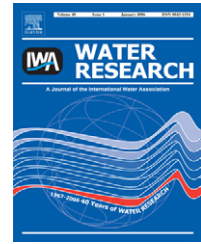


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# The effects of temperature, pH, and ammonia concentration on the inactivation of *Ascaris* eggs in sewage sludge

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## ABSTRACT

The reported inactivation of *Ascaris* eggs during alkaline sludge stabilization is highly variable. The objective of our research was to better understand the sources of this variability by quantifying the effects of temperature, pH, and ammonia concentration on the inactivation of indigenous *Ascaris* eggs in wastewater sludge. Primary sludge was supplemented with ammonia (0, 1000, and 5000 mg/l NH<sub>3</sub>-N) and Ca(OH)<sub>2</sub> and incubated in sealed bottles across the range of temperatures (20, 30, 40, and 50 °C) and pH (7 and 12) that may be encountered during treatment. Changes in egg viability over time were fit to a two-parameter kinetic model (shoulder and first-order region); to compare treatment conditions, the time for 99% inactivation (*t*<sub>99</sub>) was also calculated. Each 10 °C increase in temperature caused a significant decrease in *t*<sub>99</sub> at every pH and ammonia concentration tested. At 50 °C, the effect of temperature was dominant, such that no effect of pH or ammonia was observed. At 30 and 40 °C, raising the pH from 7 to 12 decreased *t*<sub>99</sub>, but at 20 °C no pH effect was seen over 80 d (very little inactivation occurred). At 20, 30, and 40 °C, the addition of ammonia dramatically decreased *t*<sub>99</sub>. The effect of pH could not be completely separated from that of ammonia, as the unamended sludge samples contained 100–200 mg/l indigenous ammonia. Because temperature, pH, and ammonia all contributed to *Ascaris* egg inactivation, it is essential that these parameters are measured and accounted for when assessing the effectiveness of alkaline stabilization. Furthermore, inactivation by ammonia could be exploited to improve the effectiveness of alkaline sludge stabilization.

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## 1. Introduction

Land application is often the preferred option for the disposal of treated sewage sludge (biosolids). To minimize risks to the environment and public health, the US Environmental Protection Agency (USEPA) requires that land applied biosolids be treated to reduce the threat of disease-causing

pathogens. The two classes of biosolids, Class A and B, have no detectable or reduced levels of selected pathogens, respectively. Land application of Class B solids requires additional management to prevent public exposure, but application of Class A and Class B biosolids with management are expected to be equally protective of public health (National Research Council, 2002; USEPA, 1994). Because there

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is often greater public acceptance for Class A biosolids, and because their land application is unrestricted, there is an increasing movement to convert sludge stabilization processes from Class B to Class A (Fackelmann, 2002; Lewis and Gattie, 2002; National Research Council, 2002).

Of the classes of pathogens present in biosolids, helminth eggs are the most resistant to many types of inactivation. Eggs of the genus *Ascaris* have the highest resistance and survive under numerous treatment conditions (Feachem et al., 1983; Gaasenbeek and Borgsteede, 1998; Reimers et al., 1986b). To produce Class A biosolids, the USEPA requires that concentrations of viable helminth ova be reduced to less than one egg per 4 g total solids (TS). Thus, the inactivation of helminth eggs is frequently monitored for regulatory purposes and to measure treatment efficiency (Brewster et al., 2003; Capizzi and Schwartzbrod, 2001; USEPA, 1994).

Alkaline stabilization is one option for sludge treatment, but the reported effectiveness of this option varies greatly. To achieve >90% inactivation of helminth eggs, the reported minimum exposure times vary from 2 h to >180 d (Table 1). Factors that may contribute to this variability include temperature, the type and dose of alkalinizing agent, the maximum pH attained, and the pH profile during storage. In many studies, however, these data are not reported. Inactivation rates of *Ascaris* eggs vary widely over the temperature range used for sludge treatment (20–80 °C), so the maximum temperature attained and the temperature profile during treatment are critically important (Feachem et al., 1983). The choice of alkalinizing agent may also affect the temperature profile. Of the three chemicals most often used for stabilization (CaO, Ca(OH)<sub>2</sub>, and ash), only CaO undergoes an exothermic hydration reaction that produces heat and raises sludge temperatures (Girovich, 1996). The magnitude of this temperature spike is a function of the total solids concentration (%TS), though the profile of the spike is rarely reported. Likewise, the maximum pH attained after alkaline addition and the pH profile during treatment are often not reported. The maximum pH is affected by the quantity of alkalinizing agent added, the %TS and the composition of the sludge itself. Chemical and biological reactions within sludges often decrease pH levels. If inactivation is a function of pH, then treatment efficiencies will vary with the pH profile.

Another factor that may cause variability is ammonia. The concentration of ammonia in sludges varies widely, depending on such factors as the source of sludge, the amount of dilution, and prior treatment and storage. The range of concentrations reported in the literature range from negligible levels up to 7.4 g/l in anaerobically stored agricultural manures (Sutton et al., 1999). Though ammonia concentrations in sludge are rarely reported, its presence could have an important effect on treatment efficiency. The high pH conditions of lime treatment convert NH<sub>4</sub><sup>+</sup> to NH<sub>3</sub>, a chemical species known to inactivate many organisms (Cramer et al., 1983; Jenkins et al., 1998; Warren, 1962).

We previously showed that uncharged ammonia causes inactivation of *Ascaris* eggs, and inactivation was directly proportional to the activity of NH<sub>3</sub> (Pecson and Nelson, 2005). Under the conditions tested (maximum exposure time of 72 h), high pH alone did not cause inactivation, but played an indirect role by converting ammonia into its uncharged form.

These experiments were conducted using buffered laboratory solutions and *Ascaris suum* eggs extracted from pig intestines. The goal of the research reported here was to test these findings with actual sludge containing indigenous helminth eggs, over longer exposure times (up to 80 d), and a wider range of temperatures (20–50 °C). The specific objective was to isolate and quantify the effects of temperature, pH, and ammonia on *Ascaris* egg inactivation in sludge.

## 2. Experimental methods

Plastic bottles were filled with sludge, Ca(OH)<sub>2</sub>, and ammonia and incubated in a water bath at various temperatures. Helminth eggs were extracted from the samples at eight different times during the exposure period, and the number of viable eggs was determined microscopically. The inactivation kinetics for each experimental group were determined and compared to the other groups.

Samples of municipal sludge were collected from an advanced primary treatment plant (using Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> as coagulant) in a peri-urban area near Mexico City. One major advantage of using this sludge was the high concentration of indigenous helminth eggs that allowed us to detect >1.5 log units of inactivation without needing to spike eggs into the reactors. Basic physico-chemical properties of the sludges were measured, including the pH and %TS (determined by Standard Method 2540G (Eaton et al., 1995)) and ammonia concentration (determined by Standard Method 4500-NH<sub>3</sub> by ABC Laboratory, Mexico City). The %TS of the sludges was adjusted to 5% by addition of water. Analytical grade calcium hydroxide (Ca(OH)<sub>2</sub>) was added to achieve various pH levels (J.T. Baker, Mexico, >95% purity). An ammonia stock of 30,000 mg/l as N was prepared from granular NH<sub>4</sub>Cl (J.T. Baker, Mexico, 99.5% purity) and stored at low pH to prevent volatilization. Sludge samples were amended with this stock to achieve supplemental concentrations of 0, 1000, or 5000 mg/l NH<sub>3</sub>-N.

The amended sludge samples were loaded into 125-ml plastic bottles. To prevent volatilization of gases out of the sludge, the headspace was minimized (<1% of the total volume), and the bottles were sealed four times: with a plastic plug, an inner layer of Parafilm, a screw cap, and an outer layer of Parafilm. To compare inactivation in open and closed systems, additional amended samples were made and left unsealed and open to the atmosphere at 20 °C. Samples were placed in a digital water bath and maintained at a constant temperature (Model 1228 Heated Water Bath, VWR, West Chester, PA). After each sampling period, the pH of the sample was measured (Accumet Model 25 pH/ion meter, Fisher Scientific, Pittsburgh, PA) and the samples were immediately processed for determination of viable helminth ova by a modification of the USEPA method (SEMARNAT, 2003). In brief, eggs were isolated from 2 g TS of sludge by blending, sieving, sedimentation, flotation in ZnSO<sub>4</sub>, and extraction with ethyl acetate and acid-alcohol (sulfuric acid/ethyl alcohol), taking care to minimize the exposure of the eggs to the extraction solutions (Nelson and Darby, 2001). Concentrated eggs were incubated for 30 d at 28 °C and viewed microscopically. Eggs containing larvae were counted as viable, while those at all

**Table 1 – Summary of published research on *Ascaris* egg inactivation by alkaline treatment**

Reference	Material	Total solids %	T <sub>max</sub>	Temp. range	Alkaline agent	Dose	pH <sub>max</sub>	pH range	Duration	<i>Ascaris</i> egg inactivation	pH effect isolated? <sup>c</sup>
Mendez et al. (2002) <sup>a</sup>	Sludge	4.7	20 °C	20 °C	NH <sub>4</sub> OH	50% w/w	10.7	NR <sup>b</sup>	2 h	94%	No
	Sludge	4.7	25.1 °C	20.3–25.1 °C	CaO	30% w/w	12.5	NR	2 h	90%	Yes
Gantzer et al. (2001)	Sludge	NR	NR	NR	Ca(OH) <sub>2</sub>	26% w/w	11.9	NR	<1 d	ARL <sup>d</sup>	No
	Sludge	NR	NR	NR	Ca(OH) <sub>2</sub>	65% w/w	11.5	NR	<1 d	ARL	No
	Sludge (dewatered)	NR	NR	NR	CaO	25% w/w	12.4	NR	<1 d	ARL	No
Polprasert and Valencia (1981) <sup>a</sup>	Excreta	10	25 °C	NR	CaO	19 g/l	12	<1 unit	48 h	26.5%	Yes
Kato et al. (2001) <sup>a</sup>	Sludge (digested)	NR	37 °C	37 °C	NaOH+1% (w/v) (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	NR	13	NR	48 h	>99%	No
Plachy et al. (1996)	Sludge (primary)	5	25 °C	21–25 °C	Ca(OH) <sub>2</sub>	10 g/l	12	<1 unit	7 d	3.6%	No
Reimers et al. (1986a) <sup>a</sup>	Sludge (digested)	NR	Amb. <sup>e</sup>	NR	NaOH+50 mg/g TS of (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3200 mg/ g TS	NR	NR	10 d	62%	Yes
Ghiglietti et al. (1997) <sup>a</sup>	Sludge	10	22 °C	22 °C	NH <sub>4</sub> OH	4% v/v	10.5	NR	21 d	>99%	No
Brewster et al. (2003) <sup>a</sup>	Sludge (digested)	28–30	NR	>20 °C	CaO	10% w/w	NR	NR	40 d	>99%	Yes
Eriksen et al. (1996)	Sludge (1ry+2ry)	20	45 °C	Amb.-45 °C	CaO	10% w/w	>12	Always >12	70 d	>99%	No
Schuh et al. (1984)	Sludge (digested)	6	Amb.	NR	Ca(OH) <sub>2</sub>	NR	12.5	8.7–12.5	98 d	98.50%	Yes
Gantzer et al. (2001)	Sludge	NR	NR	NR	Ca(OH) <sub>2</sub>	62% w/w	11.2	NR	180 d	BRL <sup>f</sup>	No

<sup>a</sup> Additional treatment conditions were tested that are not listed in the table.

<sup>b</sup> NR: not reported.

<sup>c</sup> The effect of pH was isolated by studying inactivation over a range of pH levels or by comparison with a neutral pH sludge control.

<sup>d</sup> ARL: concentration above regulatory limit of <3 viable nematode eggs/10 g dry material.

<sup>e</sup> Amb.: ambient temperature.

<sup>f</sup> BRL: concentration reduced below regulatory limit of <3 viable nematode eggs/10 g dry material.

pre-larval stages were not. The experimental conditions for each sample are summarized in Table 2.

Inactivation rate kinetics for all data sets were analyzed using a model for shouldered survival curves (Harm, 1980). This model is used to analyze concave inactivation curves including those exhibiting a lag before a first-order inactivation region.

$$N = N_0[1 - (1 - \exp(kt))^m], \quad (1)$$

$$\text{Lag period} = (\ln(m))/k, \quad (2)$$

where  $N_0$  is the number of viable eggs at time zero,  $N$  is the number of viable eggs at time  $t$ ,  $k$  is the first-order rate constant, and  $m$  is an empirical value used to determine the lag period. Statistical analyses were performed using Kaleidagraph (Reading, Pennsylvania) and JMP Statistical Software (SAS Institute, Inc., Cary, NC). Inactivation parameters were determined by fitting the model to graphs of  $\ln(N/N_0)$  and

minimizing the residual sum of squares. Many samples had no statistically significant lag-period according to this model. For these data sets, the model was constrained with  $m$  equal to one, which reduced the model to a linear regression with the  $y$ -intercept set to zero.

### 3. Results

The basic physico-chemical characteristics of the sludges and their helminth egg concentrations are listed in Table 3. Sludge 1 was used for the 20 and 30 °C experiments while Sludge 2 was used at 40 and 50 °C. At high pH, the hydrolysis of organic nitrogen sources can increase the total ammonia concentration within sludges (Bremner, 1949; Siddaramappa et al., 1994). Therefore, ammonia concentrations were measured in all of the unamended pH 12 samples (Table 3) at the end of

**Table 2 – Summary of experimental conditions for sludge samples**

Temperature	pH	Supplemental ammonia conc. (mg/l as N)	Sampling times	Other
20 °C	7	0	0–40 d (every 5 days), 80 d	Closed
	12	0	0–40 d (every 5 days), 80 d	Closed
	12	1000	0–40 d (every 5 days), 80 d	Closed
	12	1000	0–40 d (every 5 days), 80 d	Open
	12	5000	0–40 d (every 5 days), 80 d	Closed
	12	5000	0–40 d (every 5 days), 80 d	Open
30 °C	7	0	0–24 d (every 3 days)	Closed
	12	0	0–24 d (every 3 days)	Closed
	12	1000	0–16 d (every 2 days)	Closed
	12	5000	0–8 d (every day)	Closed
40 °C	7	0	0–16 d (every 4 days)	Closed
	12	0	0–36 h (every 4.5 h)	Closed
	12	5000	0–12 h (every 1.5 h)	Closed
50 °C	7	0	0–2 h (every 15 min)	Closed
	12	0	0–2 h (every 15 min)	Closed
	12	5000	0–2 h (every 15 min)	Closed

**Table 3 – Physical/chemical and pathogenic characteristics of sludges used**

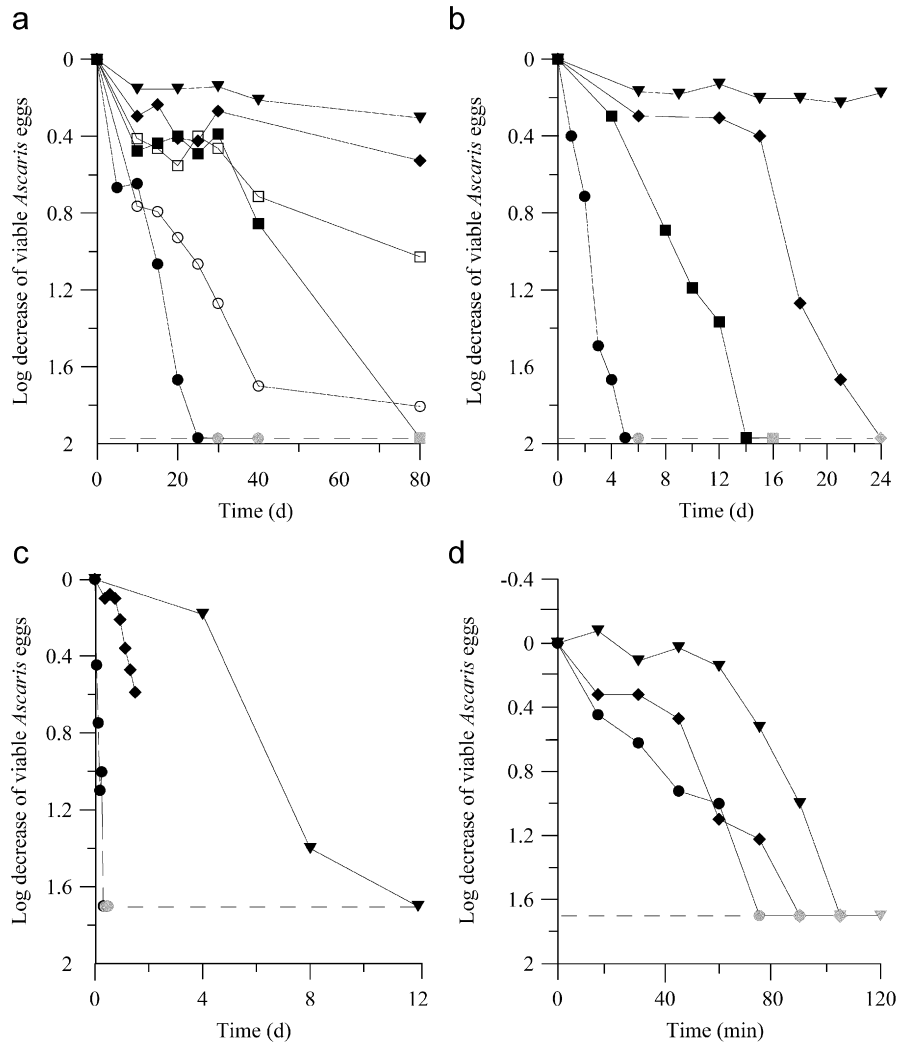
Sample	pH	TS% initial	Ammonia conc. of pH 12 samples (mg/l)	Total helminth eggs/2 g total solids	Viable helminth eggs/2 g total solids	% of viable eggs by species		
						Ascaris spp.	Toxocara spp.	Trichuris spp.+others
Sludge 1	5.5	6.5	240 (20 °C)	173.5	105.4	88.2	4.1	7.7
			220 (30 °C)					
Sludge 2	5.2	6.0	170 (40 °C)	77.7	54.7	92.1	3.7	4.3
			130 (50 °C)					

each exposure period. Total ammonia concentrations ranged from 130 to 240 mg/l NH<sub>3</sub>-N. The difference in ammonia concentrations could be due to the longer exposure times used at the lower temperatures (Sludges 1 and 2 were treated for longer times at 20 °C than 30 °C, and at 40 °C than 50 °C, respectively). This may have allowed for more complete hydrolysis of organic nitrogen, resulting in higher ammonia concentrations at the lower temperatures. The amended sludge samples contained an additional concentration of 1000 or 5000 mg/l NH<sub>3</sub>-N.

The inactivation kinetics were only determined for *Ascaris* spp., which represented ~90% of the total viable helminths (Table 3). Because the number of non-*Ascaris* helminth eggs in the sludges was very low (<10 eggs of other species in any sample), it was not possible to develop inactivation profiles or perform statistical analyses. Because the criterion for *Ascaris* egg viability was the presence of a larva after 30-d incubation, it was important to quantify the number of larvated eggs in the raw sludges before incubation. Triplicate samples of each

sludge averaged <0.2 larvated *Ascaris* eggs/g TS before incubation. These eggs would have represented only 0.36% and 0.66% of the viable *Ascaris* eggs after incubation, in Sludge 1 and 2, respectively. Thus, their presence did not have a significant effect on our assessment of the effectiveness of treatment on viability.

The inactivation profiles for all samples are shown in Fig. 1. The length of the lag-period and the pseudo-first-order inactivation rate constants were determined for every treatment condition according to Eqs. (1) and (2) (Table 4). Some of the treatments only caused a minimal decrease in viable helminth eggs over the period tested (samples unamended with ammonia at pH 7 and 12 at 20 °C, and at pH 7 at 30 °C; Fig. 1a and b). Ideally, measurements would have been taken over a longer exposure period to capture the inactivation profiles of these samples. A priori we believed that the exposure times tested would have been sufficient for the pH 12 samples. However, because the process of determining viable helminth eggs in sludges takes >30 d, we could not verify that



**Fig. 1 – Log decrease of viable *Ascaris* eggs after treatment at various temperatures: (a) 20 °C, (b) 30 °C, (c) 40 °C, (d) 50 °C. Symbols: pH 7, 0 amm: (▼); pH 12, 0 amm: (◆); pH 12, 1000 mg/l amm, closed system: (■); pH 12, 1000 mg/l amm, open system: (□); pH 12, 5000 mg/l amm, closed system: (●); pH 12, 5000 mg/l amm, open system: (○). Dotted line depicts detection limit. Gray symbols depict values below limit of detection.**

**Table 4 – Pseudo first-order inactivation rate constants and lag periods for *Ascaris* egg inactivation (Eqs. (1) and (2)). Parentheses indicate 95% confidence interval. <sup>a</sup>All data were rounded to two significant figures**

Sample pH	Added ammonia (mg/l)	$k$ (day <sup>-1</sup> )	$m$	Lag period	$t_{99}^b$	Comparison of $t_{99}$ with pH 12 samples <sup>d</sup>		
						0	1000	5000
20 °C								
7	0	0.010 (0.004)	1	NS	450 d <sup>c</sup>	NS	5.1 ×	18 ×
12	0	0.020 (0.011)	1	NS	230 d <sup>c</sup>			
12	1000	0.053 (0.009)	1	NS	87 d			
12	5000	0.18 (0.03)	1	NS	25 d			
30 °C								
7	0	0.025 (0.008)	1	NS	180 d <sup>c</sup>	7.8 ×	11 ×	38 ×
12	0	0.38 (0.15)	84	12 (9) d	24 d			
12	1000	0.28 (0.04)	1	NS	16 d			
12	5000	0.96 (0.11)	1	NS	4.8 d			
40 °C								
7	0	0.33 (0.18)	1	NS	14 d	4.1 ×		36 ×
12	0	1.8 (0.5)	4.3	0.81 (0.44) d	3.4 d			
12	5000	12 (2)	1	NS	0.39 d			
50 °C								
7	0	130 (20)	406	64 (19) min	110 min	NS		NS
12	0	56 (10)	1	NS	120 min			
12	5000	68 (12)	1	NS	97 min			

<sup>a</sup> NS = not statistically significant ( $\alpha = 0.05$ ).

<sup>b</sup>  $t_{99}$  = time needed for 99% inactivation of *Ascaris* eggs.

<sup>c</sup>  $t_{99}$  may be overestimated if a higher inactivation rate occurs at longer exposure periods (beyond the 80-d experimental period at 20 °C, or the 24-d period at 30 °C).

<sup>d</sup> Difference in  $t_{99}$  between samples is shown.

inactivation was occurring during the course of the experiment. This slow response time underlines the need for more rapid methods for determining helminth egg viability (Pecson et al., 2006).

Using the inactivation parameters, the time needed to inactivate 99% of the *Ascaris* eggs ( $t_{99}$ ) was calculated (Table 4). In many countries, Class A solids are defined by the number of viable helminth eggs per mass of solids (Norwegian Ministry of Health and Social Welfare and Ministry of the Environment, 1995; SEMARNAT, 2003; USEPA, 1994). A 99% reduction would be sufficient to bring commonly encountered concentrations of helminth ova below regulatory limits in the US and Mexico (Jimenez et al., 2004; Reimers et al., 2001). Therefore, these data can be used as a rough estimate to compare treatment conditions needed to produce Class A biosolids by alkaline stabilization. The model parameters listed in Table 4 can be used to estimate the times needed to achieve other treatment goals.

### 3.1. Effect of temperature

The temperature of the samples was tightly controlled by incubation in water baths. Ca(OH)<sub>2</sub> was used instead of CaO to avoid the temperature spike generated by reaction of CaO with water. The inactivation rate of helminth eggs was strongly dependent on temperature, in agreement with ear-

lier studies. In all cases, each 10 °C increase in temperature caused a statistically significant decrease in  $t_{99}$ . At pH 7 and 12 with zero added ammonia,  $t_{99}$  decreased from several hundred days at 20 °C to about 100 min at 50 °C (Table 4). In the samples amended with ammonia, large decreases were also seen as the temperature was increased. At 50 °C, the effect of temperature became dominant, and no significant difference in  $t_{99}$  values was observed with a change in pH or with addition of 5000 mg/l ammonia.

### 3.2. Effect of pH

All of the pH 12 closed samples maintained their initial pH levels throughout the entire exposure period; in pH 7 samples it was necessary to add Ca(OH)<sub>2</sub> periodically. At 30 and 40 °C, there was a significant increase in inactivation rates between the unamended pH 7 and 12 samples (Table 4; Fig. 1b and c). It should be noted that the effect of pH at 20 °C remains somewhat unclear, however, because only a minimal decrease in viable eggs was observed at pH 7 and 12 in the unamended samples. In addition, if an increase in the inactivation rate occurs at longer exposure periods, the  $t_{99}$  values of unamended samples at 20 °C, and at pH 7 at 30 °C, may be significantly lower than our estimates. As stated above, no pH effect was observed at 50 °C, because temperature was dominant (Fig. 1d).

### 3.3. Effect of ammonia in sludge

The addition of ammonia to sludges caused a significant increase in inactivation rates that was dose-dependent. The addition of 1000 mg/l  $\text{NH}_3\text{-N}$  to pH 12 samples caused a significant decrease in  $t_{99}$  at 20 °C, as did the addition of 5000 mg/l  $\text{NH}_3\text{-N}$  at all temperatures except 50 °C. With 5000 mg/l  $\text{NH}_3\text{-N}$ , pH 12 samples had a  $7.5 \times$  average decrease in  $t_{99}$  compared to samples with no added ammonia over the range from 20 to 40 °C (Table 4).

At 20 °C, both open and closed samples were used to test if covering the samples affected the inactivation rate. The closed samples experienced increased inactivation compared to the open samples at both ammonia concentrations tested (Fig. 1a). The open pH 12 sample amended with 1000 mg/l  $\text{NH}_3\text{-N}$  maintained its initial pH level throughout the experiment, but the sample amended with 5000 mg/l  $\text{NH}_3\text{-N}$  decreased from pH 12.1 to 11.1 at day 10, to pH 10 at day 40, and to pH 9.8 at day 80. This pH decrease was most likely the result of ammonia volatilization. Thus, the open samples were subjected to reductions in at least two important mechanisms of inactivation (pH and ammonia), which contributed to their decreased levels of inactivation.

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## 4. Discussion

The presence of ammonia, at concentrations that may be encountered in sludges, significantly increased *Ascaris* egg inactivation at 20, 30, and 40 °C. The effect of ammonia was dose-dependent and separate from the effect of pH, as ammonia-amended samples had significantly faster inactivation than unamended samples at the same pH. At 50 °C, the inactivating effect of heat dominated such that no pH or ammonia effect was observed.

In most cases, the inactivation rates of indigenous *Ascaris* eggs in sludges were faster than those of eggs isolated from pig intestines inactivated in buffered solutions. We previously measured inactivation in buffered solutions at three pH levels (7, 9 and 11) (Pecson and Nelson, 2005). The highly basic pH 11 used in that study and the pH 12 treatment used here both converted  $\geq 99\%$  of the total ammonia into the uncharged form at temperatures above 30 °C. The use of pH 11 in the current work was not feasible because sludge pH typically decreases if not maintained at or above pH 12 (Lue-Hing, 1998). We wanted to avoid opening and adjusting the pH of the ammonia-amended samples to prevent ammonia loss from volatilization; thus pH 12 was used instead. We believe, however, that the conditions are similar enough for direct comparison.

With 5000 mg/l  $\text{NH}_3\text{-N}$ , less than 34% *Ascaris* egg inactivation occurred after 72 h in a buffered solution at 30 °C (pH 11, unpublished results (Pecson and Nelson, 2005)), whereas 94% inactivation occurred after the same exposure in sludge (pH 12). Increased rates in sludge were observed not only in ammonia-containing samples, but also in the high pH controls. For example, with no added ammonia, less than 57% inactivation occurred after 72 h in buffered solutions at 40 °C (pH 11), whereas  $\sim 98\%$  inactivation occurred in sludge (pH 12). These findings are supported by Schuh et al. (1984), who also documented higher inactivation in sludge samples

compared to control solutions of equal pH. At higher temperatures ( $\geq 50$  °C), the lack of a significant pH effect in sludges was in agreement with our results using buffered laboratory solutions (Pecson and Nelson, 2005). Thus, laboratory studies conducted with controlled solutions and *A. suum* eggs from pig intestines are useful for elucidating inactivation mechanisms, but may underestimate inactivation rates of indigenous *Ascaris* in actual sludge in most cases. We suspect that the higher rates in sludge are due to the presence of additional compounds (organic acids, aldehydes, and alcohols) that contribute to inactivation (Reimers et al., 2001). However, we cannot rule out the possibility that the indigenous sludge eggs were more susceptible to inactivation than those isolated from pig intestines.

The ammonia present within the sludges may not all have been available for helminth inactivation. Ammonia is known to undergo various reactions in sludge, including ion-exchange onto substrates such as clays, adsorption onto organic matter, and biological nitrification in the presence of oxygen (Gerardi, 2002; Hedstrom, 2001). These reactions would decrease the amount that is available for inactivation. In addition, the presence of other sludge chemicals may increase inactivation (Reimers et al., 2001). Thus, comparing inactivation at varying ammonia concentrations allowed us to isolate and quantify its effect on inactivation rates.

It is important to note that the effect of ammonia may not have been completely separated from the effect of pH due to the presence of ammonia in the unamended samples. Ideally, high pH control samples that were completely devoid of ammonia would have been used. However, it was decided that any process to remove all of the ammonia from the sludge would have introduced too much variability. For example, the entire batch of raw sludge could have been stripped of ammonia by stirring at high pH. However, this process may have altered the resistance of the helminth eggs and changed the chemical composition of the sludge. Furthermore, it may not have removed all the organic nitrogen that might subsequently have been mineralized into ammonia at high pH. Neutralizing the high pH sludge to produce pH 7 control samples would require additional chemicals that would add one more layer of variability to the experiment. Therefore, the raw sludges were not stripped of ammonia but were used unaltered.

It is possible that the indigenous ammonia present in the sludges could have contributed to inactivation in the unamended control samples. In an earlier study, we found that 200 mg/l of ammonia caused an increase in inactivation over a narrow range of temperatures (45–46 °C) at pH 11, whereas samples amended with 100 mg  $\text{NH}_3\text{-N/l}$  were not statistically different from ammonia-free controls (Pecson and Nelson, 2005). These experiments were only conducted over a 24-h period. It is possible that over longer exposure periods, these low ammonia concentrations may increase inactivation, even at lower temperatures.

The effect of ammonia addition was not tested at pH 7 because ammonia inactivation is dependent on the activity of  $\text{NH}_3$ , and this species is only present at 0.4–1.5% of the total ammonia at temperatures from 20 to 40 °C (Pecson and Nelson, 2005). The concentration of  $\text{NH}_3$  at pH 7 amended with 5000 mg/l  $\text{NH}_3\text{-N}$  would range from 20 to 76 mg/l.

Our results are consistent with earlier studies that investigated the effect of ammonia addition on *Ascaris* egg survival during alkaline treatment (Table 1). Reimers et al. (1986a) reported ~60% inactivation after 10 d in high pH sludges amended with 50 mg/g TS  $(\text{NH}_4)_2\text{SO}_4$  (~2000 mg/l  $\text{NH}_3$ ), a level of inactivation that falls in between our results for samples with 1000 and 5000 mg/l  $\text{NH}_3$  at pH 12 and 20 °C (Reimers et al., 1986a). Likewise, Kato et al. (2001) reported >99% inactivation after 48 h in 37 °C pH 13 sludge amended with 2600 mg/l  $\text{NH}_3$ , which also falls in between our 0 and 5000 mg/l results at pH 12 and 40 °C (Kato et al., 2001).

Two other studies also investigated the effect of ammonia on *Ascaris* egg inactivation (Ghiglietti et al., 1997; Mendez et al., 2002) (Table 1); in these experiments, however, high pH controls were not used so it was difficult to separate the effect of ammonia from pH. Both studies used  $\text{NH}_4\text{OH}$  to alkalize the sludge, so all high pH samples also contained high concentrations of ammonia. While the high pH control used in the current study contained some ammonia, the concentrations were at least 10–20 times lower than the two earlier studies (130–240 mg/l in the current study compared to minimum ammonia concentrations of ~3000 mg/l in Ghiglietti et al. (1997) and ~5000 mg/l in Mendez et al. (2002)). Thus, the results of the current study can be used to reexamine the earlier studies to determine if pH and ammonia effects can be separated.

Ghiglietti et al. (1997) analyzed *Ascaris* egg inactivation in sludge with one of the samples containing an  $\text{NH}_3$  concentration comparable to the 5000 mg/l sample used here. At 22 °C, their sludge sample with 2%  $\text{NH}_4\text{OH}$  v/w (containing ~4800 mg/l of uncharged  $\text{NH}_3$  at pH 10) had 96% inactivation after 40 days. Under similar conditions, we found 99% inactivation after 26 d with 5000 mg/l  $\text{NH}_3\text{-N}$ , and ~70% inactivation after 80 d with 0 mg/l  $\text{NH}_3\text{-N}$  (pH 12 samples at 20 °C). Because the sample in Ghiglietti et al. (1997) was inactivated much faster than our high pH control (~40 d vs. >80 d), it suggests that they observed inactivation caused not by pH alone, but also ammonia.

Mendez et al. (2002) also treated one sludge sample with ~5000 mg/l  $\text{NH}_3\text{-N}$ . Despite the fact that this concentration of  $\text{NH}_4\text{OH}$  only raised the pH to 9.74 (at 20 °C), they found ~69% inactivation after 2 h treatment; conversely, we found no significant decrease in eggs under similar conditions (pH 12, 5000 mg/l added ammonia, incubated for 2 h at 20 °C). This discrepancy may be an effect of differences in the experimental protocol. In Mendez et al. (2002) the samples were stirred at 300 rpm during the entirety of the exposure period (2 h), whereas in the current study, the samples were not stirred after initial mixing. This stirring effect may also explain the discrepancy between the pH controls of the two studies. In that study, sludge adjusted to pH 12 with CaO and stirred for 2 h caused 88% inactivation at 24.3 °C, whereas our unamended pH 12 sample had no significant decrease after 2 h at 20 or 30 °C. Additional research is currently underway to more thoroughly describe the effect of stirring on helminth egg viability.

Most earlier studies on alkaline treatment efficiency did not measure the effect of ammonia, but focused mainly on temperature and pH. In some cases, the results of our unamended high pH samples were consistent with previously

published results. Polprasert and Valencia (1981) found ~27% inactivation at 25 °C after 48 h treatment of excreta whereas in the same period we found 29% inactivation at 30 °C. The use of CaO by Polprasert and Valencia (1981) may have caused a temperature spike. Plachy et al. (1996) found only ~4% inactivation after 7 d treatment at 21–25 °C, whereas we found ~7% after the same exposure time at 20 °C. Eriksen et al. (1996) found 99% inactivation after 70 d at an average temperature between ambient levels (exact temperature not reported) and 30 °C which was consistent with our results (results of Eriksen et al. (1996) fall in between the 24 d needed for 99% inactivation at 30 °C and >80 d needed at 20 °C).

In other cases, our results were not consistent with previous studies. Brewster et al. (2003) and Schuh et al. (1984) required 40 and 98 d, respectively, for >99% inactivation at ~20 °C. Our unamended pH 12 sample had little inactivation after 80 d, and required 230 d for 99% inactivation based on kinetic estimates. The increased rate of inactivation observed in these studies cannot be accounted for by pH alone, but may be the effect of the presence of ammonia, temperature spikes after CaO addition, desiccation, or other inactivating chemicals in the sludges that were not present in the sludges we used. According to our research, differences in sludge ammonia concentrations alone could explain the variation in inactivation rates (Fig. 1).

The different conditions used in sludge experiments may also lead to varying results. In the developed world, where many studies on sludge treatment are performed, the incidence of helminthiasis is typically low. Consequently, sludges in these countries generally contain far lower concentrations of helminth eggs compared to those of the developing world (Jimenez et al., 2002, 2004; Reimers et al., 2001; Stott et al., 1994; Theis et al., 1978). Due to the low concentrations of eggs in sludges in the developed world, many studies measure inactivation over a narrow range (<1 log unit) (Gantzer et al., 2001; Keller et al., 2004; Mignotte-Cadiergues et al., 2001). The validity of extrapolating these results to higher levels of inactivation (>1 log unit) is unclear. To measure higher levels of inactivation, studies are often performed by spiking reactors with large numbers of helminth eggs that have been extracted from the uteri of worms. However, it is not clear that the resistance of these dissected eggs is as high as those that are excreted naturally or isolated from the intestines of their hosts (Oksanen et al., 1990; Wharton, 1980, 1983), so the use of purified egg stocks may overestimate treatment efficiency. This study avoided these potential limitations by using sludges with relatively high concentrations of indigenous helminth eggs. Because this research was performed under these conditions, the results should be most applicable to actual treatment conditions.

Given the importance of ammonia on helminth egg inactivation, future studies on alkaline treatment should consider the effects of not only pH and temperature, but also ammonia. The effect of each of these parameters should be isolated as much as possible by the use of appropriate controls. At a minimum, the values of pH, temperature (including any temperature spike due to addition of CaO), and ammonia should be reported, including their variation throughout the treatment period.

To maximize the inactivation of *Ascaris* eggs during alkaline stabilization of sludges containing ammonia, reactors should be closed to prevent ammonia volatilization (Mendez et al., 2002). Other potential benefits of covering reactors include retaining heat and capturing ammonia off-gases for use as fertilizers (Girovich, 1996). In some cases, it may be necessary to cover reactors just to control the release of the malodorous and potentially toxic ammonia gas (Girovich, 1996).

Due to the importance of ammonia in alkaline systems, it would be beneficial to reassess the USEPA treatment requirements for the production of Class A biosolids by alkaline treatment. Currently, they require specific temperature, time, and pH conditions, but they do not account for the effect of differences in ammonia concentrations. Incorporating ammonia inactivation may be a way to reduce other requirements, such as shorter treatment times, lower temperatures, or even lower pH (as long as most ammonia is in neutral form). Further research is needed to characterize the effect of ammonia on viruses in sludge (Cramer et al., 1983) to ensure that virus inactivation is still adequate under alternative treatment conditions.

## 5. Conclusions

- The inactivation rate of indigenous *Ascaris* eggs in wastewater sludge was dependent on temperature, pH, and ammonia concentration.
- At every pH and ammonia concentration tested, each 10 °C increase in temperature (20–50 °C) caused a significant decrease in  $t_{99}$ .
- At 30 and 40 °C, increasing the pH from 7 to 12 caused a significant decrease in  $t_{99}$ .
- At 20, 30, and 40 °C, ammonia caused a dose-dependent increase in inactivation and led to a 7.5 × decrease in  $t_{99}$  at the highest ammonia concentration tested (5000 mg/l NH<sub>3</sub>-N) compared to unamended sludge.
- Differences in temperature, pH, and ammonia may help to explain some of the variability in *Ascaris* inactivation observed in previous studies.
- Inactivation by ammonia could be exploited to improve the effectiveness of alkaline sludge stabilization.

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