Current time-temperature relationships for thermal inactivation of *Ascaris* eggs at mesophilic temperatures are too conservative and may hamper development of simple, but effective sanitation

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**A R T I C L E  I N F O**

Article history:
Received 1 March 2019
Received in revised form 19 August 2019
Accepted 30 August 2019
Available online 3 September 2019

**Keywords:**
Ascaris
Pathogens
Mesophilic
Thermal inactivation
Sanitation
Fecal sludge

**A B S T R A C T**

Ascaris eggs are commonly used as indicators for pathogen inactivation during the treatment of fecal sludge and wastewater due to their highly resistant lipid membrane and ability to survive in the environment for long periods of time. Current guidelines suggest that thermal treatment alone cannot inactivate *Ascaris* eggs at temperatures below 45 °C, although some evidence in the literature suggests this to be incorrect. Here, we performed a controlled experiment to test the effect of mesophilic temperatures on *Ascaris* inactivation. We exposed *Ascaris suum* eggs to a temperature gradient between 34 °C and 45 °C under anaerobic and aerobic conditions to observe the required exposure times for a 3-log reduction. Indeed, we found that temperatures lower than 45 °C did inactivate these eggs, and the required exposure times were up to two orders of magnitude shorter than suggested by current guidelines. Results from the anaerobic exposures were used to develop a time-temperature relationship that is appropriate for *Ascaris* inactivation at mesophilic temperatures. Data from the literature demonstrated that our relationship is conservative, with faster inactivation occurring under environmental conditions when *Ascaris* eggs were suspended in fecal sludge or manure. A specific aerobic relationship was not developed, but we demonstrated that aerobic conditions cause faster inactivation than anaerobic conditions. Therefore, the anaerobic relationship provides a conservative guideline for both conditions. We demonstrate that relatively low temperatures can considerably impact *Ascaris* viability and suggest that mesophilic temperatures can be used in waste treatment processes to inactivate pathogens. The development of safe, low-input, mesophilic treatment processes is particularly valuable for ensuring universal access to safe sanitation and excreta management.

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1. Introduction

The intestinal roundworm *Ascaris lumbricoides* is widely considered to be the most resistant pathogen to disinfection processes in human fecal sludge and wastewater (Feachem et al., 1983; US EPA, 2003). *A. lumbricoides* infects approximately 1.3 billion people worldwide and is spread by fecal-oral transmission, particularly in areas with poor access to sanitation (De Silva et al., 1997). Eggs are passed into the environment through feces and have been shown to survive and retain infectivity for many years (Brudastov et al., 1970). Due to their extreme resistance, *Ascaris* eggs are often used as indicators of pathogen inactivation when testing treatment methods for wastewater and fecal sludge.

The resistance of *Ascaris* eggs to thermal inactivation is of particular interest here. Time-temperature recommendations for using heat to inactivate fecal pathogens are provided by Feachem et al. (1983) and the US Environmental Protection Agency (US EPA) 40 CFR Part 503 regulations (US EPA, 2003). Both guidelines consider *Ascaris* eggs to be among the most resistant pathogens to thermal inactivation (Feachem et al., 1983; US EPA, 2003). According to the US EPA regulations, *Ascaris* viability is not substantially reduced at temperatures between 32 °C and 38 °C, and complete reduction at 38°C–50°C is still not guaranteed (US EPA, 2003). Therefore, Feachem et al. and the US EPA suggest a minimum temperature of 45 °C and 50 °C, respectively, for any thermal
Ascaris eggs have been shown to form mobile larvae when incubated aerobically at temperatures up to 34 °C (Arene, 1986). For each treatment, 1000 A. suum eggs were suspended in 1.5 mL 0.1 N H₂SO₄ in a 2.0-mL microcentrifuge tube. Anaerobic and aerobic conditions were tested. The tube caps for all treatments were left open and sealed with Parafilm (Bemis NA; Neenah, WI) to allow for gas exchange. Tubes for anaerobic treatments were kept in gas-tight chambers that were made anaerobic using GasPak EZ Anaerobe Sachets (BD, Sparks, MD), and indicator strips (BD, Sparks, MD) were used to confirm anaerobic conditions. Tubes were stored in the dark in incubators, and temperatures were monitored using data loggers (HOBO UX100-003, Bourne, MA and Lascar Electronics EL-USB-1, Eerie, PA). All treatments were performed in duplicate.

After the designated exposure times, eggs were removed from incubators and transferred to 24-well plates, where they were suspended in 0.1 N H₂SO₄ and incubated aerobically at 28 °C for 3 weeks in the dark to promote larval development. The 24-well plates were monitored throughout the incubation period, and additional 0.1 N H₂SO₄ was added as needed to replace evaporated solution. Eggs were examined microscopically before and after the 3-week incubation. For eggs treated under anaerobic conditions, pre-incubation examination confirmed that they had not begun developing during treatment but would have had limited oxygen exposure. For all treatments, viability was quantified after the 3-week incubation period. Approximately 500 eggs were examined in each well. Eggs that had formed larvae were considered viable, and all others were considered nonviable. If no viable eggs were found in the initial 500, the remaining eggs in the well were examined, to a maximum of 1000 eggs. Reported percent viabilities are normalized to the baseline viability of the A. suum eggs used for a given trial (Table 1). Baseline viability was determined by incubating A. suum eggs aerobically at 28 °C for 3 weeks with no prior treatment.

When chemically or thermally treating Ascaris eggs, a lag period is expected with minimal loss of viability followed by a region of exponential inactivation (Nordin et al., 2009; Pecson et al., 2007). For most treatments in the present study, the initial sampling time occurred after the lag period. Therefore, only exponential decline was observed, and inactivation was modeled using linear regression,

\[
\ln \left( \frac{N}{N_0} \right) = -kt + b
\]  

(1)

where \(N_0\) is the baseline percent viability of the A. suum eggs used for the trial (Table 1), \(N\) is the percent viability of the A. suum eggs exposed to a given temperature for time \(t\) in days, \(k\) is the first-order inactivation constant, and \(b\) is the y-intercept, which gives some indication of the expected but unobserved lag period. If viability was below the detection limit for multiple exposure times, then only the shortest time was used in the regression analysis, and viability at that time was assumed to equal the detection limit. The linear regression equations for each temperature were then used to calculate the exposure time required to achieve a 3-log reduction in viability (\(t_3\)).

Next, linear regression of the base-ten logarithms of the anaerobic \(t_3\) values versus temperature were used to develop a new time-temperature relationship for Ascaris inactivation at mesophilic temperatures. The upper limit of the 95% confidence interval for each \(t_3\) value was used to develop a conservative relationship, and a literature review was performed to compile additional evidence of Ascaris inactivation at temperatures below 45 °C. All statistical analysis was performed using R (R Core Team, 2018), and confidence intervals were calculated using the chemCal package (Ranke, 2018).
Finally, we compared our time-temperature relationship to relationships provided by the Feachem guidelines and the US EPA Part 503 guidelines for producing Class A biosolids (Feachem et al., 1983; US EPA, 2003). Feachem et al. (1983) provides a figure with a “zone of safety” of time-temperature combinations that are expected to cause inactivation of Ascaris eggs based on literature studies, but the detection limits or degrees of inactivation in these studies are not known. The equation for the boundary of the “zone of safety” is also not given but was derived elsewhere (Equation (2)), where \( t_{\text{Feachem}} \) is the required exposure time in hours, and \( T \) is the temperature in °C (Vinnerås et al., 2003).

\[
t_{\text{Feachem}} = \left( 1.77 \times 10^2 \right) \times 10^{-0.1944(T-45)}
\]  

(2)

The US EPA recommended exposure time was calculated based on the time-temperature equation for sewage sludge with less than 7% total solids and a contact time of at least 30 min (Equation (3)), where \( t_{\text{EPA}} \) is the required exposure time in days, and \( T \) is the temperature in °C (US EPA, 2003).

\[
t_{\text{EPA}} = \left( 5.007 \times 10^7 \right) \times 10^{-0.147T}
\]  

(3)

Equation (3) is expected to create Class A biosolids that contain less than 1 viable Ascaris egg per g of biosolids (dry weight basis). Equation (3) represents the least stringent of the four time-temperature relationships given by the EPA and was chosen here because our experimental data was collected with Ascaris eggs suspended in aqueous solution. The corresponding log reduction would depend on the initial concentration of viable eggs in the biosolids. It should be noted that the Feachem equation and the US EPA equation were only intended for use at temperatures \( \geq 45 \) °C and \( \geq 50 \) °C, respectively, but we have extended them here below the recommended minimums for comparison purposes.

### 3. Results and discussion

#### 3.1. Ascaris inactivation under anaerobic conditions

Under anaerobic conditions, we observed Ascaris inactivation at temperatures between 37 °C and 45 °C when no additional inactivating agents were present (Fig. 1A), and we used linear regression of graphs of \( \ln(N_0/N) \) versus time to determine inactivation parameters \( (k \) and \( b) \) and the time required for a 3-log reduction in Ascaris viability (\( t_3 \)) (Table 2). At 45 °C, we observed rapid inactivation under anaerobic conditions, with viability reduced below the detection limit within 2 days (Fig. 1A, Table S1). Correspondingly, the \( t_3 \) calculated by linear regression was 2.12 days (Table 2). At 40 °C under anaerobic conditions, we observed a 2.19-log reduction after a maximum exposure time of 16 days (Fig. 1A, Table S1), and the calculated \( t_3 \) from linear regression was 20.5 days (Table 2). We did not test longer exposure times to confirm the \( t_3 \) value, but we have high confidence in the regression because all four time points occurred along the exponential portion of the inactivation curve (Fig. 1A), and the regression provided a good fit to the data with all inactivation parameters significant at \( p < 0.05 \) and \( R^2 = 0.954 \) (Table 2). Under anaerobic conditions at 39 °C, the exponential portion of the inactivation curve was fully captured, and we found no viable eggs after a 32-day exposure time (Fig. 1A, Table S1). Our linear regression fit the data well and yielded a \( t_3 \) of 31.8 days (Table 2).

The longest exposure time tested under anaerobic conditions at 37 °C was 40 days, at which point the results from the duplicate samples varied. Viability was reduced below detection (2.84-log reduction) for one sample, but only a 1.37-log reduction was observed for the second sample (Fig. 1A, Table S1). Correspondingly, the \( t_3 \) calculated by linear regression (57.5 days) was longer than 40 days (Table 2). The regression line fit the data well (\( R^2 = 0.759 \)) but would be improved with an additional time point beyond 40 days to demonstrate viability below detection in both replicates and an additional point between 21 and 40 days to refine the rate of decline. Unfortunately, the three-week incubation time required between the end of the exposure period and examining the eggs for viability prevented us from recognizing the need for these treatments until after the experiment was completed. Longer exposure times were also needed to improve inactivation predictions under anaerobic conditions at 36 °C. The longest exposure time we tested was 42 days, and we observed minimal inactivation (0.321-log reduction), indicating that the exponential portion of the inactivation curve was not observed (Fig. 1A, Table S1). As expected, the resulting linear regression provided a poor fit to the data (Table 2).

#### 3.2. Ascaris inactivation under aerobic conditions

Under aerobic conditions, we observed Ascaris inactivation at temperatures between 34 °C and 45 °C, with Ascaris viability reduced below detection during the tested exposure times at all temperatures. Inactivation at 45 °C under aerobic conditions was similar to the inactivation observed under anaerobic conditions with no viable eggs found after a 2-day exposure time (Fig. 1B, Table S1). For temperatures between 34 °C and 40 °C, mesophilic inactivation of Ascaris eggs was even more effective under aerobic conditions than under anaerobic conditions (Fig. 1B). Under aerobic conditions at 40 °C, no viable eggs were found in samples with an 8-day exposure time (detected limit = 2.69-log reduction). However, low viability (2.33-log reduction) was still observed in samples with a 12-day exposure time, and exposure times longer than 12 days were not tested (Fig. 1B, Table S1). The corresponding \( t_3 \) value calculated by linear regression was 12.6 days, which was

| Trial | Baseline Percent Viability (SE) | Temperature (°C) | Anaerobic/Aerobic | Exposure Times (d) |
|-------|-------------------------------|-----------------|-------------------|-------------------|
| 1     | 73.6 (2.1)                    | 36              | Anaerobic         | 10, 15, 20, 24, 30, 42 |
| 1     | 73.6 (2.1)                    | 36              | Aerobic           | 10, 15, 30        |
| 2     | 67.5 (1.6)                    | 40              | Anaerobic         | 4, 8, 12, 16      |
| 2     | 67.5 (1.6)                    | 40              | Aerobic           | 2, 4, 8, 12       |
| 2     | 67.5 (1.6)                    | 45              | Anaerobic         | 0.75, 2, 4.5      |
| 2     | 67.5 (1.6)                    | 45              | Aerobic           | 0.75, 1, 2.4      |
| 3     | 67.5 (1.6)                    | 36              | Aerobic           | 2, 5, 8, 12       |
| 4     | 69.1 (1.2)                    | 34              | Aerobic           | 3, 5, 10, 15, 20  |
| 4     | 69.1 (1.2)                    | 37              | Anaerobic         | 6, 11, 16, 21, 40 |
| 4     | 69.1 (1.2)                    | 39              | Anaerobic         | 5, 11, 16, 21, 32 |

*a* Standard error of replicate samples tested for baseline viability. Three samples of 500 eggs were counted for Trial 1, and two samples of 500 eggs were counted for Trials 2–4.


Fig. 1. Inactivation profiles of Ascaris eggs when exposed to mesophilic temperatures between 34 °C and 45 °C under anaerobic (A) and aerobic (B) conditions. Error bars show the range between duplicate samples. Open symbols indicate that no viable eggs were found in either sample, and log reduction is plotted as equal to the detection limit of the individual treatment. Symbols with a shaded fill (anaerobic, 37 °C, 40 d and anaerobic, 45 °C, 4 d) indicate that no viable eggs were found in one of the two duplicate samples. The dashed gray line marks the cumulative average detection limit of 2.81-log reduction. Raw data for each treatment is included as supplemental information (Table S1).

3.3. We developed a time-temperature relationship for mesophilic temperatures and anaerobic conditions that is reasonable and conservative

Our results demonstrate that thermal inactivation of Ascaris eggs consistently occurs at temperatures between 37 °C and 45 °C under anaerobic conditions and between 34 °C and 45 °C under aerobic conditions, which directly contradicts current guidance from Feachem et al. (1983) and the US EPA (2003) that require minimum temperatures of 45 °C and 50 °C, respectively. Even if lower temperatures were considered sufficient, the time-temperature relationships provided by these guidelines predict substantially longer inactivation times than we found from our $t_3$ values (Table 2). Under anaerobic conditions, the Feachem guidelines suggest exposure times that are 3–5 times larger than our $t_3$ values, and the US EPA guidelines suggest times that are up to 12 times larger than ours (Table 2). Under aerobic conditions, the differences are even larger, particularly at lower temperatures of 34 °C and 36 °C. The recommended exposure time by Feachem et al. at 34 °C is two orders of magnitude larger than our $t_3$ value (Table 2). Therefore, a new time-temperature relationship specific for mesophilic temperatures is clearly necessary. We developed this relationship using linear regression of the base-ten logarithm of our anaerobic $t_3$ values as a function of temperature, rearranged to produce Equation (4),

$$t_{Harroff} = (2.20 \times 10^0) \times 10^{-0.199T}$$  \hspace{1cm} (4)

where $t_{Harroff}$ is the exposure time in days required for a 3-log reduction, and $T$ is temperature in °C. The upper limit of the 95% confidence interval was used for each $t_3$ value to develop a more conservative relationship. The $t_3$ calculated for anaerobic inactivation at 36 °C was excluded due to the poor fit of the linear regression model. Therefore, the model should only be applied with substantially shorter than the 20.5 days calculated for anaerobic conditions at the same temperature (Table 2). Inactivation rates under aerobic conditions at 34 °C and 36 °C were similar, with no viable eggs found after exposure times of 15 days for both temperatures (Fig. 1B, Table S1). The corresponding $t_3$ values calculated by linear regression were 16.6 and 16.5 days, respectively, which are less than half of the longest exposure time tested for 36 °C under anaerobic conditions that resulted in minimal inactivation (Fig. 1, Table 2).

Table 2

Inactivation parameters determined by linear regression of experimental data and inactivation times recommended by three time-temperature relationships ($t_{Feachem}$, $t_{EPA}$, and $t_{Harroff}$). Standard errors for inactivation parameters are given in parentheses. Exposure time required for a 3-log reduction ($t_3$) was calculated using the inactivation parameters, and the lower and upper bounds of the 95% confidence interval are given in parentheses. Inactivation times required by the Feachem ($t_{Feachem}$) and US EPA ($t_{EPA}$) guidelines are given as comparison, as well as inactivation times given by the time-temperature relationship developed in the current study ($t_{Harroff}$) (Equation (4)).

| Anaerobic/Aerobic | Temperature (°C) | Linear regression of experimental data | Inactivation times predicted by time-temperature relationships |
|-------------------|-----------------|----------------------------------------|-------------------------------------------------------------|
|                   | $k$ (SE)        | $b$ (SE)                              | $t_3$ (d)  | 95% CI | $t_{Feachem}$ (d) | $t_{EPA}$ (d) | $t_{Harroff}$ (d) |
| Anaerobic         | 36              | 0.0148 * (0.00976)                    | 0.0285 * (0.251) | 0.187 | 465 $^{\text{b}}$ (187; 1120) | 414 | 457 | 151 |
|                   | 37              | 0.147 (0.0292)                       | 1.53 (0.648) | 0.759 | 57.5 $^{\text{b}}$ (32.4; 82.7) | 265 | 331 | 95.4 |
|                   | 39              | 0.270 (0.0326)                       | 1.67 (0.567) | 0.907 | 31.8 $^{\text{b}}$ (23.2; 40.4) | 108 | 174 | 38.1 |
|                   | 40              | 0.406 (0.0363)                       | 1.41 (0.397) | 0.954 | 20.5 (16.8; 24.2) | 69.2 | 126 | 24.1 |
|                   | 45              | 4.71 (0.0703)                        | 3.12 (0.106) | 0.999 | 2.12 (2.03; 2.23) | 7.38 | 25.1 | 2.44 |
| Aerobic           | 34              | 0.521 (0.0583)                       | 1.72 (0.541) | 0.930 | 16.6 (12.1; 21.1) | 1014 | 870 | 377 |
|                   | 36              | 0.501 (0.0435)                       | 1.35 (0.422) | 0.930 | 16.5 (13.1; 19.8) | 414 | 457 | 151 |
|                   | 40              | 0.618 (0.140)                        | 0.900 $^{\text{c}}$ (1.06) | 0.763 | 12.6 (5.40; 19.9) | 69.2 | 126 | 24.1 |
|                   | 45              | 3.94 (0.331)                         | 1.50 (0.450) | 0.973 | 2.13 (1.74; 2.52) | 7.38 | 25.1 | 2.44 |

a Parameter is not significant at $p < 0.05$.

b Viability was not reduced below the detection limit during the exposure times tested. Inactivation times are therefore extrapolated.

c Feachem et al. (1983).

d US EPA (2003).

e Current study.
inactivation is assumed to occur primarily due to temperature, as well as other studies that are discussed here, are evidence when combined. Details about each literature data point about mesophilic thermal inactivation, but they provide convincing evidence when combined. Details in Table S2 include exposure conditions, how inactivation times were determined, and the degree of inactivation that was measured in each study.

With all literature data points falling at or below our time-temperature relationship and the \( t_3 \) values generated from regression of experimental data, we conclude that the time-temperature relationship developed here is more reasonable than the Feachem and US EPA relationships for temperatures between 37 °C and 45 °C, while still being conservative. The conservative nature of our time-temperature relationship can be attributed to the use of aqueous solutions for exposing eggs in our experimental results versus the use of manure or fecal sludge matrices for the literature points. Past work looking at Ascaris inactivation in autoclaved versus non-autoclaved anaerobic fecal material at 30 °C showed that compounds may be biologically produced during anaerobic fermentation that contribute to Ascaris inactivation (Harroff et al., 2017). Other studies examining Ascaris inactivation by ammonia have also found that results from exposures in aqueous solutions are conservative compared to those in manures and fecal sludge (Nordin et al., 2009; Peeson et al., 2007; Schuh et al., 1985). Here, the relationship that we developed using aqueous exposures appears more conservative at lower temperatures than at higher temperatures, indicating that intrinsic inactivating compounds in sludge became less important at warmer temperatures within the mesophilic spectrum (Fidjeland et al., 2015).

Even though overly conservative guidelines may hamper the use of simple treatment, the use of a conservative time-temperature relationship is still important for protecting public health when developing waste treatment strategies. For this reason, our time-temperature relationship should still be confirmed at temperatures less than 37 °C using exposures in aqueous solutions. Although literature values demonstrate inactivation at 34 °C and 35 °C at exposure times less than those predicted by our relationship (Cruz Espinoza et al., 2012; Manser et al., 2015; Nordin et al., 2009), intrinsic factors in those treatments, such as ammonia and solids concentration, may have affected inactivation times. For example, Manser et al. (2015) observed 99% inactivation of Ascaris eggs after 24 days in an anaerobic digester at 35 °C, but another study observed only 50% inactivation after 35 days in a 35 °C digester (Johnson et al., 1998). Both times are far below the 238 days required for a 3-log (99.9%) inactivation by our time-temperature relationship, but they suggest a need for more information.

3.4. The time-temperature relationship we developed also provides conservative recommendations under aerobic conditions

Recent work by Manser et al. (2015) compared inactivation times required for Ascaris inactivation under anaerobic and aerobic conditions at 34 °C (24 days and 16 days, respectively) and concluded that aerobic conditions cause faster inactivation. An earlier study compared anaerobic and aerobic digestion at 37 °C and 47 °C and reached the opposite conclusion that anaerobic conditions caused faster inactivation. However, the authors acknowledged that the actual temperatures in the aerobic digesters were uncertain due to the use of circulating air to keep them oxygenated. Therefore, the results of that study are inconclusive (Kato et al., 2003). Our experimental data supports the conclusion of Manser et al. (2015) at a range of mesophilic temperatures between 34 °C and 40 °C (Fig. 1), which has not been shown before. In addition, our data shows that the effect of oxygen exposure decreases as temperature increases. Three temperatures (36 °C, 40 °C, and 45 °C) were tested under both anaerobic and aerobic conditions. We cannot directly compare the 36 °C treatments because...
exponential decline was not observed under anaerobic conditions, but the $t_2$ calculated for 37 °C under anaerobic conditions is 3.5 times larger than the $t_2$ predicted for 36 °C under aerobic conditions (Table 2). Meanwhile, at 40 °C the $t_2$ under anaerobic conditions is only 1.6 times larger than the $t_2$ under aerobic conditions (Table 2). The $t_2$ values at 45 °C for anaerobic and aerobic conditions are equal, which indicates that the effect of oxygen becomes negligible at this temperature (Table 2). Aerobic conditions likely cause faster inactivation because the larvae undergo embryonic development when they have access to oxygen, and they consume lipids and carbohydrates within the eggshell to cause structural and chemical changes that may leave the larvae more susceptible to environmental conditions (Arene, 1986; Fairbairn, 1957; Manser et al., 2015). However, inactivation at 45 °C is likely too rapid (2.1 days) to allow for significant embryonic development, which causes the effect of oxygen presence to be negated.

Previous studies have shown aerobic inactivation at times and temperatures consistent with ours, but they did not directly compare inactivation rates under anaerobic and aerobic conditions (Fig. 2B, Table S2). In control treatments of a pH 7 saline solution, Ghiglietti et al. (1995) observed 99% inactivation of Ascaris eggs after exposure to 40 °C for 14 days (Fig. 2B). Aerobic conditions were not explicitly indicated in the Ghiglietti study, but embryonic development was observed during the exposure time for treatments at lower temperatures so we can assume that oxygen was present in the treatments (Ghiglietti et al., 1995). In comparison, our $t_2$ for 40 °C under aerobic conditions was slightly lower at 12.6 days. Tharaldsen and Helle (1989) found that Ascaris eggs in mechanically aerated pig manure slurry at 37 °C were 99% inactivated between 14 and 22 days, which is shown in Fig. 2B as 22 days, and we observed similar inactivation at 36 °C with a predicted $t_2$ of 16.5 days.

Nordin et al. (2009) examined Ascaris inactivation from different ammonia concentrations at 34 °C and reported results that also indicate an effect of aerobic conditions. In one treatment of fecal material containing 43 mM NH$_3$ ammonia (maximum pH = 8.3, total ammonia [NH$_3$+NH$_4$] = 247 mM), 99% inactivation was predicted after 21 days using linear regression. For a treatment using diluted urine containing 40 mM NH$_3$ ammonia (maximum pH = 8.7, total ammonia [NH$_3$+NH$_4$] = 131 mM), 99% inactivation was predicted after only 8.5 days. With similar NH$_3$ ammonia concentrations, the study concluded that unmonitored factors contributed to the different inactivation rates. We propose here that oxygen was the primary unmonitored factor. For the fecal material treatment, eggs were contained in mesh bags and inserted in 200 g of fecal material in sealed containers, leading to anaerobic conditions. For the urine treatment, the mesh bags were suspended in urine contained in a sealed 50-mL tube. However, the tube was not evenly heated, where required exposure times for inactivation may be affected by uneven heating, fluctuating temperatures, and shielding effects of solids (Popat et al., 2010; Senecal et al., 2018). For example, composting systems often do not reach expected temperatures, leading to poor pathogen inactivation (Mehl et al., 2011). Those that do reach proper temperatures have large spatial and temporal variations that make thermal inactivation difficult to predict. In one study, measured temperatures ranged between <30 °C and nearly 50 °C in different portions of a compost heap over a 120 day period, and over 100 days of exposure was required to observe >99% Ascaris inactivation (Jensen et al., 2009). Another study observed temperatures >60 °C. Ascaris inactivation would typically be expected within minutes at such high temperatures, but 6 days were required to reduce viability below detection, indicating pockets with a lower temperature (Szabóvá et al., 2010). For this reason, the US EPA guidelines require longer exposure times for sewage sludges with higher solids content (US EPA, 2003). In addition to variation within treatment systems, Pecson and Nelson (2005) also have observed variability in temperature resiliency between batches of Ascaris eggs. Temperature must be closely controlled and monitored for any treatment system relying on thermal inactivation of pathogens. For mesophilic systems in particular, pathogen inactivation should be verified for each unique system.

3.5. Limitations

Caution must be used when applying the results and time-temperature relationship shown here to large-scale systems, where required exposure times for inactivation may be affected by uneven heating, fluctuating temperatures, and shielding effects of solids (Popat et al., 2010; Senecal et al., 2018). For example, composting systems often do not reach expected temperatures, leading to poor pathogen inactivation (Mehl et al., 2011). Those that do reach proper temperatures have large spatial and temporal variations that make thermal inactivation difficult to predict. In one study, measured temperatures ranged between <30 °C and nearly 50 °C in different portions of a compost heap over a 120 day period, and over 100 days of exposure was required to observe >99% Ascaris inactivation (Jensen et al., 2009). Another study observed temperatures >60 °C. Ascaris inactivation would typically be expected within minutes at such high temperatures, but 6 days were required to reduce viability below detection, indicating pockets with a lower temperature (Szabóvá et al., 2010). For this reason, the US EPA guidelines require longer exposure times for sewage sludges with higher solids content (US EPA, 2003). In addition to variation within treatment systems, Pecson and Nelson (2005) also have observed variability in temperature resiliency between batches of Ascaris eggs. Temperature must be closely controlled and monitored for any treatment system relying on thermal inactivation of pathogens. For mesophilic systems in particular, pathogen inactivation should be verified for each unique system.

4. Conclusions

- Given adequate exposure times, Ascaris eggs are consistently inactivated under anaerobic conditions at mesophilic temperatures between 37 °C and 45 °C and under aerobic conditions at
mesophilic temperatures between 34 °C and 45 °C without the addition of external inactivating agents.

- The relationship $\ln(0.1997) = (2.20 \times 10^4) \times 10^{-0.1997}$ can be used to conservatively estimate exposure times (t, days) required to achieve a 3-log reduction of Ascaris viability at temperatures (T) between 37 °C and 45 °C under both anaerobic and aerobic batch conditions, when uniform temperature occurs.

- This relationship is still conservative, but much less conservative than current relationships that are used as standards. Therefore, reduction of minimum temperature thresholds in regulations for thermal waste treatment processes should be considered.

- Mesophilic waste treatment processes should be given greater consideration when considering how to treat the excreta of the 4.5 billion people globally who currently lack access to safely managed sanitation services (WHO and UNICEF, 2017). Mesophilic systems can be less expensive and easier to operate than thermophilic systems, and they are shown here to potentially provide adequate pathogen treatment.

- Thermal inactivation of Ascaris eggs is faster under aerobic conditions than under anaerobic conditions. Therefore, presence of oxygen should be monitored more closely in research. New waste treatment systems should be developed that use this advantage of oxygen to inactivate pathogens.

- Ascaris is less resistant to thermal inactivation than is often stated, and more work is needed to evaluate the circumstances under which it can be used as a reliable indicator organism.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

LAH thanks Lucinda Li for valuable laboratory assistance and acknowledges support from the Cross-Scale Biogeochemistry and Climate NSF IGERT program (Award #1069193) and the NSF Graduate Research Fellowship Program (Award #1650441). LTA acknowledges support from the Alexander von Humboldt Foundation in the framework of the Alexander von Humboldt Professorship endowed by the Federal Ministry of Education and Research in Germany. We acknowledge support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of University of Tübingen.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wroa.2019.100036.

References

Aitken, M.D., Sobsey, M.D., Blauth, K.E., Shehee, M., Crunk, P.L., Walters, G.W., 2005. Inactivation of Ascaris suum and poliovirus in biosolids under thermophilic anaerobic digestion conditions. Environ. Sci. Technol. 39 (15), 5804–5809.

Arene, F. 1986. Ascaris suum: influence of embryonation temperature on the viability of the infective larva. J. Therm. Biol. 11 (1), 9–15.

Brudastov, A., Krasnonos, L., Lemeev, V., Kholmukhamedov, S., 1995. Infectivity of Ascaris lumbricoides eggs to man and Guinea-pigs after 10 years in the soil. Med. Parasitol. Parasit. Bolezni 39 (4).

Cruz Espinosa, L.M., Vinnerås, B., Rajaram, L., Whiteford, L., Convin, J., Izurieta, R., 2012. Inactivation of Ascaris suum by ammonia in feces simulating the parameters of the solar toilet. J. Appl. Sci. Environ. Sanit. 7 (3).

De Silva, N.R., Chan, M.S., Bundy, D.A.P., 1997. Morbidity and mortality due to ascariasis: re-estimation and sensitivity analysis of global numbers at risk. Trop. Med. Int. Health 2 (6), 513–518.

Faiyaz, D., 1957. The biochemistry of Ascaris. Exp. Parasitol. 6 (5), 491–554.

Feachem, R.G., Bradley, D.J., Henda, G., Mara, D.D., 1983. Sanitation and Disease: Health Aspects of Excreta and Wastewater Management (Washington, DC).

Fidjeland, J., Nordin, A., Pecson, B.M., Nelson, K.L., Vinnerås, B.R., 2015. Modeling the inactivation of Ascaris eggs as a function of ammonia concentration and temperature. Water Res. 83, 153–160.

Gaspard, P., Wiart, J., Schwartzbrod, J., 1996. A method for assessing the viability of nematode eggs in sludge. Environ. Technol. 17 (4), 415–420.

Ghiglietti, R., Rossi, P., Ramos, M., Colombo, A., 1995. Viability of Ascaris suum, Ascaris lumbricoides and Trichuris muris eggs to alkaline pH and different temperatures. Parasitologa 37 (2–3), 229.

Harroff, L.A., Liotta, J.L., Bowman, D.D., Angenent, L.T., 2017. Inactivation of Ascaris eggs in human fecal material through in situ production of carboxylic acids. Environ. Sci. Technol. 51 (17), 9729–9738.

Jensen, P.K., Phuc, P.D., Konradsen, F., Klaan, L.T., Dalgaard, A., 2009. Survival of Ascaris eggs and hygienic quality of human excreta in Vietnamese composting latrines. Environ. Health 8 (1), 57.

Johnson, P., Wasi, D., Ross, A., 1998. An in-vitro test for assessing the viability of Ascaris suum eggs exposed to various sewage treatment processes. Int. J. Parasitol. 28 (4), 627–631.

Kato, S., Fogarty, E., Bowman, D.D., 2003. Effect of aerobic and anaerobic digestion on the viability of Cryptosporidium parvum oocysts and Ascaris suum eggs. Int. J. Environ. Health Res. 13 (2), 169.

Kranz, N.D., Wald, I., Ergas, S.J., Izurieta, R., Mihelic, J.R., 2015. Assessing the fate of Ascaris suum ova during mesophilic anaerobic digestion. Environ. Sci. Technol. 49 (5), 3128–3135.

Maya, C., Torner-Morales, F., Lucario, E., Hernández, E., Jiménez, B., 2012. Viability of six species of larval and non-larval helminth eggs for different conditions of temperature, pH and dryness. Water Res. 46 (15), 4770–4782.

Mehl, J., Kaiser, J., Hurtado, D., Gibson, D.A., Izurieta, R., Mihelic, J.R., 2011. Pathogen destruction and solids decomposition in composting latrines: study of fundamental mechanisms and user operation in rural Panama. J. Water Health 9 (1), 187–199.

Nordin, A., Nyberg, K., Vinnerås, B.R., 2009. Inactivation of Ascaris eggs in source-separated urine and feces by ammonia at ambient temperatures. Appl. Environ. Microbiol. 75 (3), 662–667.

Pandey, P.K., Gao, W., Wang, Y., Vaddella, V., Castillo, A.R., Souza, A., de Lis, N.S., 2016. Simulating the effects of mesophilic anaerobic and aerobic digestions, lagoon system, and composting on pathogen inactivation. Ecol. Eng. 97, 633–641.

Pecson, B.M., Barrios, J.A., Jiménez, B.E., Nelson, K.L., 2007. The effects of temperature, pH, and ammonia concentration on the inactivation of Ascaris eggs in sewage sludge. Water Res. 41 (13), 2893–2902.

Pecson, B.M., Nelson, K.L., 2005. Inactivation of Ascaris suum eggs by ammonia. Environ. Sci. Technol. 39 (20), 7909–7914.

Popat, S.C., Yates, M.V., Deshusses, M.A., 2010. Kinetics of inactivation of indicator pathogens during thermophilic anaerobic digestion. Water Res. 44 (20), 5965–5972.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranke, J., 2018. chemCal: Calibration Functions for Analytical Chemistry. R package version 0.2.1.

Schulz, R., Philipp, W., Strauch, D., 1985. Influence of Sewage Sludge with and without Lime Treatment on the Development of Ascaris suum Eggs. Elsevier.

Seamster, A.P., 1950. Developmental studies concerning the eggs of Ascaris lumbricoides var. suum. Ann. Midl. Nat. 43 (2), 450–470.

Senecal, J., Nordin, A., Simha, P., Vinnerås, B.R., 2018. Hygiene aspect of treating human urine by alkaline dehydration. Water Res. 144, 474–481.

Szcána, E., Juris, P., Papajová, L., 2010. Sanitation composting process in different seasons. Ascaris suum as model. Waste Manag. 30 (3), 426–432.

Thalassides, J., Helle, O., 1989. Survival of parasite eggs in livestock slurry utilized for compost heat. Acta Agric. Scand. 39 (4), 381–387.

US EPA, 2003. Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) under 40 CFR Part 503 (Cincinnati, OH).

Vinnerås, B., Björklund, A., Jonsson, H., 2003. Thermal composting of faecal matter as treatment and possible disinfection method—laboratory-scale and pilot-scale studies. Bioresour. Technol. 88 (1), 47–54.

WHO, UNICEF, 2017. Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines (Geneva, Switzerland).