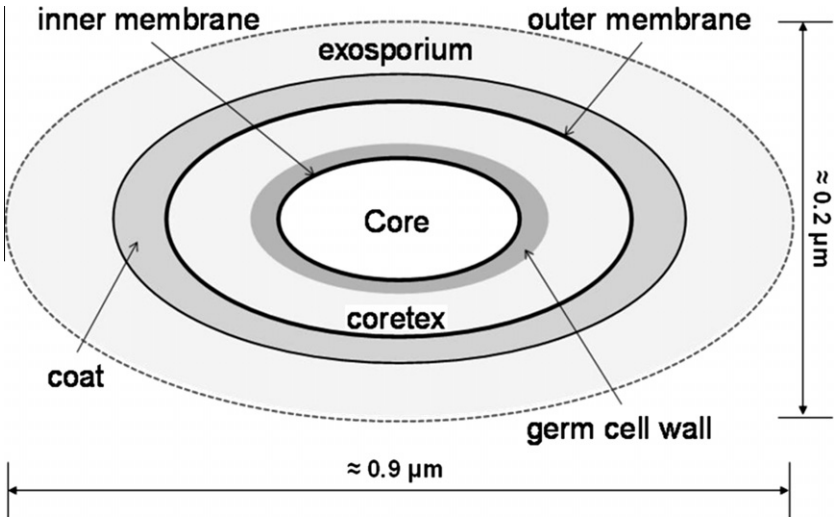

Fig. 5. Structure of *B. subtilis* spore (modified from Setlow, 2006).

to the species distribution over pH, namely, at high pH unprotonated Fe(VI) which is a relatively weak oxidant exists.

In terms of inactivation efficiency, ozone could inactivate the spores almost completely with $\overline{C}T$ of less than 10 mg L^{-1} min at normal condition (i.e., pH 7 and 20 °C). However, Fe(VI) could not achieve the similar level of spore inactivation with ozone even at lower pH (e.g., pH 6), where protonated Fe(VI) would exist.

The inferior spore inactivation by Fe(VI) to that by ozone was attributed to the followings. With its relatively large molecule size and hydrophilicity, Fe(VI) could be consumed at the spores reactive barrier made of the complex of petidoglycans and proteins, and could not inactivate the spores. In addition, Fe(III) formed from Fe(VI) decomposition would coagulate spores and make it more difficult for residual Fe(VI) to access to live spores locating inside of the spore aggregations.

On the other hand, continuous supply of ozone could result in the complete oxidation of spore coat layer, direct exposure of decoated spores to free ozone, and subsequent spore inactivation. In addition, the small size of ozone could help it to penetrate into the center of a spore.

From the result of our study, Fe(VI) application to exclusion of spores from drinking water sources is not advisable, since it did not work as a disinfectant but did as a coagulant. On the other hand, ozone can be effectively applied to inactivate spores as long as its aqueous concentration is maintained at certain level through continuous bubbling, which is now common in practice.

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