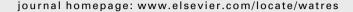


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## Kinetics of inactivation of indicator pathogens during thermophilic anaerobic digestion

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#### ABSTRACT

Thermophilic anaerobic sludge digestion is a promising process to divert waste to beneficial use, but an important question is the required temperature and holding time to achieve a given degree of pathogen inactivation. In this study, the kinetics of inactivation of Ascaris suum and vaccine strain poliovirus type 1 (PVS-1), selected as indicators for helminth ova and enteric viruses respectively, were determined during anaerobic digestion at temperatures ranging from 51 to 56 °C. Inactivation of both indicator organisms was fast with greater than two log reductions achieved within 2 h for A. suum and three log reductions for PVS-1, suggesting that the current U.S. regulations are largely conservative. The first-order inactivation rate constants k followed Arrhenius relationship with activation energies of 105 and 39 KJ mol<sup>-1</sup> for A. suum and PVS-1, respectively indicating that A. suum was more sensitive to temperature. Although inactivation was fast, the presence of compounds in the sludge that are known to be protective of pathogen inactivation was observed, suggesting that composition-dependent time—temperature relationships are necessary.

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#### 1. Introduction

Land application of biosolids is of considerable interest to the wastewater treatment community, because it provides the opportunity to put sewage sludge, which otherwise needs to be disposed of, towards beneficial use such as crop growth (National Research Council Committee on Toxicants and Pathogens in Biosolids Applied to Land, 2002). The use and disposal of biosolids in the United States is regulated by the U.S. Environmental Protection Agency (EPA) under 40 CFR Part 503 (U.S. Environmental Protection Agency, 2003). Treated biosolids need to meet either of two requirements in terms of pathogen presence: Class A or Class B. Class A requires that treated biosolids contain no detectable levels of pathogens from three

classes: viable helminth eggs, enteric viruses and Salmonella spp. Land application of Class B biosolids, which require only reduction in the density of pathogens but not necessarily complete removal, requires additional management and thus most treatment facilities want to move towards production of Class A biosolids.

The CFR Part 503 regulations have identified six alternatives in treating sewage sludge in order to meet Class A requirements for biosolids (U.S. Environmental Protection Agency, 2003). One of the alternatives is classified as "processes to further reduce pathogens" (PFRP) and is assumed to result in complete inactivation of pathogens. PFRP include treatment techniques such as composting, heat drying, heat treatment, thermophilic aerobic digestion, beta

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#### Nomenclature

- C concentration of indicator pathogen at time t
- C<sub>0</sub> concentration of indicator pathogen at time zero
- k first-order rate constant for inactivation at temperature T
- $k_0$  pre-exponential constant
- E activation energy

ray and gamma ray irradiation, and pasteurization. Heat drying and heat treatment require temperatures of at least 80 °C, while thermophilic aerobic digestion needs to be carried out in the presence of oxygen. These significantly increase the treatment cost, and can result in major releases of odors and thus are not popular. Thermophilic anaerobic digestion is comparatively less expensive, but it is not classified as a PFRP. In order to use thermophilic anaerobic digestion, treatment facilities must use holding at a specified time-temperature combination to meet the CFR Part 503 regulations. Thermophilic anaerobic digestion is an attractive solution for the plant operators (Iranpour et al., 2002). However, the current time-temperature relationships specified by the EPA may require excessively long holding times depending on the temperature. Thus, better information on the inactivation of pathogens and indicator organisms during thermophilic anaerobic digestion is warranted.

Aitken et al. (2005) critically evaluated the basis of the current EPA time—temperature relationships by determining the kinetics of inactivation of two indicator pathogens during thermophilic anaerobic digestion. They used the swine pathogen Ascaris suum as an indicator for viable helminth eggs, and the vaccine strain poliovirus type 1 (PVS-1) as an indicator for human enteric viruses. They found that indicator microorganism inactivation was rapid and the effect of temperature was prominent, and concluded that the current EPA guideline was extremely conservative. While most other researchers have evaluated the kinetics of inactivation of helminth eggs and enteric viruses in aqueous media, the study by Aitken et al. (2005) remains one of the very few that was aimed at quantifying inactivation kinetics in a sewage sludge matrix as well as studying the effect of temperature. While the Aitken et al. study suggested that a revision of the EPA time-temperature relationships was necessary, additional studies are needed for several reasons. First, several kinetic constants in the Aitken et al. study were determined with only two or three data points and confirmation of their validity is warranted. Second, the variability of conditions or of sewage sludge composition, especially the presence of virucidal or protective agents at different facilities may have a significant impact on the kinetics of inactivation of pathogens.

Thus, the objective of our study was to determine the kinetics of inactivation of A. suum and PVS-1 during thermophilic anaerobic digestion of first-stage digested sludge obtained from a major wastewater treatment facility, evaluate the effect of temperature on inactivation kinetics, and compare the results with those of Aitken et al. (2005) and with EPA guidelines. An additional objective was to test for the presence of sodium dodecyl sulfate (SDS) and cysteine in the sludge as possible agents protecting the indicator organisms from inactivation.

#### 2. Materials and methods

#### 2.1. Microorganism spikes

A. suum and PVS-1 spikes were obtained from Hoosier Microbiological Laboratory (HML, Inc., Muncie, IN), an EPA-approved commercial laboratory that was also selected to perform the microbial analyses in samples from all experiments. A. suum spikes were prepared by the manufacturer by suspending eggs obtained from feces of naturally infected pigs into a sterile medium containing deionized water and 0.2% Tween 80. PVS-1 spikes were suspended in a maintenance medium containing per 100 mL: 45.0 mL Eagles MEM, 45.0 mL Leibovitzs L-15, 0.70 mL NaHCO<sub>3</sub>, 7.5%, 5.0 mL Fetal Calf Serum, 5.0 mL Sterile Water, 0.10 mL Penicillin-Streptomycin, 0.05 mL Tetracycline, B 0.02 mL Amphotericin. This medium was designed to result in minimal viability loss prior to use in spiking. A. suum spikes for inactivation experiments were 25 mL in volume and contained 10<sup>6</sup> ova, while those for recovery tests were 10 mL in volume and contained  $5 \times 10^4$  ova. PVS-1 spikes for inactivation experiments were 25 mL in volume and contained 108 PFU, while those for recovery tests were 10 mL in volume and contained 5  $\times$  10<sup>5</sup> PFU. A. suum spikes were stored at 4  $^{\circ}$ C and PVS-1 spikes at −18 °C.

#### 2.2. Microorganism recovery tests

Microorganism recovery tests consisted of spiking known concentrations of A. suum and PVS-1 individually into 1 L sterile polyethylene screw-capped bottles containing either first-stage digested sludge from a major wastewater treatment facility or a simple basal salt medium (BSM, 40 g L<sup>-1</sup>Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O in water). Both the sludge and the BSM were at room temperature (22 °C) for the recovery tests. After shaking for 10 min, replicate samples were taken and analyzed for each organism. Recovery of A. suum in sludge averaged 30%, while that of PVS-1 was 15%. Recovery of A. suum in BSM was 64%, while that of PVS-1 was 78%. The lower recovery in sludge indicates significant matrix effects from the complex composition of sludge. Thus, the amount of organisms to be spiked into the digesters for the inactivation experiments had to be significantly higher, especially since  $\geq$ 2-log reduction of A. suum and  $\geq$ 3-log reduction of PVS-1 needed to be demonstrated.

#### 2.3. Digesters setup

To determine time—temperature relationships relevant to practical conditions, six glass digesters (Kimble Chase Life Science and Research Products LLC, Vineland, NJ), each 22 L in volume and equipped to run at a constant temperature and mixing speed, were established (Fig. 1). Each digester was

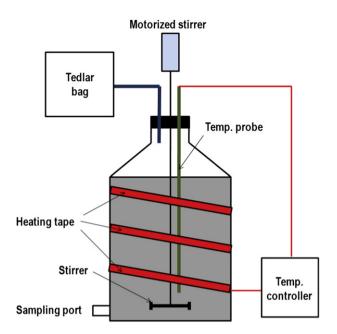


Fig. 1 -Schematic diagram of the digesters used in this study. A total of six parallel digesters were used.

equipped with a heating tape, a temperature probe and a temperature controller (Omega Engineering, Inc., Stamford, CT). The temperature probes and controllers were calibrated against a general purpose NIST-traceable laboratory thermometer. Stirrers were used to continuously mix the contents of the digesters. Two sampling ports, one at the top and one at bottom, were located on the digesters. The top port was used to connect a 10 L Tedlar bag, while the bottom was used for sample collection. The Tedlar bag inflated or deflated depending on the increase or decrease in digester headspace volume due to production of biogas and displacement of volume during sampling, respectively. The Tedlar bags were initially filled with 5 L nitrogen, and refilled as needed if sampling caused complete deflation.

#### 2.4. Inactivation experiments

Inactivation experiments were performed at temperatures of 51.1, 53.3 and 55.5 °C in sets of two replicate digesters. Samples were taken at 0, 0.5, 1, 1.5, 2, 4 or 12, and 8 or 16 h. This sampling schedule was developed based on preliminary experiments that suggested that pathogen inactivation was very rapid, resulting in complete inactivation within 4 h at these temperatures. The selected sampling schedule also ensured that at least 5 data points would be available for least squares regression while fitting the data to a first-order inactivation kinetic model.

Experiments were conducted with first-stage digested sludge obtained from a major wastewater treatment facility. The retention time and temperature in the primary digesters at that facility were 13–15 days and 53.3 °C, respectively. The primary digested sludge is further digested in a second stage at 53.6 °C for an average holding time of 16.5 h. Inactivation experiments were started with the transfer of 20 L of first-stage digested sludge containing approximately 2% solids to each

digester, and adjusting it to the desired temperature. The sludge was collected the same day and used in the laboratory digesters within 4 h of collection. After transferring the sludge into the digesters, the headspace was flushed with nitrogen. Microorganism spikes were transferred to room temperature 2–3 h prior to spiking. All spikes were inserted into the digesters through the top port using 60 mL syringes. The spikes corresponded to an initial concentration in the digesters of A. suum and PVS-1 of  $10^4$  ova  $(4 \text{ g dry wt})^{-1}$  and  $10^6$  PFU  $(4 \text{ g dry wt})^{-1}$ , respectively. Tedlar bags were removed from the top port while spiking, and were immediately replaced once the microorganisms had been spiked into the digesters. The stirrer speeds were increased for 1 min in order to ensure rapid mixing after spiking the test organisms. The first sample was collected at the 1 min mark after spiking the first organism, and set as time zero. The good consistency for all samples collected at time zero indicates that the spikes were well mixed into the bulk sludge volume.

The initial samples at time zero were taken to validate the microorganism recovery during analysis as determined previously. Measured concentrations of the microorganisms in the initial samples indicated that the recovery of A. suum was 22–24%, and of PVS-1 was 10–11%. This is only slightly lower than that determined in earlier recovery tests (see Section 2.2), and because this does not affect the calculation of inactivation rate constants, the possible reasons for this were not further pursued. All equipment was cleaned and disinfected as per EPA-approved methods after completion of the experiments (U.S. Environmental Protection Agency, 2003).

#### 2.5. Sample collection and storage

Two L of sample was collected for each sampling event in two 1L sterile polyethylene screw-capped bottles. The samples were immediately cooled to 4 °C by submerging and hand mixing in an ice water bath. 1 L of sample each was used for A. suum and PVS-1 analysis. For digesters at 51.1, 53.3 and 55.5 °C, it took 1.9, 2.2 and 2.5 min for the samples to reach 35 °C in the ice water bath. 35 °C is referenced as the temperature at which it takes about an hour to see appreciable inactivation (EPA, 2003), and thus it was assumed that any inactivation during cooling would essentially stop at 35 °C. The effect of the small time required for cooling samples to 35 °C on the kinetics of inactivation determined using measured concentrations is discussed in the Supporting Information. The samples were shipped overnight in coolers with ice packs to HML for analysis after no more than two days of storage (A. suum at 4 °C and PVS-1 at -18 °C).

#### 2.6. Microbial analyses

A. suum and PVS-1 in samples were extracted and analyzed by HML, after storage of no more than 2 weeks, using EPA-approved methods (U.S. Environmental Protection Agency, 2003). This storage time was within the allowable limits specified by the EPA.

#### 2.7. Chemical analyses

The pH of the sludge after the inactivation experiments was determined as per Method 4500 B, total solids as per Method 2540 G, volatile solids as per Method 2540 G, total alkalinity as

per Method 2320 B and total volatile fatty acids (VFAs) as per Method 5560 C (Clesceri et al., 1998). Additionally, concentrations of sodium dodecyl sulfate (SDS) and cysteine in first-stage sludge were analyzed by another commercial laboratory, Adamson Analytical Laboratories, Inc. (Corona, CA), using high-pressure liquid chromatography following extraction from the sludge using previously described methods (Ward and Ashley, 1978a).

#### 2.8. Data analysis

Concentration vs. time data obtained from the inactivation experiments was fitted to a first-order kinetic model represented as follows.

$$\frac{C}{C_0} = e^{-kt} \tag{1}$$

The effect of temperature on the inactivation rate was modeled using the Arrhenius equation as presented below.

$$k = k_0 e^{-\frac{E}{RT}} \tag{2}$$

Linear regression was performed using Microsoft Excel 2007, and first-order rate constants (k) and activation energies (E) were determined.

#### 3. Results and discussion

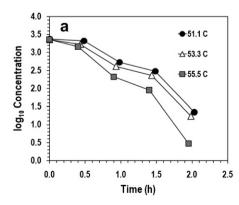
The effect of temperature on the inactivation of A. suum in replicate digesters is shown in Fig. 2. The concentrations (in ova per 4 g dry weight) are plotted as logarithmic values so as to easily observe time required for  $\geq$ 2-log reduction. As expected, inactivation was the slowest at 51.5 °C and the fastest at 55.5 °C. Irrespective of the temperature, however, greater than 2-log reduction of A. suum was observed within 2 h of treatment. It should be noted that there was some "lag" time for the inactivation of A. suum. This is characteristic of A. suum during thermal inactivation and was also observed by Aitken et al. (2005) and Pecson et al. (2007). The effect of temperature on the inactivation of PVS-1 in replicate digesters is shown in Fig. 3. The concentrations (in PFU per 4 g dry weight) are plotted as logarithmic values so as to easily

observe time required for  $\geq$ 3-log reduction. The effect of temperature on the rate of inactivation of PVS-1 was not as prominent as for A. suum. Nonetheless, greater than 3-log reduction of PVS-1 was observed by 2 h of treatment at all temperatures. Inactivation for both A. suum and PVS-1 followed first-order kinetics as indicated by straight lines on the log-linear plots.

The first-order rate constants for the inactivation of A. suum and PVS-1 determined from the data collected during the inactivation experiments are listed in Table 1, along with a comparison with the study by Aitken et al. (2005). The linear fits of  $\ln(C/C_0)$  vs. time for PVS-1 had  $R^2 > 0.9$ , but for A. suum some had  $R^2$  values between 0.8 and 0.9. This is because of the lag time observed during A. suum inactivation, resulting in the first data point deviating slightly from the expected first-order kinetics trend. Correcting for the lag time resulted in values within 10-15% of the estimated values without correction, and thus to maintain consistency, no changes were made to the data analysis protocol.

Rate constants for A. suum in our studies were all lower than those for PVS-1, suggesting that A. suum was more resistant to thermal inactivation than PVS-1, despite the inactivation rate being more sensitive to temperature. Aitken et al. (2005) also observed the same trend. However, the rate constants determined by them were statistically different from those determined in this study, based on 95% confidence intervals. It should be noted that because they did not conduct preliminary tests to determine rough inactivation rates, their experiments resulted in only 2-3 samples with detectable levels of pathogens, and thus the rate constants were determined through linear regression with only a limited number of points. Our sampling schedule was such that we could obtain at least five data points for each regression. Even so, they observed ≥3-log reduction of A. suum within 30 min, whereas it took 2 h in our experiments. Possible reasons for this difference are discussed later.

Arrhenius plots for A. suum and PVS-1 inactivation are shown in Fig. 4. The slopes of the fitted lines correspond to activation energies of 105 and 39 KJ mol<sup>-1</sup> for A. suum and PVS-1 inactivation, respectively. The values are consistent with the enhanced effect of temperature on the inactivation of A. suum compared to that of PVS-1. Although the primary inactivation mechanism in A. suum at high temperatures is not known,



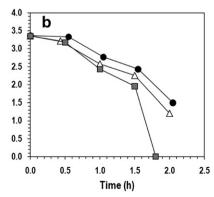


Fig. 2 – Effect of temperature on inactivation of A. suum in replicate digesters (a and b). Concentrations (ova (4 g dry weight)<sup>-1</sup>) are plotted as logarithmic values. Samples collected at 4 and 12 (a) and 8 and 16 (b) had undetectable levels of ova.

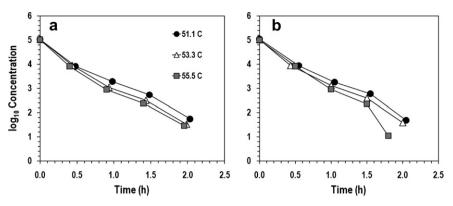


Fig. 3 — Effect of temperature on inactivation of PVS-1 in replicate digesters (a and b). Concentrations (PFU (4 g dry weight) $^{-1}$ ) are plotted as logarithmic values. Samples collected at 4 and 12 (a) and 8 and 16 (b) had undetectable levels of PFU.

enteroviruses, especially polioviruses and its vaccine strains have been studied in detail to determine mechanisms of their inactivation at high temperatures. It has been reported that inactivation of enteroviruses at high temperatures can occur either through RNA or capsid protein denaturation (Woese, 1960). For each individual strain of virus, there is a threshold temperature beyond which the mechanism shifts from RNA to capsid protein denaturation (Dimmock, 1967). For polioviruses, this is reported to occur at temperatures around 50 °C (Woese, 1960; Dimmock, 1967). The activation energy for inactivation of PVS-1 determined in this study falls in the range reported for RNA inactivation, even though we worked with temperatures higher than 50 °C. It should, however, be noted that all the other studies were done in aqueous medium, and thus significant matrix effects may be occurring during inactivation in sludge.

Considerable focus has been given to the presence of ammonia in sludge and its effect on poliovirus and A. suum inactivation (Ward and Ashley, 1977; Ward, 1978; Burge et al., 1983; Pecson and Nelson, 2005). Ward and Ashley (1977) indicated that the presence of ammonia significantly increased the rate of inactivation of poliovirus type 1 strain CHAT, but only at pH values above 8. This suggested that free ammonia rather than ammonium ions contributed to inactivation. The pH of the sludge after the inactivation experiments was 7.6 (see Table 2 for

Table 1- Summary of inactivation rate constants (with 95% confidence intervals) obtained in this study and comparison with previous study by Aitken et al. (2005).

Temperature (°C)	$k_{A. suum} (h^{-1})$	$k_{PVS-1} (h^{-1})$
This study		
51.1	$\textbf{1.79} \pm \textbf{0.16}$	$3.74\pm0.07$
53.3	$2.10\pm0.02$	$4.09\pm0.22$
55.5	$3.05\pm0.19$	$4.54\pm0.25$
Aitken et al., 2005		
49	1.25	3.15
51	$4.40\pm0.19$	$20.85\pm9.31$
53	$17.30 \pm 4.31$	$39.10\pm0.39$
55	13.30	ND
ND = not determined.		

detailed chemical analyses of sludge). At this pH, it is not expected that a significant proportion of ammonia will exist in its nonionic form. Additionally, at higher temperatures, others have attributed the primary phenomenon of inactivation of pathogens to temperature rather than ammonia, irrespective of the pH of the sludge (Pecson et al., 2007).

Lesser attention has been paid to agents present in sludge that may contribute to protection of pathogens against thermal inactivation. Our observation of a comparatively smaller rate constant for inactivation of PVS-1 at high temperatures compared to Aitken et al. (2005) stimulated a detailed literature survey to determine if other researchers have identified agents in sludge that may protect polioviruses. In two papers published in 1978 (Ward and Ashley, 1978a,b), it was determined that the presence of anionic detergents in sludge can significantly reduce the rate of inactivation of poliovirus type 1 strain CHAT at higher temperatures. For experiments conducted at 45 °C, Ward and Ashley observed that in aqueous medium containing individually octanoic acid, SDS, Clindrol 200-L, lauroyl sarcosine and Standapol ES-40, virus inactivation was slower than controls. They determined that a cumulative concentration of 1 g  $L^{-1}$  of anionic detergents resulted in the maximum protective effect. It was further identified that this protective effect was more important at near neutral pH values, because anionic detergents exist in their uncharged form under these conditions. Ward et al. (1976) had earlier determined that the protective effect of the agents later identified as anionic detergents on inactivation of poliovirus type 1 strain CHAT was more prominent in raw sludge, but reduced in digested sludge. They concluded that this was due to the formation of ammonia during digestion. We determined the concentration of SDS, as an indicator anionic detergent, in our first-stage sludge to be 5.8 mg  $L^{-1}$ (Table 2). Although this is lower than the concentrations reported by Ward and Ashley, note that this is only the concentration of SDS, and not a cumulative concentration of all anionic detergents that could be present in the sludge. Thus, some protective effect is expected, especially as the pH of the sludge during digestion was 7.6. Free ammonia would be minimum at this pH, and further, although total ammonia was not measured in the current experiment, its concentration has historically been low in the sludge that was used.

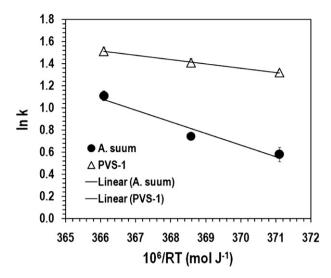


Fig. 4 – Arrhenius plots for inactivation of A. suum and PVS-1 between 51.1 and 55.5 °C. Both linear fits have  $R^2 > 0.9$ . From the plot, the activation energy for A. suum inactivation is calculated as 105 KJ mol<sup>-1</sup>, while that for PVS-1 inactivation is calculated as 39 KJ mol<sup>-1</sup>. Errors bars show standard deviations in k.

Thus free ammonia was not expected to decrease the possible protective effect of anionic detergents, and this may have steered the inactivation mechanism to RNA denaturation despite temperatures of 51.1–55.5 °C. This is consistent with the observation of Ward and Ashley (1979) that poliovirus type 1 strain CHAT that was inactivated with SDS at pH 7.0 still had a stable capsid protein.

In addition to anionic detergents, the presence of certain food additives and amino acids in sludge has been documented to result in a protective effect on enteroviruses inactivation. Milo (1971) studied the effect of cholesterol, lecithin, collagen and  $\beta$ -carotene on thermal inactivation of poliovirus type 1 strain LS-a at 121 °C, and found that collagen significantly reduced the rate of virus inactivation. It was also concluded that the mechanism of inactivation was still RNA denaturation. The effect of the other food additives studied was not as significant. Collagen is the main protein of connective tissues in animals, and thus is expected to be present in sludge to some extent. In addition, sometimes collagen is added to raw sludge prior to digestion for

#### Table 2 – Summary of chemical analyses of sludge.

First-stage digested sludge Sodium dodecyl sulfate  $5.8 \text{ mg L}^{-1}$  $2.7 \text{ mg L}^{-1}$ Cysteine Sludge after inactivation experiments (average of six parallel digesters) рН 3300 mg(eq,CaCO<sub>3</sub>)  $L^{-1}$ Alkalinity Volatile acids 225 mg(eq,CH $_3$ COOH) L $^{-1}$ Total solids 1.85% Volatile solids 71%

dewatering purposes (Yin et al., 2004). Collagen is expected to be converted further to amino acids when present in sludge, through biotic and abiotic degradation. Pohjanpelto (1958) showed that poliovirus type 1 strain R68 was significantly protected at temperatures approaching 50  $^{\circ}\text{C}$  in the presence of amino acids L-cystine or L-cysteine, especially between pH values of 7.0 and 9.0. It was determined that cystine molecules attach to virus protein, forming cross links in the protein capsid, thus making the protein structure firmer and resistant to uncoiling and splitting during thermal stress. We determined that the concentration of cysteine in first-stage digested sludge was 2.7 mg L<sup>-1</sup> Pohjanpelto (1958) noted that cysteine may not directly be protective, but could be through its oxidation to cystine. The lowest concentration of cystine that resulted in appreciable protection in the study by Pohjanpelto (1958) was 6 mg  $L^{-1}$ , or 3 mg  $L^{-1}$  equivalent of cysteine assuming complete oxidation. Lower concentrations were not tested by Pohjanpelto. Even so, at the concentrations of cysteine we report, it is reasonable to assume that some protective activity occurred.

Despite the presence of potentially protective agents in the sludge used in our study,  $\geq$ 2-log reduction of A. suum and  $\geq$ 3-log reduction of PVS-1 at all temperatures tested was achieved significantly faster than the time—temperature relationships as per the current EPA guidelines for production of class A biosolids. Fig. 5 shows a comparison of the time—temperature relationships per current EPA guidelines (U.S. Environmental Protection Agency, 2003), from this study and from the study by Aitken et al. (2005). Note that Aitken et al. (2005) report a more significant impact of temperature on inactivation than this study, and thus the steeper slope for the time—temperature relationships. They did not evaluate the presence of protective agents in sludge, but the faster inactivation rates may possibly be attributed to the absence of these.

On the other hand, other components possibly present in their sludge that are known to increase inactivation rates, but absent or present in low concentration in this study, may have resulted in faster inactivation rates in the study by Aitken et al. (2005). It is possible that the sludge used by them contained high concentrations of VFAs, although in the absence of any documentation related to sludge composition this is only speculation. VFAs are known to be inhibitory to enteric and other pathogens as determined by Kunte et al. (2004). At neutral pHs, this inhibition is significant only at high concentrations (>3000 mg L<sup>-1</sup> of equivalent acetate) (Kunte et al., 2000). The concentration of VFAs in the sludge used in the present study was an order or magnitude lower than this (Table 2), and thus it is unlikely that VFAs contributed significantly to inactivation.

Microbial inactivation thus may be a strong function of the composition of the sludge. While ammonia and VFAs can accelerate inactivation, anionic detergents and amino acids may provide a protective effect. The results from this study indicate that to optimize time—temperature relationships, it may thus be necessary to characterize pathogen inactivation under various sludge compositions, and develop composition-dependent time—temperature relationships. Eventually, investment into developing composition-dependent time—temperature relationships would depend primarily on the cost and energy savings resulting from possible shortening of

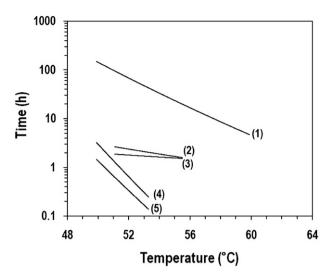


Fig. 5 – Time—temperature relationships for thermal inactivation of A. suum and PVS-1: (1) according to current EPA guideline, (2) for 2-log reduction of A. suum according to this study, (3) for 3-log reduction of PVS-1 according to this study, (4) for 2-log reduction of A. suum according to Aitken et al. (2005), and (5) for 3-log reduction of A. suum according to Aitken et al. (2005).

sludge holding times as a result of better defined relationships vs. resources necessary for evaluating sludge compositions especially for components like anionic detergents and amino acids, which are not frequently analyzed.

Nonetheless, it appears that the current EPA guideline is extremely conservative. For example, for a temperature of 55.5 °C, we determine, in conditions where agents contributing to inactivation other than temperature may not be active and where agents contributing to protection are present, the holding time necessary for 2-log reduction of A. suum and 3-log reduction of PVS-1 to be 1.5 h, while according to the EPA time-temperature relationship, the holding time for this temperature is 20 h. Closer inspection of the EPA guideline reveals that the time-temperature relationships are similar to, and justified by, that suggested by a U.S. Food and Drug Administration (FDA) regulation for pasteurization of eggnog (U.S. Environmental Protection Agency, 2003). It is even more cautious than the largely conservative estimates given by Feachem et al. (1983) for conditions leading to safe inactivation of A. suum and enteroviruses. Further, the FDA regulation is based on an extrapolation between two temperatures, one high and the other low, and thus it does not consider changes in inactivation mechanisms with temperature. Also, because the FDA regulation was derived for pasteurization of eggnog, which has a significantly different matrix compared to sludge, using it as a reference to justify the EPA time-temperature relationships may not necessarily be correct.

#### 4. Conclusions

In this study the kinetics of inactivation of the indicator microorganisms, A. suum and PVS-1, during thermophilic anaerobic digestion were studied. The following conclusions can be derived from the results obtained in this study:

- >2-log reduction of A. suum and >3-log reduction of PVS-1 was obtained within 2 h of treatment at temperatures of 51.1-55.5 °C.
- 2. The activation energies for inactivation of A. suum and PVS-1 as determined from Arrhenius plots were 105 and 39 KJ mol<sup>-1</sup>, suggesting that A. suum was more sensitive to temperature. Both activation energies correspond to the primary inactivation mechanism being nucleic acid denaturation.
- 3. The presence of anionic detergents and amino acids in sludge may result in significant protective activity on the thermal inactivation of PVS-1 and A. suum.
- 4. The inactivation rates observed here are significantly faster than those of the time—temperature relationships from current EPA guideline for obtaining class A biosolids, suggesting that further study is needed in order to provide guidelines that would not be overly conservative.

#### Acknowledgments

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#### Appendix. Supplementary data

Supplementary data associated with the article can be found in online version, at doi:10.1016/j.watres.2010.07.045.

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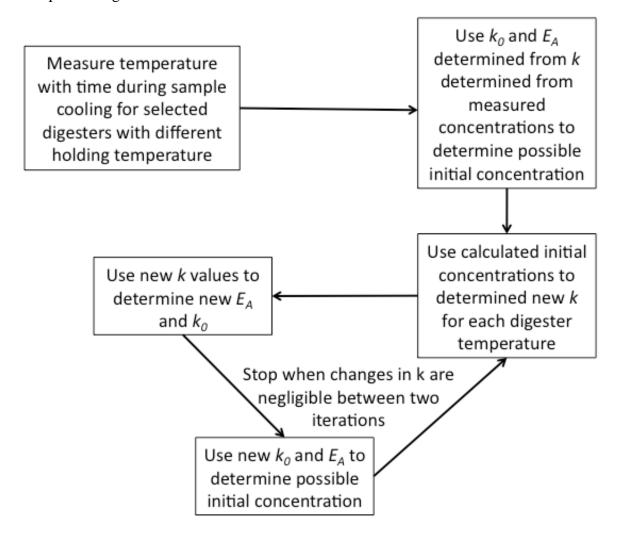
#### Supporting Information for:

### Kinetics of inactivation of indicator pathogens during thermophilic anaerobic digestion

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#### Effect of sample cooling time on sampling results and on inactivation kinetics:

Deactivation of indicator pathogens may occur during cooling of samples. The effect of sample cooling time on inactivation kinetics was determined as follows:



A comparison of the k values determined from measured concentrations (without correction) against those determined from calculated initial concentrations after three iterations of the above process is shown in the table below:

Temperature (*C)	k <sub>A. suum</sub> (h <sup>-1</sup> )	k <sub>PVS-1</sub> (h <sup>-1</sup> )	
Original for one dataset			
51.1	1.87	3.77	
53.3	2.09	4.21	
55.5	2.94	4.41	
After 3 iterations for one dataset			
51.1	1.86	3.77	
53.3	2.09	4.20	
55.5	2.94	4.41	

Note that it was assumed that after the samples reached 35 °C, there is no appreciable inactivation. Thus measured concentrations were assumed as concentrations when the samples reached 35 °C. For samples taken from digesters at 51.1, 53.3 and 55.5 °C, it took 1.9, 2.2 and 2.5 minutes for the temperature to decrease to 35 °C.

The overall conclusion of this exercise is that inactivation during the sample cooling period has very little effect on value of the inactivation kinetic parameters since k values determined using measured concentrations or corrected concentrations are virtually the same.