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The Phenomenon of Medical Waste Recycling in Indonesia: Contact Time and Chlorine Dose as a Disinfectant with the Bio-Indicator *Bacillus subtilis* and *Bacillus stearothermophilus*

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ABSTRACT

Efforts that have been done to manage the Hazardous and Toxic waste into non-Hazardous and Toxic waste are done by emptying, shredding, washing, and rinsing at least 3 times and disinfecting using chlorine. This is in accordance with the Indonesian Regulation of Ministry of Health No. 27 of 2017, which has stated that surface cleaning is permitted to use 0.05% chlorine during the process. Furthermore, in the Indonesian Regulation of the Ministry of Environment and Forestry No.56 of 2015, the chemical disinfection process is permitted to use an additional 3–6% sodium hypochlorite (NaOCl). However, there are still differences in dosage and it has not been mentioned regarding the immersion period during the disinfection process on both of the regulations. The purpose of this study was to determine the difference in contact time and the dose of chlorine as a disinfectant on the number of *Bacillus subtilis* and *Bacillus stearothermophilus* in the medical waste recycling process. The research design used in this study was a Randomized Factorial Design with experimental research type. A total of 104 recyclable medical waste samples were taken, using 3 treatments and 6 repetitions. The average temperature of the chlorine solution at the contact time of 15 minutes, 30 minutes, and 45 minutes was 24.34 °C; 24,53 °C; and 24,54 °C respectively, while the average pH of the chlorine solution at the contact time of 15 minutes, 30 minutes, and 45 minutes was 8.344; 8,375; and 8,461 respectively. The results showed that there was no difference in the duration of contact and the dose of chlorine as a disinfectant in the medical waste recycling process with a p-value of 0.377. The percentage reduction in the number of *Bacillus subtilis* and *Bacillus stearothermophilus* before and after treatment was 99.99% based on 4 positive controls. The findings in this study were the duration of contact time and the effective dose used in the recycling process of medical waste, which was at a dosage of 0.03% with 45 minutes of contact time. The government needs to conduct a re-assessment regarding the recommended ideal dosage in the surface disinfection process so that it would not cause a potential risk to humans or the environment.

Keywords: recycling, medical waste, dosage, duration of contact, chlorine, disinfection.

INTRODUCTION

Hospital medical waste can be considered as a link in the chain of spreading infectious diseases. A dump of waste usually becomes a place for pathogenic organisms to accumulate and become a nest for insects and rodents. Moreover, the waste also contains various toxic chemicals and sharp objects that can cause health problems and injuries. Dust particles in the waste can cause air pollution which will cause disease and contaminate medical equipment and food (Adisasmito,

2009; Akter and Trankler, 2003; Bokhoree, C. et al, 2014; Emilia et al, 2015).

Hazardous and Toxic Waste produced by health facilities is also referred to as medical waste (Windfeld and Brooks, 2015) and (Ministry of Environment and Forestry, 2018). The types of hazardous waste in health facilities include infectious properties, sharp materials, expired chemicals products, pathological substances, radioactive materials, pharmaceuticals products, cytotoxic properties, and medical equipment that have high heavy metal content (Indonesian Ministry of Environment and Forestry, 2015; Indonesian Ministry of Health, 2019; Mantzaras and Voudrias, 2017; Chartier et al., 2014; Manga et al., 2011).

In Indonesia, besides health facilities that have medical waste treatment facilities, most medical waste is handled by third parties. Based on data from the Ministry of Environment and Forestry in April 2020, the total capacity for medical waste treatment in Indonesia is 314.29 tons/day. In detail, there are as many as 21 provinces that provide health facilities that are capable of processing medical waste with a capacity of 70.21 tons per day and there are as many as 7 provinces that provide 7 Hazardous and Toxic waste treatment services with a capacity of 244.08 tons/day (Sinta Saptarina Soemarno, 2020).

Hospitals and other health installations have an "obligation to maintain" the environment and public health condition and have particular responsibilities related to the waste that is produced by the installations (Ojuolape and Afon, 2016; Omar et al, 2012). Among the obligations borne by these installations are the obligation to ensure that the handling, treatment, and disposal of the waste are done without causing any adverse health and environmental impacts (Akum, 2014; Al-Khatib, 2013; Ali et al, 2017). By implementing policies regarding health care waste management systems, medical facilities and research institutions are becoming closer to fulfilling the goal of creating a healthy and safe environment for their employees and the surrounding community (Ali et al, 2017).

Nowadays, there are still many hospitals that do not pay serious attention to their waste management system. The waste management system is still marginalized by the hospital management (Almuneef and Memish, 2003; Cheng et al, 2010). Based on the news from the Central Java Tribun newspaper, a case of selling jerry cans which have previously been used in the hemodialysis process was found out to be sold by the State Civil Apparatus at the Salatiga City Hospital. Indeed it is an effort to reduce medical waste, but it is not eligible for the existing regulatory procedures. The jerry cans are not being treated beforehand as to how Hazardous and Toxic Waste should be treated.

There should be proper and safe medical waste management, and the handling of solid medical waste must be immediately addressed to ensure the health and safety of workers and other people around the hospital (Bokhoree et al, 2014). Hence, a policy is required in accordance with The Occupational Health and Safety Management by carrying out management and monitoring activities of hospital medical waste as an important indicator that requires attention (Ali et al, 2017; Riyanto, 2013).

According to the Indonesian Ministry of Environment and Forestry No. P.56, 2015 Regarding Procedures and Technical Requirements for The Management of Hazardous and Toxic Waste from Health Service Facilities, based on article 38, hospitals can carry out hazardous waste processing themselves, such as used Hazardous and Toxic waste packaging, used syringes, used infusion bottles other than blood infusions and/or body fluids; and/or used hemodialysis fluid packaging. Hazardous waste treatments are done by emptying, cleaning, disinfecting, and crushing or shredding. Hence, the result after the treatment is finally categorized into non-Hazardous and Toxic waste (Gunawan, 2019).

The processing process of Hazardous and Toxic waste into non-Hazardous and Toxic waste was carried out as a means of reducing the Hazardous and Toxic waste generated by the hospital (Gupta and Boojh, 2006; Hamid et al, 2013). However, there are still only a few of hospital that processes Hazardous and Toxic waste into non-Hazardous and Toxic waste, because based on the previous studies regarding medical waste management, including the research conducted by (Wulandari and Kusnoputranto, 2015), Dr. Soedirman Hospital still manages medical waste through the use of the container and doing things such as collection, sorting, transportation, storage, and processing.

One of the hospitals that have processed Hazardous and Toxic waste into non-Hazardous and Toxic waste is the Santo Borromeus Hospital. The management/minimization of hazardous and toxic waste activities are carried out by the Sanitation Installation of the St. Borromeus Hospital,

as stated in the Regulation of the Indonesian Regulation of Ministry of Environment and Forestry No. P.56, 2015 in article 38, waste such as: used Hazardous and Toxic waste packaging, used infusion bottles other than blood and/or body fluids and used hemodialysis fluids. Among the three wastes, 50% of them was an infusion spike waste.

Efforts that have been done to manage the Hazardous and Toxic waste into non-Hazardous and Toxic waste are done by emptying, shredding, washing, and rinsing at least 3 times and disinfecting using chlorine. This is in accordance with the Indonesian Regulation of Ministry of Health No. 27 of 2017, which has stated that surface cleaning is permitted to use 0.05% chlorine during the process. Further, in the Indonesian Regulation of the Ministry of Environment and Forestry No.56 of 2015, the chemical disinfection process is permitted to use an additional 3–6% sodium hypochlorite (NaOCl). However, there are still differences in dosage and it has not been mentioned regarding the immersion period during the disinfection process on both of the regulations.

MATERIALS AND METHODS

Type of research

The type of research used in this research is experimental design (Randomized Factorial Design), which is used to find out the effect of certain treatments towards others under controlled conditions. The research method uses the post-test design with control, which means that the researcher will carry out directly on a group of subjects with two conditions being implemented, as well as a comparison group. (Arikunto, 2010; Notoatmodjo, 2014)

Sampling technique and sample size

The sample in this study is the amount of recycled medical waste materials used for testing *Bacillus subtilis* and Bacillus stearothermophilus. The sample size taken in this study was based on the number of treatments and treatment repetitions used in the study. The treatments used in this study were 3 treatments using a comparison of contact time for 15 minutes, 30 minutes. and 45 minutes, as well as using doses of chlorine. The sample size calculation uses the Gomez formula (Sabri and Hastono, 2014), namely:

$$(t-1)\,(r-1) \geq 20$$
$$(9-1)\,(r-1) \geq 20$$
$$8r-8 \geq 20$$
$$8r \geq 28$$
$$r \geq 3.5$$

The number of repetitions in the study is 4 times, so the total sample in the study was 108 samples with 8 research control samples. The sampling technique in this study was carried out by grab sampling, the samples will be taken at certain times in one location and it is able to represent the entire medical waste material for the recycling process.

Research steps (Figures 1–4):

- Prepare a medical waste recycling reactor according to the dose and duration of contact time.
- Making the dosage (disinfectant solution) according to the required dose in the study.
- Inserting the medical waste recycle materials into the reactor
- Checking the required time (duration of contact) between the disinfectant solution and medical waste recyclable materials.
- Taking samples of each material for the *Bacillus subtilis* and *Bacillus stearothermophilus* test in the medical waste recycling process.
- Conducting Laboratory Examination using Blood Agar and Soy Broth Trypticase.

Bivariate analysis

Bivariate analysis was carried out on variables that were thought to be related or had an influence and by seeing the influence magnitude of the independent variable towards the dependent variable. The bivariate analysis used in this study was two-way ANOVA.

RESULTS AND DISCUSSION

Temperature measurement result after the given treatment shown in Table 1. Based on these results at each contact time and the dose of chlorine in the medical waste recycling process, the average temperature of the chlorine solution at the contact time of 15 minutes, 30 minutes, and 45 minutes was 24.34 °C; 24,53 °C; and 24.54 °C.

The temperature of the chlorine solution during the experiment did not show a significant increase or decrease in its temperature. The temperature range at the time of the study was

Figure 1. Aseptic technique during the swabbing process of used spike infusion

Figure 2. Measurement of temperature and Ph during the medical waste recycling process

Figure 3. Used spike infusion swabbing process

Figure 4. Bacterial culture/microbial isolation from samples of recyclable medical waste

22.8–26.6^{0}C. Changes in temperature at any time in the reactor can be affected due to changes in air temperature around the research area. According to Soebagio (2011) in Handayani and Sugeng (2015), states that the higher water temperature can increase the effectiveness of chlorine for disinfection. This condition also affects the amount of chlorine for disinfection, when the water temperature decreases, the use of required chlorine increases, and when the temperature increases the use of chlorine is relatively less.

However, Sari's research, 2018 states that the higher the water temperature, the greater the chlorine decay. If the water temperature increases, it will affect the presence of calcium hypochlorite, the efficiency of calcium hypochlorite will decrease as the water temperature increases. Parameter pH measurement results after the given treatment shown in Table 2.

Based on the results of pH measurements at each contact time and the dose of chlorine in the medical waste recycling process, the average pH of the chlorine solution at the contact time of 15 minutes, 30 minutes, and 45 minutes was 8.344; 8,375; and 8,461. It can be seen that the pH of the chlorine solution at each concentration has increased. The greater the concentration or dose of chlorine used, the pH will increase or the solution

Table 1. Temperature measurement result after the given treatment

Chlorine dosage	Repetition	Temperature (^{0}C)		
		15 minutes	30 minutes	45 minutes
0.03%	1	25.6	25.8	25.9
	2	23.3	23.5	24
	3	26.6	26.5	26.6
	4	23.6	23.8	23.8
0.05%	1	25.7	25.9	26.1
	2	23.2	23.5	23.6
	3	25.7	25.7	25.6
	4	23.6	23.7	23.8
0.07%	1	25.8	25.9	26.1
	2	23.3	23.4	23.5
	3	25.7	25.8	25.8
	4	23.2	23.4	23.8
0.3%	1	25.8	25.7	25.8
	2	23.1	23.6	23.4
	3	24.4	24.8	24.9
	4	23.2	23.6	23.9
0.5%	1	25.6	25.9	25.7
	2	22.8	23.5	23.5
	3	24.3	24.7	25
	4	23.2	23.4	23.6
0.7%	1	25.6	25.7	25.6
	2	22.9	23.2	23.5
	3	24.7	25.2	25.1
	4	23.4	23.8	23.5
3%	1	25.7	25.6	25.6
	2	23	23.2	23.5
	3	24.7	25.1	25.2
	4	23.2	23.6	23.5
5%	1	25.5	25.3	24.9
	2	23.7	23.7	23.2
	3	24.5	24.8	24.9
	4	23.3	23.5	23.6
7%	1	25.5	25.3	25.0
	2	24.1	24.1	23.2
	3	24.6	25	25
	4	24.2	23.9	23.9
Average (N=108)		**24.342**	**24.531**	**24.544**
Standard Deviation		**1.1284**	**1.0362**	**1.0338**
Maximum		**26.6**	**26.5**	**26.6**
Minimum		**22.8**	**23.2**	**23.2**

becomes more alkaline. This is because if the chlorine $Ca(OCl)_2$ is in water, hence the following reaction will occur:

$$Ca(OCl)_2 + 2(H_2O) \leftrightarrow 2HOCl + Ca(OH)_2$$

The reaction of chlorine with water will cause an increase in the pH of the water because it produces $Ca(OH)_2$ which is an alkaline or strong base and HOCl which is a weak acid. $Ca(OH)_2$ immediately decomposes to form Ca^{2+} and OH^- ions so that the ratio of OH ions$^-$ is greater than H ions$^+$ which causes the solution to be alkaline. HOCl is the remaining free chlorine which is most effective as a disinfectant compared to OCl^- which is produced as free chlorine from the use of NaOCl and Cl disinfectants in the form of gas (Aziz et al., 2013). Residual chlorine measurement result shown in Table 3.

Table 2. pH measurement results after the given treatment

Chlorine dosage	Repetition	pH		
		15 minutes	30 minutes	45 minutes
0.03%	1	8	8	8
	2	8.2	8.3	8.4
	3	7.7	7.7	7.7
	4	7.6	7.7	7.7
Average (N=12)		7.950	7.875	7.875
0.05%	1	8	8	8
	2	8.3	8.3	8.4
	3	7.7	7.7	7.7
	4	7.6	7.7	7.7
(N=12)		7.950	7.9	7.9
0.07%	1	8	8	8
	2	8.2	8.3	8.4
	3	7.7	7.7	7.8
	4	7.7	7.7	7.7
Average (N=12)		7.975	7.9	7.9
0.3%	1	9	9	9
	2	8.5	8.6	8.6
	3	7.9	7.9	7.9
	4	7.6	7.6	7.6
Average (N=12)		8.725	8.25	8.25
0.5%	1	9	9	9
	2	8.4	8.6	8.7
	3	8	8	8
	4	7.6	7.6	7.6
Average (N=12)		8.325	8.25	8.25
0.7%	1	9	9	10
	2	8.7	8.7	8.8
	3	8	8	8.1
	4	7.6	7.7	7.7
Average (N=12)		8.65	8.325	8.325
3%	1	9	9	10
	2	9	9	9.1
	3	8.2	8.3	8.3
	4	7.7	7.7	7.7
Average (N=12)		8.775	8.475	8.475
5%	1	10	10	10
	2	9.5	9.5	9.5
	3	8.5	8.5	8.6
	4	7.8	7.8	7.9
Average (N=12)		9	8.95	8.95
7%	1	10	10	10
	2	10	10.1	10.2
	3	8.8	8.8	8.8
	4	7.9	8	8
Average (N=12)		9.175	9.225	9.25
Average (N=108)		**8.344**	**8.375**	**8.461**
Standard Deviation		**0.7229**	**0.7201**	**0.8058**
Maximum		**10**	**10.1**	**10.2**
Minimum		**7.6**	**7.6**	**7.6**

Table 3. Chlorine residual measurement results data at doses of 0.05% and 0.5%

Chlorine dosage	Repetition	Residual chlorine (%)
		45 Minutes
0.05%	1	-
	2	0.03
	3	0.03
	4	0.02
0.5%	1	-
	2	0.49
	3	0.49
	4	0.41

In addition to temperature and pH factors, the effectiveness of the disinfection process is also influenced by residual chlorine. Following are the results of measuring the residual chlorine content for 4 consecutive days. The examination of the chlorine content at a concentration of 0.05% and 0.5%. The chlorine content in the two concentration variations decreased with each repetition. The chlorine concentration of 0.05% decreased to 0.03% on the 2nd repetition, and the 3rd and 0.02% on the 4th repetition. While the chlorine concentration of 0.5% decreased to 0.49% in the 2nd and 3rd repetitions, then decreased again in the 4th repetition to 0.41%. The decrease in chlorine levels in this study can be influenced by several factors, including temperature and water pH. According to Sari's research (2018), the higher the temperature, the greater the chlorine decay. In the results of temperature measurement, it was found that the longer the contact time, the temperature of the chlorine solution in the reactor increased so that the chlorine decay process was getting faster causing the chlorine level to decrease. So that if the chlorine level decreases, the effectiveness of disinfection will also decrease.

In addition, the reduction in residual chlorine is also influenced by the duration of water storage time. During the chlorination process, the chlorine will be reduced to chloride (pure Cl) which has no killing power at all (Giyantini, 2004 in Anam). The longer the water storage time, the more chlorine residual is reduced into chloride. Measurement of residual chlorine carried out in the 2nd to 4th repetitions of 45 minutes contact time showed that the residual chlorine had decreased, meaning that the chlorine was reduced into chloride.

Total plate count of *Bacillus* sp. test result

Figure 5 shows *Bacillus* sp. found in the medical waste recycling process, viewed on a microscope with 1000× magnification. *Bacillus sp* with shape: round, size: 1–8 mm diameter, pigment: white-gray-greenish, elevation: flat/slightly convex, property: haemolysis, surface: rough-dry, and margin: serrated. The colony of the suspect *Bacillus sp.* was grown on biochemical test media, namely semi-solid, glucose, mannitol, arabinose, gelatin, nitrite, urea, and Simmons' citrate, continued by incubating at 37 °C for 18–24 hours, and then observing the reaction results in the biochemical test, and lastly was to determine the bacterial species.

In this study, the treatment given was in the form of nine variations of chlorine/ chlorine concentration and three variations of contact time. 138 samples were taken and examined from 4 repetitions. The existing quality standards related to the presence of *Bacillus* sp. Spore was stated in the Indonesian Regulation of Minister of Environment

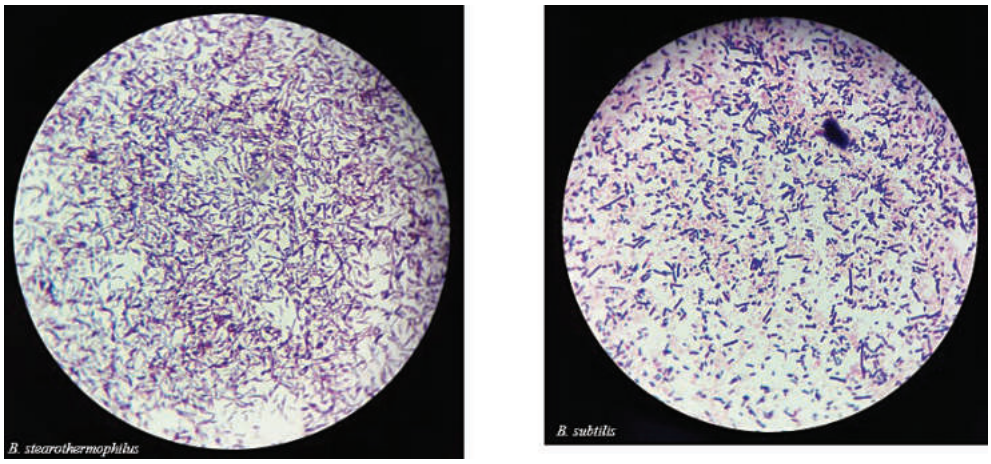

Figure 5. *Bacillus* sp.

Table 4. Total plate count of *Bacillus* sp. (*Bacillus subtilis* and *Bacillus stearothermophilus)* test result

No	Sample code	TPC test result	Unit	Description
1	C. A	67×10^5	CFU/ml	*Bacillus stearothermophilus*
2	B. 45'	300	CFU/ml	*Bacillus subtilis*
3	C. B	$>300\times10^5$	CFU/ml	*Bacillus stearothermophilus*
4	C. 30'	100	CFU/ml	*Bacillus subtilis*
5	C3. 30'	47×10^4	CFU/ml	*Bacillus stearothermophilus*
6	C. 45'	10	CFU/ml	*Bacillus stearothermophilus*
7	C. C	$>300\times10^5$	CFU/ml	*Bacillus subtilis*
8	D3. 45'	100	CFU/ml	*Bacillus subtilis*
9	F3. 15'	1×10^4	CFU/ml	*Bacillus stearothermophilus*
10	F4. 30'	negative	CFU/ml	–
11	F4. 45'	negative	CFU/ml	–
12	G. 15'	negative	CFU/ml	–
13	C. G	50×10^5	CFU/ml	*Bacillus subtilis*
14	H. 30'	1000	CFU/ml	*Bacillus stearothermophilus*
15	H3. 30'	1000	CFU/ml	*Bacillus stearothermophilus*
16	I. 30'	100	CFU/ml	*Bacillus subtilis*
17	C. I	$>300\times10^5$	CFU/ml	*Bacillus subtilis*

and Forestry No. P.56, 2015 concerning Procedures and Technical Requirements for Management of Hazardous and Toxic Waste from Health Care Facilities, as a concentration of 1×10^4.

In Table 4, there are 14 positive samples of Bacillus sp, and there are 3 (three) negative samples of Bacillus sp. The presence of Bacillus sp. in medical waste treatment is a bioindicator of the success or failure of the disinfection process. The number of TPC on Control A (CA), CB, CC, CG, and CI exceeds the requirements on The Indonesian Regulation of Minister of Environment and Forestry No. P.56, 2015 concerning Procedures and Technical Requirements for Management of Hazardous and Toxic Waste from Health Service Facilities, which exceeds the concentration of 1×10^4. 14 samples were positive for Bacillus sp. with a minimum value at a concentration of 1×10^1 and a maximum of 47×10^4, meaning that the 8 samples that were positive for Bacillus sp, was still below the required quality standard, while one positive sample of Bacillus sp. did not meet the requirements on the 3rd repetition, namely at a concentration of 0.07% and a contact time of 30 minutes.

Normality test

In Table 5 the output results of the normality test indicated. Based on these results, it was found that Kolmogorov Smirnov's p-value was 0.001. The analysis conditions are if the p-value> α (0.05) then the data is normally distributed or H0 is rejected. In the table, the p-value results for the number of Bacillus sp. is smaller than α (0.05) thus, it is categorized that the data is not normally distributed or H0 is accepted. If the data is not homogeneous, then a non-parametric analysis is carried out.

Homogeneity test

Table 6 show the output results of the homogeneity test. Based on these results it was found that the p-value was 0.001. The analysis conditions are if the p-value> α (0.05) then the data is homogeneous or H0 is rejected. In table 6, the p-value for the number of Bacillus sp. obtained are smaller than α (0.05) thus, the data is categorized as not homogeneous or H0 is accepted. If the data is not homogeneous, then a non-parametric analysis is carried out.

Table 5. Normality test results

No	Variation	Kolmogorov-Smirnov test	Shapiro-Wilk test
		p-Value	
1	15 minutes	0.001	0.001
2	30 minutes	0.001	0.001
3	45 minutes	0.001	0.001

Table 6. Homogeneity test results

Levene Statistic	df1	df2	Sig.
4.808	26	81	0.001

Bivariate data analysis

Bivariate analysis used the Kruskal-Wallis test because the test results showed that the data were not normally distributed. The test in this study was to determine the difference in the duration of contact time and the dose of chlorine as a disinfectant against the number of *Bacillus subtilis* and *Bacillus stearothermophilus* in the medical waste recycling process. The output results of the Kruskal-Wallis test show Table 7.

In the Table 7, the p-value is 0.377 consecutively. The analysis conditions are if the p-value < α (0.05) then HO is rejected or the variable is said to have a significant difference. The p-value is 0.377> 0.05, it can be concluded that H0 is accepted, which means there is no difference in the duration of contact time and the dose of chlorine as a disinfectant on the number of *Bacillus subtilis* and *Bacillus stearothermophilus* in the medical waste recycling process.

The results of the bivariate statistical test showed that there was no difference in the duration of contact time and the dose of chlorine as a disinfectant on the number of *Bacillus subtilis* and *Bacillus stearothermophilus* in the medical waste recycling process. However, descriptively, on the three-contact time at a chlorine concentration of 0.03%, there was no Bacillus sp found. While the smallest average number of Bacillus sp TPC is found in the contact time of 45 minutes namely 15,185. If the results are viewed as descriptive analysis, then from this study it can be concluded that the effective concentration of chlorine as a disinfectant in the recycling process of medical waste is on a concentration of 0.03% with an effective contact time of 45 minutes.

Table 7. Kruskal-Wallis test results

Statistic	Total of *Bacillus* sp.
Chi-Square	8.597
Df	8
Sig	0.377

Minimum bactericidal concentration test result

Based on Table 8 it is known that the Minimum Bactericidal Concentration (MBC) for *Bacillus stearothermophilus* is at a concentration of 0.03% in 45 minutes, and for *Bacillus subtilis* at 0.03% in 30 minutes and 45 minutes. Thus, on further tests to determine the MBC in these two bacteria, for a while it can be concluded that the most effective concentration is at 0.03% with 45 minutes contact time (0 CFU/ml).

The working mechanism of chlorine as a disinfectant in the recycling process of medical waste

Chlorine or calcium hypochlorite is a type of disinfectant used in the management or recycling of *medical* waste in hospitals. The alternative choice of using chlorine as a disinfectant is because it is easy to find, it has a cheap price and relatively more stable and can be stored longer. If chlorine is dissolved in water it will produce the following reaction:

$$Ca(OCl)_2 + 2(H_2O) \leftrightarrow 2HOCl + Ca(OH)_2$$

$$2HOCl \rightarrow 2\,HCl + 2O_2$$

$$Ca(OCl)_2 2H_2 \rightarrow Ca(OH)_2 + 2H_2O + 2O_2$$

Chlorine reacts with water to produce $Ca(OH)_2$ (which is alkaline) and HOCl, where this HOCl will form oxygen atoms. The more HOCl that is formed, the more oxygen atoms will be released so that the disinfectant will increase.

Table 8. Minimum bactericidal concentration test result

Chlorine (%)	*Bacillus stearothermophilus*			*Bacillus subtilis*		
	Total colony number (1×10^5 CFU/mL)			Total colony number (1×10^5 CFU/mL)		
	15’	30’	45’	15’	30’	45’
0.01	5	5	5	3	3	5
0.02	3	3	3	2	2	2
0.03	2	2	0	1	0	0

However, the pH of the solution needs to be considered so that chlorine disinfection is more optimal (Rohim, 2006).

Determination of the optimum dose of chlorine for water samples based on the chlorination breakpoint curve. The advantage of achieving a breakpoint is that the ammonium compound is completely oxidized (Joko, 2010), completely kills pathogenic bacteria, and prevents algae growth. The contact time is thought to be the most important factor in the disinfection process. The longer the contact time between the disinfectant and microbes in the water, the greater the killing power (Budiyono and Sumardiono, 2013).

Free chlorine damages the membrane of the bacterial cell, this causes the cell to lose its permeability (ability to penetrate) and damage other cell functions (Figure 6). Chlorine exposure causes leakage of protein, RNA, and DNA. Damage to permeability is the cause of the destruction of bacterial spores by chlorine (Bitton, in Said, 2007). Chlorine also destroys bacterial nucleic acids, as well as enzymes. One result of the reduction in catalyst activity is inhibition by the accumulation of hydrogen peroxide. How chlorine works against viruses depends on the type of virus (Busyairi, 2016).

The macroscopic (A) and microscopic (B) form of the bacterial cells forming the lender biofilm, the size of the colony, and the bacterial cells were not on a proper scale, because the image had automatically undergone a digital enlargement. The application of active chlorine was able to reduce the MPN of *Bacillus subtilis* and *Bacillus stearothermophilus* according to the quality standards of hospital wastewater.

CONCLUSIONS

There were no differences in the duration of contact time and the dose of chlorine as a disinfectant on the number of *Bacillus subtilis* and *Bacillus stearothermophilus* in the medical waste recycling process with a p-value of 0.377. The percentage reduction in the number of *Bacillus subtilis* and *Bacillus stearothermophilus* before and after treatment was 99.99% based on 4 positive controls. The duration of contact time and the effective dose used in the recycling process of medical waste in RS Santo Yusuf Boromeus is 0.03% with a contact time of 45 minutes.

The government needs to conduct a review related to the recommended ideal dosage in the surface disinfection process, so that it would not cause a potential risk to humans or the environment.

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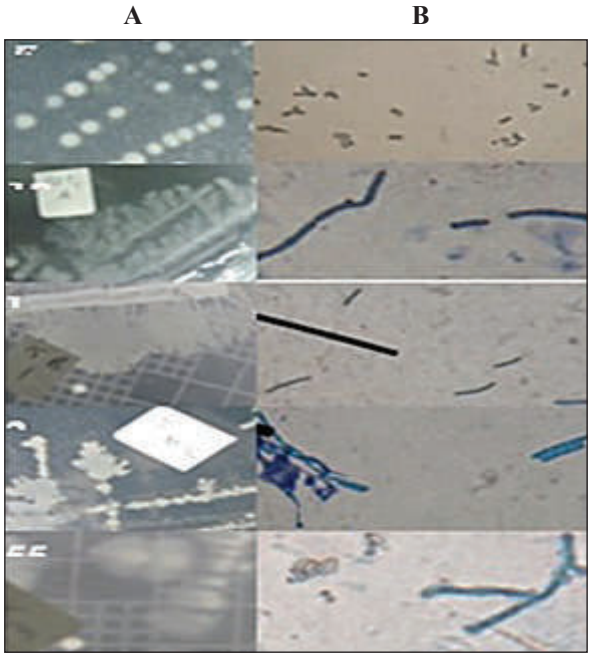

Figure 6. Mechanism of bacterial damage by chlorine

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