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THE EFFICIENCY OF ASCARIS SPP. EGGS INACTIVATION IN SEWAGE SLUDGE BY LIME DOSAGE, AMMONIA CONCENTRATION, AND TEMPERATURE VARIATION

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Abstract

Introduction: *Ascaris spp. eggs threaten sludge reuse because they are usually more environmentally resistant than other microorganisms are. Consequently, the WHO recommended an acceptable limit of <1 helminth egg per gram of total solid as a health-based target for treated feces and fecal sludge and aimed to ascertain the effectiveness and reduction rate of Ascaris spp. eggs in sludge at various lime doses, ammonia concentrations, and temperature values.* ***Methods:*** *Eggs were detected and enumerated using the flotation and sedimentation principle. The reduction process was performed in batches with quick lime doses of 30% and 50% w/w, ammonia concentrations of 1,000 and 5,000 mg/L, and temperatures of 30°C and 50°C.* ***Results and Discussion:*** *The number of eggs of Ascaris spp. fertile in the ponds was 1.38 ± 1.38 eggs/gram to 42.98 ± 13.09 eggs/gram, and the infertile amount was 0.46 ± 0.80 eggs/gram to 17.82 ± 6.55 eggs/gram. Using a temperature of 40°C, 50% CaO, and 5,000 ppm ammonia resulted in the highest percentage of reduction, 97.98 - 98.09% but 1.12 eggs/gram was remaining.* ***Conclusion:*** *Higher pH and ammonia levels primarily affect egg decrease in Ascaris spp. To reduce 99% of Ascaris spp. eggs until it reaches <1 egg/g, a dose of 50% CaO is required, with 5,000 ppm ammonia at a temperature of 40°C for 2.7 hours.*

INTRODUCTION

Sewage sludge is a byproduct of solids that have been separated from wastewater treatment. Because of its high organic and inorganic substance contents, sewage sludge is commonly reused as a soil enhancer in Indonesia (1). However, sludge also contains pathogenic pollutants, including soil-transmitted helminth (STH) eggs, which have adverse effects and threaten human health. The incidence of infection reflects the normally high quantity of STH eggs in sludge and is concerning, especially in reuse circumstances. Sludge reuse can be a significant way to expose people to eggs, which primarily affects farmers, but it can also have an indirect effect on populations, especially crop consumers (2). To prevent human health issues, the World Health Organization (WHO) has published guidelines for the reuse of wastewater, fecal material, and greywater for agriculture and aquaculture. The equivalent limit of helminth eggs in treated feces and fecal sludge is less than 1 egg/g of total solids for a large-scale treatment system of fecal sludge reuse in agriculture as a health-based target. Health-based targets specify an appropriate amount of health protection for each threat (3).

One of the soil-transmitted helminths (STH) is *Ascaris* spp. It is a parasitic nematode (family Ascarididae), with the two most important species being *Ascaris lumbricoides* and *Ascaris suum*. The eggs of this nematode are considered the primary constraint to wastewater and sludge reuse because of their high survival rate in the environment, persistence in the environment, low infectious doses, and high resistance to conventional disinfection processes (3-4).

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Ascaris spp. eggs can infect humans when fertilized eggs are ingested or when infected larvae penetrate the exposed skin. Ascariasis, the infection caused by *Ascaris* spp. eggs, is categorized as a Neglected Topical Disease (NTD). It is proved that the infections are related to direct contact with wastewater or sludge and the consumption of vegetables grown on soil treated with contaminated wastewater (2). The parasite may have an impact on long-term, chronic nutritional morbidity and cognitive development (5).

Several methods have been investigated for helminth egg reduction in sludges. One of the methods for treating conventional domestic sludge is limited. Liming is the process of adding lime to liquid sludge to achieve a pH of more than 12. Another parameter that effectively reduced helminth eggs in sludge was ammonia. Ammonia is naturally present in sewage through urea hydrolysis and protein degradation. The most significant factor influencing the inactivation of viable *Ascaris* eggs in sludge was the temperature-time factor. However, other processes, including microbial rivalry, antibiotic activity brought on by local bacteria, and toxic byproducts such as free ammonia, have also played a significant role in the inactivation of *Ascaris* eggs in fecal sludge (6).

Ascariasis has the highest prevalence in South and Southeast Asia, including Indonesia (7). *Ascaris* spp. eggs were also found in higher amounts than helminth eggs of other species in many countries (8). There is not much information about the incidence of *Ascaris* spp. eggs in the sludge, as well as the sludge treatment to inactivate the eggs in Indonesia. Therefore, it is necessary to perform experiments related to the reduction of *Ascaris* spp. eggs in the environment, in this case the sludge, before being reused or disposed of in the environment. This study focused on the quantification of *Ascaris* spp. eggs and the effectiveness and reduction rate of sludge stabilization in lowering *Ascaris* eggs at various lime doses, ammonia concentrations, and temperature values. The sludge was obtained from the Bojongsoang Wastewater Treatment Plant in Bandung, Indonesia, which uses a lagoon treatment system.

METHODS

Location of Study

The Bojongsoang wastewater treatment plant (WWTP) has a total area of 85 ha. The treatment capacity of the WWTP is approximately 243,000 m^3 with an average discharge inlet of 77,931.58 m^3/day (9). The treatment process starts with the inlet, physical, and biological processes of the lagoon, as shown in Figure 1.

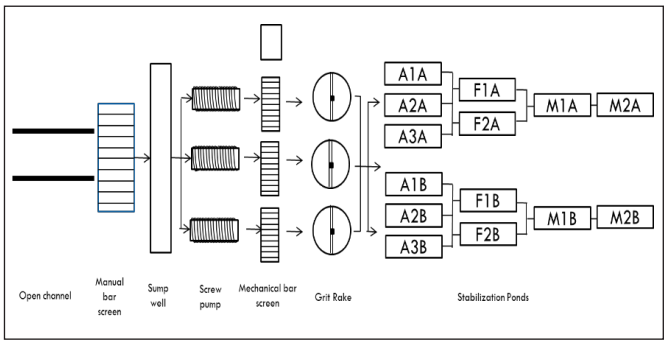


Figure 1. Schematic process of the Bojongsoang WWTP (9)

Sampling

The sludge samples were taken from set A ponds: anaerobic 1A, facultative 2A, and maturation 2A with a total volume of ponds, 38,993, 150,782, and 111,886.3 m^3 respectively. Sampling took place in January and September 2020. Samples were collected from the margins and center of the ponds using an Eckman grab dradge sampler, mixed, and kept in a 1,000 ml wide-mouthed plastic bottle. Two samples were collected from each of the ponds. The samples were stored at 4°C. The pH, temperature, and moisture content of the samples were measured.

Detection and Quantification of *Ascaris* spp. Eggs

The number of *Ascaris* eggs was counted for each sludge sample. A floation-sedimentation approach based on the modification by Amoah et al. was used for detection and quantification (10). A 0.1% Tween80 detergent solution was added to 20 g of sludge sample, homogenized, and filtered through a 100 and 20 µm screen. The filtered samples were washed and sedimented, and the supernatant was collected. A $ZnSO_4$ flotation solution with a specific gravity (SG) of 1.3 was then added. A 20 µm sieve was used to filter the supernatant once more before it was rinsed and sedimented. After removing the supernatant, the sediment was placed on a McMaster slide and examined under a microscope a magnification of 200x to count the number of helminth eggs. Identification of *Ascaris* spp. eggs under the microscope referred to physical characteristics, which are the form and size of the eggs, matched with the reference from the WHO (11). Each sample was obtained in 3-5 tubes and examined under a microscope. The number of eggs per gram was calculated as follows:

$$N = \frac{A\,X}{P\,V}$$

Explanation :

N = number of eggs (per gram)
A= number of the eggs (eggs)
X = final product volume (ml)
P= slide volume, McMaster = 0.3 ml
V = dry weight of the initial sample (gram)

Reduction

The process was carried out on a laboratory-scale in batches (Table 1). Two sludge samples from anaerobic pond 1A were used in the reduction test. Ammonia at concentrations of 1,000 and 5,000 mg/L and quick lime containing 70% CaO were adjusted to 26% and 39% of the wet weight of the sludge, respectively. This percentage was based on a preliminary experiment to increase the pH of the sludge to 12. Sludge was added to each batch along with quicklime and ammonia, mixed to homogeneity, and then placed into a cap-tight plastic bottle. The sample mixture-containing bottles were then incubated in a water bath at 30°C and 40°C. At 0, 30, 60, and 90 min, pH, temperature, and quantity of *Ascaris* spp. fertile eggs of *Ascaris* spp. were recorded. Testing was performed in duplicate for each sample. After the reduction treatment, the samples were analyzed to determine the number of *Ascaris* eggs remaining using the same quantification method.

Table 1. Variations of the Experiments Performed

Variation	Temperature	Lime dosage (% wet weight)	Additional ammonia concentration (mg / L)	Sampling time
1	30°C	26	1,000	0 – 90 min (every 30 min)
2		26	5,000	
3		39	1,000	
4		39	5,000	
5	40°C	26	1,000	0 – 90 min (every 30 min)
6		26	5,000	
7		39	1,000	
8		39	5,000	

Kinetics

The reduction rate of decreasing *Ascaris* spp. egg concentration was plotted into the first-order kinetics model in Equation (2).

$$\ln\left(\frac{C}{C_0}\right) = -kt$$

Explanation :

C = number of *Ascaris* spp. eggs in sludge at time t (eggs/g dry weight)
C_0= initial number of *Ascaris* spp. eggs (eggs/g dry weight)
k = reduction kinetic coefficient (per minute)
t = time (minutes)

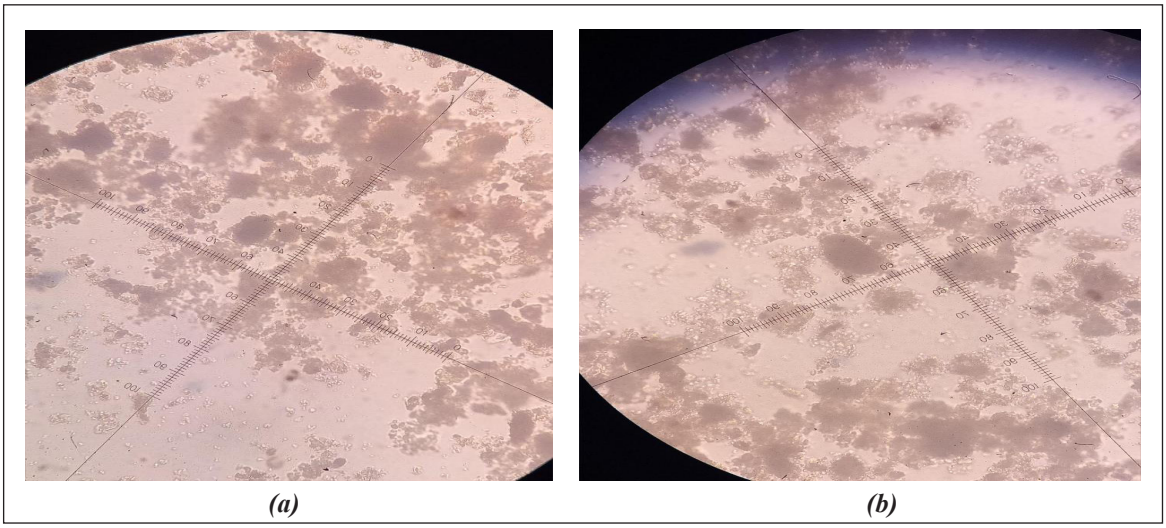
RESULTS

Characteristics

The average pH of the sludge samples increased from the anaerobic pond to the maturation pond samples by 6.77, 6.89, and 7.81, respectively. The average temperature of all sludge samples was 26.6°C. The lowest temperatures were observed in the anaerobic and maturing ponds. This value was recorded during the dry season in January when the ambient temperature was relatively high. The moisture content of the samples was measured. The water content of the sludge was relatively high:85.16% for anaerobic pond sludge, 85.80% for facultative pond sludge, and 75.92% for maturation pond sludge. This occurred because the sludge samples were taken directly from the pond without compaction or drying.

Quantification of *Ascaris* spp. Eggs

Ascaris spp. eggs were discovered in all sludge samples from the inlet to the maturation ponds, in the form of infertile and fertile eggs. Figure 2 shows the *Ascaris* spp. found in the samples.



(a) *(b)*

Figure 2. *Ascaris* spp. Eggs *(a)* Fertile *(b)* Infertile

The number of fertile eggs was higher than that of infertile eggs in all samples (Figure 3). The average number of viable *Ascaris* spp. eggs in anaerobic, facultative, and maturation pond set A were 221±27.15 eggs/gram, 28±14.28 eggs /gram, and 55±24.94 eggs/ gram respectively. The infertile eggs on average were 84±20.42 eggs/gram, 4±3.53 eggs/gram, and 32±2.68 eggs/gram. Overall, fertile eggs overall reached 71.75-80% of the total *Ascaris* spp. eggs found in the sludge.

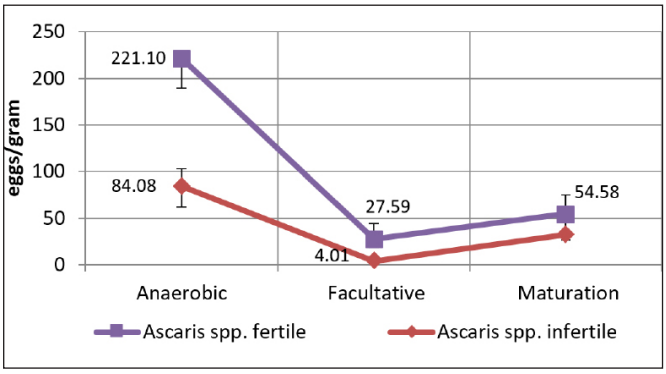


Figure 3. Number of *Ascaris* spp. eggs in the Sludge

Reduction

Treatment with a temperature of 30ºC has a recorded sample temperature range of 26.5ºC - 32.1ºC and an average of 29.48ºC. At a treatment temperature of 40 °C, the sample temperature was 33.4ºC - 39.2ºC and the average was 37.6ºC. For comparison, sample temperature was measured without incubation in a water bath (under ambient conditions). With an average ambient temperature of 26ºC, the sample mixture, 26% quicklime, and ammonia both 1,000 and 5,000 mg/L had an average temperature fluctuating of 27.25ºC at 0 minutes; 31.15ºC in 30 minutes; 31°C in 60 minutes, and 29.6°C in 90 minutes. This was relatively close to the temperature of 30°C in a water bath.

The results of the egg reduction test of *Ascaris* spp. fertile at 30°C and 40°C are shown in Figure 4. The graph shows that the reduction, in general, increased over time. At 30°C, after 90 min, the reduction reached 64.59 to 88.87%, with the number of *Ascaris* spp. eggs remaining at 6.18 to 19.67 eggs/gram.

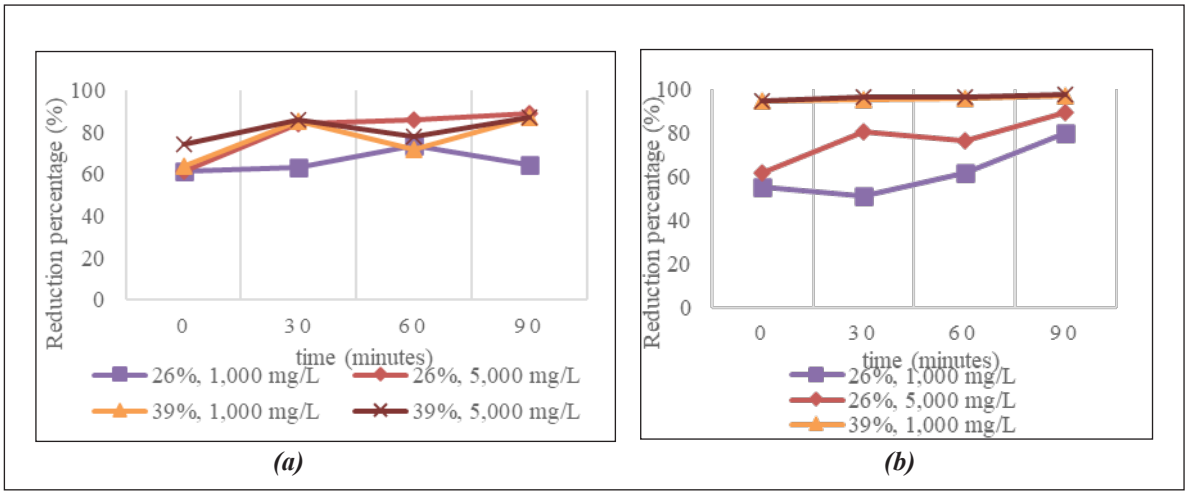


Figure 4. Reduction Percentage of *Ascaris* spp. Fertile at a Temperature of *(a)* 30° C and *(b)* 40° C

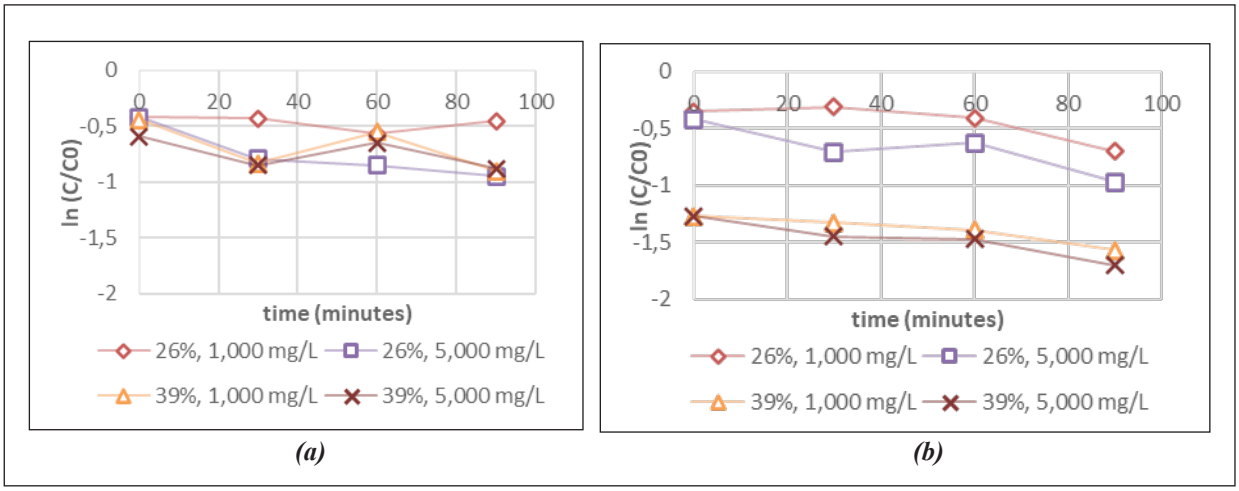


Figure 5. Kinetics Profile at *(a)* 30° C and *(b)* 40° C

The highest reduction percentage occurred when using a 26% quicklime dose and 5,000 mg/L ammonia. The addition of 5,000 mg/L ammonia resulted in a greater reduction than 1,000 mg/L for both quicklime doses. The number of infertile *Ascaris* spp. eggs was counted under the same conditions. There were some differences in the reduction in fertile eggs. In the test using the 26% quicklime dose, the reduction increased only until the first 60 min. The highest percentage of reduction was observed using 39% quicklime and 5,000 mg/L ammonia, which was 98.09% and only 0.28 eggs/gram remaining.

At a temperature of 40°C, the percentage of reduction of *Ascaris* spp. fertile using a 26% quicklime dose was lower than when using a 39% dose. The highest percentage of removal (97.98%) was achieved with 39% quicklime and 5,000 mg/L ammonia. However, there were still 1.12 eggs/gram remaining. The highest reduction was 98.09%, with an infertile eggs 0.28 eggs/gram. Among all variation tests, the condition using 40°C temperature, quicklime dose of 39%, and ammonia concentration of 5,000 mg/L reached the highest reduction percentage which was 97.98 – 98.09% but unfortunately, 1.12 eggs/gram of *Ascaris* fertile eggs was still found in the sample.

Kinetics

The reduction kinetics coefficient k values for all treatment variations are listed in Table 2.

Table 2. Reduction Kinetics Coefficient

Var.	Temp	CaO Dose (%)	Ammonia Dose (ppm)	k (/min)	t_{99} (hour)
1	30°C	26	1,000	0.002	30.1
2		26	5,000	0.0128	3.5
3		39	1,000	0.0084	6.8
4		39	5,000	0.0052	10
5	40°C	26	1,000	0.0088	7.5
6		26	5,000	0.0121	5
7		39	1,000	0.0074	3.9
8		39	5,000	0.01	2.7

The reduction parameters can be used to calculate t_{99}, which is the time required to reduce 99% of *Ascaris* spp. eggs. It was found from the calculation that it would take 2.7 hours to reach 99% of the reduction of *Ascaris* spp. The eggs used 39% quicklime and 5,000 mg/L of ammonia. The removal rate coefficient k was determined by plotting the data of natural logarithm (ln) concentration per initial concentration (C/C_0) against time, as shown in Equation (2). Reduction kinetics were determined only for eggs of *Ascaris* spp. that were fertile with a reduction profile at temperatures of 30 °C and 40°C, as shown in Figure 5. From this graph, it can be seen that the kinetic coefficient (k) is the first-order coefficient of the regression equation for changes in the number of eggs of *Ascaris* spp. before and after reduction.

DISCUSSION

The temperature, pH, and moisture content of the sludge encouraged the growth of the *Ascaris* eggs. The ideal temperature for the development of an *Ascaris* egg is 17°C–32°C. Because helminth eggs can develop into an infectious phase and hatch within a pH range of 4.6-9.4, a neutral pH of approximately 7 was also beneficial for the resistance of the eggs (12). *Ascaris* spp. eggs can develop for 3–6 weeks depending on the temperature in the right environmental circumstances, and they can withstand the environment for more than 6 years (8). This resistance is derived from the layers of the eggshell that serve as a barrier to larval exposure to harsh external circumstances (12). *Ascaris* spp. eggs are easily recognized because of the composition of the egg wall, which is relatively thick and has an uneven exterior. The egg wall is composed of three layers: a thick, impermeable albuminoid layer on the outside, an impermeable hyaline layer in the middle (which gives the egg its form), and an extremely impermeable vitelline layer that serves as a protective coating for the egg cells (13).

Fertile and infertile eggs of *Ascaris* spp. were found in samples from all ponds. The total number of *Ascaris* spp. eggs found in the anaerobic, facultative, and maturation ponds was approximately 305, 32, and 107 eggs/gram, respectively. This could have occurred because the eggs were attached to the suspended solids and removed from the wastewater during the process. Therefore, the eggs settled and accumulated in sludge. The highest number of *Ascaris* spp. eggs, both fertile and infertile, was found in anaerobic ponds and decreased in facultative and maturation ponds. This might be related to the location of the anaerobic pond where the initial raw wastewater sedimentation occurred. This was related to a previous study where *Ascaris* spp. eggs found in wastewater and sludge decreased from anaerobic ponds to maturation ponds in the Bojongsoang WWTP (14). This was also related to several studies conducted in Ghana, where helminth eggs were found to be more concentrated in the anaerobic pond compared to primary and secondary facultative ponds, and maturation ponds at concentrations of 23.6,20.8, 13.3, and 10.7 eggs/L (15).

A study reported that *Ascaris* spp. eggs had the highest load of helminth eggs (47.7%) found in sludge from the lagoon system of Chichaoua City, Morocco (16). Another study in Tunisia also discovered 56.9% of

Ascaris spp. eggs in sewage sludge (17). This discovery is also related to research in which helminth eggs were more frequently discovered in sludge in developing nations, where the concentrations can range from 7 to 80 times greater than in developed countries (4). The abundance of *Ascaris* spp. eggs indicates that ascariasis is a common condition in the city. This was associated with a study conducted in 2020 that discovered abundant helminth eggs, ranging from 4 to 617 eggs/L, including *Ascaris* spp. eggs, in fecal waste collected from septic tanks in the urban area of Bandung City (18).

The number of fertile eggs was greater than that of the infertile eggs. This indicates that there were many infectious and viable eggs. Both males and females deliberately isolate fertilized eggs when they are nearby. They can carry on biological processes until they reach the larval stage, at which point they are ready for infection. The stages of embryo development include cells, morula (cluster of cells), gastrula, buds, and larvae at the end of the process. When to 1-2 female human *Ascaris* are present in the body, only unfertilized eggs are isolated in cases of non-intensive ascariasis, and their detection aids in the clinical picture of ascariasis (19).

Ascaris spp. eggs in sludge can be reduced using a method that generally applies the effects of a high pH and temperature. Alkaline/lime post-stabilization is a technique frequently used in wastewater treatment facilities. It is frequently used to treat sludge in both large and small plants. Lime kills cells by altering the colloidal composition of the protoplasm. Because the pH can rise over 12 for at least two hours, this technique is also excellent for stabilizing and deodorizing sludge (20). Alkaline stabilization has been proven to reduce helminth eggs to one or zero with sludge dewatering treatment in Colombia. Alkaline stabilization also produces biosolids that can be used as fertilizers (21). Lime treatment increases the dehydration of sludge, temperature, and alkalinity, which contributes to sludge disinfection. It is also effective in removing odor from sludge (22).

The pH reached in each variation test was mostly higher than 13 and did not change drastically from the beginning to the end of the test. Determination of the quicklime dose, 26% and 39% wet weight of the sludge was based on the initial experiment performed before the reduction process, and it was found that the pH reached after adding the quicklime was higher than 12 for 2 hour, which accordingly fulfilled the requirement to maintain sludge hygiene. However, regulation is needed to specify quality parameters such as the concentration of *Ascaris* spp. eggs in sludge reuse, especially for use as fertilizer. Pathogens such as *Ascaris* spp. eggs were found to be more relevant to demonstrate sanitation than indicator organisms (23).

The temperature of the environment was maintained by placing a bottle filled with the sample mixture in a water bath. The temperature measured in this study was influenced by the internal and external conditions. In addition to using a water bath as a source of external heating, quicklime was added to the sludge, which increased the temperature of the mixture. Lime reacts with water and increases the temperature inside the mixture, as shown in the following reaction:

$$CaO_{(s)} + H_2O_{(l)} \rightarrow Ca(OH)_{2\ (s)}, \text{ Exothermic.}$$

This was also proven in the experiment at ambient temperature without using a water bath, where the temperature was relatively close to the temperature reached using a water bath. However, without a water bath, the temperature decreased after 60 min. When the experiment was performed at 40°C in a water bath, the temperature of the mixture increased, but the maximum temperature achieved was maximum 38.28°C. It was proven that external heating affected the temperature of the mixture, but unstable heating occurred in the water bath.

A recent study found that at temperatures of 28°C and 34°C, viable *Ascaris* spp. eggs could develop into larval stages, which made them more infectious, while quick incubation at 60°C for 1 hour could reduce the number of eggs (24). The effect of temperature was also found in research using a solar drying process for sludge with a daily maximum temperature of 40°C – 68.8°C can endanger helminth egg viability as it can remove most helminth eggs contained (25). The temperature effect itself can be lethal to eggs. When *Ascaris* eggs were suspended in fecal sludge, inactivation occurred faster under environmental conditions. *Ascaris* eggs are inactivated at temperatures between 37°C and 45°C (26). However, the time required to achieve a higher inactivation rate should be up to 40 days. To reduce the amount of time required, an additional requirement is required.

Higher ammonia levels generally increase the reduction process. However, when a quicklime dose of 39% was used, the differences in ammonia concentration did not cause significant differences. Meanwhile, the percentage reduction of infertile eggs increased with higher quicklime doses and ammonia concentrations. Ammonia was measured as nitrogen in the sludge. Ammonia in sludge is generated from nutrients that enter wastewater, such as urine. The toilets used by most residents do not separate urine, and black fecal water

also consists of urine containing ammonia. Mechanism of reduction in *Ascaris* spp. eggs. using ammonia is not fully understood, but based on an experiment conducted in Egypt in 2018, it was believed that ammonia as NH_3 gas, as the dominant nitrogen species at pH values above 9.30 can be toxic to nematode eggs since the research showed that in a condition where ammonia is zero, the inactivation of nematode eggs never reached 100 percent (27).

A study conducted in Sweden showed that alkaline pH itself could not inactivate *Ascaris* spp. eggs, but could enhance the effect of ammonia, which is likely to be present in organic waste (28). This means that a higher pH and ammonia should be available to effectively remove *Ascaris* spp. eggs. After treatment, the surviving *Ascaris* spp. in the sludge demonstrated resistance. Several researchers have also discovered that the resilience of *Ascaris* spp. eggs as total removal of *Ascaris* spp. eggs was not possible despite treatment with drying, liming, and co-composting (25,29).

In this study, it was found from the calculation that it will take 2.7 hours to reach a 99% reduction in *Ascaris* spp. eggs using 39% quicklime and 5,000 mg/L ammonia. Another study found that the t_{99} value of *Ascaris* eggs in fecal sludge was 429 days in an onsite sanitation system without sludge (30). It can be concluded that using alkaline and ammonia stabilization for sludge treatment is efficient for removing *Ascaris* eggs because it decreases the time needed.

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CONCLUSION

The pH values for the sludge samples in the anaerobic, facultative, and maturation ponds were 6.96, 7.18, and 7.60, respectively; the temperatures were 26.5, 26.6, and 26.5°C, respectively; and the moisture contents were 85.16, 85.80, and 75.92%, respectively. In all samples, the number of *Ascaris* spp. fertile was higher than that of *Ascaris* spp. eggs, with a fertile percentage of 71.75 - 80%. Of all the variations in the treatment, the use of temperature 40°C, 39% CaO dose, and 5,000 ppm ammonium dose reached the highest percentage removal, 97.98% but there were still 1.12 eggs/gram of *Ascaris* spp. fertile so that it does not meet the requirements of WHO. To reduce 99% of *Ascaris* spp. eggs to <1 egg/gram, a dose of 39% CaO is required, ammonia 5,000 ppm at a temperature of 40°C for 2.7 hours. The temperature measured in this experiment was influenced by both internal and exterior variables. Quicklime can raise the temperature in the mixture, in addition to using a water bath as the external heating source. A higher pH and ammonia should be available to effectively remove *Ascaris* spp. eggs. Alkaline and ammonia stabilization for sludge treatment is efficient for removing *Ascaris* eggs because it will decrease the time needed, rather than only applying a higher temperature.

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