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The temperature-dependent kinetics and bacteria regrowth by performic acid and sodium hypochlorite disinfection

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ABSTRACT

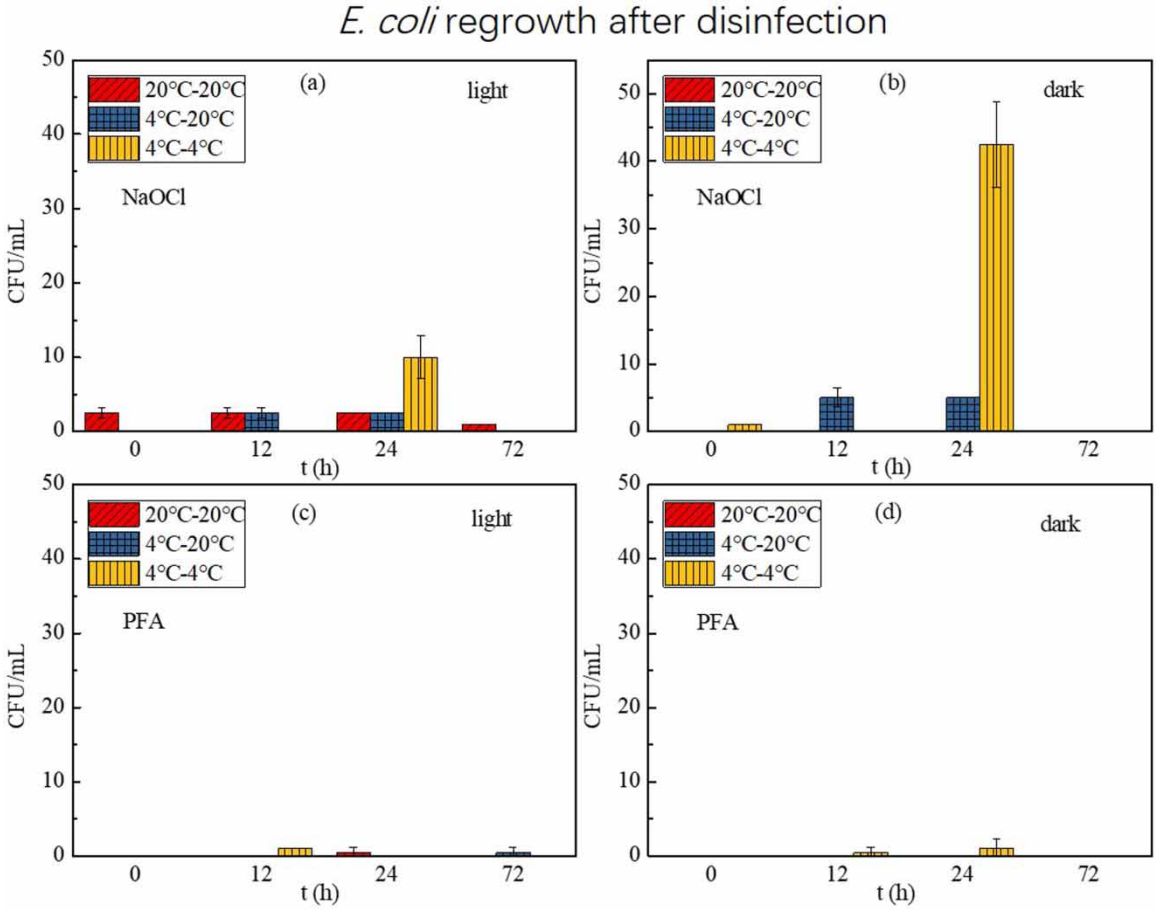
Sodium hypochlorite (NaOCl) has been widely used as a disinfectant in water and wastewater treatment, because of its high efficiency and low cost, whereas the bio-toxicity of its disinfection byproducts (DBPs) raised great concern. Performic acid (PFA) produces less DBPs and shows strong oxidation abilities. In this study, the effect of temperature on NaOCl and PFA disinfection as well as bacteria regrowth were evaluated. First, the inactivation of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* by NaOCl and PFA at 4 and 20 °C, detected by cell cultured-based plate counting were fitted to kinetic models, and the predicted CTs were calculated. The results showed that NaOCl was more effective than PFA for *E. coli* and *S. aureus* inactivation, and the temperature was positively correlated to disinfection. Second, bacteria regrowth was evaluated at different temperatures (4 and 20 °C) of disinfection and storage. The results showed that the bacteria inactivated by NaOCl regrew prominently, especially for those inactivated at 4 and stored at 4 °C, probably through the mechanism of reactivation of viable but non-culturable (VBNC) bacteria. PFA was superior in suppressing bacteria regrowth, and it may be used as an alternate disinfectant in water treatment in cold environment.

Key words: disinfection, performic acid, regrowth, sodium hypochlorite, temperature

HIGHLIGHTS

- Performic acid was more efficient than NaClO on *B. subtilis* inactivation.
- Performic acid was superior than NaClO in the inhibition of bacteria regrowth.
- Bacteria regrowth was prominent after NaClO disinfection.
- Performic acid could be used as an alternate disinfectant in cold environments.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Sodium hypochlorite (NaOCl) has been the most widely used disinfectant in water and wastewater, as well as in food industries, due to its convenient handling and storage (compared to chlorine gas), high efficiency, and low cost (U.S. EPA 1999; Fukuzaki 2006). However, there are several disadvantages associated with NaOCl disinfection, such as the possibilities of producing toxic chlorine gas, decreased efficiency in the presence of organic loads, and deleterious effects on some metals. One of the most severe drawbacks is the formation of mutagenic and carcinogenic halogenated disinfection byproducts (DBPs), which may result in high toxicity to humans and animals (Monarca *et al.* 2000; Crebelli *et al.* 2005).

Recently, the long-term presence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on cold-chain food packaging has been shown (Chi *et al.* 2021), and the concern of disinfection at low temperatures has thus raised great research interest. NaOCl was once believed to be a very effective disinfectant against *Bacillus subtilis* (*B. subtilis*) spores at low temperatures, even at subzero with the addition of ethylene glycol to prevent freezing (Jones *et al.* 1968). However, a slight effect on HOCl dissociation was related to temperature reduction (Gray 2014). In the aqueous solution, HOCl, OCl^-, and a small portion of Cl_2 were in equilibrium. Both HOCl and OCl^- have disinfection abilities, but the former has a much higher ability to penetrate cell walls and membranes (McDonnell & Russell 1999). It has also been reported that the log reduction of *Escherichia coli* (*E. coli*) and *Enterococcus* spp. inactivated by NaOCl was lower at 4 than at 20 °C, and the log reduction could not be increased by extending the contact time to 4 °C (Hassaballah *et al.* 2020).

Performic acid (PFA) belongs to the family of aliphatic peracids. It has the highest oxidation potential among the peracids, thus it is industrially relevant, such as in food processing and fine chemical production industries (Luukkonen & Pehkonen 2017). PFA can be prepared by mixing formic acid and hydrogen peroxide, with or without a catalyst. It has always been prepared on-site due to its unstable characteristics and safety concerns, and the temperature for storage was recommended below

20 °C (Gehr *et al.* 2009). PFA has been reported to efficiently inactivate some enteric bacteria, such as *E. coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella enteritidis* at 2.5 °C, thus it could be used as a disinfectant in low-temperature food processing and storage rooms (Heinonen-Tanski & Miettinen 2010).

A study has shown that no brominated DBPs were formed in a full-scale PFA disinfection experiment, other than a stoichiometric increase of formic acid (Ragazzo *et al.* 2013). Therefore, the lower likelihood of producing DBPs than chlorine has made PFA a disinfectant of great interest in water and wastewater industries (Luukkonen & Pehkonen 2017). PFA was able to successfully inactivate pathogens including *E. coli*, fecal coliforms, *Enterococci*, intestinal *Enterococci*, *Aeromonas spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus (S. aureus)*, somatic coliphages, and murine norovirus in combined sewer overflows and treated wastewater (Chhetri *et al.* 2014; Tondera *et al.* 2016; Maffettone *et al.* 2020; Ding *et al.* 2023), whereas the inactivation of resistant microorganisms such as *Bacillus subtilis (B. subtilis), Clostridium*, and *Giardia* in the secondary effluents required much higher doses than the above-mentioned pathogens (Karpova *et al.* 2013; Ragazzo *et al.* 2013; Luukkonen *et al.* 2015; Ding *et al.* 2023). Although showing effectiveness, most laboratory-scale PFA disinfection experiments were conducted at room temperature, and the effect of temperature on pathogen inactivation and regrowth has never been analyzed. The significance of this study was to evaluate PFA as a potential alternate disinfectant for water and wastewater industries, especially in a cold environment. In this study, the common pathogenic bacteria *E. coli* and *S. aureus* were selected as representatives of Gram-negative and Gram-positive bacteria, respectively, and *B. subtilis* as a resistant microorganism, to evaluate the disinfection efficacy of PFA.

The objectives of this study were to (a) establish disinfection kinetics of NaOCl and PFA at 4 and 20 °C, and (b) analyze the effect of temperature on bacteria regrowth after disinfection.

2. MATERIALS AND METHODS

2.1. Chemicals

NaOCl solution (10%) was purchased from Fuchen Tianjin Chemical Reagent Factory. The residual chlorine after disinfection was tested using a residual chlorine detector (DR 300, Hach, USA). PFA was synthesized before each experiment by mixing formic acid and hydrogen peroxide (H_2O_2), with the addition of sulfuric acid as the catalyst. The concentration of PFA was detected by titration. Detailed procedures refer to Ding *et al.* (2023).

2.2. Bacteria cell culture

The lyophilized powder of *E. coli* (BNCC 133264), *S. aureus* (BNCC 186335), and *B. subtilis* (BNCC 109047) was dissolved in beef peptone solution medium in an ultra-clean bench to form the bacterial broth. *E. coli* and *S. aureus* were incubated at 37 °C for 24 h, and *B. subtilis* for 48 h. The bacteria were passed for at least three generations before the disinfection experiment. *B. subtilis* spores were detected under a microscope via staining, and a percentage of 20–40% sporulation was observed. The cultured bacterial suspension was centrifuged at 10,000 r/min for 10 min, and the precipitate was re-suspended in the sterilized phosphate-buffered saline (PBS) by a vortexer (VM-300, Qunan, China), resulting in approximately 10^8–10^9 colony forming unit (CFU)/mL.

2.3. Disinfection experiment

The disinfection experiment was conducted by adding 1 mL bacterial suspension into 500 mL deionized water (pH 7.09 $\pm$ 0.01) in a 1-L conical flask. The flask was either submerged in ice water (4 $\pm$ 1 °C) or directly mounted on top of a magnetic stirrer at room temperature (controlled at 20 $\pm$ 1 °C). Magnets were put into each reaction flask to ensure even mixing during the reaction.

The effect of the temperatures of 4 and 20 °C on disinfection was analyzed. The temperature of 4 °C was controlled by submerging the conical flask into ice water, and 20 °C was controlled by conducting the experiment in an air-conditioned laboratory with the temperature set at 20 °C. A 2 mL water sample was withdrawn at regular time intervals. Half of the withdrawn sample was immediately neutralized by adding excess sodium thiosulfate ($Na_2S_2O_3$) to terminate the reaction of NaOCl, and excess $Na_2S_2O_3$ (1 M) and Catalase (Macklin, 3,500 units/mg) were applied to terminate the reaction of PFA. The sample was sent for determination of the bacterial CFU by cell culture-based plate counting, as described in Section 2.2. The other half portion was used to analyze the concentration of the residual disinfectant, as described in Section 2.1. The disinfection was evaluated by the survival of pathogenic bacteria in water samples at different contact times, calculated

as given in the following equation.

$$\text{Survival} = \log_{10}(N/N_0) \tag{1}$$

where N is the CFU of pathogenic bacteria in water samples at time t, and N_0 is the initial microbial CFU in water samples before the disinfection experiment. All experiments were conducted in triplicate. Control experiments were conducted without adding the disinfectants.

2.4. Disinfection kinetic simulation

The decomposition of NaOCl and PFA with initial disinfectant demand followed the first-order kinetic equation, as shown in the following equation.

$$C = (C_0 - D) \times e^{-k't} \tag{2}$$

where C and C_0 are the concentration (mg/L) of disinfectant at time t and 0, D is the initial demand (mg/L) of the disinfectant, and k' is the first-order decomposition rate constant (min^{-1}) of the disinfectant. The values of k' and D were determined by nonlinear regression analysis using Origin 8.5 software (OriginLab, USA).

The inactivation of *E. coli*, *S. aureus*, and *B. subtilis* by NaOCl and PFA, respectively, were fitted into the Chick–Watson and Selleck models, as shown in the following equations.

$$\log_{10}\frac{N}{N_0} = \frac{-\text{k}(C_0 - D)^n}{nk'} \times (1 - e^{-nk't}) \tag{3}$$

$$\log_{10}\frac{N}{N_0} = -n \times \ln\left[1 + \frac{C_0}{\text{kk}'} \times (1 - e^{-k't})\right] \tag{4}$$

where N and N_0 are as presented in Equation (1), k is the inactivation rate constant, and n is the constant referred to as the coefficient of dilution. C_0, D, t, and k' are as presented in Equation (2). To determine the unknown parameters in Equations (3) and (4), the nonlinear regression analysis function of Origin 8.5 was used to minimize the sum of squares of the differences (error sum of squares (ESS)) between observed (Equation (1)) and predicted (Equations (3) and (4)) survival under each condition. The fit of each parameter was reflected by the standard error, and the fit of the model was examined by the R^2 value.

The CTs (concentration of the disinfectant × contact time) were estimated for the purpose of assessing the efficacy of each disinfectant. Assuming that disinfectant decomposition follows pseudo-first-order kinetics after initial demand, the CTs ($\text{mg}\cdot\text{L}^{-1}\cdot\text{min}$) were estimated by the area under the disinfectant decomposition curve at the specific time, using the following equation.

$$\text{CT} = \int C(t)dt = \frac{C_0 - D}{k'}(1 - e^{-k't}) \tag{5}$$

where C, C_0, D, k', and t are the same as described in Equation (2).

2.5. Bacterial regrowth after disinfection

The disinfection experiment was carried out for 1 h at 4 and 20 °C, followed by adding $Na_2S_2O_3$ to quench NaOCl, and $Na_2S_2O_3$ and peroxidase to neutralize PFA, as described in Section 2.3. The water samples disinfected at 20 °C were stored at 20 °C, and those disinfected at 4 °C were stored at 4 and 20 °C, both in the dark and under ambient light. Water samples were withdrawn at 12, 24, and 72 h during the storage, and sent for cell culture for bacterial regrowth analysis, as described in Section 2.2. All analyses were conducted in triplicate.

3. RESULTS AND DISCUSSION

3.1. Bacteria inactivation kinetics

Preliminary experiments showed that an initial NaOCl concentration of 0.3 mg/L could completely inactivate *E. coli* and *S. aureus* in 10 min, whereas a much higher concentration of 7.5 mg/L with a contact time of 30 min was required for

B. subtilis inactivation (Figure 1(a), 1(c) and 1(e)). There was no significant reduction ($p > 0.05$) of the bacteria in the zero-control groups. NaOCl was extremely effective on *E. coli* inactivation, which achieved 6-log within 5 min (Figure 1(a)). Both Chick-Watson and Selleck models were fitted on the experimental data; the Selleck model showed a better fit (higher R^2) on the disinfection of all tested bacteria at both temperatures (Figure 1(b), 1(d) and 1(f)). This was consistent with the research findings of Lee & Nam (2002) that the Selleck model more closely resembled total coliform disinfection than the Chick-Watson and Hom models. The kinetic modeling parameters are shown in Table S1 in the supplementary material. A prominent tailing effect was detected for the three bacteria. For *E. coli* and *S. aureus* which were comparatively susceptible to NaOCl, the tailing effect could be partially due to the highest inactivation close to the detection limit. *B. subtilis* was present

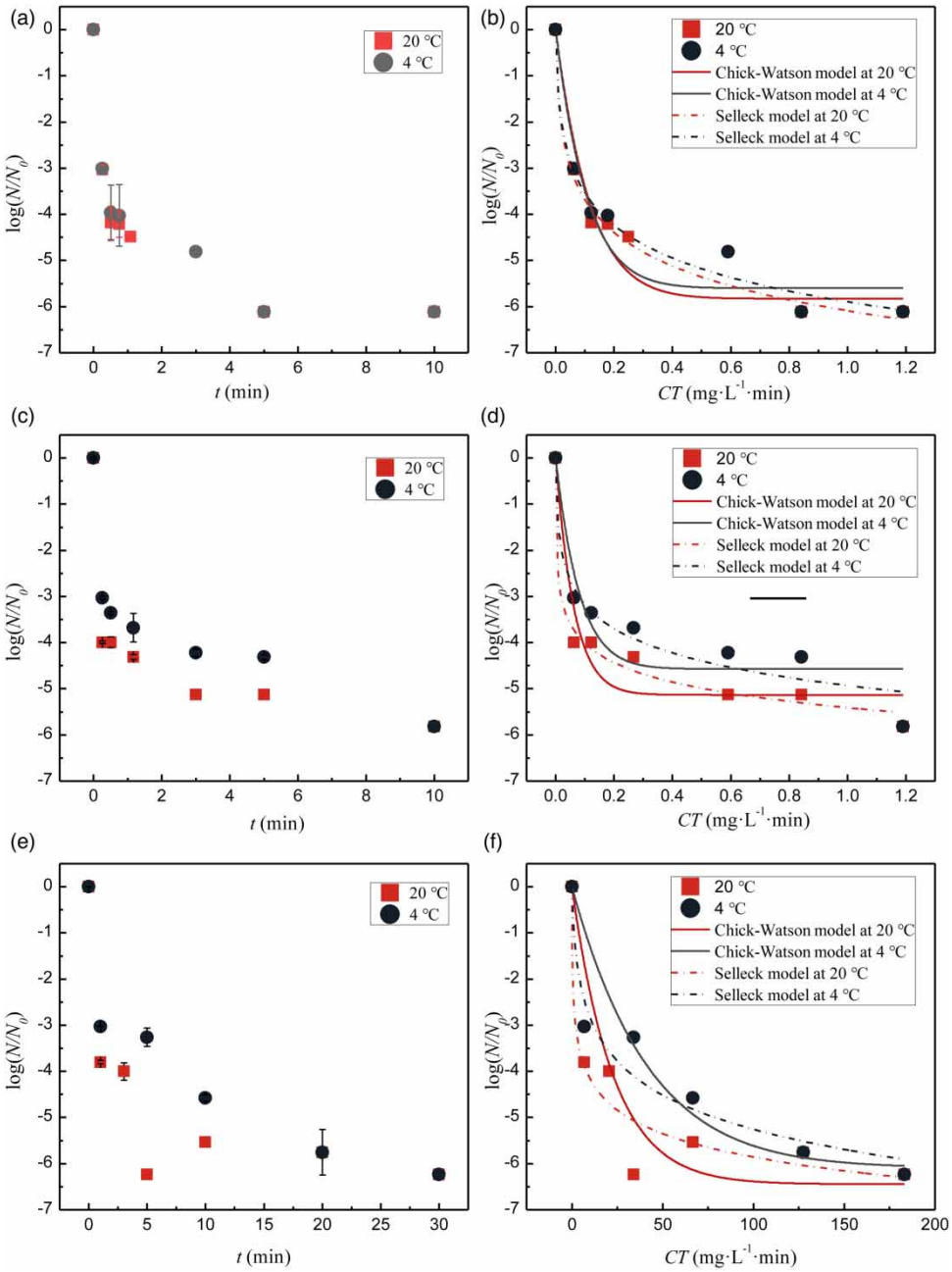


Figure 1 | Observed inactivation of *E. coli* (a), *S. aureus* (c), and *B. subtilis* (e) by NaOCl at 4 and 20 °C. The projected inactivation curves of *E. coli* (b), *S. aureus* (d), and *B. subtilis* (f) by NaOCl at 4 and 20 °C.

as a mixture of vegetative cells and spores, and the latter was extremely resistant to disinfection (Cho & Chung 2020), which might induce the tailings. The disinfection efficacy of NaOCl was higher at 20 than 4 °C. Other studies have shown similar results (Le Dantec *et al.* 2002). The rate constant for the inactivation of *B. subtilis* spores showed an Arrhenius-type temperature dependence (Sagripanti & Bonifacino 1996). For most chemical disinfectants, the disinfection rate constant is higher when temperature increases, which is interrelated with the higher decomposition rate of the disinfectant. Changes in the temperature only within the ambient ranges (<45 °C) do not affect the structure of microorganisms (Dias *et al.* 2017).

PFA was less effective than NaOCl in the inactivation of *E. coli* and S. *aureus*, especially at the low temperature (Figure 2(a) and 2(b)). Chick-Watson fitted the experimental data better than the Selleck model (Figure 2(c) and 2(d)). However, PFA was

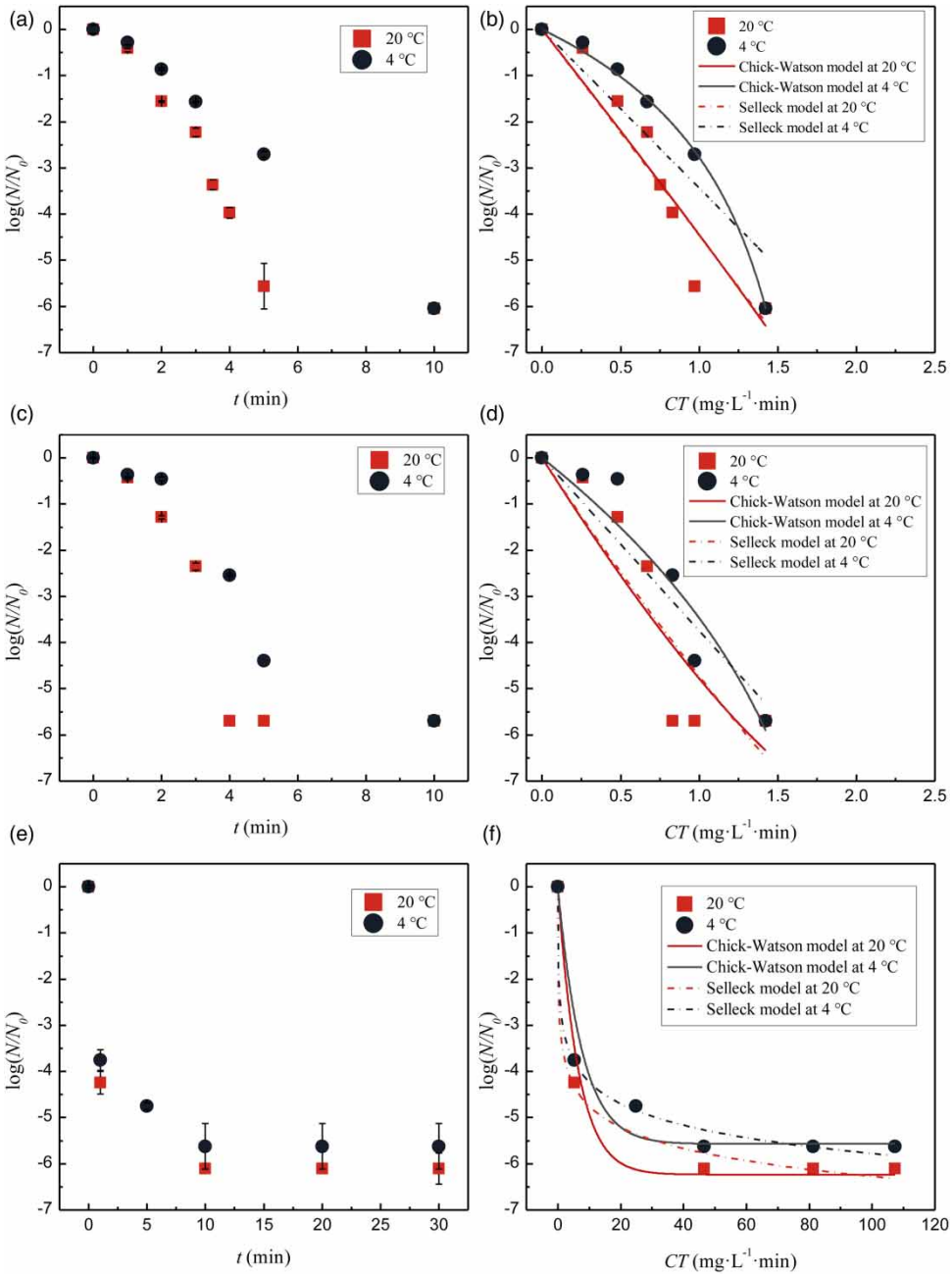


Figure 2 | Observed inactivation of *E. coli* (a), *S. aureus* (c), and *B. subtilis* (e) by PFA at 4 and 20 °C. The projected inactivation curves of *E. coli* (b), *S. aureus* (d), and *B. subtilis* (f) by PFA at 4 and 20 °C.

more effective in the degradation of *B. subtilis* than NaOCl (Figure 2(e)), and the Selleck model fitted the experimental results better than the Chick-Watson model. Apparently, when the inactivation was fast at the initial stage and the tailing effect was dominant, the Selleck model fitted better (Figures 1 and 2). The kinetic modeling parameters are shown in Table S2 in the supplementary material. The inactivation of *E. coli* and S. *aureus* by PFA, especially at 4 °C, showed apparent shoulders (Figure 2(b) and 2(d)), indicating a slower inactivation than NaOCl. This was also apparent by a comparison of the kinetic parameters in Tables S1 and S2. Similarly to NaOCl, raising the temperature from 4 to 20 °C had a positive effect on disinfection, although further temperature elevation may accelerate the decomposition of the disinfectant (Kunigk *et al.* 2001).

The calculated *CTs* based on the optimal model at each condition are listed in Table 1. At an initial concentration of 0.3 mg/L, NaOCl was extremely efficient at inactivating *E. coli* at both 4 and 20 °C, with CTs of 0.1372 and 0.1588 mg/L·min to achieve a 4-log inactivation, respectively. PFA required much higher CTs than NaOCl to achieve the same inactivation of *E. coli* and *S. aureus*. However, with a higher initial concentration of 7.5 mg/L, PFA was more efficient on *B. subtilis* than NaOCl, and required 2–4 times lower *CTs* to achieve 4-log inactivation. A slightly lower effectiveness of PFA toward *E. coli* and *S. aureus* in the secondary effluent was also observed in one of our previous studies (Ding *et al.* 2023). Ragazzo *et al.* (2013) also reported that at a CT of 10–15 $mg{\cdot}L^{-1}{\cdot}min$, NaOCl disinfection resulted in higher removal of *enterococci* in the secondary effluent than PFA at an initial dose of 1 mg/L. Nevertheless, by increasing the contact time or initial disinfection dose, the effectiveness of PFA against *enterococci* reached the same level as NaOCl, and that against *E. coli* exceeded that of NaOCl (Ragazzo *et al.* 2013; Ragazzo *et al.* 2020).

3.2. Bacteria regrowth tests

E. coli was inactivated for 1 h with an initial concentration of 0.3 mg/L of NaOCl or PFA, prior to the regrowth test. As shown in Table 1, NaOCl was extremely effective in the inactivation of *E. coli*, and required nearly eight times lower *CTs* than PFA to achieve the same level of removal. However, the regrowth of *E. coli* was obvious, both under light and in dark (Figure 3). When *E. coli* was inactivated by NaOCl at 20 °C and stored at 20 °C, it was found that a small portion of bacteria regenerated under light, but no regrowth was found in the dark. Since regrowth of *E. coli* also happened with the bacteria without storage ($t = 0$) under light (Figure 3(a)), the appearance at a longer time might be due to the incomplete inactivation. When *E. coli* was inactivated at 4 °C and stored at 20 °C under light, a small portion regrew after 12 and 24 h. Slightly more colonies appeared when incubated in the dark, but no colonies show up after 72 h both under light and in the dark. The most prominent regrowth of *E. coli* happened for those disinfected by NaOCl at 4 °C and stored at 4 °C. In contrast, despite that PFA showed a lower efficacy than NaOCl on *E. coli* inactivation, its ability to inhibit *E. coli* regrowth was remarkable. Minimal regrowth of *E. coli* was observed, either disinfected at 20 or 4 °C, and stored for regrowth under light or in the dark at 20 or 4 °C (Figure 3(c) and 3(d)). The regrowth of *E. coli* after chlorine disinfection has been reported by several studies. Wang *et al.* (2022) found that a simulated treated wastewater sample chlorinated for 30 min at 25 °C with an initial dose of 0.2 mg/L was not able to inhibit *E. coli* regrowth at day 3 at 25 °C; however, a higher dose of 0.5 mg/L could suppress its regrowth.

Table 1 | The predicted CTs for bacteria inactivation by NaOCl and PFA

Disinfectant	Bacteria	Temperature (°C)	Model	CT ($mg{\cdot}L^{-1}{\cdot}min$)		
				2-log	3-log	4-log
NaOCl	*E. coli*	20	Selleck	0.0181	0.0515	0.1372
		4		0.0203	0.0586	0.1588
	S. aureus	20	Selleck	0.0035	0.0187	0.0981
		4		0.0207	0.0802	0.2972
	B. subtilis	20	Selleck	0.4898	2.0094	7.9331
		4		4.2018	11.7735	30.8370
PFA	*E. coli*	20	Chick-Watson	0.4555	0.6798	0.9014
		4		0.8234	1.0517	1.2149
	S. aureus	20	Chick-Watson	0.3846	0.5902	0.8080
		4		0.6392	0.8937	1.1089
	B. subtilis	20	Selleck	0.1459	0.6917	3.1809
		4		0.3401	1.5751	7.0692

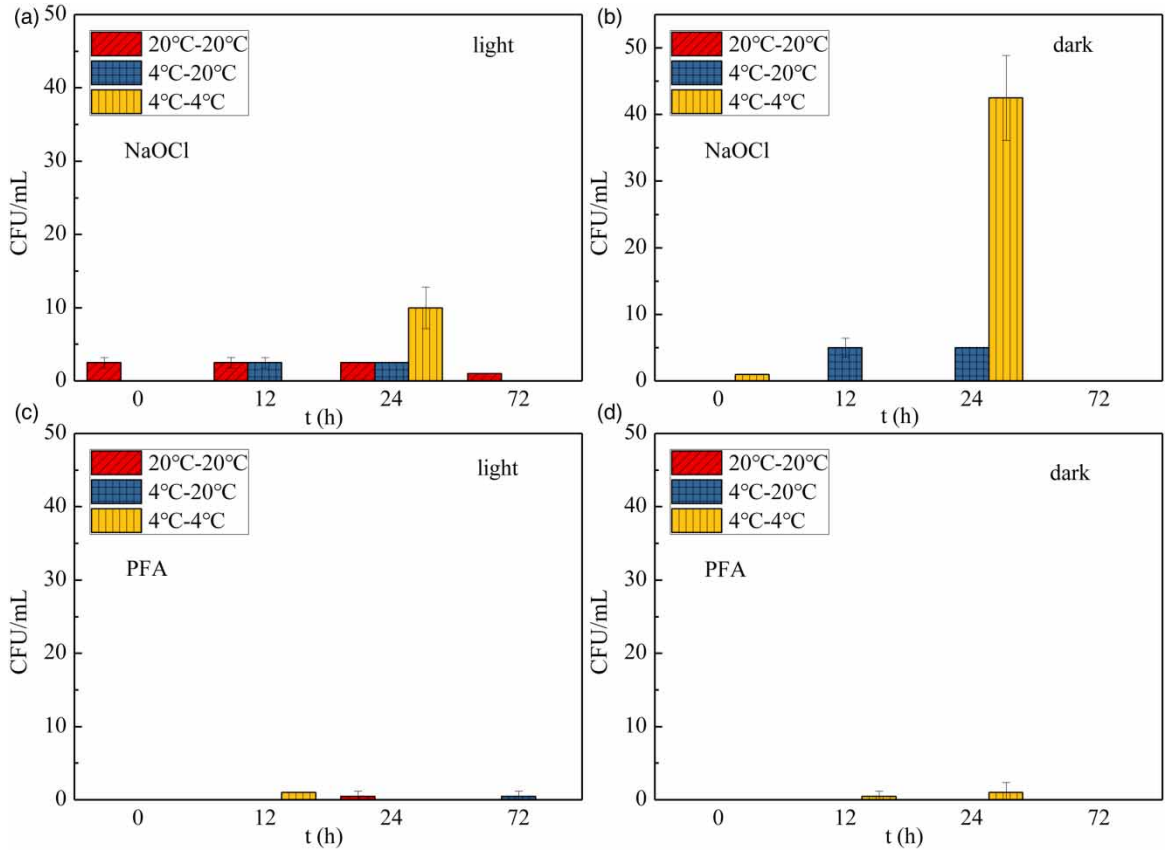


Figure 3 | *E. coli* regrowth under ambient light (a,c) and in dark (b,d) after disinfection by NaOCl and PFA, t represents the time of storage. The first and second temperature in the legend indicates the disinfection and regrowth temperature, respectively, i.e. 4–20 °C indicates disinfection at 4 °C and storage at 20 °C.

Regrowth of *E. coli* has always been observed in the reclaimed water distribution system after chlorine disinfection (Lin *et al.* 2016).

The regrowth of *S. aureus* after disinfection by NaOCl and PFA is shown in Figure 4. There was essentially no regrowth of *S. aureus* inactivated by NaOCl at 20 °C with continued storage at 20 °C. When disinfected at 4 °C and stored at 4 °C, a small number of colonies appeared at 24 h under light, and more colonies appeared at 12 h in the dark, though with a large standard error. For those inactivated by PFA at 20 °C and stored at 20 °C, *S. aureus* regrowth occurred at 0 and 12 h under light, indicating an incomplete inactivation in this situation. Disappearance of the bacteria at longer storage time could be due to lack of nutrients or uneven dispersion of the bacteria in water samples. Little regrowth of *S. aureus* happened when inactivated and stored at 4 °C.

The regrowth of *B. subtilis* after disinfection is shown in Figure 5. Clearly, regrowth of those inactivated by NaOCl at 4 °C and stored at 4 °C under ambient light showed apparent regrowth after 12-h storage time. Reactivation in the dark also happened to the bacteria inactivated at 4, and stored at 4 and 20 °C, respectively (Figure 5(a) and 5(b)). A discussion of this phenomenon is given below. On the other hand, little bacteria regrowth happened after PFA disinfection, in most cases (Figure 5(c) and 5(d)). *B. subtilis* was more resistant to chemical inactivation than *E. coli* and *S. aureus*, therefore a higher initial dose was applied in this study. Apparently, although shown to effectively inactivate *B. subtilis*, NaOCl was not effective at inhibiting its regrowth at 4 °C. A recent study has reported that chlorine was able to prevent the regrowth of *B. subtilis* at a low CT dose of < 45 mg·L^{-1}·min; however, the disinfection experiment was conducted at room temperature, and *B. subtilis* used was supposed to be solely vegetative cells (Zhang *et al.* 2019).

According to the literature, there are basically three mechanisms involved in bacteria regrowth after disinfection, which are reproduction, repair, and reactivation (Wang *et al.* 2021). Reproduction refers to the viable bacteria that maintain the

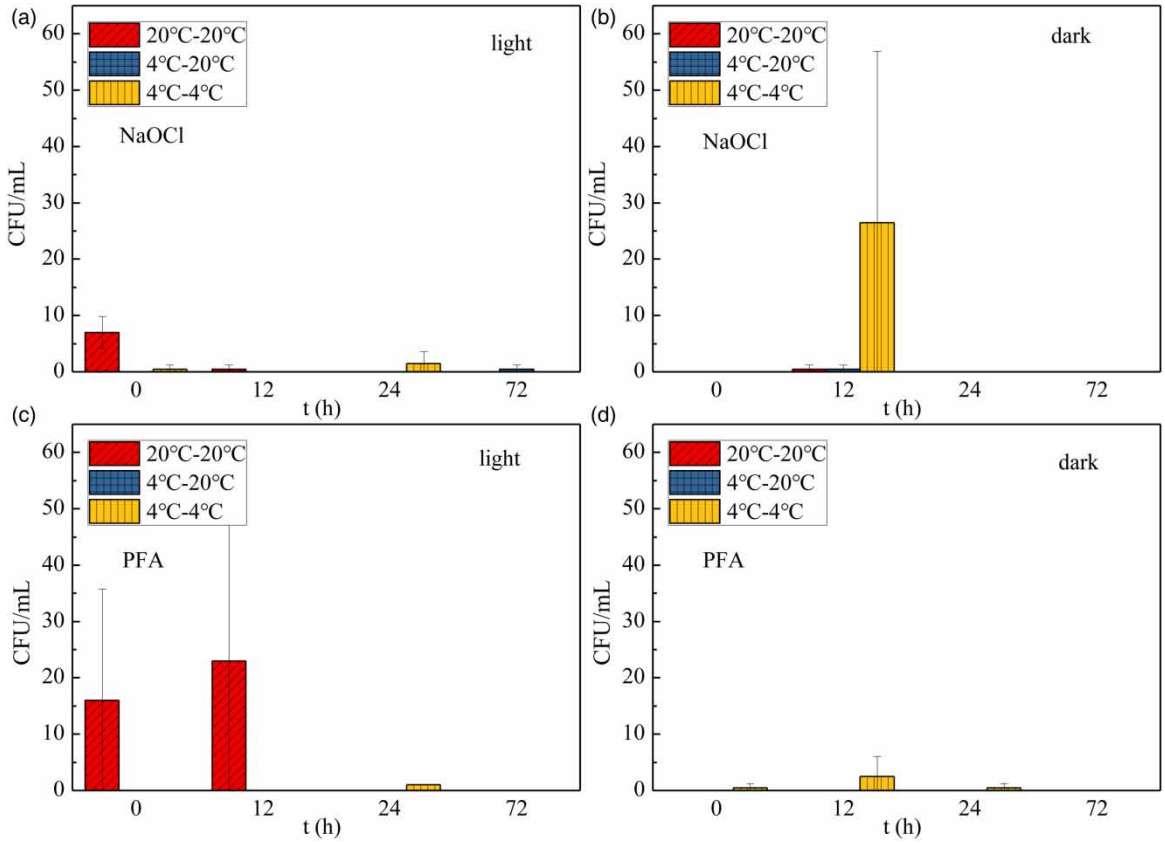


Figure 4 | *S. aureus* regrowth under ambient light (a,c) and in dark (b,d) after disinfection by NaOCl and PFA, t represents the time of exposure. The first and second temperature in the legend indicates the disinfection and regrowth temperature, respectively, i.e. 4–20 °C indicates disinfection at 4 °C and regrowth at 20 °C.

inherent reproducibility with intact bacteria cells, which can be interpreted as non-inactivated bacteria. Repair includes dark-repair and photo-reactivation of damaged DNA by photo-irradiation (Sinha & Häder 2002; Kraft *et al.* 2011). The mechanism of reactivation mostly refers to the reactivation of viable but non-culturable (VBNC) state bacteria (Chen *et al.* 2018). VBNC state is an adaptive strategy for the survival of bacteria under stress. They may retain the ability to reactivate and regrow when external stress disappears (Ayrapetyan *et al.* 2018). The chemical disinfectants used in this study aimed at attacking or permeating cell walls and membranes, but not specifically damaging the DNA of the bacteria, thus the bacteria repair mechanism involving photo-reactivation and dark-repair might not apply, as shown in this study that there was no specific trend showing bacteria regrowth under light or in dark (Figures 3–5). Possibly, the regrowth was induced by the reactivation of VBNC bacteria.

Previous studies have reported that the regrowth of bacteria after disinfection was caused by the bacteria in the VBNC state. Over 99.95% *E. coli* was in the VBNC state when chlorinated at 0.2 mg/L for 30 min (Wang *et al.* 2022), and a slightly higher dose (0.5 mg/L) also could not fully inactivate *E. coli* but reduced its culturability to the VBNC state (Lin *et al.* 2017). Similarly in this study, inactivation by an initial NaOCl concentration of 0.3 mg/L for 1 h, the regrowth of *E. coli* was prominent. Other than chlorination, disinfection treatments using chloramination or UV irradiation also induced the VBNC state of bacteria (Zhang *et al.* 2015; Chen *et al.* 2018). In our previous study, we found that the inactivation of *E. coli*, *S. aureus*, and *B. subtilis* detected by cell culture-based plate counting and flow cytometry were significantly different, indicating the presence of VBNC state bacteria after disinfection (Ding *et al.* 2023). However, the regrowth of PFA-inactivated bacteria was not as evident as NaOCl disinfected bacteria, as shown in this study, and the reason might be related to the inactivation mechanisms of the disinfectants.

HOCl is the predominant reactant accounting for NaOCl disinfection. It has strong permeability to cell walls and membranes (McDonnell & Russell 1999); however, it shows a lower redox potential than PFA (Zhang *et al.* 2018). Therefore,

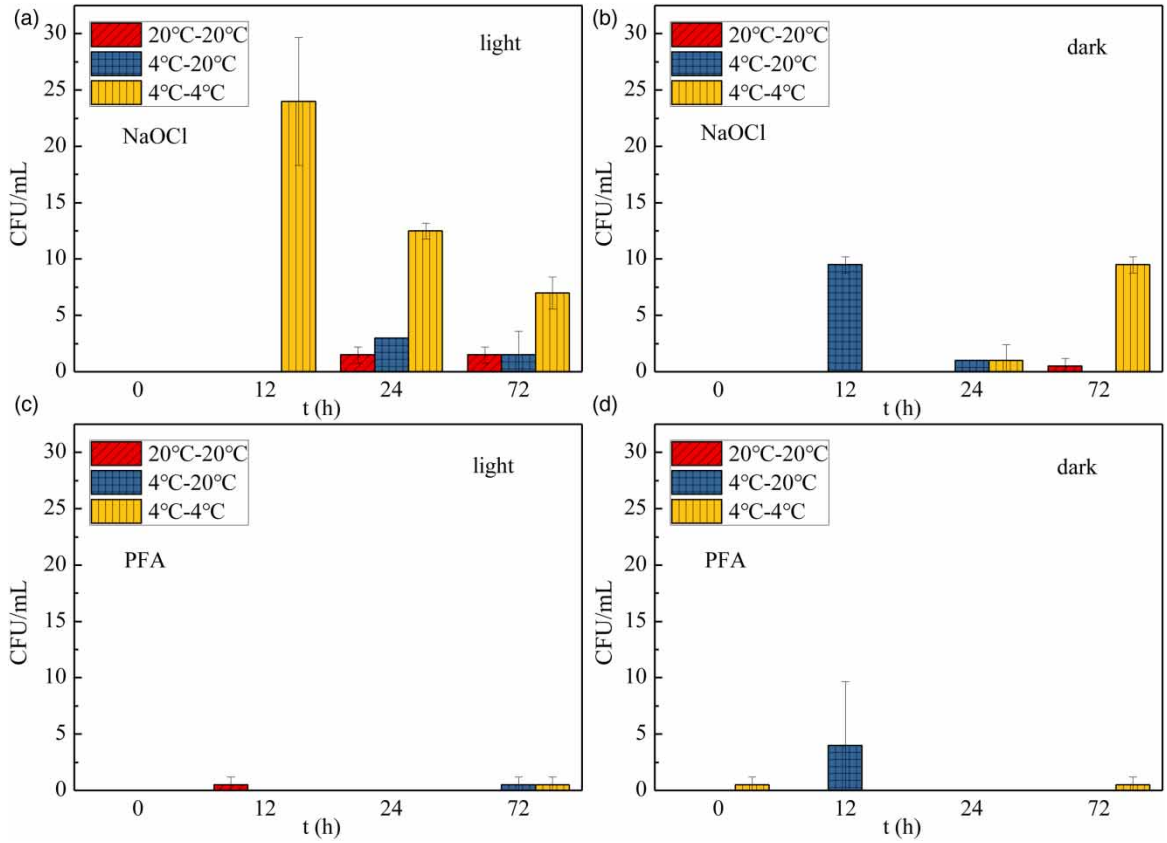


Figure 5 | *B. Subtilis* regrowth under ambient light (a,c) and in dark (b,d) after disinfection by NaOCl and PFA, t represents the time of exposure. The first and second temperature in the legend indicates the disinfection and regrowth temperature, respectively, i.e. 4–20 °C indicates disinfection at 4 °C and regrowth at 20 °C.

although showing a much higher inactivation by cell culture-based detection, the regrowth of bacteria after NaOCl disinfection was more prominent than that after PFA disinfection (Figures 3–5). Peracetic acid (PAA), another widely used peracid, has also shown more effective inactivation of *E. coli* than NaOCl, with lower induction of VBNC state bacteria (Teixeira *et al.* 2020). In this study, the inactivation rate of NaOCl and PFA on the three tested bacteria was higher at 20 °C than at 4 °C (Figures 1 and 2). Temperature has been proved to positively correlate with bacteria inactivation by chlorine and its derivatives, and PAA (Stampi *et al.* 2001; Fukuzaki 2006).

However, there have been controversies on the effect of temperature on bacteria regrowth. In this study, the regrowth of the bacteria inactivated by NaOCl at 4 °C and stored at 4 °C was more prominent than those stored at 20 °C. It was believed that adverse conditions such as low temperature are inducers of bacteria entering the VBNC state (Arana *et al.* 2010). The low temperature (<10 °C) and low nutrient conditions may induce a set of specific proteins to tune cell metabolism and readjust to the new conditions (Barria *et al.* 2013). While storing at low temperatures, the metabolism of bacteria has been restricted to the lowest level, along with the delayed cell damage (Orruno *et al.* 2017). On the other hand, when maintained at 20 °C, *E. coli* populations were more prone to damage (Arana *et al.* 2010). Wu *et al.* also demonstrated that in an artificial seawater sample, live *Vibrio cholerae* decreased from 10^8 CFU/mL to 10^6 and 10^5 when stored at 22 and 37 °C, respectively, while all of those maintained at 4 °C entered into the VBNC state (Wu *et al.* 2016) and remained alive. In contrast, other studies reported that low temperatures hindered bacteria regrowth after disinfection (Giannakis *et al.* 2014). However, this finding was primarily substantiated by bacterial regrowth after UV disinfection (Wang *et al.* 2021); and the temperature was considered an influential factor of photo-reactivation (Lindenauer & Darby 1994).

Compared with NaOCl, PFA showed a superior ability in the suppression of bacteria growth. Previous studies also reported that bacteria regrowth happened less frequently when treated by peracids. Hassaballah *et al.* (2020) inactivated *E. coli* and *Enterococcus* spp. in the secondary effluent by PAA at 2 mg/L for 24 h; no bacteria regrowth was observed thereafter. In

addition, the authors also reported that for *E. coli* inactivation at 4 °C, increasing the contact time from 10 to 30 min had no significant effect on NaOCl but enhanced PAA disinfection. With a higher redox potential than PAA, PFA at 0.8 mg/L and a contact time of 10 min allowed for stable disinfection of *E. coli* and intestinal *Enterococci*, with no growth in the dark after 24 h (Pigot 2021), suggesting PFA irreversibly reacted with cell membrane components, most likely by means of chemical reaction with the disulfide and sulfide components in the protein residuals and unsaturated fatty acids (Voet & Voet 1995).

4. CONCLUSIONS

According to the optimal disinfection models of *E. coli*, *S. aureus*, and *B. subtilis* inactivated by NaOCl and PFA, respectively, *E. coli* and *S. aureus* were extremely susceptible to NaOCl, whereas PFA seems to be more effective on *B. subtilis*. For both disinfectants, inactivation was more effective at 20 than 4 °C, detected by cell culture-based plate counting. Although NaOCl showed a higher efficacy on *E. coli* and *S. aureus* inactivation, bacteria regrowth was prominent, especially for those inactivated at 4 °C and stored at 4 °C, possibly through the mechanism of reactivation of VBNC bacteria. PFA was superior on suppressing bacteria regrowth, which suggested that it could be used as a wastewater disinfectant in a cold environment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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