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Reduction and regrowth of total coliform bacteria, fecal coliform bacteria, and *E. coli* after chlorine and peracetic acid disinfection in the hospital effluent

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ABSTRACT: Hospital wastewater is an important source of pollutants resulting from medical activities. It has a high vulnerability towards the outbreak of several diseases. The aim of the study was to investigate the disinfection efficiency of different dosages and contact times of chlorine and peracetic acid (PAA) to reduce the contaminated microbes. The consequences after treatments such as regrowth capacity, disinfection by-products, and water quality were also observed. The result showed that in all fourth-time samplings of effluent, its physical, chemical, and biological characteristics exceeded the standard requirement and needed further treatment before discharge. The disinfection experiments were run in parallel among 3 chlorine dosages (1.388, 1.588, and 1.988 mg/l) and 3 PAA dosages (5, 10, 15 mg/l) at contact times of 15 and 30 min. PAA performed faster than chlorine from the beginning, after that, they all provided similar microbial reduction around 2.38–4.47 log 10 MPN/100 ml. Chlorine provided higher efficiency in reducing total coliform while PAA was greater for *E. coli*. The efficiencies increased as contact time was increased. PAA exhibited higher physical and chemical treating capacities than those of chlorine. The study suggested that chlorine at 1.528 mg/l and/or PAA at 5 mg/l, at a contact time of 15 min, are suitable microbial treatments for this effluent. These suggested conditions could improve all water quality parameters to meet the standard requirement and inhibit regrowth of microbes during the three-day incubation period. The stakeholders should also continue to monitor effluent characteristic variation and treatment conditions regarding the application of these infectants.

KEYWORDS: hospital effluent, chlorine, peracetic acid, regrowth capacity, disinfection by-product

INTRODUCTION

The water resources in Thailand usually receive drainage from many water-use activities. Water quality is lowered and prone to public health risks for further users. In 2020, a report by World Health Organization (WHO) pointed out that there was an illness from polluted water of around 74 million people and more than 1 million deaths per year worldwide [1]. The diarrheal disease was the second-ranked cause of death for children aged under 5 years and was responsible for the deaths of 525,000 children. As compared to Thailand, the Bureau of Epidemiology, Department of Disease Control, and Ministry of Public Health (MOPH) reported the number of diarrheal disease patients from the epidemiology surveillance report in 2022 was 833,540 cases and 4 deaths and diarrhea are most common among children aged under 5 years, 4,819.82 per 100,000 population [2].

Hospital effluent is one of the major pollution contributors to water resources. It consists of harmful pollutants such as pathogenic microorganisms as well as pharmaceutical and chemical substances. In 2020, the Research and Laboratory Development Center, Department of Health, MOPH, Thailand, reported that 69.1% of all studied hospital effluent (6,408 samples) did not meet the standard requirement [3]. The majority of microorganisms were total coliform bacteria of 38.7% and fecal coliform bacteria of 38.4%. Both microorganisms exceeded the standard requirement. It imposes a potent threat to the security of human health due to its high vulnerability towards the outbreak of several diseases. Furthermore, the outbreak of the COVID-19 pandemic demanded global attention towards monitoring viruses and other infectious pathogens in hospital wastewater and their removal. Detection of non-infective RNA fragments of SARS-CoV-2 in untreated wastewater and/or sludge has been reported in several settings and countries such as Italy, Spain, Australia, Netherlands, United States of America, France, and Pakistan [4]. The transmission of this coronavirus into the environment, especially due to the risk associated with becoming infected with SARS-CoV-2 in waters where untreated or inadequately treated wastewater is discharged, was also proven in Thailand. From wastewater surveillance in the Bangkok Metropolitan Region in November 2020 and February 2022 from 23 sites in the Bangkok Metropolitan Region to detect the presence of SARS-CoV-2 of the 102 samples, 86 were polymerase chain reaction (PCR)-positive for all 3 genes, 15 were positive for 2 genes, and one was positive for only the ORF1ab gene (PCR Ct value = 33.8) [5]. Therefore, it is vital importance to pay more attention to hospital effluent, especially the microbiological characteristics.

Almost all hospital effluent in Thailand has improved its microbial quality by chlorination process before discharge. The improper application results remained microbial burden while overdose and/or misapplication of chlorine also creates the formation of disinfection by-products (DBPs), which are classified as carcinogenicity chemical Group 2B by the International Agency for Research on Cancer (IARC) [6]. Thus, it is necessary to study other alternatives for microbial disinfection. Many studies suggested the use of peracetic acid (PAA) for the sterilization of equipment in hospitals, pharmaceutical, cosmetic, and food industries as well as water and wastewater disinfection [7–10]. PAA, which is soluble in water, has a pungent odor and is a colorless liquid. In the disinfection process PAA breaks down into hydrogen peroxide, oxygen, water, and acetic acid with much less DBP production than that of chlorine. As a result, PAA is classified as a safe and environmentally friendly disinfectant since it presents a challenge for chlorine substitution, provides effective microbial hyalinization (using *E. coli* and Salmonella as indicators), and prevents odor formation [11].

The aim of this study is to determine the disinfection efficiency of chlorine and PAA to reduce total coliform bacteria and *E. coli* in the hospital effluent and microbial regrowth capacity, the disinfection by-product, and others after treatment. It provides more information for use as a selection criterion for alternatives and caution for microbial treatment. These will help not only increase treatment efficiency, but also reduce public health risks from wastewater and water resources.

MATERIALS AND METHODS

Water sampling and analysis

The studied water sample was effluent from an advanced-level hospital of MOPH in Nonthaburi province, Thailand. This hospital has 515 beds, a high referral system level, and various medical activities. The effluent samples were collected from the wastewater treatment plant at a sanitary sewer after passing the sedimentation pond. Samples were not treated with chemical disinfectant or other disinfecting provisions and were treated by an activated sludge treatment system. They were analyzed as soon as possible. In case of time limitation, it was preserved at a temperature below 4 °C. The analytical procedure was following a standard method for the examination of water and wastewater of American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) [12]. Properties measured for physical and chemical characteristics were pH (electrometry method), biochemical oxygen demand (BOD) (azide modification method), settleable solids (volumetric test by Imhoff cone), total dissolved solid (dried at 103–105 °C), sulfide (ion chromatography), total Kjeldahl nitrogen (TKN) (Kjeldahl method), oil and grease (soxhlet extraction method), and chemical oxygen demand (COD) (open reflux method).The disinfection by-products (trihalomethanes (THMs)), chloroform, bromodichloromethane, dibromochloromethane, and bromoform were determined by headspace coupled to gas chromatography/mass spectrometric (GC/MS) method (5975C Insert XL EI/CI MSO, Agilent Technologies Inc., Santa Clara, California, United States). The biological characteristics were total coliform bacteria and fecal coliform bacteria using multiple-tube fermentation technique (5 tubes). *E. coli* was determined by using fluorogenic substrate method (EC-MUG medium). The assay of positive control, negative control, and an un-inoculated medium control was applied for laboratory quality assurance.

Chemical disinfection preparation

Chlorine: It consisted of 10% w/w liquid sodium hypochlorite (Merck KGaA, Darmstadt, Germany). The designed concentration was done after determining the breakpoint chlorination of chlorine (x) and studied hospital effluent by DPD ferrous titrimetric method [12].

PAA: 15% commercial PAA (MIKROKIL Stellar Unity®, Stellar Unity Co., Ltd., Bangkok, Thailand) was used. It consisted of 17% acetic acid (CH_3COOH) and 15% hydrogen peroxide (H_2O_2) in a water solution. The concentration of PAA was tested by Kemio™ a Palin tests (ELS untility and municipal products, London, United Kingdom).

Experimental methods

Chlorine and PAA disinfection study

Bacterial disinfection efficiency by chlorine and PAA were studied as a factorial design of chlorine concentration ($x \pm 0.2$, $x \pm 0.4$, and $x \pm 0.8$ mg/l) and PAA (5, 10, and 15 mg/l). They were completely mixed in the reactor volume of 1,000 ml and run in duplicate at 3 contact times (initial, 15, and 30 min) and room temperature conditions (29 °C). The disinfection efficiency of chlorine and PAA to reduce bacterial contamination including total coliform bacteria, fecal coliform bacteria, and *E. coli* in the hospital effluent was examined based on log 10 reduction of the bacterial [13] after treatment as this equation: Log reduction $= \log 10\ (N_0/N)$, where N_0 is the number of viable microorganisms before treatment and N is the number of viable microorganisms after treatment.

Microbial regrowth capacity study

The two best microbial treatment efficiencies of each disinfectant were investigated for microbial regrowth capacity by incubation study. The incubation times were 4 durations: initial on day 0, 1, 2, and 3. Each treatment was operated in duplicate at room temperature conditions (26–29 °C). They were analyzed for bacteriological characteristics, and the most probable number (MPN) of coliform organisms by multiple-tube fermentation technique (5-tube) was determined.

RESULTS AND DISCUSSION

Hospital effluent characteristics

The fourth time samplings of the effluent were different in terms of their physical and chemical characterization (Table 1). There were many parameters such as TKN, sulfide, COD, and BOD exceeding the effluent standard requirement [14]. Moreover, some amount of chlorine residual (0.11–0.15 mg/l) was measured even though there was no chlorine application in the wastewater treatment process. This chlorine might come from wastewater containing substances used in medical treatments and housekeeping cleaning processes. However, this amount of chlorine was not enough to destroy the existing microbes. The biological characteristics revealed that the indicator of contamination such as coliform bacterial group was still high. This study found breakpoint chlorination dosages (x) were between 0.856–1.128 mg/l. These biological characteristics revealed sub-standard wastewater treatment operations and risky situations of waterborne diseases. Many waterborne pathogens can also be acquired by consuming contaminated food or beverages and from contacting animals or their environment [15].

Chlorine disinfection

The preliminary step to determine the appropriate chlorine dosage (x) was performed, and breakpoint chlorination was 1.188 mg/l for the effluent samples of first and second-time samplings. The second time effluent sample was used in this study, and experimental chlorine dosages were CL1 (1.388 mg/l), CL2 (1.588 mg/l), and CL3 (1.988 mg/l). The profile of microbial changes after chlorine treatment at various contact times and dosages is shown in Table 2 and Fig. 1

The microbial reduction profile of all chlorine treatments was similar. At initial contact time, the total coliform was rapidly reduced almost 10 times, which is equal to a reduction between 0.52–4.47 log 10 MPN/100 ml. The increasing contact time of 15 and 30 min resulted in a decreasing phenomenon of total coliform bacteria of 4.47 log 10 MPN/100 ml, fecal coliform bacteria of 3.94 log 10 MPN/100 ml, and *E. coli* of 3.70 log 10 MPN/100 ml (Fig. 1). The existing number was under the standard criterion [14].

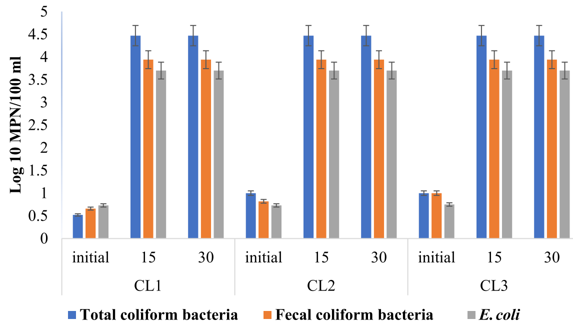

Fig. 1 The disinfection efficiency of chlorine at different concentrations and contact times of log to reduce total coliform bacteria, fecal coliform bacteria, and *E. coli*.; Error bars represent 95% CI.

Chlorine application dosage has contributed to a variation of disinfection efficiencies since initial application; however, it still requires more contact time to react and destroy contaminated microbes. The residual chlorine left in treatment CL1, CL2, and CL3 was increased from the initial of 0.10 mg/l to 0.23, 0.30, and 0.58 mg/l, respectively.

A study on chlorine tolerance and inactivation of *E. coli* recovered from wastewater treatment plants in the Eastern Cape, South Africa [13] explained that the reduction of *E. coli* after 30 min was within a range of 3.88–6.0 log at chlorine residuals ranging from 0.14–0.44 mg/l. A higher application dose marked a significant reduction ($p < 0.05$) in the viability of *E. coli* isolates greater than 7.3 log inactivation of the bacterial population. In addition, inactivation kinetics revealed a high rate of bacterial kill over time ($R^2 >$ 0.9) at a chlorine dose of 1.5 mg/l, which matches with this study dosage. A study of suitability of using chlorine dioxide as a tertiary treatment for municipal wastewater [16] reported residual chlorine between < 0.02–0.33 mg/l at a contact time of only 6 min had efficacy to reduce *E. coli* to 2 logs CFU/100 ml. It agreed with this study that rapid disinfection efficacy of chlorine could occur during the initial period. Chlorine dioxide is known as one of the efficient disinfectants with high oxidization capability even under acidic conditions [17]. It is an effective disinfectant in both liquid and gas states and can trigger the denaturation of enzymes and proteins. It destroys the anabolic pathways of protein and thus kills the microorganism, including bacteria, viruses, fungi, spores, and *Clostridium botulinum*. The chlorine dioxide has the ability of decoloring, deodorization, oxidation, and increasing the oxygen content in wastewater. Moreover, there are many factors which have been found to exert great impacts on bacteria and virus inactivation rates. pH and temperature, for example, are factors that inactivate bacteria and viruses [15]. The residual chlorine levels from studied treatments (0.23–0.58 mg/l) were in the

Table 1 Characteristics of hospital effluent sample at each sampling time.

Parameter	1st time	2nd time	3rd time	4th time	Mean ± S.D.	Effluent standard†
Physical						
Oil & grease (mg/l)	4.10	14.10	12.20	18.00	12.10 ± 5.85	Less than 20
Total dissolved solid (mg/l)	594.00	485.00	313.00	531.00	480.75 ± 120.42	Less than 500
Suspended solid (mg/l)	8.00	72.00	28.00	20.00	32.00 ± 27.90	Less than 30
Settleable solid (mg/l)	< 0.1	< 0.1	< 0.1	< 0.1	0.10 ± 0.00	Less than 0.5
Chemical						
pH (pH at 25 °C)	7.70	7.20	7.30	7.40	7.40 ± 0.21	5.0–9.0
TKN (mg/l)	4.81	42.49	42.06	61.8	37.79 ± 23.83	Less than 35
Sulfide (mg/l)	ND	0.09	0.27	135.00	33.84 ± 67.44	Less than 1.0
COD (mg/l)	33.00	224.00	135.00	295.00	171.75 ± 113.31	Less than 120
BOD (mg/l)	3.00	113.00	21.00	81.00	54.50 ± 51.31	Less than 20
Residual chlorine (mg/l)	0.15	0.10	0.11	0.11	0.11 ± 0.02	0.2–0.5
Chlorine dose (mg/l) at chlorination (x)	1.188	1.188	0.856	1.128	1.09 ± 0.15	0.2–0.5 mg/l
Biological						
Total coliform bacteria (MPN/100 ml)	92×10^6	54×10^3	22×10^6	22×10^6	3.40 ± 4.00	Less than 5,000
Fecal coliform bacteria (MPN/100 ml)	54×10^6	16×10^3	22×10^6	22×10^6	2.45 ± 2.22	Less than 1,000
E. coli (MPN/100 ml)	54×10^6	92×10^2	79×10^5	79×10^5	1.74 ± 2.46	–

† Source: Announcement of the Ministry of Science, Technology, and the environment regarding the establishment of control standard of drainage water from certain types and sizes of building type A (hospital with 30 beds and above) [14].

Table 2 Profile of total coliform bacteria, fecal coliform bacteria, and *E. coli* changes in hospital effluent by chlorine disinfection at different concentrations and contact times.

Treatment	Microbe (MPN/100 ml)											
	Total coliform bacteria				Fecal coliform				*E. coli*			
	Effluent	Contact time (min)			Effluent	Contact time (min)			Effluent	Contact time (min)		
		Initial	15	30		Initial	15	30		Initial	15	30
CL1 (1.388 mg/l)	54×10^3	16,000	< 1.8*	< 1.8	16×10^3	3,500	< 1.8*	< 1.8	92×10^2	1,700	< 1.8*	< 1.8
CL2 (1.588 mg/l)	54×10^3	5,400	< 1.8*	< 1.8	16×10^3	2,400	< 1.8*	< 1.8	92×10^2	1,700	< 1.8*	< 1.8
CL3 (1.988 mg/l)	54×10^3	5,400	< 1.8*	< 1.8	16×10^3	1,600	< 1.8*	< 1.8	92×10^2	1,600	< 1.8*	< 1.8

* Statistically significant difference between initial contact time and 15 min at $p < 0.05$.

general guideline range of the WHO criterion [15].

PAA disinfection

Parallel operations of chlorine treatments and PAA disinfection were carried out. The detailed results are in Table 3. The disinfection efficiency of 3 PAA dosages at initial contact time was higher than that of the chlorine and reached the maximum of almost 1,000-fold microbial reductions or 2.38–4.47 log 10 MPN/100 ml (Fig. 2), which met standard requirements [14]. PAA has the dominant disinfection efficiency against *E. coli*, which has been reduced to almost non-detected since initial application. The increased contact time to 15 and 30 min expressed a similar direction as that of chlorine. Nevertheless, the residual PAA left in treatment PAA1, PAA2, and PAA3 were decreased to non-detected, < 5, and < 5 mg/l, respectively.

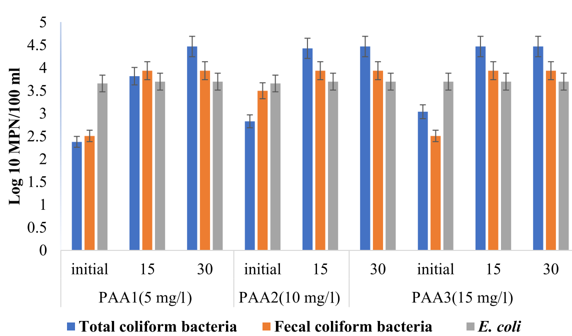

Fig. 2 The disinfection efficiency of PAA at different concentrations and contact times of log to reduce total coliform bacteria, fecal coliform bacteria, and *E. coli*.; Error bars represent 95% CI.

The results agreed with the study of PAA disinfection for decentralized wastewater at concentrations of 5, 10, and 15 mg/l and contact times of 5, 10, and 15 min [7], from which the best condition of PAA disinfection to reduce total coliform bacteria, fecal coliform, and *E. coli* was at a concentration of 15 mg/l and a contact time of 15 min. In addition, the yield of bacterial reduction still occurred at lower PAA dosages

Table 3 Profile of total coliform bacteria, fecal coliform bacteria, and *E. coli* changes in hospital effluent by PAA disinfection at different concentrations and contact times.

Treatment	Microbe (MPN/100 ml)											
	Total coliform bacteria				Fecal coliform				*E. coli*			
	Effluent	Contact time (min)			Effluent	Contact time (min)			Effluent	Contact time (min)		
		Initial	15	30		Initial	15	30		Initial	15	30
PAA1 (5 mg/l)	54×10^3	220	8*	< 1.8	16×10^3	49	< 1.8*	< 1.8	92×10^2	2	< 1.8	< 1.8
PAA2 (10 mg/l)	54×10^3	79	2*	< 1.8	16×10^3	5	< 1.8*	< 1.8	92×10^2	2	< 1.8	< 1.8
PAA3 (15 mg/l)	54×10^3	49	< 1.8*	< 1.8	16×10^3	49	< 1.8*	< 1.8	92×10^2	< 1.8	< 1.8	< 1.8

* Statistically significant difference between initial contact time and 15 min at $p < 0.05$.

reported by many studies. There was a feasibility of using PAA as a substitution for sodium hypochlorite in tap water and wastewater which is aimed for discharging to surface water and for agricultural reuse [8]. The efficacy of PAA at 5 to 10 mg/l and contact time of 35–50 min could inactivate 4-log of fecal coliform and *E. coli*. PAA dose lower than 5 mg/l at a contact time of 12 min was more appropriate, especially for *E. coli* which is matched with this study dosage. Similar to this study, PAA presents better disinfection, especially for *E. coli* and pathogenic organisms. A review study [9] reported that PAA is a stronger disinfectant with a wide spectrum of antimicrobial activity. Due to its bactericidal, virucidal, fungicidal, and sporicidal effectiveness as demonstrated in various industries, the use of PAA as a disinfectant for wastewater effluents presents a challenge. It was found that PAA concentration of 5 mg/l at a contact time of 45 min could provide average bacterial log-inactivation to -0.59 ± 0.12 [10]. PAA concentration of 5 mg/l and 5 min of contact time could reduce resistant and susceptible *Enterococcus faecalis* and *Escherichia fergusonii* [18]. All strains were inactivated within 5 min. They showed gradual log-inactivation overtime at 1 and 2 mg/l of initial PAA.

A comparison of disinfection efficiency between chlorine and PAA at different concentrations and contact times to reduce total coliform bacteria, fecal coliform bacteria, and *E. coli*

The selection of the two best microbial treatment efficiencies of each disinfectant (treatment A, B for chlorine and treatment C, D for PAA) was done based on 2 criterions. The first was disinfecting experimental treatment which provides the lowest remaining bacteriological characteristics. The second was that the number of remaining bacteria should be under the standard criterion of 5,000 MPN/100 ml for total coliform bacteria and 1,000 MPN/100 ml for fecal coliform bacteria and *E. coli*.

Regarding chlorine treatment, chlorine displayed efficient disinfection performance. Moreover, the residual chlorine left in the effluent (0.3 mg/l) was not higher than the standard criterion of 0.5 mg/l. It was then selected, and the treatments were $x\pm0.4$ mg/l (1.388 mg/l) at contact times of 15 and 30 min for treatment CL(A) and CL(B), respectively (Table 2). Since the treatments were further repeated with the third effluent sample (0.856 mg/l (Table 1)), the studied chlorine dosage was 1.256 mg/l (0.856+0.4 mg/l). Besides the PAA treatments, all treatments showed high disinfecting efficiencies. The selected dosage was the lowest dose of 5 mg/l and at contact times of 15 and 30 min (Table 3). They were then called PAA (C) and PAA (D), respectively. All these treatments were studied to re-confirm the microbial disinfection efficiency, together with the determination of the microbial regrowth capacity at different incubation periods.

The microbial regrowth capacity after being treated with chlorine and PAA

The third hospital effluent sample had a smaller number of contaminated microbes than the previous samples. Table 4 displays the change of studied microbes of each treatment at studied incubation times. Without any treatment, all microbes naturally died off and accounted for around 500–1,000 folds from the beginning. However, the existing amount is still very high and needs treatment.

The selected disinfectants provided treatment efficiency quite well. They could destroy all studied microbes to an acceptable level of standard of drainage water from certain types and sizes of building type A (hospital with 30 beds and above) [14]. Moreover, those microbes remaining from the initial day were starting to die off at day 1 of incubation and remained lower than 1.8 MPN/100 ml. They continued to observe until day 3 and found no regrowth capacity. It presented that both chlorine and PAA could prolong their regrowth capacity. However, in order to investigate more precisely microbial growth, the use of a most-probable-number loop-mediated isothermal amplification (MPN-LAMP) method for the enumeration of total coliforms and *E. coli* should be applied since it is rapid and highly sensitive than MPN method [19].

Various studies confirm the efficiency of bacterial

Table 4 The remaining total coliform bacteria, fecal coliform bacteria, and *E. coli* (MPN/100 ml) in hospital effluent before and after treatment with selected treatment of chlorine and PAA at studied incubation periods.

Incubation	Total coliform bacteria (MPN/100 ml)					Fecal coliform bacteria (MPN/100 ml)					*E. coli* (MPN/100 ml)				
period	Before	CL(A)	CL(B)	PAA(C)	PAA(D)	Before	CL(A)	CL(B)	PAA(C)	PAA(D)	Before	CL(A)	CL(B)	PAA(C)	PAA(D)
Initial	22×10^6	23	49	3,300	1,700	22×10^6	2.00	4.5	70	49	79×10^5	2.00	<1.8	70	49
Day 1	16×10^5	1.8	1.8	1.8	1.8	22×10^3	<1.8	<1.8	<1.8	<1.8	16×10^5	<1.8	<1.8	<1.8	<1.8
Day 2	46×10^4	1.8	1.8	1.8	1.8	21×10^3	<1.8	<1.8	<1.8	<1.8	21×10^3	<1.8	<1.8	<1.8	<1.8
Day 3	92×10^3	2	1.8	1.8	1.8	22×10^3	<1.8	<1.8	<1.8	<1.8	14×10^3	<1.8	<1.8	<1.8	<1.8

regrowth inhibition of chlorine. The observation of the inactivation and regrowth of multi-drug resistant (MDR) bacteria in urban wastewater [20] demonstrated that chlorine concentration of 1.0 mg/l at 15 min was effective in achieving total inactivation of MDR *E. coli* and could control regrowth within 48 h. Besides this, the application of chlorine concentration (0.2, 0.5, and 1.0 mg/l) could prevent bacterial regrowth of more than 99.95% of *E. coli* in environmental waters. Chlorine also affects the tolerance profile of other bacterial species. The Gram-negative bacteria recovered from secondary treated wastewater in Jaipur, India, such as *Citrobacter freundii*, *Klebsiella* sp., and *Stenotrophomonas maltophilia* had completely inhibited regrowth after 6 h of chlorine application at the dosage between 0.75–1.75 mg/l [21]. A study of the inactivation kinetic experiments on chlorine-tolerant bacteria [22] indicated a strong correlation (R^2 > 0.89–0.99) between log reduction values and contact times. Reactivation and regrowth of bacteria most likely occurred after exposure to lower chlorine doses, and the extent of reactivation decreased gradually with increasing chlorine doses. In contrast, they found that chlorination contributes to the selection of chlorine-resistant pathogenic bacteria. The regrowth of pathogenic bacteria after chlorination in reclaimed water with a long retention time could threaten public health security during wastewater reuse.

Regarding the treatment performance of peracetic treatments, they had lower efficiencies than those of the previous experiment but no statistical difference. The variation of treatment performance depends primarily on the effluent characteristics such as turbidity, sulfide, and pH [23]. However, despite it did not leave the remaining amount of PAA after treatment like chlorine, the existing microbes could not express regrowth ability until the end of incubation. The study of the inhibition of the regrowth of planktonic and biofilm bacteria after PAA and chlorine disinfection [23] pointed out that both disinfectants are strong oxidants disrupting the cell membrane. A result from environmental scanning electron microscopy (ESEM) revealed that PAA made holes in the center of the cells whereas free chlorine desiccated the cells. Finally, they concluded that PAA is a powerful disinfectant to prevent bacterial regrowth even in the presence of organic matter.

Temperature and pH after treated with chlorine and PAA

Temperature is a critical parameter to monitor any biological wastewater treatment system and many living organisms. Management of bacterial capacity survival post chlorine disinfection is vital for safe wastewater reuse for irrigation, as the presence of microorganisms in large numbers may lead to subsequent contamination in treated effluents for drinking water or reclaimed water. Meanwhile, for conventional disinfection, pH and temperature after treatment are important factors of several physical and chemical factors, which influence the disinfectant process. Furthermore, the rapid increase in temperature causes the disinfectant to degrade and weakens its germicidal activity and thus might produce a potential health hazard. Proteins, particularly enzymes, are affected by temperature. On the other hand, temperature below a specific minimum temperature enhances the reduction activity of microbes.

The ambient room temperature during the experiment ranged between 26.6–29.7 °C while the effluent was 25.7–26.9 °C. The natural died off of microbes in the effluent was occured and resulted in decreased of effluent temperature to around 1.2 °C. The *in-situ* temperature of all treatments rose to almost 3 °C at incubation day 1 (Fig. S1a). It then decreased to normal on the second and third days afterward. This noticeable change of temperature implies the steady stage of the environmental condition and matches suitable water temperature for the wastewater treatment process (25–35 °C) for tropical countries like Thailand.

The pH is a value that indicates the acidity and alkalinity of wastewater. It influences antimicrobial activity by altering the disinfectant molecules. An increase in pH improves the antimicrobial activity of some disinfectants. The pH affects organisms on the molecular level by influencing biomolecular structures. A pH that is too high or low in water will damage the aquatic ecosystem and aquatic animals and cause corrode pipes or containers, and plants cannot live. Generally, the aquatic organisms or microorganisms in the treatment tanks could survive and grow well in neutral conditions with pH of 6.0–8.0.

The pH of hospital effluent ranged between 7.3–8.3. It slightly decreased with the increase of the

Table 5 The physical and chemical characteristics of hospital effluent before and after disinfection with selected chlorine (CL (A)) and PAA (PAA (C)) treatments.

Parameter	Before treatment hospital effluent	After treatment Chlorine	After treatment PAA	Standard†
Physical characteristic				
Oil & grease (mg/l)	18.00	1.20	1.20	Less than 20
Total dissolved solid (mg/l)	531.00	394.00	350.00	Less than 500
Suspended solid (mg/l)	20.00	1.00	1.00	Less than 30
Settleable solid (mg/l)	< 0.1	< 0.1	< 0.1	Less than 0.5
Chemical characteristic				
pH (pH at 25 °C)	7.40	7.60	7.70	5.0–9.0
TKN (mg/l)	61.80	6.02	5.79	Less than 3.5
Sulfide (mg/l)	0.17	0.00	0.00	Less than 1.0
COD (mg/l)	295.00	30.00	23.00	Less than 120
BOD (mg/l)	81.00	3.00	1.00	Less than 20
Residual chlorine (mg/l)	0.11	0.31	ND	0.2 and 0.5
Trihalomethane (THM)				
Chloroform	20.8	N/D	N/D	200 µg/l
Bromodichloromethane	16.3	N/D	N/D	60 µg/l
Dibromochloromethane	4.6	N/D	N/D	100 µg/l
Bromoform	< 2.7	N/D	N/D	100 µg/l
Biological characteristic				
Total coliform bacteria	22×10^6	17	3,300	Less than 5,000
Fecal coliform bacteria	22×10^6	< 1.8	< 1.8	Less than 1,000
E. coli	79×10^5	< 1.8	< 1.8	

† Source: Announcement of the Ministry of Science, Technology, and the environment regarding the establishment of control standard of drainage water from certain types and sizes of buildings type A (hospital with 30 beds and above) [14].

incubation time. The changing of pH of all treatments presented more challenges (Fig. S1b). The derivatives of disinfectant such as chlorine dioxide from chlorine and acetic acid and hydrogen peroxide from PAA reacted with microbes and naturalized the effluent. In addition, the applied dosage of both disinfectants was minimized from the design. The effluent pH after treatment had risen to 7.6–8.2. A lower pH of 6.5 enhanced more PAA inactivation of multidrug-resistant *E. coli* compared with pH 7.5 [24]. This might give more room for increasing the PAA dosage in case there is a requirement for ensuring the disinfection process. However, within the scope of this study, the treated effluent has complied with the standard pH of 5.0–9.0 [14].

Table 6 The log 10 reduction of microbes in hospital effluent before and after disinfection with selected chlorine (CL (A)) and PAA (PAA (C)) treatments.

Parameter	Microbe in hospital effluent log 10 (MPN/100 ml)			Sig.
	Before treatment	After treatment Chlorine	After treatment PAA	
Total coliform bacteria	22×106	6.11	3.82	0.00*
Fecal coliform bacteria	22×106	7.08	7.08	1.00
E. coli	79×105	6.64	6.64	1.00

* The data are expressed as t-test and statistically significant different at $p < 0.05$.

The hospital effluent characteristics after disinfection with chlorine and PAA

The fourth time hospital effluent sample was studied by using disinfection conditions of CL(A) and PAA(C). The characteristics before and after treatment were determined, and the results are shown in Table 5. This hospital effluent was still not well treated since its physical, chemical, and biological qualities were over the standard requirements. It might be caused by an improper sludge settling process which resulted in a high total dissolved solid content. They contributed high TKN, COD, and BOD as a consequence. There were also some amounts of trihalomethane substances in which the chloroform was mainly found. This disinfectant by-product did not surprisingly occur since it is well understood that various disinfectants are used for medical activities and residual chlorine of 0.11 mg/l was found. However, the biological characteristics confirmed the requirement of further treatment.

Typically, effluent treatment in this tertiary step has a main purpose to destroy the microbes left from the previous secondary step. This study results offered more advantages of treatment for physical and chemical qualities. It is remarkable that chlorine, at this time, gave statistically higher total coliform reduction than PAA (Table 6). PAA provided less effective than free chlorine in killing bacteria within biofilms and/or the presence of organic matter due to its slower reaction with organic matter and/or slower self-decomposition [23]. However, PAA exhibited higher physical and chemical treating capacities than those of chlorine since it caused clear solutions, of which the laboratory results were confirmed. Finally, all studied parameters were better and met the standard requirement of the hospital effluent control from certain types and sizes of buildings [14].

CONCLUSION

This studied hospital effluent still contained many contaminants even after passing the activated sludge treatment process. The biological characteristics might contribute to a risky environment and threaten public health wellness if there is no suitable disinfection application. Chlorine, a normal disinfectant used in tertiary wastewater treatment process, and PAA were then studied to find out suitable disinfecting condition for this effluent. The design of disinfectant dosage was based on minimizing the concentration and contact time. The breakpoint chlorination was done during each sample. Although it had varied characteristics, the chlorine requirement for breakpoint was not much changed (from 0.856 to 1.128 mg/l). The disinfection study of all treatments from both chlorine and PAA presented a good performance of microbial reduction. Chlorine could destroy higher total coliform bacteria and, at the same time, PAA reacted well with *E. coli*. Chlorine is suitable for effluent which has low total solids and turbidity. Moreover, the wastewater treatment system should allow enough time for microbial-destroying reaction. It provides residual effect for disinfecting the re-contamination or microbial regrowth. PAA can be used for improving the effluent which has low physical and chemical qualities and destroying a high number of microbes at the same time. Nevertheless, it cannot provide residual effect for additional microbial contamination. However, these 2 disinfectants could also inhibit the regrowth capacity of all studied microbes. Chlorine dosage of $x \pm 0.4$ mg/l (1.528 mg/l) and/or PAA at 5 mg/l at contact time of 15 min are suggested to be suitable disinfectants for this effluent. The disinfectant by-product which was found as some amount of contamination disappeared to non-detected level after treatment. These treated effluent characteristics were better as a whole and passed the standard requirement.

Limitation: The hospital effluent characteristics had high variation, and treatment efficiency depends on appropriate applied dosage and contact time. The selection of disinfectant and application should be performed as tailor-made study which is specific to each hospital effluent.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.026.

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Appendix A. Supplementary data

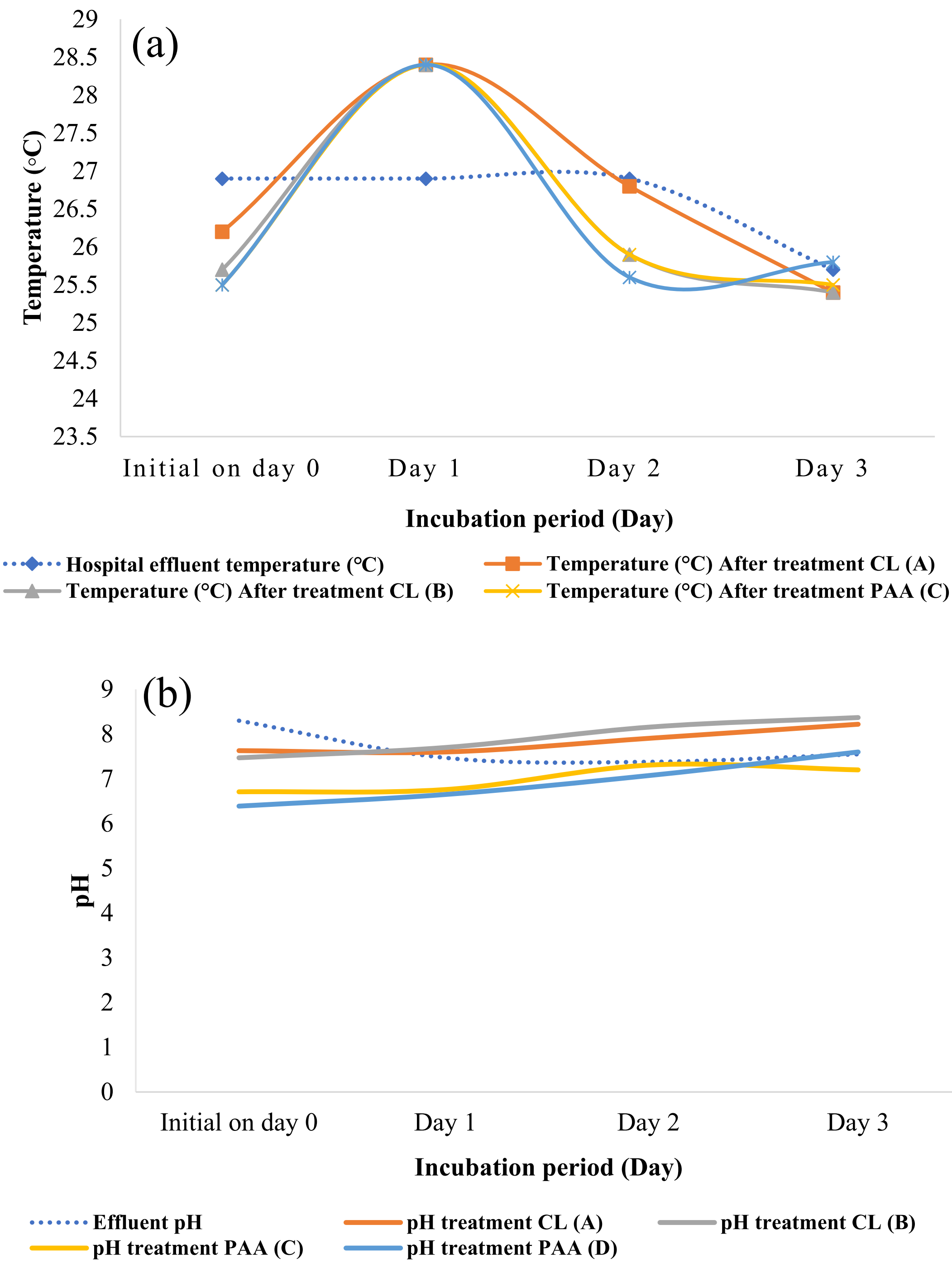

Fig. S1 *In-situ* temperature (a) and pH (b) of hospital effluent before and after treatment with selected disinfectants at studied incubation periods.