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Evaluation of disinfection efficacy of performic acid (PFA) catalyzed by sulfuric and ascorbic acids tested on *Escherichia coli* (ATCC, 8739)

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ABSTRACT

Performic acid (PFA) is an oxidizing agent which has recently attracted attention its for use as disinfectant in the medical field and food industry. This study investigated the efficacy of PFA catalyzed by both sulfuric and ascorbic acid. Tap water in one experiment and sterilized activated sludge effluent (ASE) in another, were used for the preparation of aqueous solutions. *Escherichia coli* was used as the target micro-organism to investigate the efficacy of catalyzed PFA. The results were analyzed to set up the pertinent mathematical relationship according to Hom's model. Applying the highest initial dose of 15 mg l^{-1}, showed the superior catalytic action of sulfuric acid and 7-log inactivation was achieved in 30 min of disinfection period. Application of PFA on *E. coli* in our experiment caused CT value of 12.16 mg min l^{-1} for 99% inactivation and CT value of 23.82 mg min l^{-1} for 99.9% inactivation. PFA produced 4-log inactivation at a CT value of 35.48 mg min l^{-1}. The results showed that, the promoting action of sulfuric acid is expected in higher initial dose of PFA when synthetic liquid medium was prepared of sterilized ASE. The disinfection efficacy of PFA is proved to be less than free chlorine and more than chloramines.

Keywords: Performic acid; Disinfection; Sulfuric acid; Ascorbic acid; *E. coli*

1. Introduction

Untreated wastewater and secondary treated effluents contain a wide range of pathogenic micro-organisms that pose a potential risk to the health of humans and livestock [1]. Hence the disinfection with the aim of removing or inactivating pathogenic microorganisms is considered as an essential process.

Bacteria are the most common microbial pathogens found in wastewater. They are often used as an

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indicator of microbial contamination and as a surrogate indicator to assess the efficacy of treatment and disinfection methods [1].

Disinfection is one of the main processes carried out in wastewater treatment plants. In recent years, various methods have been proposed for this purpose and many of these methods were used in various treatment plants around the world for years. Along the application of chemical disinfection, formation of byproducts such as trihalomethanes, haloacetic acids, and other unwanted byproducts became a crisis for the human health; hence searching for innovative and effective methods of disinfection to replace the current methods seems necessary.

In the last few decades, oxidation compounds such as hydrogen peroxide (HP) have been introduced for wastewater disinfection. HP has disinfection capability and does not leave any unfavorable environmental effects. The disinfection potential of HP is due to the production of free radicals ($OH^{\bullet}$) in the absence of a catalyst; however, the rate of radical production is slow [2].

Another possible method for the disinfection of wastewater is the use of some synergistic agents in combination with HP [3]. This technique leads to extremely better production of hydroxyl radicals. One of these processes is Fenton reaction with the oxidation potential of 2.7 eV compared with 1.8 eV for HP [4].

It is known that HP is a moderately effective and mild disinfecting agent with bacteriostatic properties. Where H_2O_2 concentration of $25\,mg\,l^{-1}$ inhibits the growth of some bacteria, an effective lowering of the bacterial count requires many hours even at much higher H_2O_2 concentrations or an additional UV irradiation process [5]. Unfortunately, according to the results of related studies, the application of HP in combination with some substances such as Ag, Cu, and Fe has shown only mild disinfection ability, that in some cases such as disinfection of raw sewage cannot provide the related standards [2–4]. This means that high dosages of this disinfectant are needed to meet the relevant effluent standards. This has led us to design another study on a new disinfectant, performic acid (PFA), to achieve maximum impact with minimum dosage of chemical disinfectant.

PFA (CH_2O_3) is a colorless liquid, that because of its strong oxidizing potential is used for cleaving disulfide bonds in protein mapping [6]. PFA is a wide-spectrum disinfectant. It inactivates viruses, bacteria and bacterial spores, mycobacteria as well as microscopic fungi [7]. Thus PFA was found to be considered as an interesting potential disinfectant in many industrial processes, such as medical and food industries, being effective at low temperature [8]. As the contact time in the study presented by Gehr et al. (2009) was set to 45 min, CT values for 4–6 log removal of *Enterococcus* were about $225–270\,mg\,min\,l^{-1}$ [9]. In disinfection using free chlorine, CT value for 99% inactivation of *Giardia lamblia* cysts are reported to be $47–150\,mg\,min\,l^{-1}$ and for chloramines disinfection, it is determined as $2{,}200\,mg\,min\,l^{-1}$ [10].

The results from experiments on the kinetics of PFA (reactions 1–5) have shown that it can be successfully interpreted through a kinetic mechanism, consisting of the reversible formation of the PFA and the irreversible decomposition to CO_2 and H_2O, both catalyzed by hydrogen ions [9].

$$H_2O_2 + HCOOH \rightleftharpoons HCOOOH + H_2O \quad (1)$$

$$HCOOOH \xrightarrow{H^+} CO_2 + H_2O \quad (2)$$

$$2HCOOOH \xrightarrow{H^+} 2HCOOH + O_2 \quad (3)$$

$$HCOOOH + H_2O_2 \xrightarrow{H^+} HCOOH + H_2O + O_2 \quad (4)$$

$$H_2O_2 \xrightarrow{H^+} 2H_2O + O_2 \quad (5)$$

None of the products are considered toxic to aquatic life at doses which would be used for disinfection, and in fact tests with FA have yielded LC50 values of $46–175\,mg\,l^{-1}$ for fish, $120–150\,mg\,l^{-1}$ for Daphnia, and $25\,mg\,l^{-1}$ for algae [12,9]. But, its main drawback is its instability, which emphasizes on a logical necessity for it to be prepared from its components HCOOH and H_2O_2 prior to use [7].

PFA has also recently found a growing importance in chemical industries due to the versatile oxidizing properties in several applications [11]. The effects of conventional disinfectants such as chlorine, ozone, chloramines, and chlorine dioxide on target microbial strains such as *Escherichia coli* have been sufficiently studied. To compare the efficacy of PFA to other conventional disinfectants, the power of its disinfection on microbial strains should be studied. As the effect of PFA on microbial strains is not specifically presented, this study aimed to determine the disinfection efficacy of PFA on *E. coli*.

2. Materials and methods

The materials used in this study involved formic acid (AppliChem, No. A0749), HP (50 wt.%, Sigma-Aldrich, 516813), sulfuric acid (Merck, No. 112080), ascorbic acid (AppliChem, No. A1052), sodium

thiosulfate pentahydrate (Merck, No. 1.06516), potassium iodide (Merck, No. 1.05043), and A1-Medium (Merck, No. 1.00415). The necessary stock solutions were made from the above compounds and doubled distilled water. The glassware was washed and autoclaved at 121 °C for 20 min before use.

All analytical measurements followed conventional procedures in standard methods. The standard test for the *E. coli* was carried out by the multiple-tube fermentation technique. The method is referred to as, method 9221 E-2 (*E. coli* procedure) [12]. This test is considered to distinguish those total coliform organisms that are fecal coliforms.

2.1. Preparation of microbial culture

E. coli (ATCC, 8739) was selected as the target bacteria in this study, since it has been used as an indicator of fecal pollution in other similar studies. *E. coli* was cultivated on Tryptic soy agar (TSA, Merck) slant for 24 h at 35–37 °C. After incubation, the colonies of bacteria were washed by 2 ml of Tryptic soy broth (TSB, Merck) that contain 3% of glycerol and were kept in sterile vials in the refrigerator. Then for each experiment, one of these vials was used. For the final culture, 1 vial containing 2 ml of frozen strain was poured into 100 ml of TSB medium. Then, the flask containing the culture medium was incubated for 24 h at 37 °C.

For providing the raw sewage characteristics, the microbial loads of 10^8–10^9 per 100 ml were needed. Hence, the number of bacteria in our nutrient medium was determined by plate count method and it was about 1.25×10^9. After adding aliquots of 1 ml from microbial suspensions into the desired media, the initial rates of 10^5–10^6 per 100 ml were prepared.

2.2. Preparation of disinfectant

In this study, the role of two catalysts (sulfuric acid and ascorbic acid) in the enhancement of PFA disinfection efficiency was investigated. PFA was prepared daily and stored in room temperature. Preparation of PFA was as follows:

Step 1: 15 ml deionized water was added to 35 ml of HP (50 wt.%). This solution is equal to 50 ml of HP (35 wt.%).

Step 2: 50 ml of formic acid (85 wt.%) was prepared from formic acid (AppliChem, 98–100 wt.%) and was added to a 100-ml Erlenmeyer flask.

Step 3: 4.7 ml of sulfuric acid or ascorbic acid were used as catalysts and added to the prepared solution described in step 2. The salt of ascorbic acid was used and its solution was prepared with the highest degree of solubility (333 g l^{-1} of H_2O). Due to the exothermic reaction between PFA and HP, the temperature of the pot should be kept below 20 °C [14]. To solve this problem, an erlenmeyer flask was located into a beaker while the space between them were filled with water and ice cubes.

Step 4: Prepared HP solution was slowly added to the formic acid by using a burette. The content of the flask was gently stirred [9]. The mixture was maintained at 20 °C for about 60–90 min prior to the application of PFA solution.

This solution, prepared according to the mentioned method has a density of 1.18 g cm^{-3} for sulfuric acid and 1.16 g cm^{-3} for ascorbic acid as a catalyst.

2.3. Sampling and preparation of samples

Tap water and sterilized activated sludge effluent (ASE) were used for preparation of aqueous solutions. Plastic containers, each of 4 liter volume, were used for ASE sampling. The ASE samples were taken from a municipal wastewater treatment plant with activated sludge process, located in the north of Tehran city, Iran and after reaching to the laboratory was poured in bottles with 1 l volume and autoclaved at 121 °C for 30 min to be sterilized. Water samples were taken from the tap water in the laboratory. The physicochemical properties of the tap water and ASE samples used during the period of this research, are shown in Table 1.

2.4. Procedure

One liter of the desired media (tap water or sterilized ASE) was poured into a beaker and a magnetic stirrer was used for stirring, then 1 ml of microbial suspension was added to it. After complete mixing, a few milliliters of this mixture were used for the cultivation of control blank samples. Due to the high microbial load of the prepared sample (10^5–10^7 per 100 ml), the blank samples were diluted proportionally to be able to quantify the microbial contamination.

Ninty minute after the preparation of PFA stock solution, experimental solutions were prepared so that the amounts of added disinfectant did not exceed 2.5% of final sample volume plus added disinfectant. As shown in Table 2, doses of the disinfectants were gradually increased to determine the trend of disinfection activity. It should be mentioned that the applied doses for ASE media were higher because of probable interference of organic matters and other constituents in wastewater. Contact times were 5, 10, 20, and 30 min. Each combination was at least triplicated in

Table 1
average of some physico-chemical properties of tap water and ASE sample

The parameters in tap water		The parameters in ASE	
pH	7.44	pH	7.42
T (℃)	17.8	T (℃)	19.68
Turbidity (NTU)	0.615	Turbidity (NTU)	4.67
TDS (mg/l)	342.45	TSS (mg/l)	31
EC (μS/cm)	470	COD (mg/l)	28.51
Na^+ (mg/l)	14.866	BOD5 (mg/l)	17.08
K^+ (mg/l)	0.541		
Ca^+ (mg/l)	51.073		
Cl^- (mg/l)	22.695		
NO_3^- (mg/l)	7.906		
HCO_3^- (mg/l)	162.37		

Table 2
Experimental applied doses in each media

Dose (mg l^{-1})	
Drinking water	Sterile ASE
2	6
4	8
6	10
8	12.5
10	15

different days. After elapsing the detention times, the residual disinfectant concentration was neutralized by adding sodium thiosulfate based on mole/mole ratio.

2.5. Microbiological analysis

Microbiological analyses of the drinking water and ASE were performed before and after the disinfection process according to the doses shown in Table 2. Microorganisms were analyzed by *E. coli* procedure. For this step, the multiple-tube method (15-tubes) was used. Tubes of A1-Medium (with 2 dilutions, double & single) are inoculated with samples measuring 10 ml, 1 ml, and 0.1 ml. The samples were incubated at 37℃ for 3 h and the tubes were then transferred to a 44.5℃ water bath for 19–21 h [13]. During incubation, EC organisms produce gas and turbidity. If no gas is present at the end of 48 h, the test is negative. If gas in any amount is present, it is a positive presumptive test.

2.6. Measurement of disinfectant residual concentration

For this purpose, a 10% potassium iodide KI solution, 3% ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) solution, 1% starch solution, and 0.001 N $Na_2S_2O_3$ solution were prepared.

Five ml of KI solution was added to 50 ml of the disinfected sample. After adding KI, a yellowish-brown color appeared due to release of iodine. The color was enhanced by adding a few drops of ammonium heptamolybdate solution. The released iodine was titrated with a 0.001 N $Na_2S_2O_3$ solution from yellowish-brown to light yellow. After the addition of 1 ml of a 1% starch solution, a blue color appeared and titration was continued until a colorless solution was achieved. The residual concentrations of PFA were calculated as follows:

$$V_{\mathrm{PFA}}\ (\mathrm{mg}^{-1}) = \frac{N_2 \times V_2 \times \mathrm{eqw}_{\mathrm{PFA}} \times 1,000}{V_{\mathrm{sample}}} \tag{6}$$

The same method was used for measuring the weight percentage (wt.%) of PFA. Due to the higher concentration of disinfectant, 0.1 N $Na_2S_2O_3$ solution was used and the wt.% was calculated as follows:

$$\mathrm{HCOOOH}\ (\mathrm{wt.}\%) = \frac{N_2 \times V_2 \times \mathrm{eqw}_{\mathrm{PFA}} \times 100}{V_{\mathrm{sample}}} \tag{7}$$

where N_2: actual normality of $Na_2S_2O_3$ solution; V_2: consumption of $Na_2S_2O_3$ solution (ml); $\mathrm{eqw}_{\mathrm{PFA}}$: equivalent weight of HCOOOH (62/2 = 31); V_{sample}: sample size (here is 50 ml).

2.7. Hom disinfection model

Disinfection model, represented in equation 8, was proposed by Hom (1972) and used in this study for data analysis. It provides for a relationship between disinfectant concentration and contact time, and empirical constants m and n [15].

$$\ln(N/N_0) = -kC^n t^m \quad (8)$$

where N = number of infective organisms at any given time; N_0 = Initial number of infective organisms; C = residual disinfectant concentration, M/V; k, m, n: Empirical constants for Hom model; t = contact time.

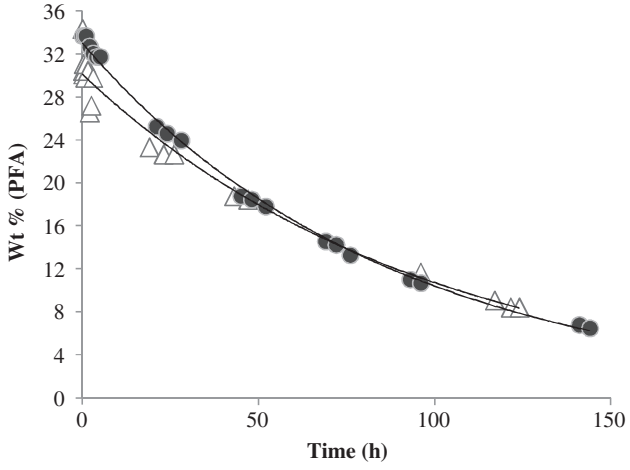

Fig. 1. Natural decline (Break down) of PFA solution catalyzed by sulfuric acid (Δ) and by ascorbic acid (●). Sulfuric acid's equation: wt.% PFA = $30.108e^{-0.01t}$, R^2 = 0.9887. Ascorbic acid's equation: wt.% PFA = $32.992e^{-0.012t}$, R^2 = 0.9977.

3. Results

As previously mentioned, two PFA solutions were prepared, in which sulfuric acid and ascorbic acid were used as catalysts. The natural declines of PFA in the prepared solutions are illustrated in Fig. 1. Exponential model proved to be the best fit for PFA decay. As shown, the trend of PFA decline is almost equal for sulfuric acid catalyzed PFA and ascorbic acid catalyzed PFA. PFA demonstrated to have a half life of 58 h. By extrapolation of the curves, it was found that 90% of PFA would be disintegrated in 192 h. Accordingly, PFA solutions are not stable enough to be preserved for a long time.

The microbial inactivation capability of PFA (with synthetic liquid medium prepared of sterilized ASE) is illustrated in Fig. 2, to show the role of sulfuric and ascorbic acids as catalysts. In the initial PFA concentration of 6 mg l^{-1}, there is no significant difference in microbial inactivation up to 20 min detention time. In the mentioned dose of PFA, ascorbic acid proved to act slightly stronger than sulfuric acid; since the log-inactivation reached about 3.6. As shown in Fig. 2(b), the stronger catalyst activity of ascorbic acid, appeared in initial PFA dose of 10 mg l^{-1} in which about 1.5 extra microbial log-inactivation was achieved. In higher PFA dose of 12.5 mg l^{-1}, microbial inactivation changed in favor of sulfuric acid as catalyst (Fig. 2(a)). Application of the highest initial dose of 15 mg l^{-1} in this study showed the superior catalytic action of sulfuric acid, since 7-log inactivation was achievable in 30 min of disinfection period. Accordingly, the considerable promoting action of sulfuric acid is expected in higher initial dose of PFA.

Tables 3 and 4 were show the descriptive statistics of triplicates. In these tables the minimum, maximum, and the mean of all data that was used for charting Figs. 2 and 3 are presented.

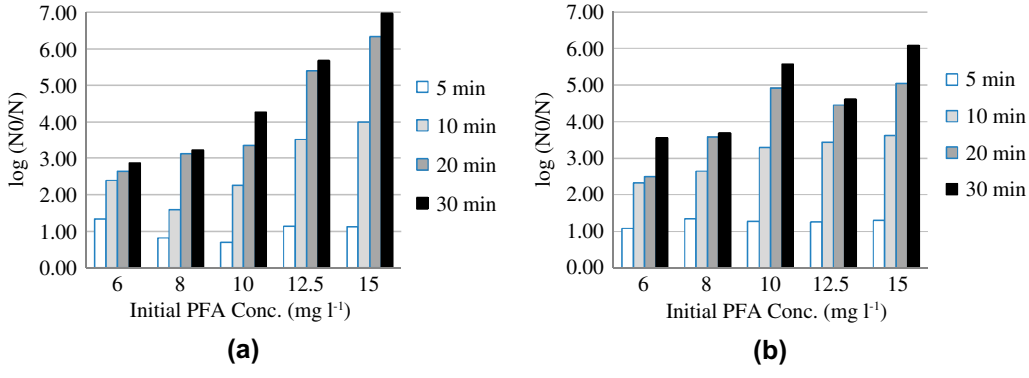

Fig. 2. Log-inactivation resulting from PFA disinfection with synthetic liquid medium prepared of sterilized ASE: (a) catalyzed by sulfuric acid; (b) catalyzed by ascorbic acid.

Table 3
Descriptive statistics on log N_0/N (sterilized ASE disinfection with PFA catalyzed by sulfuric & ascorbic acid)

Statistical analysis	Initial PFA concentration																			
	6 mg/l				8 mg/l				10 mg/l				12.5 mg/l				15 mg/l			
	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min
Sulfuric acid																				
Mean	1.33	2.39	2.65	2.87	0.82	1.59	3.14	3.22	0.69	2.26	3.36	4.26	1.14	3.53	5.40	5.69	1.11	4.01	6.34	6.97
Standard deviation	0.10	0.04	0.10	0.05	0.04	0.02	0.06	0.00	0.06	0.01	0.04	0.04	0.01	0.08	0.02	0.06	0.04	0.06	0.01	0.10
Minimum	1.23	2.36	2.56	2.81	0.77	1.57	3.09	3.22	0.63	2.25	3.31	4.21	1.13	3.47	5.37	5.62	1.07	3.94	6.33	6.86
Maximum	1.43	2.44	2.76	2.90	0.85	1.62	3.21	3.23	0.73	2.28	3.40	4.29	1.16	3.63	5.41	5.73	1.14	4.05	6.34	7.05
Con. level (95%)	0.25	0.11	0.25	0.12	0.10	0.06	0.16	0.01	0.14	0.03	0.11	0.11	0.04	0.20	0.06	0.15	0.09	0.15	0.02	0.24
Ascorbic acid																				
Mean	1.09	2.32	2.50	3.57	1.36	2.66	3.58	3.70	1.28	3.28	4.91	5.58	1.26	3.43	4.45	4.62	1.30	3.63	5.04	6.08
Standard deviation	0.06	0.06	0.11	0.17	0.07	0.12	0.05	0.05	0.15	0.02	0.04	0.08	0.05	0.04	0.02	0.10	0.12	0.05	0.02	0.07
Minimum	1.02	2.28	2.39	3.44	1.29	2.53	3.53	3.65	1.10	3.27	4.88	5.51	1.22	3.39	4.43	4.56	1.16	3.58	5.02	6.02
Maximum	1.14	2.40	2.60	3.76	1.43	2.74	3.63	3.74	1.38	3.31	4.95	5.66	1.31	3.46	4.46	4.73	1.37	3.68	5.05	6.16
Con. level (95%)	0.15	0.16	0.26	0.42	0.17	0.29	0.13	0.12	0.38	0.05	0.09	0.19	0.12	0.10	0.04	0.24	0.29	0.12	0.04	0.17

Table 4
Descriptive statistics on log N_0/N (tap water disinfection with PFA catalyzed by sulfuric & ascorbic acid)

Statistical analysis	Initial PFA concentration																			
	2 mg/l				4 mg/l				6 mg/l				8 mg/l				10 mg/l			
	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min	5 min	10 min	20 min	30 min
Sulfuric acid																				
Mean	1.62	3.08	3.84	4.05	2.89	3.01	4.80	5.33	3.04	3.15	5.18	5.39	4.11	4.99	5.18	5.28	4.63	5.13	5.33	5.42
Standard deviation	0.07	0.02	0.03	0.09	0.04	0.02	0.02	0.04	0.08	0.07	0.12	0.10	0.06	0.11	0.09	0.13	0.08	0.02	0.06	0.05
Minimum	1.56	3.06	3.81	3.97	2.86	2.99	4.78	5.28	2.96	3.08	5.05	5.32	4.04	4.88	5.08	5.13	4.56	5.12	5.30	5.36
Maximum	1.69	3.10	3.88	4.15	2.94	3.03	4.83	5.37	3.12	3.21	5.30	5.50	4.15	5.10	5.26	5.36	4.72	5.16	5.40	5.46
Con. level (95%)	0.17	0.05	0.09	0.23	0.11	0.05	0.05	0.11	0.20	0.16	0.31	0.24	0.16	0.27	0.23	0.31	0.21	0.05	0.14	0.13
Ascorbic acid																				
Mean	0.57	1.48	2.87	3.07	1.74	2.73	5.00	5.09	2.17	3.48	6.12	6.12	4.04	4.71	6.12	6.12	3.97	6.26	6.26	6.26
Standard deviation	0.08	0.03	0.07	0.01	0.04	0.03	0.04	0.05	0.07	0.03	0.10	0.10	0.10	0.08	0.11	0.11	0.02	0.09	0.09	0.09
Minimum	0.48	1.45	2.80	3.06	1.69	2.69	4.96	5.03	2.09	3.46	6.05	6.05	3.92	4.62	6.02	6.02	3.95	6.18	6.18	6.18
Maximum	0.63	1.51	2.93	3.07	1.76	2.74	5.04	5.12	2.24	3.51	6.24	6.24	4.11	4.78	6.24	6.24	3.98	6.36	6.36	6.36
Con. level (95%)	0.20	0.08	0.17	0.01	0.10	0.08	0.10	0.13	0.18	0.07	0.26	0.26	0.26	0.21	0.27	0.27	0.05	0.23	0.23	0.23

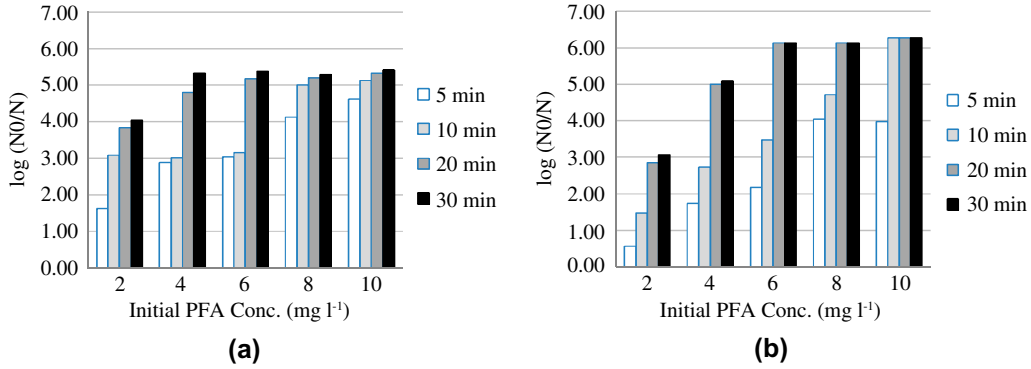

Fig. 3. Log-inactivation resulting from PFA disinfection with synthetic liquid medium prepared of tap water: (a) catalyzed by sulfuric acid; (b) catalyzed by ascorbic acid.

Table 5
Data used in calculating of the Hom's relation in sterilized ASE

C_0 (mg l^{-1})	Time (min)	Sulfuric acid		Ascorbic acid	
		$-\text{Ln}(N/N_0)$	C_t (mg l^{-1})	$-\text{Ln}(N/N_0)$	C_t (mg l^{-1})
6	5	3.06	0.777	2.49	0.777
	10	5.50	0.745	5.34	0.713
	20	6.08	0.68	5.76	0.616
	30	6.61	0.616	8.20	0.551
8	5	1.88	1.296	3.11	1.199
	10	3.67	1.231	6.08	1.01
	20	7.20	1.101	8.24	1.004
	30	7.43	1.037	8.50	0.939
10	5	1.59	1.361	2.92	1.717
	10	5.20	1.263	7.56	1.6035
	20	7.71	1.231	11.32	1.5065
	30	9.81	1.134	12.84	1.3765
12.5	5	2.62	2.462	2.88	2.073
	10	8.11	2.268	7.90	1.976
	20	12.43	2.138	10.25	1.782
	30	13.08	2.073	10.61	1.685
15	5	2.58	2.786	2.99	2.559
	10	9.21	2.754	8.34	2.462
	20	14.60	2.689	11.61	2.43
	30	16.07	2.656	14.02	2.332

Table 6
Data used in calculating of the Hom's relation in tap water

C_0 (mg l^{-1})	Time (min)	Sulfuric acid		Ascorbic acid	
		$-\text{Ln}(N/N_0)$	C_t (mg l^{-1})	$-\text{Ln}(N/N_0)$	C_t (mg l^{-1})
2	5	3.73	0.421	1.31	0.356
	10	7.09	0.356	3.41	0.324
	20	8.82	0.292	6.59	0.292
	30	9.30	0.295	7.07	0.162
4	5	6.63	0.616	4.01	0.583
	10	6.94	0.551	6.29	0.389
	20	11.05	0.454	11.51	0.194
	30	12.27	0.421	11.70	0.097
6	5	7.00	0.972	5.01	1.166
	10	7.25	0.81	8.02	1.101
	20	11.90	0.745	14.10	1.037
	30	12.39	0.713	14.10	1.004
8	5	9.49	1.361	9.30	1.425
	10	11.51	1.231	10.86	1.328
	20	11.96	1.166	14.10	1.231
	30	12.18	1.101	14.10	1.166
10	5	10.63	1.425	9.15	2.626
	10	11.81	1.328	14.42	2.332
	20	12.26	1.263	14.42	2.17
	30	12.47	1.166	14.42	2.008

The microbial inactivation capability of PFA (with synthetic liquid medium prepared by tap water) is illustrated in Fig. 3 to compare the capability of sulfuric and ascorbic acids as catalysts. As shown in initial PFA dose of 2 mg l^{-1}, log inactivation was higher when sulfuric acid was used as catalyst.

The results revealed that in higher applied doses of PFA (more than 4 mg l^{-1}), application of ascorbic acid leads to 6-log inactivation after 20 and 30 min of disinfection practice. Furthermore it was shown that for the mentioned higher dose of PFA, catalytic effects of ascorbic acid were stronger than sulfuric acid. Hence, the considerable promoting action of ascorbic acid is expected in higher initial doses of PFA when tap water is used as the liquid medium.

Disinfection efficacy was modeled using of Hom model based on data presented in Tables 5 and 6. Higher initial concentration of PFA was applied in the experiments in which synthetic liquid medium was prepared of sterilized ASE. The higher applied doses

were considered because in ASE medium there are some constituents, capable of interacting with the disinfectant. Equations according to Hom model are presented as follow (9–12):

(1) Sulfuric acid (in the ASE):

$$\mathrm{Ln}(N/N_0) = -0.54C^{0.48}t^{0.87} \quad (9)$$

R^2 : 0.84

(2) Ascorbic acid (in the ASE):

$$\mathrm{Ln}(N/N_0) = -0.86C^{0.36}t^{0.75} \quad (10)$$

R^2 : 0.89

(3) Sulfuric acid (in tap water):

$$\mathrm{Ln}(N/N_0) = -4.25C^{0.35}t^{0.35} \quad (11)$$

R^2 : 0.76

(4) Ascorbic acid (in tap water):

$$\mathrm{Ln}(N/N_0) = -1.67C^{0.38}t^{0.67} \quad (12)$$

R^2 : 0.68

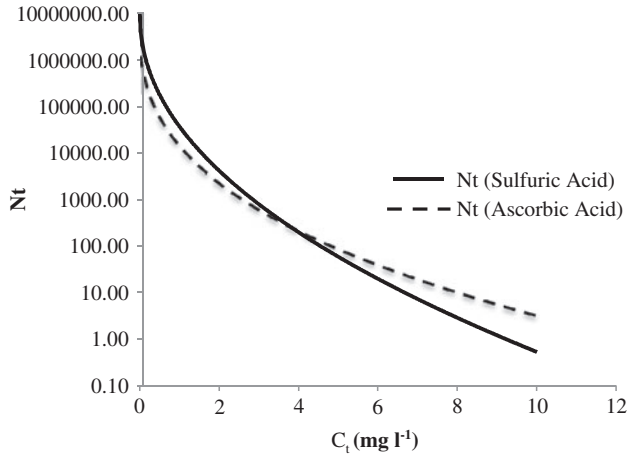

Fig. 4. Comparing data from Hom's equation in the ASE media for 15 min contact time and various C_t.

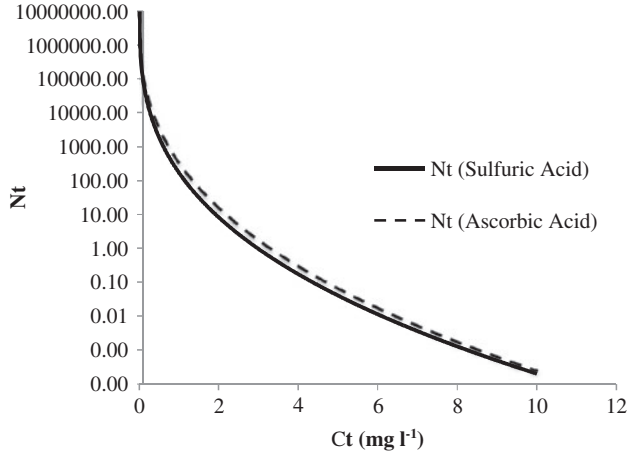

Fig. 5. Comparing data from Hom's equation in the tap water media for 15 min contact time and various C_t.

4. Discussion

The results of this work showed that, the considerable promoting action of sulfuric acid is expected in higher initial doses of PFA when synthetic liquid medium is prepared of sterilized ASE. As shown in Fig. 4, the lowest number of remaining *E. coli* is expected using sulfuric acid as catalyst above initial doses of 4 mg l^{-1} of PFA. It was also shown that for the higher doses of PFA when synthetic liquid medium was prepared of tap water, catalytic effects of ascorbic acid were stronger than sulfuric acid. Hence, the considerable promoting action of ascorbic acid is expected in higher initial doses of PFA when the tap water is used as liquid medium.

It should be mentioned that for initial applied doses of 6 mg l^{-1} and more, the MPN/100 ml was practically less than 1.8 when applying a contact time of more than 20 min. But as shown in Fig. 5 which is derived from the Hom's model, for lower detention time of 15 min there is no significant difference between the catalytic power of sulfuric acid and ascorbic acid. As there are always some constituents in liquid media such as wastewater, the application sulfuric acid as a catalyst for PFA disinfection is preferred.

The efficacy of disinfection is predicted based on residual concentration of disinfectant, temperature, pH, and contact time. This relationship is commonly referred to as the CT concept and is used as a tool for ensuring adequate inactivation of organisms during disinfection practice [16]. The variations of $\log_{10}$ inactivation of *E. coli* vs CT values are presented in Fig. 6. Calculated CT values in other studies for 99% inactivation of *E. coli* using chlorine, chlorine dioxide, chloramines, and ozone are presented in Table 7 [15].

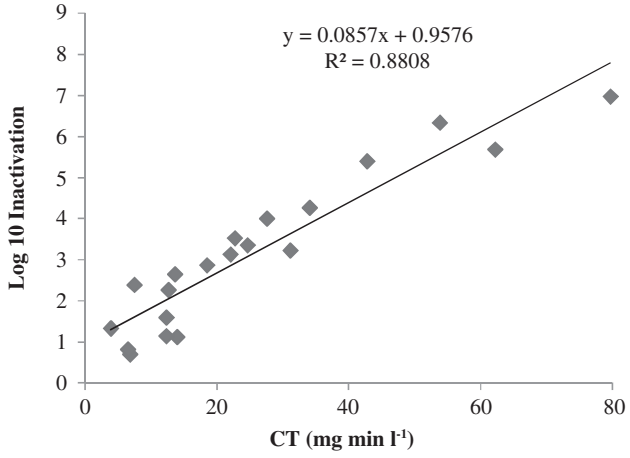

Fig. 6. Log inactive vs. C_t (mg min l^{-1}) in ASE media catalyzed by sulfuric acid.

Table 7
CT values for 99% inactivation at 5°C

Disinfectant agent	pH	*E. coli* (mg min l^{-1})
Free chlorine	6–7	0.034–0.05
Preformed chloramines	8–9	95–180
Chlorine dioxide	6–7	0.4–0.75
Ozone	6–7	0.02

In this study, CT value for 99% inactivation of *E. coli* was determined as 12.16 mg min l^{-1}. Chauret, Smith et al. (2008) showed that for *E. coli*, chlorine produced approximately 4-log inactivation at a CT value of 0.13 mg min l^{-1}, whereas monochloramine resulted in 4-log inactivation at a CT value of approximately 9.2 mg min l^{-1} [17].

In our study, PFA produced 4-log inactivation at CT value of 35.48 mg min l^{-1}. Accordingly, the disinfection efficacy of PFA is proved to be less than that for free chlorine and more than that for chloramines. *Encephalitozoon intestinalis* is known as microsporidian pathogen of humans and animals which has been detected in surface water and is on the Contaminant Candidate List of potential emerging waterborne pathogens for the US EPA. Disinfection study using chlorine and ozone on *E. intestinalis* spores revealed that Chlorine CT values varied with pH such that 99% (2-$\log_{10}$) CT values ranged from 12.8 at a pH value of 6 to 68.8 mg min l^{-1} at a pH value of 8. Application of PFA on *E. coli* in our experiment caused CT value of 12.16 mg min l^{-1} for 99% inactivation, and CT value of 23.82 mg min l^{-1} for 99.9% inactivation. It was mentioned that *E. intestinalis* is more resistant to chlorine than enteric bacteria and viruses, but not as resistant as *Giardia*. The effect of chlorine on *E. intestinalis* presented by John et al. (2005) and the efficacy of PFA on *E. coli* especially for 99% inactivation presented in the present study are almost identical [18]. The disinfection of *Enterococcus*, *coliphage* and *Clostridium* microorganisms with PFA was also evaluated by Gehr et al. (2009). Their research showed that 4–6 log removal of *Enterococcus* could be achieved at 5 to 6 mg l^{-1} PFA. They also revealed that for *Coli phage* and *Clostridium*, 1–2 log could be removed at the same dose [9].

As the contact time in the study presented by Gehr et al. (2009) was set to 45 min, CT values for 4–6 log removal of *Enterococcus* were about 225 to 270 mg min l^{-1} [9]. Results of the present study on PFA efficacy showed that for *E. coli* (which is more sensitive to disinfectants than *Enterococcus*) CT values for 4–6 log inactivation are 35.48–58.81 mg min l^{-1}.

The comparison of the results of the present study shows that efficacy of PFA is less than free chlorine and more than chloramines. Therefore, for PFA disinfection, CT value for 99% inactivation of *G. lamblia* cysts is supposed to be less than 2,200 mg min l^{-1}.

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