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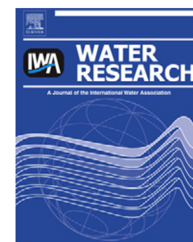

Kinetics of inactivation of indicator pathogens during thermophilic anaerobic digestion

Sudeep C. Popat [a,1], Marylynn V. Yates [b], Marc A. Deshusses [c,*]

[a] Department of Chemical & Environmental Engineering, University of California, Riverside, CA 92521, USA
[b] Department of Environmental Sciences, University of California, Riverside, CA 92521, USA
[c] Department of Civil & Environmental Engineering, Duke University, Durham, NC 27708, USA

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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^{-1} 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

* Corresponding author. Tel.: +1 919 660 5480; fax: +1 919 660 5219.
E-mail address: marc.deshusses@duke.edu (M.A. Deshusses).
[1] Present address: Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA.
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Nomenclature

C	concentration of indicator pathogen at time t
C_0	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 $NaHCO_3$, 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 10^6 ova, while those for recovery tests were 10 mL in volume and contained 5×10^4 ova. PVS-1 spikes for inactivation experiments were 25 mL in volume and contained 10^8 PFU, while those for recovery tests were 10 mL in volume and contained 5×10^5 PFU. *A. suum* spikes were stored at 4 °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 $L^{-1} Na_2HPO_4 \cdot 7H_2O$ 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 ≥2-log reduction of *A. suum* and ≥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

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