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Inactivation of *Escherichia coli* and *Enterococcus faecalis* by sulfate radical and hydroxyl radical using Fe(II) based advanced oxidation systems

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ABSTRACT

In this study, the inactivation of *Escherichia coli* and *Enterococcus faecalis* by sulfate radical ($SO_4^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) using Fe^{2+}/peroxydisulfate (PDS) and Fe^{2+}/H_2O_2 systems under neutral pH condition were investigated. 6 log of *E. coli* and 2.71 log of *E. faecalis* were inactivated in Fe^{2+}/PDS system, respectively, compared to 0.98 log and 0.51 log in Fe^{2+}/H_2O_2 system. *E. coli* was much easier to be inactivated than *E. faecalis*. And the inactivation rate of *E. coli* within the first 30 min was higher than that in 30–120 min, while the *E. faecalis* was the opposite. This suggests that the different inactivation patterns of bacteria by advanced oxidation treatments were due to the variation of microbial structures. In addition, the inactivation ability of Fe^{2+}/PDS on *E. coli* and *E. faecalis* in the wastewater effluent decreased by 23.5% and 23.6%, respectively, while the inactivation ability of Fe^{2+}/H_2O_2 decreased by 88.8% and 78.2%, respectively. Higher inactivation efficiencies by $SO_4^{\bullet-}$ were obtained than that by $^{\bullet}OH$. Therefore, inactivation efficiency by $SO_4^{\bullet-}$ based advanced oxidation treatment was less affected by the actual reclaimed water matrix for disinfection.

Keywords: Inactivation; Advanced oxidation treatment; Sulfate radical; Hydroxyl radical

1. Introduction

Reusing the treated wastewater is one of the necessary options to augment available water supplies in the world [1,2]. However, wastewater contains certain pathogenic bacteria that originally exist in the intestines of humans and animals [3,4]. In general, chlorine, ultraviolet (UV), or ozone disinfection is used to control pathogens pollution [5–7]. But concerns have been increasingly raised about the formation of disinfection by-products (DBPs) during chlorination or ozonation and the emergence of pathogens resistant to common disinfectants [6,8–10]. Therefore, in order to completely guarantee the safety of water quality, it is necessary to develop disinfection technologies that efficiently inactivate pathogenic microorganisms and generate less toxic DBPs.

Advanced oxidation processes (AOPs) have been studied in water treatment for decades due to its high rate of reaction to many pollutants. Feng et al. [11] employed bacterial mutations for elucidation of photo-Fenton disinfection process and found that the disinfection process was intracellular. Rodríguez-Chueca et al. [12] investigated the effectiveness of a mild solar photo-Fenton system for the removal of fecal bacteria and found that solar photo-Fenton treatment at pH 3 achieved complete inactivation of *Escherichia coli*

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and *Enterococcus faecalis* in simulated effluents. However, hydroxyl radical ($^{\bullet}OH$, non-selective oxidation ability) has common been recognized as the main subject for inactivation.

In recent years, sulfate radical-based advanced oxidation processes (SR-AOPs) have also been proposed as an alternative oxidation process in water treatment, due to their high efficiency in degrading a wide range of recalcitrant micro-contaminants and even inactivating harmful organisms [13–19]. Sulfate radical ($SO_4^{\bullet-}$) has a redox potential of 2.5–3.1 V which is a selective oxidant with higher reactivity to electron-rich contaminants and has a longer half-life [20,21].

Sulfate radical is generated through activation of persulfate (PS) by heating, microwave, UV and the addition of transition metal or carbon materials. Among them, transition metals (zero valent iron, Co_3O_4, CuO/Fe_3O_4, etc.) consume neither energy nor energy during the activation process, thus obtaining extensive research. Wordofa et al. [22] reported that iron-activated persulfate (PS) could efficiently induce the viability loss of pathogenic *E. coli* O157:H7. Xia et al. [23] investigated natural occurring pyrrhotite (NP) as an alternative catalyst to activate PS for *E. coli* inactivation and found that the optimum inactivation rate attained at a NP dose of 1 g/L and a PS dose of 1 mM. However, these studies were either focused on $^{\bullet}OH$ or $SO_4^{\bullet-}$ inactivation in one process. Considering the different characteristics between $^{\bullet}OH$ and $SO_4^{\bullet-}$, the distinct inactivation efficiency of $^{\bullet}OH$ and $SO_4^{\bullet-}$ need to be further studied.

Besides, the microorganisms with various structures have different performances in AOPs. Rodríguez-Chueca et al. [24] investigated the inactivation of different iron species combined with PMS or PS/UV-A on *E. coli* and *Enterococcus* sp. under neutral pH, and found that the *E. coli* was completely inactivated whereas *Enterococcus* sp. inactivation efficiency was notably lower. Rodríguez-Chueca et al. [25] also investigated the disinfection of simulated winery wastewater and found that the photolytic activation of PMS by UV-A LED radiation allowed the *E. coli* to be completely inactivated, followed by *Staphylococcus aureus* (4 log) and *Bacillus mycoides* (3 log). Qi et al. [26] reported that *Listeria monocytogenes* had more resistance to the persulfate treatment compared with *Escherichia coli* O157: H7. Table 1 shows the sterilization effects of different sterilization methods. These studies all illustrated that the difference in inactivation of pathogens is also depended on the type of bacteria. To better evaluate the sterilization performance of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments, *E. faecalis* and *E. coli* were used as indicator of gram-positive and gram-negative bacteria in this experiment.

The main objective of this work was to comprehensively compare the sterilization performance of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments (the comparable reaction rate of Fe^{2+}/H_2O_2 (76 $M^{-1}\cdot s^{-1}$) and the Fe^{2+}/PDS process (27 $M^{-1}\cdot s^{-1}$)) under neutral condition through the inactivation rates of *E. coli* and *E. faecalis* due to. The inactivated effect of pH and concentrations of activator and oxidant were investigated. Through analysis of free radical action and the determination of extracellular protein and DNA, the inactivation mechanisms of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments to bacteria were investigated. In addition, the disinfection ability of the two treatments was further compared in the effluent matrix of the water from V-shaped filter (FW) of urban domestic sewage treatment plant, which provided technical support for the practical application.

2. Materials and methods

2.1. Bacterial cultivation and preservation

Escherichia coli (gram-negative, *E. coli*) and *Enterococcus faecalis* (gram-positive, *E. faecalis*) were chosen as model bacterium to synthetically evaluate the inactivation ability of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments. The bacterial cells were cultured in nutrient broth (AOBOX, Beijing, China) at 37°C with shaking, then collected in the late exponential phase of growth. The collected bacteria were centrifuged at 5,000 rpm

Table 1
Comparison of inactivation results of *E. coli* and *E. faecalis* by different methods

Disinfection method	Results	Experimental condition	Microbial species	References
Solar/Fe(III)-EDDS/PDS, solar/Fe(III)-EDDS/H_2O_2	6 log within 180 min	Initial cell density: 1 × 10^6 CFU/mL; initial pH: 8.0 ± 0.2; initial temperature: 26°C–28°C; Fe(III)EDDS: 0.1 mM; H_2O_2 or $S_2O_8^{2-}$: 0.5 mM	*E. faecalis*	[27]
Visible light/PDS	6 log within 40 min and 7 log of within 120 min	Initial cell density: 1 × 10^7 CFU/mL; initial pH: 6.0; initial temperature: 30°C; PDS: 2 mM; visible light wavelength: 420 nm	*E. coli*	[28]
Fenton like reaction (magnetic Fe_3O_4–deposited flower-like MoS_2 (MF)/H_2O_2)	6 log within 75 min	Initial cell density: 1.2 × 10^6 CFU/mL; initial pH: 9.5; initial temperature: 25.0°C ± 0.2°C; MF: 2.0 g/L; H_2O_2: 5 mM	*E. coli*	[29]
Solar/heat/PDS	6 log within 80 min for both bacteria	Initial cell density: 1 × 10^6 CFU/mL; initial pH: 8.15; temperature: 50°C; PDS: 0.5 mM	*E. coli* *E. faecalis*	[30]
Iron ion/PDS	6 log within 15 min for both bacteria	Initial cell density: 1 × 10^6 CFU/mL; pH: 7.3–7.6; temperature: 25°C ± 2°C; iron ion 30 mg/L; PDS: 200 mg/L	*E. coli* *E. faecalis*	[31]

for 5 min two times, then the final pellets were resuspended in ultrapure water for experiment. The culture method of *E. faecalis* was similar to that of *E. coli*, except that the nutrient broth was replaced with Luria-Bertani Broth (AOBOX, Beijing, China) [27]. The bacteria stock solution was stored in a refrigerator at 4°C and re-cultured before each experiment to ensure its fresh and viability.

2.2. Chemicals and materials

$Na_2S_2O_8$ and H_2O_2 were used as oxidants in different treatments. $FeSO_4·7H_2O$, $Na_2S_2O_3·5H_2O$ and *tert*-butyl alcohol (TBA) were purchased from Kermel. Na_2SO_4 was purchased from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. The working solution was prepared with 100 mL ultrapure water containing 5 mM Na_2SO_4. The sodium thiosulfate was prepared weekly. All reagents were used at least analytical grade and prepared with ultrapure water from a Milli-Q device (Heal Force, 18.2 Ωcm). Reclaimed water was collected from the FW in a municipal sewage treatment plant.

2.3. Experimental procedures

A 100 mL suspension including oxidant of 0.5 mM and *E. coli* of 1 × 10^7 CFU/mL or *E. faecalis* of 1 × 10^6 CFU/mL in a flask was vigorously dispersed by a magnetic stirrer, followed by adjusting the initial pH to 7 and adding Fe^{2+} (0–0.5 mM) to start the reaction. Aliquot samples were collected at the same time interval. Control experiments with Fe^{2+} or oxidants alone were also conducted in triplicate. Appropriate amounts of H_2SO_4 (0.1 M) or NaOH (0.1 M) was added to adjust the initial pH. TBA was chosen as a probe and added to the reactor with the molar ratio of oxidant/probe/$FeSO_4·7H_2O$ at 5/5/2. And the initial pH was adjusted to 5. After the sterilized working solution was purged with N_2 for 2 h to remove dissolved oxygen (DO), the experiment was conducted under the same conditions. Sodium thiosulfate (0.1 M) was added into above solution to quench the residue oxidant, then centrifuged at 10,000 rpm for 5 min. After that, extracellular protein and DNA were determined using spectrophotometric method. Bacterial inactivation was also conducted in authentic water matrix. Prior to use, the water samples were filtered by the 0.45 μm membrane. Each experiment was conducted with three times.

2.4. Determination of microbial concentration

Microbial concentrations were determined by the dilution method of plate counting. Samples were diluted serially with sterilized saline solution. Each step was repeated three times. 0.1 mL of the sample was spread on the nutrient agar (AOBOX, Beijing, China) plate after half an hour and incubated at 37°C for 24 h. Clear colonies were counted by the standard plate counting method.

The colony effective detection range is 1–300 CFU on a petri dish. The inactivation efficiency was measured by the logarithmic inactivation rate $\log(N_0/N_t)$, The detection limit (DL) was 10 CFU/mL. Inactivation rate constant: $k = \log(N_0/N_t)/t$, N_0 represents the bacterial concentration before inactivation, CFU/mL; N_t refers to the bacterial concentration at t min of inactivation, CFU/mL; t represents inactivation time, min.

3. Results and discussion

The *E. coli* and *E. faecalis* inactivation by exposure to 0.5 mM Fe^{2+} alone or 0.5 mM oxidants alone treatment were conducted under the neutral condition (Fig. 1). less than 0.1 log reduction after 3 h were obtained, so that the direct sterilization was negligible. The research of Rodríguez-Chueca et al. [21] and Bianco et al. [27] also proved that Fe^{2+}, PDS and H_2O_2 alone would not affect the bacterial concentration. The inactivation rate constants k and final pH values during Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments were shown in Table 2.

3.1. Effects of concentration of Fe^{2+}

Two kinds of bacteria under a certain concentration of PDS (0.5 mM), varying the concentration of Fe^{2+} at initial neutral pH were inactivated by Fe^{2+}/PDS and Fe^{2+}/H_2O_2

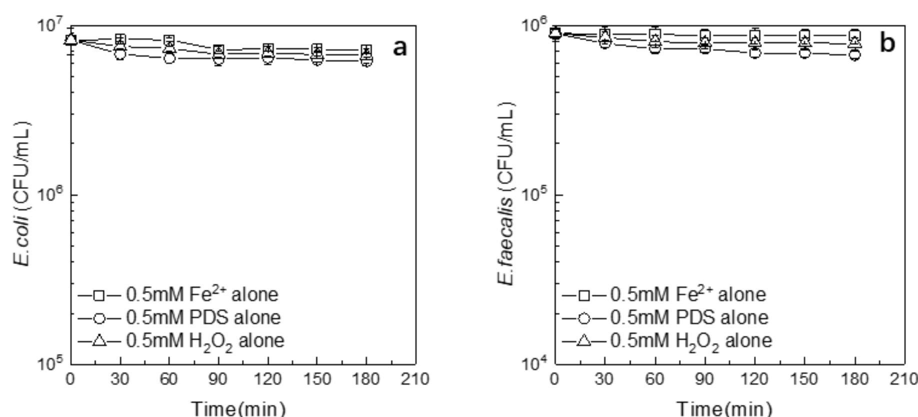


Fig. 1. Inactivation of (a) *E. coli* and (b) *E. faecalis* under oxidants and Fe^{2+} alone. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentration of *E. coli* and *E. faecalis* were about 1 × 10^7 CFU/mL and 1 × 10^6 CFU/mL, and initial pH 7.

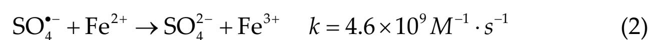
Table 2
Inactivation rate constants *k* and final pH values during Fe^{2+}/PDS and Fe^{2+}/H_2O_2

Parameter	Concentration (mM)	*k* (log/min)				Final pH			
		Fe^{2+}/PDS		Fe^{2+}/H_2O_2		Fe^{2+}/PDS		Fe^{2+}/H_2O_2	
		E. coli	*E. faecalis*	*E. coli*	*E. faecalis*	*E. coli*	*E. faecalis*	*E. coli*	*E. faecalis*
Fe^{2+} (pH = 7)	0.1	0.027	0.012	0.002	0.004	3.66	3.49	3.64	3.85
	0.2	0.033	0.015	0.005	0.003	3.37	3.16	3.48	3.39
	0.3	0.061	0.021	0.011	0.003	3.24	3.02	3.30	3.22
	0.4	0.300	0.023	0.017	0.004	3.17	2.94	3.24	3.13
	0.5	0.600	0.023	0.019	0.005	3.06	2.87	3.13	3.04
PDS (pH = 7)	0.25	0.019	0.005	0.003	0.001	3.71	3.54	3.82	3.89
	0.50	0.023	0.012	0.009	0.004	3.66	3.49	3.64	3.85
	0.75	0.024	0.014	0.008	0.002	3.68	3.54	3.84	3.78
	1.00	0.026	0.015	0.010	0.004	3.68	3.58	3.85	3.77
pH	3	0.067	0.028	0.020	0.014	2.98	2.91	3.07	2.98
	5	0.040	0.020	0.009	0.005	3.19	3.09	3.43	3.34
	7	0.033	0.015	0.005	0.005	3.37	3.16	3.48	3.39
TBA (pH = 5)	0	0.036		0.008		2.99		3.26	
	0.5	0.034		0.004		3.00		3.28	
Aeration (pH = 5)		0.040		0.009		3.19		3.43	
	Aeration 2 h	0.032		0.007		2.92		3.17	

treatments for 180 min (Fig. 2). In the Fe^{2+}/PDS treatment, inactivation of *E. coli* was much faster than that of *E. faecalis* (Fig. 2a and b).

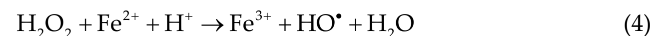
The cultivable population of both bacteria decreased with the increase of Fe^{2+} concentration from 0.1 to 0.5 mM. When the PDS/Fe^{2+} molar ratio was 1/1, the maximum inactivation rates of *E. coli* and *E. faecalis* were achieved, which were 0.6 and 0.023 log/min, respectively. The inactivation of *E. coli* was achieved from 4.8 log with 0.1 mM Fe^{2+} for 180 min to 6.0 log with 0.5 mM Fe^{2+} for only 10 min, which was regarded as complete inactivation under this experiment conditions (Fig. 2a). The inactivation rates were 0.027 and 0.6 log/min, respectively (Table 1). This was attributed to the contribution of Fe^{2+} that could activate persulfate to produce reductive oxidation active free radicals [Eq. (1)]. The inactivation of *E. faecalis* increased when the concentration of Fe^{2+} increased from 0.1 mM (2.11 log) to 0.3 mM (3.83 log) (Fig. 2b). The inactivation slightly increased with the continuous addition of Fe^{2+} to 0.5 mM (3.83 to 4.11 log). These might be due to the excessive Fe^{2+} was competed with *E. faecalis* for sulfate radicals as shown in Eq. (2) [16,32,33]. The inactivation rate of *E. faecalis* at 0.5 mM Fe^{2+} (0.023 log/min) was twice that of 0.1 mM Fe^{2+} (0.012 log/min).

$$S_2O_8^{2-} + Fe^{2+} \rightarrow Fe^{3+} + SO_4^{\bullet -} + SO_4^{2-} \quad k = 27\ M^{-1} \cdot s^{-1} \tag{1}$$


$$SO_4^{\bullet -} + Fe^{2+} \rightarrow SO_4^{2-} + Fe^{3+} \quad k = 4.6 \times 10^9\ M^{-1} \cdot s^{-1} \tag{2}$$

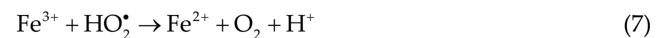
In the Fe^{2+}/H_2O_2 treatment, the inactivation of *E. coli* gradually increased along with the concentration of Fe^{2+} (Fig. 2c). When the H_2O_2/Fe^{2+} molar ratios were 5/1 and 1/1, the inactivation of *E. coli* increased from 0.41 log to 3.37 log at 180 min, and the inactivation rate increased from 0.002 to 0.019 log/min. It was because Fe^{2+} activate hydrogen peroxide to produce $^{\bullet}OH$, which could inactivate *E. coli* in water [Eq. (3)]. In analogy, excess H_2O_2 and Fe^{2+} would also consume $^{\bullet}OH$ as shown in Eqs. (4)–(8) [34]. Regardless of the Fe^{2+} concentration, the inactivation rate of *E. faecalis* was only about 0.004 log/min (Fig. 2d). According to the results of the above two treatments, the gram-negative bacteria were more likely to be inactivated than gram-positive bacteria.

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad k = 76\ M^{-1} \cdot s^{-1} \tag{3}$$


$$H_2O_2 + Fe^{2+} + H^{+} \rightarrow Fe^{3+} + HO^{\bullet} + H_2O \tag{4}$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \tag{5}$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + OH^{-} \tag{6}$$


$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^{+} \tag{7}$$

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2 \tag{8}$$

At Fe^{2+} concentration of 0.3 mM, the Fe^{2+}/PDS treatment on *E. coli* achieved a 6.0 log inactivation, compared to the 2.05 log achieved by Fe^{2+}/H_2O_2, and Fe^{2+}/PDS treatment on *E. faecalis* achieved a 3.83 log inactivation, compared to the

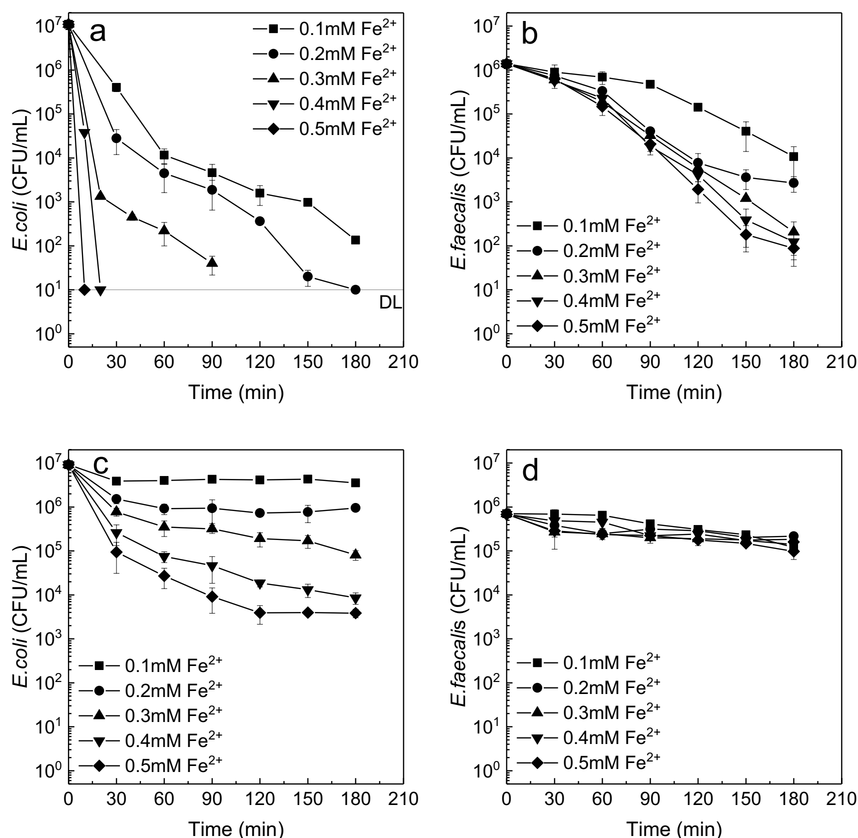


Fig. 2. Inactivation of (a) *E. coli*, (b) *E. faecalis* by Fe^{2+}/PDS, (c) *E. coli* and (d) *E. faecalis* by Fe^{2+}/H_2O_2 under different Fe^{2+} concentration. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentrations of *E. coli* and *E. faecalis* were about 1×10^7 CFU/mL and 1×10^6 CFU/mL, respectively, [oxidant] = 0.5 mM, and initial pH 7.

0.58 log achieved by Fe^{2+}/H_2O_2. The inactivation rates of Fe^{2+}/PDS for *E. coli* and *E. faecalis* were 0.061 and 0.021 log/min, respectively, which were both about 6.0–7.0 times higher than those for Fe^{2+}/H_2O_2 treatment (Table 1). These results indicate that the inactivation of microorganisms in water is more efficient by $SO_4^{\bullet-}$ based advanced treatment than that by $^{\bullet}OH$ based advanced treatment. In addition, it was noticed that the final pH values of both treatments were decreased with the continuous addition of Fe^{2+}. However, due to the acidity of PDS in solution, the final pH values of Fe^{2+}/PDS treatment were slightly lower than that of Fe^{2+}/H_2O_2 treatment, which might be the other reason of the better performance of Fe^{2+}/PDS.

The inactivation rates *k* of *E. coli* and *E. faecalis* by the treatments of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 during 0–30 min and 30–120 min were shown in Fig. 3. During the 0–30 min, the inactivation rates of *E. coli* through the Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments were 0.198 and 0.035 log/min, which were about 9.0 and 5.0 times higher than those during the 30–120 min. However, during the 30–120 min, the inactivation rates of *E. faecalis* by the Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments were 0.028 and 0.009 log/min, which were both about 2.3 times than those in the 0–30 min. This discrepancy might due to the different cell structure of gram-negative bacteria and gram-positive bacteria, which indicated that oxidation damage within the first 30 min for gram-positive bacteria accelerated the behind inactivation. Bianco et al. had mentioned that *E. faecalis* was another pathogenic indicator microorganism with higher resistance than the frequently used *E. coli* during AOPs disinfection. Overall, no matter which treatment was used, the inactivation of *E. coli* was much faster than those of *E. faecalis*, which indicated that *E. coli* is an 'easy target' to AOPs [12]. *E. faecalis* is more suitable as an alternative model microorganism given its higher resistance to AOPs.

3.2. Effects of concentration of PDS or H_2O_2

E. coli and *E. faecalis* under a certain concentration of Fe^{2+} (0.1 mM), varying the dosage of oxidants at neutral pH were inactivated by Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments for 180 min (Fig. 4). When the oxidants concentration increased from 0.25 mM to 0.5 mM, the inactivation rates of *E. coli* and *E. faecalis* through Fe^{2+}/PDS treatment increased by 0.004 and 0.007 log/min, and those of Fe^{2+}/H_2O_2 treatment increased by 0.006 and 0.003 log/min, respectively. However, with the continuous addition of oxidants, the inactivation rate increased slowly. This was consistent with the result of Xia et al. [23].

The initial enhancement (Fig. 4a) might be due to the increase of the added oxidants, which accelerated the generation of free radicals, thereby the cell structure was destroyed. The later flattening perhaps might be due to the complete conversion of Fe^{2+} (0.1 mM) to Fe^{3+}, resulting in excessive oxidants not being activated.

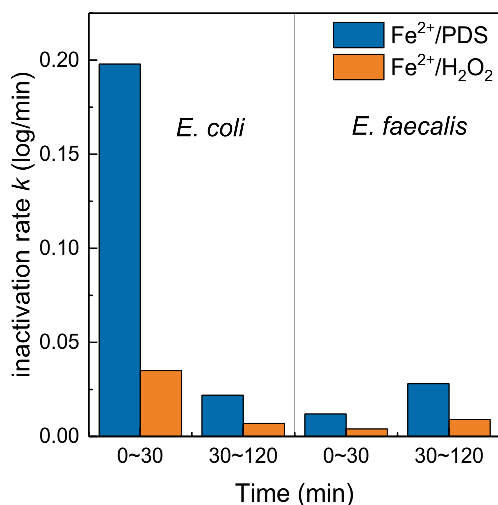


Fig. 3. Inactivation rates k of *E. coli* and *E. faecalis* by Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments during 0–30 min and 30–120 min. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentrations of *E. coli* and *E. faecalis* were about 1×10^7 CFU/mL and 1×10^6 CFU/mL, respectively, [oxidant] = 0.5 mM, $[Fe^{2+}]_0$ = 0.3 mM, and initial pH 7.

When the oxidants at a concentration of 1.0 mM, the Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments on *E. coli* achieved 4.73 and 1.73 log inactivation, compared to the 2.64 and 0.07 log achieved on *E. faecalis*. The inactivation rates of Fe^{2+}/PDS for *E. coli* and *E. faecalis* were 0.026 and 0.015 log/min, respectively, which were about 2.6 and 3.75 times higher than those for Fe^{2+}/H_2O_2 treatment. In addition, with the increase of oxidants concentration, the final pH values of Fe^{2+}/PDS treatment were also slightly lower than that of the Fe^{2+}/H_2O_2 treatment (Table 1). SO_4^{2-} produced by PDS does not exceed the standard value [35].

3.3. Effects of initial pH value

Acid pH condition is reported more favorable for the Fe^{2+}/H_2O_2 and Fe^{2+}/PDS treatments [36]. As mentioned above, excessive Fe^{2+} would consume radicals and generate precipitates, so a concentration of 0.2 mM was used. From Fig. 5, it could be seen that the inactivation effects of the two bacteria by different treatments decreased with the increasing pH. The *E. coli* was almost completely inactivated during Fe^{2+}/PDS treatment at three pH values (Fig. 5a). When the pH value decreased from 7 to 3, *E. coli* inactivation rate during the Fe^{2+}/PDS treatment was increased by 1.0 times, while it was increased by 3.0 times during Fe^{2+}/H_2O_2 (Fig. 5a and b). Similarly, the inactivation rates of *E. faecalis* was increased by 0.9 and 1.8 times during the Fe^{2+}/PDS and Fe^{2+}/H_2O_2, respectively (Fig. 5c and d). It was perhaps related to the iron precipitation at higher pH. The oxidation potential of $^{\bullet}OH$ (1.8 V) under alkaline conditions is significantly lower than that of $SO_4^{\bullet-}$ and $^{\bullet}OH$ under acidic conditions (2.5–3.1 and 2.7 V), resulting in a decrease in overall oxidation capacity. These results were similar to the previous study [23].

At pH 3, the inactivation rates of *E. coli* and *E. faecalis* during Fe^{2+}/PDS were 0.067 and 0.028 log/min, which were about 3.4 and 2.0 times higher than those during Fe^{2+}/H_2O_2 treatment (Table 1). These results indicated that even at the ideal pH for AOPs, a better performance of Fe^{2+}/PDS for bacterial inactivation could be obtained than of Fe^{2+}/H_2O_2.

3.4. Identification of active radicals

AOPs can not only generate radicals with high oxidation potential such as $^{\bullet}OH$ and $SO_4^{\bullet-}$, but they will also further convert to $O_2^{\bullet-}$ and other relatively weak radicals. To identify the main oxidizing substances, TBA and aeration of N_2 were used to determine the contribution of $^{\bullet}OH$ and $O_2^{\bullet-}$ for bacteria inactivation. TBA is a radical scavenger that is often used to quench $^{\bullet}OH$ (k = 3.8–7.6 × 10^8 $M^{-1}\cdot s^{-1}$) but not for $SO_4^{\bullet-}$ (k = 4.0–9.1 × 10^5 $M^{-1}\cdot s^{-1}$) [14,37]. In addition, aeration can reduce the DO in the system and indirectly reduce the formation of $O_2^{\bullet-}$.

When the molar ratio of PDS/TBA/Fe^{2+} was 5/5/2 and the initial pH was 5, *E. coli* inactivation was achieved 5.08 log at 150 min and the inactivate rate was 0.034 log/min (Fig. 6). Compared with no TBA treatment (0.036 log/min), it only reduced 0.002 log/min, hence the change (6%) was minimal. However, the inactivation rate of *E. coli* of Fe^{2+}/H_2O_2 was reduced from 0.008 log/min without TBA to 0.004 log/min with TBA, which was suppressed by about 50% (Table 1). The above results shown that $^{\bullet}OH$ was not the foremost free radicals in Fe^{2+}/PDS treatment to inactivate *E. coli*, but the most important radical in Fe^{2+}/H_2O_2.

The concentration of DO was reduced from 6.98 to 0.28 mg/L after exposure to nitrogen for 2 h. The inactivation effect of *E. coli* after aeration of N_2 had all deteriorated (Fig. 7). The treatment time for complete inactivation of *E. coli* was extended from 150 to 180 min in Fe^{2+}/PDS treatment, and the inactivate rates were 0.040 and 0.032 log/min (suppressed 20%), respectively. The inactivate rate of *E. coli* in Fe^{2+}/H_2O_2 was reduced from 0.009 to 0.007 log/min after aeration, which was only inhibited by approximately 22%. These results indicated that $O_2^{\bullet-}$ had a certain contribution in Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments.

$SO_4^{\bullet-}$ selectively reacts with electron-rich organic substances by the way of transferring electrons, while $^{\bullet}OH$ reacts non-selectively with organic substances through the rotes of hydrogen abstract and hydroxide addition [38,39]. Wordofa et al. [22] investigated the disinfection kinetics of $SO_4^{\bullet-}$ and $^{\bullet}OH$ on *E. coli* O157:H7 and found that they had different performance. The CT value of $SO_4^{\bullet-}$ was 9.5 × 10^{-10} M min, which was approximately 5 times faster than $^{\bullet}OH$. This also explained why the disinfection effect of Fe^{2+}/PDS treatment was better than Fe^{2+}/H_2O_2. From this point of view, $SO_4^{\bullet-}$ played a major role in the inactivation of bacteria in Fe^{2+}/PDS treatment. Their contribution ranges from $SO_4^{\bullet-} > O_2^{\bullet-} > {}^{\bullet}OH$ in this study. However, $^{\bullet}OH$ and other free radicals both played important role in the inactivation of bacteria in the Fe^{2+}/H_2O_2 treatment.

3.4. Inactivation mechanism

The extracellular proteins and DNA of *E. coli* were determined to judge whether the cell wall of *E. coli* was damaged. The concentration of extracellular protein and DNA before, after and continued inactivation of *E. coli* by Fe^{2+}/PDS and

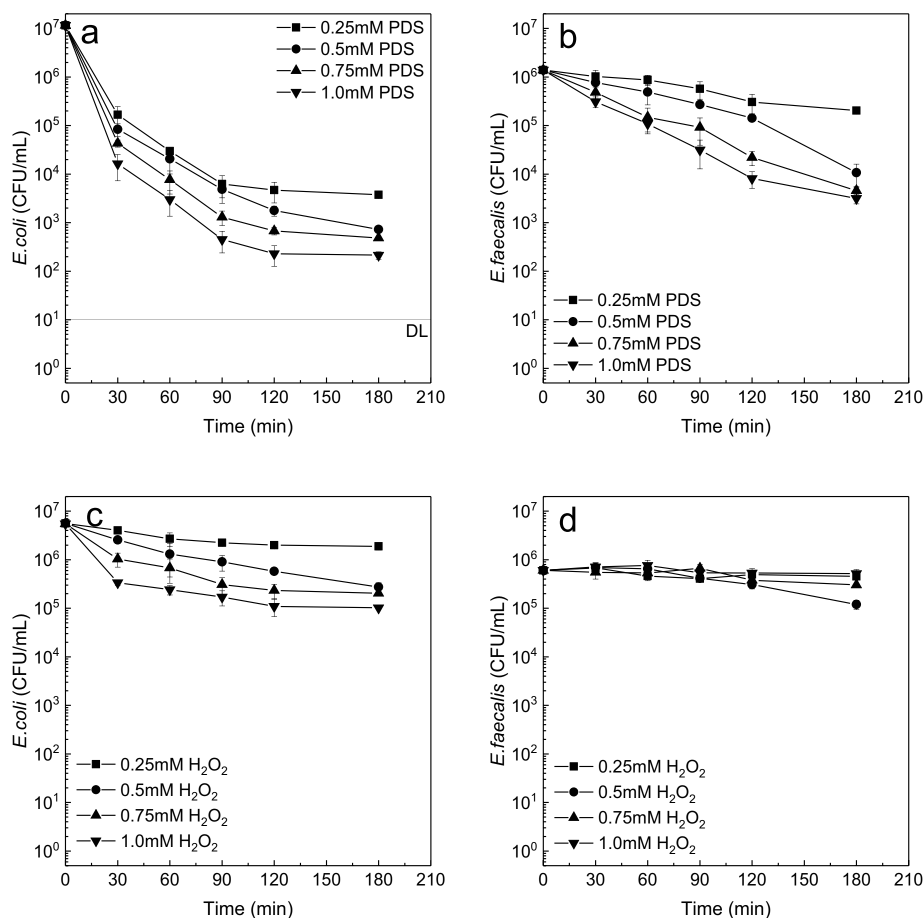


Fig. 4. Inactivation of (a) *E. coli*, (b) *E. faecalis* by Fe^{2+}/PDS, (c) *E. coli* and (d) *E. faecalis* by Fe^{2+}/H_2O_2 under different oxidant concentrations. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentrations of *E. coli* and *E. faecalis* were about 1×10^7 CFU/mL and 1×10^6 CFU/mL, respectively, $[Fe^{2+}]_0$ = 0.1 mM, and initial pH 7.

Fe^{2+}/H_2O_2 treatments were shown in Fig. 8. The extracellular protein levels were increased after *E. coli* inactivation during Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments (Fig. 8a). For example, the extracellular protein concentration of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 were 3,789 and 3,675 μg/mL at the end (120 min), respectively. It was indicated that highly reactive oxygen species could destroy the cell wall of *E. coli* and release intracellular organic compounds, resulting in the inactivation of *E. coli*. When the reaction lasted for 300 min, the extracellular protein concentration inactivated by Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments showed a decreasing trend, which were 3,475 and 3,265 μg/mL, respectively. This was because proteins were organic macromolecules that highly reactive oxygen species would react with them.

Fig. 8b was clearly showed that the extracellular DNA increased firstly and then decreased as well during the treatments of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 inactivation of *E. coli*. The extracellular DNA concentrations in Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments were 19.61 and 5.19 μg/mL at 120 min, respectively. After the prolonged inactivation, they were decreased to 15.87 and 4.42 μg/mL, respectively. These were also proved that the reactive species could attack and destroy bacterial cell wall, leading intracellular content out, and inducing bacterial inactivation. In addition, the releasing DNA was far lower in in Fe^{2+}/H_2O_2 treatment than in Fe^{2+}/PDS treatment, which might suggest that $^{\bullet}OH$ highly attacked free DNA causing lower inactivation efficiency compared to $SO_4^{\bullet-}$.

3.5. Disinfection in real water matrix

The inactivation of Fe^{2+}/PDS and Fe^{2+}/H_2O_2 against *E. coli* and *E. faecalis* in FW was shown in Fig. 9. Fe^{2+}/PDS treatment could inactivated *E. coli* for 6 log within 150 min, and the treatment time was extended by 60 min compared with that in the ultrapure water (UPW) (Fig. 9a). However, Fe^{2+}/PDS treatment inactivated 5 log and 3.82 log *E. faecalis* after 180 min in UPW and FW, respectively (Fig. 9b). The inactivation of *E. coli* and *E. faecalis* were 0.41 and 0.55 log, respectively, after Fe^{2+}/H_2O_2 treatment was treated in FW for 180 min (Fig. 9c and d). In addition, the inactivation of Fe^{2+}/PDS against *E. coli* and *E. faecalis* in water matrix were 14.6 and 6.9 times higher than those of Fe^{2+}/H_2O_2, respectively.

There were many ingredients, such as natural organic matter (NOM) and inorganic ions in the real water matrix, which had influence on Fe^{2+}/PDS and Fe^{2+}/H_2O_2 disinfection. $SO_4^{\bullet-}$ could selectively react with nitrogen-containing organics which dominate in NOM through electron transfer oxidation mechanism, while $^{\bullet}OH$ no selectively react with NOM.

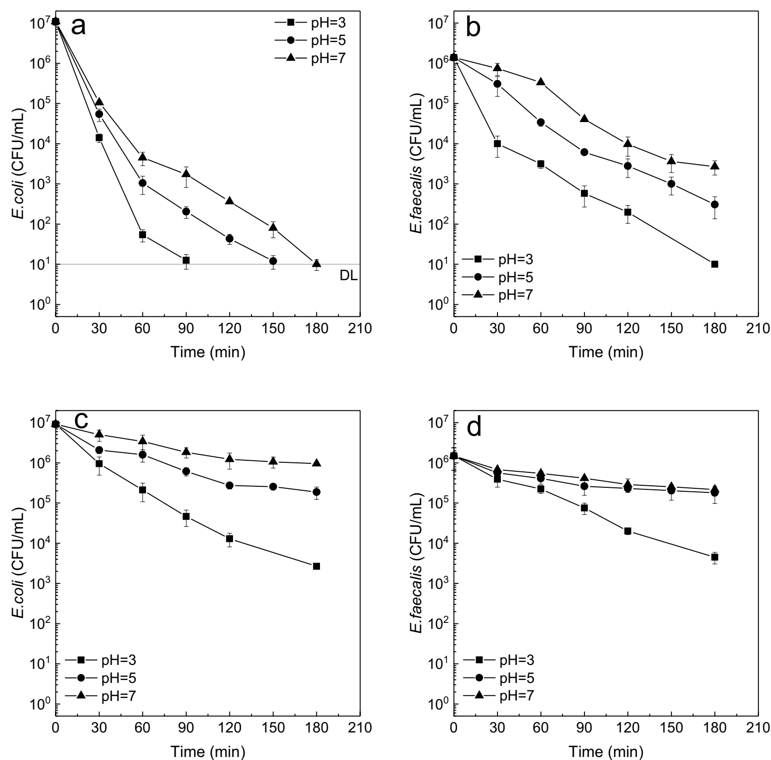


Fig. 5. Inactivation of (a) *E. coli*, (b) *E. faecalis* by Fe^{2+}/PDS, (c) *E. coli* and (d) *E. faecalis* by Fe^{2+}/H_2O_2 under different initial pH values. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentrations of *E. coli* and *E. faecalis* were about 1×10^7 CFU/mL and 1×10^6 CFU/mL, respectively, $[oxidant]_0 = 0.5$ mM, and $[Fe^{2+}]_0 = 0.2$ mM.

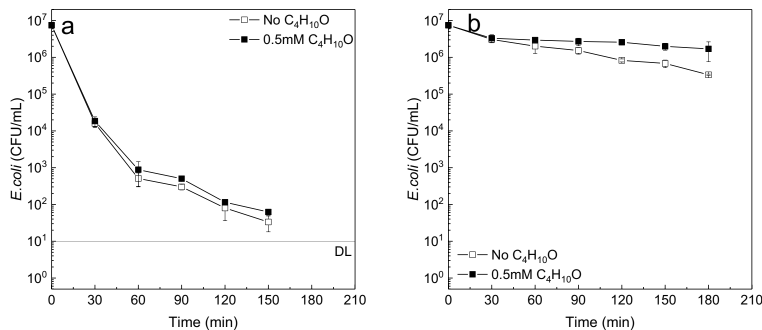


Fig. 6. Inactivation of *E. coli* by adding 0.5 mM TBA under the (a) Fe^{2+}/PDS (b) Fe^{2+}/H_2O_2 condition. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentration of *E. coli* was about 1×10^7 CFU/mL, $[oxidant]_0 = 0.5$ mM, $[Fe^{2+}]_0 = 0.2$ mM and initial pH 5.

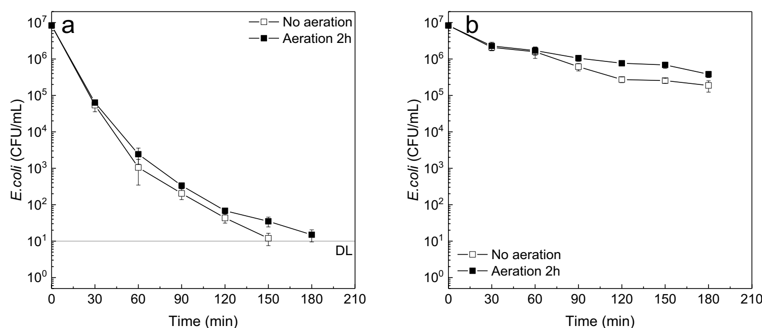


Fig. 7. Inactivation of *E. coli* through aeration of N_2 under (a) Fe^{2+}/PDS (b) Fe^{2+}/H_2O_2. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentration of *E. coli* was about 1×10^7 CFU/mL, $[oxidant]_0 = 0.5$ mM, $[Fe^{2+}]_0 = 0.2$ mM and initial pH 5.

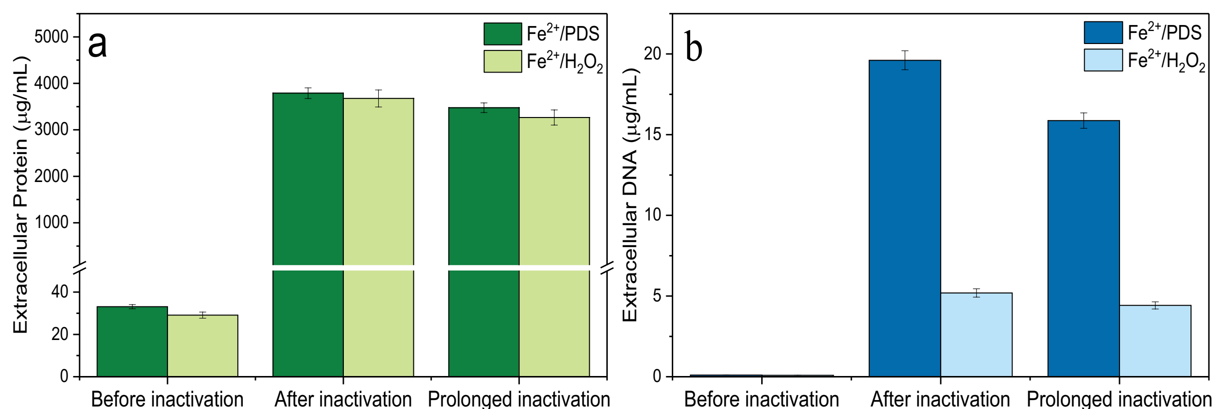


Fig. 8. Determination of (a) extracellular proteins and (b) extracellular DNA of *E. coli* before, after and prolonged inactivation by Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentration of *E. coli* was about 1×10^7 CFU/mL, $[oxidant]_0 = 0.5$ mM, $[Fe^{2+}]_0 = 0.2$ mM and initial pH 5.

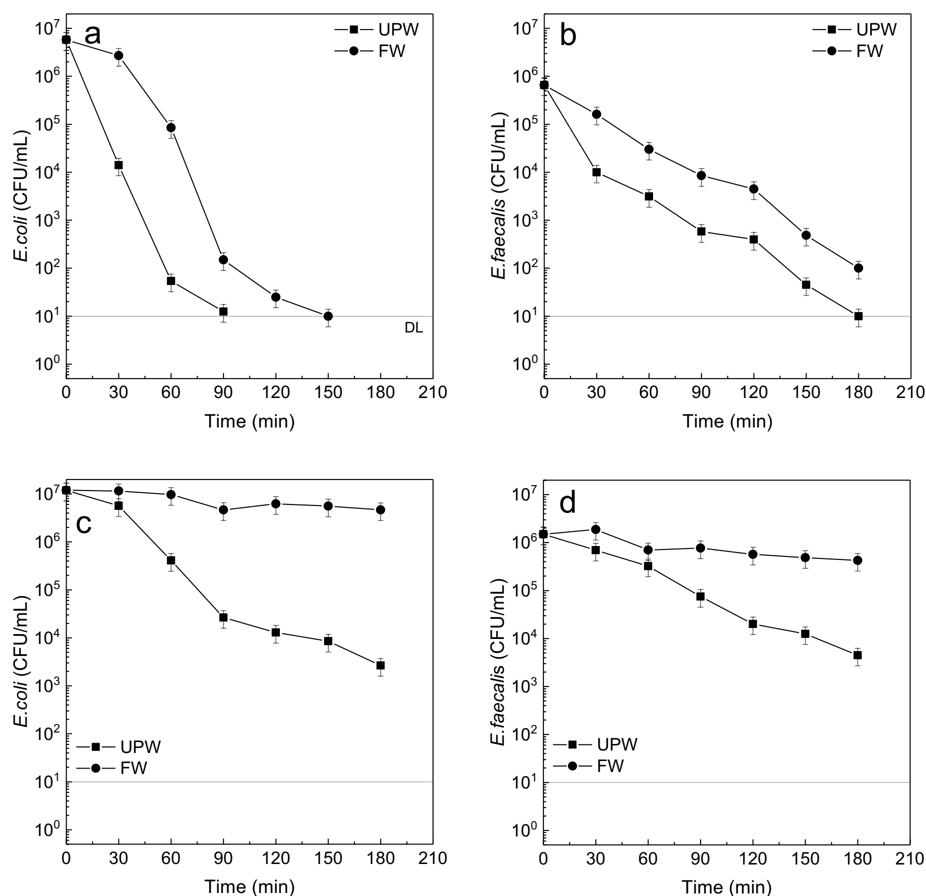


Fig. 9. Inactivation of (a) *E. coli* (b) *E. faecalis* by Fe^{2+}/PDS and (c) *E. coli* (d) *E. faecalis* by Fe^{2+}/H_2O_2 under authentic water matrix. Error bars on points represent standard deviation from 3 experimental data. Conditions: initial concentrations of *E. coli* and *E. faecalis* were about 1×10^7 CFU/mL and 1×10^6 CFU/mL, respectively, $[oxidant]_0 = 0.5$ mM, $[Fe^{2+}]_0 = 0.2$ mM, initial pH 3.

$SO_4^{\bullet-}$ and $^{\bullet}OH$ might react with carbonate in FW, which could also greatly inhibit the inactivation of bacteria. The inactivation of Fe^{2+}/PDS treatment on *E. coli* and *E. faecalis* decreased by 23.5% and 23.6% in FW, respectively compared with those in UPW. However, the inactivation of Fe^{2+}/H_2O_2 treatment on *E. coli* and *E. faecalis* decreased by 88.8% and 78.2% in FW, respectively. These results indicated that Fe^{2+}/PDS treatment was more suitable for actual reclaimed water disinfection.

4. Conclusions

This study compared the inactivation of *E. coli* and *E. faecalis*, representing as gram-negative bacteria and gram-positive bacteria, respectively, at neutral condition in Fe^{2+}/PDS and Fe^{2+}/H_2O_2 treatments. The inactivation rates of the two kinds of bacteria in the Fe^{2+}/PDS were higher than that of the Fe^{2+}/H_2O_2. And, no matter which of the two treatments was used, the inactivation effect of *E. coli* was greater than *E. faecalis*, suggested that *E. faecalis* was a better model microorganism in AOPs. It was found that radicals contribution in inactivation ranges from $SO_4^{\bullet-} > O_2^{\bullet-} > {}^{\bullet}OH$ in Fe^{2+}/PDS treatment, and the major contribution of $^{\bullet}OH$ in Fe^{2+}/H_2O_2 treatment. Moreover, the extracellular proteins and DNA of *E. coli* and *E. faecalis* were increased after inactivation. Compared with UPW, the inactivation of Fe^{2+}/PDS on *E. coli* and *E. faecalis* in FW decreased by 23.5% and 23.6%, respectively, while the inactivation of Fe^{2+}/H_2O_2 decreased by 88.8% and 78.2%, respectively. Instead of Fe^{2+}/H_2O_2, Fe^{2+}/PDS treatment was more suitable for actual reclaimed water disinfection under the same conditions.

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