WATER RESEARCH 41 (2007) 2893–2902

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

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

Brian M. Pecson[a], *José Antonio Barrios*[b], *Blanca Elena Jiménez*[b], *Kara L. Nelson*[a,*]

[a]*Department of Civil and Environmental Engineering #1710, University of California, Berkeley, CA 94720-1710, USA*
[b]*Instituto de Ingenieria UNAM, Apartado Postal 70-402, Ciudad Universitaria, 04510 Coyoacan, Mexico, D.F., Mexico*

ARTICLE INFO

Article history:
Received 4 January 2007
Received in revised form
13 March 2007
Accepted 30 March 2007
Available online 23 May 2007

Keywords:
Ascaris
Sludge
Biosolids
Alkaline stabilization
Ammonia
Pathogens

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_3-N) and $Ca(OH)_2$ 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_{99}) was also calculated. Each 10 °C increase in temperature caused a significant decrease in t_{99} 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_{99}, 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_{99}. 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.

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

*Corresponding author. Tel.: +1 510 643 5023; fax: +1 510 642 7483.
E-mail addresses: bmpecson@gmail.com (B.M. Pecson), JBarriosP@iingen.unam.mx (J.A. Barrios), bjc@pumas.iingen.unam.mx (B.E. Jiménez), nelson@ce.berkeley.edu (K.L. Nelson).
0043-1354/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.03.040

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, $Ca(OH)_2$, 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_4^+ to NH_3, 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_3 (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, $Ca(OH)_2$, 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 $Al_2(SO_4)_3$ 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_3 by ABC Laboratory, Mexico City). The %TS of the sludges was adjusted to 5% by addition of water. Analytical grade calcium hydroxide ($Ca(OH)_2$) 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_4Cl (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_3-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 $ZnSO_4$, 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