Mathematical modeling of helminth eggs inactivation in pig (*Sus domestica*) manure from a backyard farm

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Abstract: Pathogenic organisms in pig manure can cause serious environmental and health problems. Pigs are susceptible to infection by microorganisms such as fecal coliforms, *Salmonella* spp., protozoa, and helminths. The latter are found in tropical and subtropical zones, where they are a source of health risk associated with poor sanitation due to contaminated water for agricultural irrigation and the inadequate final disposal of excrements on the ground. For this reason, it is necessary to treat the waste so that it complies with the maximum permissible limits established in the official regulations and is disposed of correctly. In this work, the kinetic parameters of the alkaline inactivation process were determined with different CaO concentrations (10, 15, and 20% m/m) and different time periods (0, 30, 60, 90, and 120 min). In addition, the evaluation of the product DT (ammonia dose and temperature) was carried out for the described process, for which the increase in pH and temperature after the addition of alkaline matter was studied for the inactivation of total helminth eggs in a system open. The mathematical modeling was carried out with the Hom model modified for chemical treatments. The results showed that the process used had an efficiency of 94.7% in the destruction of whole helminth eggs, of which 5.8% was carried out thanks to the ammonia dose and the resulting temperature (9,963 mg/L °C). Although the DT factor was not the leading cause of the helminth eggs inactivation it contributed favorably to the process in addition to the applied CaO dose (20%).

Keywords: helminth eggs; pig manure; alkaline stabilization; mathematical modeling.

Introduction

Worldwide, pork production has increased more than 3.5 times over the last 40 years; the trend is to increase the number of animals per farm, even reaching values of thousands of heads. As the demand for meat increases, vast amounts of waste are generated worldwide, which in turn causes an increase in the number of excreta and wastewater (Pérez-Pérez *et al*., 2016), mainly from non-technified farms. The intensive production of pork has caused severe impacts on the environment, in addition to generating health problems and parasitic diseases such as helminthiasis (Murillo, 2016) that are caused by helminths, which are multicellular organisms that infect humans, animals, and plants, representing a severe problem for poor and developing countries (Purwandani *et al*., 2021). It is estimated that more than a third of the world population is infected by helminths, mainly in tropical and subtropical regions (Tuasha *et al*., 2019). Due to their high environmental persistence and resistance to conventional treatment processes, helminth eggs (HE) are considered the most resistant biological structures to inactivation in environmental engineering (Jiménez *et al*., 2020).

It is essential to carry out an adequate treatment of pig manure (PM), which ensures the stabilization of sludge and inactivation of pathogens it may contain. Alkaline treatment with CaO applied to sludge is one of the most common and successful process for pathogen inactivation (Fubin *et al*., 2017). However, the ammonia dose and temperature (DT product) generated in this process are associated with a more efficient inactivation of microorganisms (Méndez *et al*., 2008).

In the present investigation, the mathematical modeling of the alkaline inactivation kinetics obtained during the stabilization process with CaO applied to PM was carried out, which had the purpose of inactivating pathogenic microorganisms and determining the kinetic parameters of the inactivation of total helminth eggs (HE) to ensure the stabilization and suitable disposal of PM obtained in a backyard farm, which is associated with unsuitable sanitary conditions and the development of parasitic diseases (Pinilla *et al*., 2020). These diseases are mainly caused by the high levels of pathogenic microorganisms present in pigs. It has been shown that pigs with high levels of helminth infection can be harmed by the destruction of functional kidney tissue, which in turn triggers a series of problems that result in weight loss and weakness of the infected animal (Fernandez-Vizcaino *et al*., 2021). For this reason, the presence of helminths in pigs represents not only an environmental problem but also an economic problem for the families in charge of operating this type of farm.

Materials and Methods

Obtaining, conditioning, and characterization of PM

PM was obtained from a backyard farm located in Acultzingo, Ver., Mexico. Sampling was carried out under the conditions established in standard Annex II of NOM-004 SEMARNAT-2002. The physicochemical characterization of the sample included the determination of Total Solids (TS) content based on the Standard Method 2540B (APHA-AWWA-WEF, 2005); for this reason, a 1:6 dilution was made to have a sample with values between 2 and 3% of TS. The microbiological characterization included the quantification of total HE, fecal coliforms, and *Salmonella* spp., present in the sample, for which the identification and quantification methods established in NOM-004-SEMARNAT-2002 were used.

Alkaline inactivation tests with variable CaO dose

A unifactorial design (CaO dose) with complete blocks was carried out; the treatments were performed with 0, 10, 15, and 20% (m/m) of CaO. The alkaline treatment was carried out at laboratory level in 250 mL open reactors to which a volume of 38 mL of sample equivalent to 1 gTS was added; the reactors were kept under constant agitation at 120 rpm for 120 minutes.

Equations 1 and 2 show the modified Hom model for alkaline treatment used to model the alkaline inactivation kinetics with variable CaO doses and constant time.

$$log\frac{N}{N_0} = -k^*D^n \quad \textbf{(1)}$$

$$-k^* = -kt^m \quad \textbf{(2)}$$

Alkaline inactivation tests with variable time periods

A unifactorial design (time) with complete blocks was carried out; the treatments were performed with 20% (m/m) of CaO and exposure times of 0, 30, 60, 90, and 120 minutes. In the same way, the treatment was performed at laboratory level in 250 mL open reactors to which a volume of 38 mL of sample equivalent to 1 gTS was added.

Equations 3 and 4 show the modified Hom model for alkaline treatment with different levels of time and constant disinfectant dose.

$$log\frac{N}{N_0} = -k^{**}t^m \quad \textbf{(3)}$$

$$-k^{**} = -kD^n \quad \textbf{(4)}$$

where *N* is the final concentration of microorganisms; N_0 is the initial concentration of microorganisms; *k* is the microorganism inactivation rate constant; *D* is the concentration of disinfectant; *m* is the Hom's constant; *n* is the dilution coefficient; and t is the time.

Inactivation by DT factor

In Equation 5, the modified Hom model is shown, which was used to determine the inactivation kinetics by the DT factor. The time of 120 min is involved in k^*, and the change in dose is associated with temperature changes.

$$log\frac{N}{N_0} = -k^*(DT)^m \quad \textbf{(5)}$$

where D is the ammonia dose; and T is the temperature.

Results and Discussion

Microbiological evaluation in PM

Fecal coliforms and *Salmonella spp*. in PM were 2x10^9 NMP/gTS and 9.3x10^4 NMP/gTS, respectively. Both values were above the maximum permissible limit established in NOM-004-SEMARNAT-2002 for classes A, B and C classification. In addition, two species of HE were identified: *Ascaris suum* (Figure 1) and *Trichuris suis* (Figure 2), registering 1152 and 1051 HE/gTS, respectively, which represents a total of 2203 HE/gTS, an amount that exceeds what was reported by Murillo (2016) and Amador (2018), who counted 180 and 100 HE/gTS, respectively. Other authors, such as Khadra *et al*. (2019) report 136 HE/10 g of dry sample (DS) from sludge from a wastewater treatment plant located in Morocco. Grego *et al*. (2018) studied 29 different samples of wastewater obtained in an urban area in India; the results showed concentrations from 0 to 56 HE/L. El Hayany *et al.* (2018) analyzed samples of residual sludge from a wastewater treatment plant located in Chichaoua city; their samples, found concentrations from 15.4 to 37 HE/gDS. Konate *et al*. (2013) evaluated the content of HE in sludge accumulated in anaerobic reactors after four years of operation; this study was carried out in Burkina Faso, and the samples analyzed had a final concentration of 556 HE/gTS. The contrast with the literature mentioned above shows the extreme outbreak of HE that was found in the sample analyzed in the present investigation. The leading cause of said atypical concentration is the origin of the sample since, as mentioned by Montero-López *et al*. (2015) in backyard farms, pigs are usually fed with food waste that is collected by the producer; in addition, in this area, there are no health plans, they do not have biosecurity measures, and far fewer essential programs are carried out for diseases prevention.

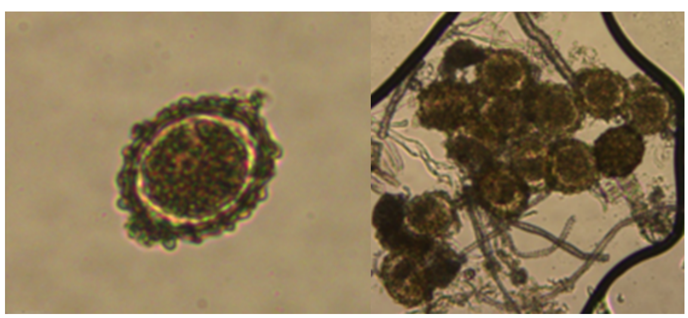

Figure 1. *Ascaris suum* eggs seen under a microscope with a 10x objective.

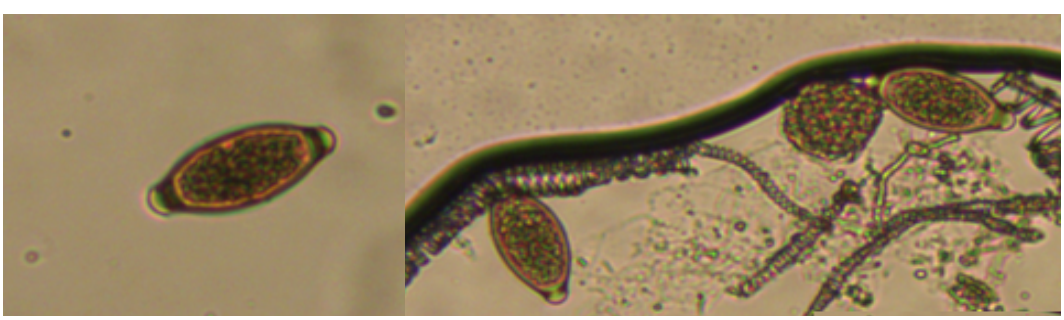

Figure 2. *Trichuris suis* eggs seen under a microscope with a 10x objective.

Alkaline inactivation with variable CaO dose

Table 1 shows the final concentration of HE after alkaline treatment with different amounts of CaO for 2 hours. Figure 3 shows a curve of pseudo first order with the behavior of valleys in which it is observed that the most significant effects occurred with the concentration of 20% CaO, which generated a pH of 12.1, a condition that managed to inactivate 94.7% of HE in both species; such a concentration was similar to that used by Senecal *et al*. (2020), who increased the pH of the sample from 7 to 12.5 for 70 days, however, concluded that pH alone did not affect the inactivation rate of *Ascaris suum* eggs at a temperature of 27.5 °C. On the other hand, Lopes *et al*. (2018) reported that the inactivation in pH> 12 was insufficient to comply with the maximum permissible limits established by the different official standards; Class A (<1 viable egg), Class B (<10), Class C (<35).

Table 1. HE concentration with alkaline treatment (variable CaO).

CaO Concentration	1	2	3	4	Average	Standard deviation
0%	2,104	1,984	2,409	2,315	2,203	193.9
10%	224	219	246	232	230	11.79
15%	176	152	188	173	172	14.97
20%	109	84	164	110	117	33.7

The percentage of HE alkaline inactivation achieved in this research (94.7%), through the destruction and physical involvement of the lipoprotein layers causing lysis that led to the release of cellular material, turned out to be similar to that reported by other authors when using different processes, such as An-nori *et al*. (2020), who used a solar drying process, recorded removal of 92.8% of HE in sludge obtained from a wastewater treatment plant. On the other hand, El Hayani *et al*. (2018) evaluated the effectiveness of composting in eliminating HE in sludge from a wastewater treatment plant for 105 days, achieving a HE reduction of 97.5%. However, Naidoo *et al*. (2020) report that the eggs of *Ascaris spp*. present in fecal sludge, are completely inactivated at temperatures above 50 °C but with much longer exposure times than those used in this research. Pompeo *et al.* (2016) also reported a higher percentage than in this work, obtaining 99.67% inactivation of *Ascaris* spp. present in sewage sludge.

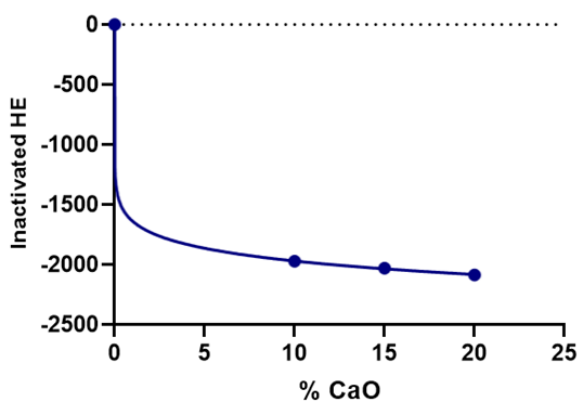

Figure 3. Kinetics of alkaline inactivation of total HE.

Alkaline inactivation with variable time periods

Once the recommended experimental dose was determined, the alkaline inactivation process was carried out with 20% CaO (m/m) and different time periods, as shown in Table 2. The alkaline inactivation kinetics with 0 to 120 minutes can be seen in Figure 4, a curve with valley behavior where it is observed that for the case of total HE, the greatest inactivation was performed with the longest exposure time (120 min); there is a notable difference with respect to inactivated HE between 90 and 120 min. Due to the atypical presence of HE in PM, it was not possible to comply with the maximum permissible limits of the Mexican standard for a class A, B, or C biosolid. However, the inactivation percentage obtained was satisfactory.

Table 2. HE concentration with alkaline treatment (variable time).

Time	1	2	3	4	Average	Standard deviation
0	2,104	1,984	2,409	2,315	2,203	193.9
30	534	354	672	603	541	136.7
60	456	337	564	510	467	97.1
90	401	280	498	447	407	93.2
120	109	84	164	110	117	33.7

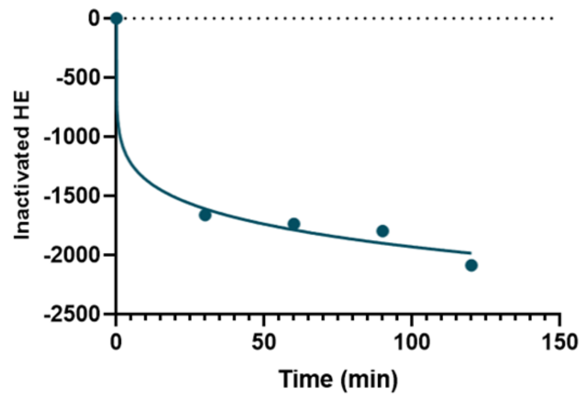

Figure 4. Kinetics of alkaline inactivation of total HE (variable time).

Inactivation by DT factor

Quantifying the pH and temperature during the alkaline inactivation process with CaO allowed obtaining actual ammonia concentrations that contributed to HE inactivation in PM. Table 3 shows the data of the HE inactivation generated by the DT product (Dose and temperature); it can be seen that a higher temperature was generated with a dose of 20% CaO. Therefore, the best result was obtained. DT product (9,963 mg/L°C) since the converted NH_3 was 99.82%, although it was lower than that reported by Méndez *et al*. (2012), who obtained 99.99% of NH_3 converted. However, the greatest inactivation by DT occurred with the 15% dose, eliminating 127 HE in the treatment.

A study by Ogunyoku *et al*. (2016) mentions the importance of the presence of non-ionized ammonia in fresh fecal sludge for the inactivation of *Ascaris* spp. eggs, because when conducting experiments with different concentrations of ammonia, they determined that the conditions to obtain 99% inactivation of HE were 14 days, pH = 12.5, 24 °C, and 6.6 g/L of $N\text{-}NH_3$; this experiment in which the highest concentration of ammonia was used.

Table 3. Relationship of the inactivation of HE generated by the DT product.

Dose CaO	$N\text{-}NH_3$ (mg/L)	pH	% NH_3 converted	Actual dose (mg/L)	Temp. (°C)	DT mg/L (°C)	N/No HE DT	N/No HE CaO	N/No HE (total)
0%	180	7.13	0.81	2	19.5	39	0	0	0
10%	190	11.30	99.09	188	24.5	4,606	-91	-1,882	-1,973
15%	370	11.77	99.69	369	25.5	9,409	-127	-1,904	-2,031
20%	370	12.01	99.82	369	27	9,963	-104	-1,982	-2,086

Figure 5 shows the graph of the total HE inactivation by the destruction or physical involvement of the lipoprotein layers. In addition, the comparison of the inactivation caused by the DT product generated in the alkaline stabilization process with CaO is observed.

As mentioned above, the treatment with CaO was able to inactivate 94.7% HE, while the highest inactivation resulting from the DT product was 5.8%, higher efficiency than that reported by Méndez *et al*. (2012), where it only reached 2%. Based on the above, it can be said that the dose of ammonia and the temperature generated by the treatment with CaO in an open system are not the leading cause of inactivation. However, they contribute insignificantly to the elimination of pathogenic microorganisms.

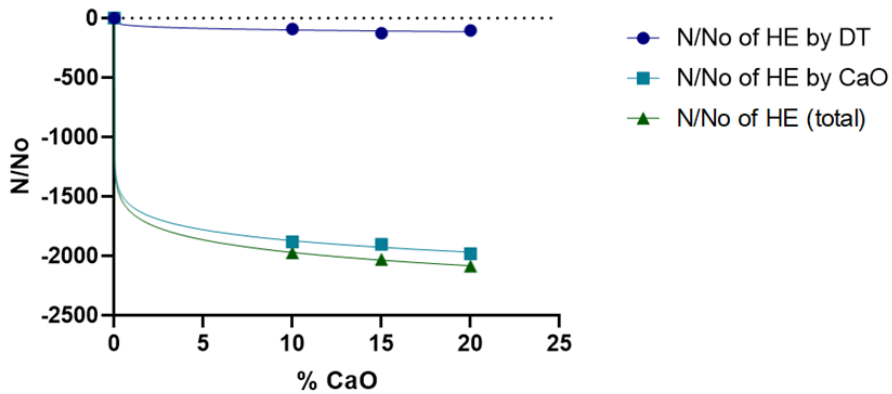

Figure 5. HE eliminated by the different factors evaluated.

Kinetic parameters of HE inactivation

Table 4 shows the kinetic parameters obtained after alkaline inactivation with variable disinfectant doses. The value of R^2 was 0.9924, which indicates that the mathematical model correctly describes the inactivation of the helminths present in PM. The k*= 0.3879 translates into an appropriate rate of inactivation of microorganisms and is higher than that reported by Atenodoro-Alonso *et al*. (2015). They obtained values of 0.01311 and 0.002126 in an improved anaerobic digestion process with pre-treatment and post-thermal treatment, respectively, for the inactivation of *Ascaris* spp. present in sewage sludge. This suggests that alkaline inactivation proceeds faster than thermal inactivation.

Regarding the kinetic parameters of the DT product (see Table 4), it is observed that according to the value of $k*$ = 0.0008479, the inactivation caused by the DT product was very slow, similar to that reported by Méndez *et al*. (2012) who obtained k^* = 0.000924. The value of the constant m^* served to modulate the deviations from the model. It is evident that the R^2 value of the DT product modeling was lower than that of the CaO inactivation modeling; however, R^2 = 0.9463 suggests that it is a reliable model for predicting the inactivation of pathogens in customary treatment conditions.

Table 4. Comparison of the kinetic parameters of the DT product and inactivation with CaO.

Disinfectant	k^*	m	R^2
DT	0.0008479	0.4540	0.9463
CaO	0.3870	0.6024	0.9924

Table 5 shows the kinetic parameters of alkaline inactivation with constant disinfectant dose and variable time; unlike previous models, the value of R^2 was 0.8745, which indicates that the model had less precision. However, it turns out to be equal to the value of R^2 obtained by Espinosa *et al*. (2020), who reported 0.874, when performing multiple regression analysis for HE inactivation data in wastewater, biosolids, and soil, through a thermal treatment with variable time.

Table 5. Kinetic parameters of alkaline inactivation (variable time).

Microorganism	k^{**}	M	R^2
HE total	0.0631	0.6024	0.8745

When performing the mathematical modeling with the Hom model, the values of k^* (alkaline treatment with constant time) and k^{**} (alkaline treatment with constant CaO dose) were obtained and with them the following calculations were made to determine the value of k, which represents the rate of HE inactivation with the complete alkaline treatment.

First stage (variable CaO dose)

$$\log\frac{N}{N_0} = -KD^n t^m \qquad t^m = Constant = 120\ min$$

$$\therefore \log\frac{N}{N_0} = -K^* D^n \qquad y \qquad K^* = Kt^m$$

$$K_1 = \frac{k^*}{t^m}$$

$$k_1 = \frac{0.387}{120^{0.6024}} = 0.0216$$

Second stage (variable time)

$$\log\frac{N}{N_0} = -KD^n t^m \qquad D^n = Constant = 20\%$$

$$\therefore \log\frac{N}{N_0} = -K^{**} t^m \qquad y \qquad K^{**} = KD^n$$

$$K_2 = \frac{k^{**}}{D^n}$$

$$k_2 = \frac{0.0631}{20^{0.3973}} = 0.0192$$

$$\therefore k = \frac{k_1+k_2}{2} = \frac{0.0216+0.0192}{2} = 0.0204$$

From the previous calculations, a k = 0.0204 was obtained, which suggests that the alkaline treatment was developed with a low inactivation rate that is characteristic of the resistance of this type of structure to stabilization processes.

It is worth mentioning that at the end of the alkaline HE inactivation process, the total elimination of fecal coliforms and *salmonella* spp. was obtained because these microorganisms are more sensitive to the different inactivation processes compared to more complex structures such as HE. Some authors report similar results, such as Jafari & Botte (2020), who mention that, by using an electrolysis method in sewage sludge, the elimination of pathogenic bacteria in the product is obtained.

Conclusions

The PM evaluated had high concentrations of pathogenic microorganisms, mainly HE of the species *Ascaris suum* and *Trichuris suis*. The maximum concentration of these microorganisms present in the sample was 2,203 HE/gTS, a value higher than that reported by other authors, which is indicative of the lack of health in raising pigs, specifically in the so-called "backyard farms", which in Mexico, represent one of the most important commercial activities within communities with low socio-economic levels, however, due to the precarious conditions in which they operate, they represent a serious environmental and health problem.

For the alkaline treatment, the dose of disinfectant with which there was a more significant HE inactivation was 20% m/m of CaO; however, it was not enough to inactivate the total concentration of said microorganisms since the treatment with CaO was able to inactivate 94.7% of the eggs of both species present in PM.

Time was an essential factor in the alkaline inactivation process since the most significant elimination of pathogenic microorganisms was recorded in the experiments where the longest exposure time to CaO was operated (120 minutes). In this way, it was inferred that the recommended conditions for the alkaline treatment were 20% m/m CaO for 120 minutes of exposure. It is worth mentioning that the biosolids obtained were close to complying with the maximum permissible limits for class C biosolids, simply a slightly higher contact time is required.

The DT product depended on the dose of CaO applied to the sample. The increase in pH in the alkaline treatment was essential for the inactivation of helminth eggs. However, although the temperature generated in the process did not play a fundamental role in reaching the inactivation achieved, it did compliment the dose of CaO and increase its efficiency. The DT product can be beneficially used in those residues with low concentrations of helminth eggs and high concentrations of pathogenic bacteria, which are significantly more sensitive to the presence of ammonia and changes in temperature in combination with CaO.

The data obtained in the studied inactivation process were used to determine the kinetic parameters with the help of the Hom model, which adequately described the inactivation of HE present in PM. For this reason, it is of the utmost importance to carry out mathematical modeling of this process to contribute to scaling and possible industrial management. Finally, the treatment and modeling strategy presented here can solve atypical and high-risk contamination problems for the population in countries such as Mexico and some other developing countries where this type of health emergency can occur.

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