Ecological Engineering 97 (2016) 633–641

Contents lists available at ScienceDirect

Ecological Engineering

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

Simulating the effects of mesophilic anaerobic and aerobic digestions, lagoon system, and composting on pathogen inactivation

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ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form 8 August 2016
Accepted 7 October 2016

Keywords:
Animal waste
Anaerobic digestion
Lagoon system
Composting
Pathogens

ABSTRACT

To improve understanding of pathogen inactivation in flushed dairy manure during anaerobic digestion, lagoon systems, and mesophilic composting, we assessed pathogen inactivation in batch-scale anaerobic digesters, aerobic lagoons, and manure piles. Further, we assessed the impact of air injection in lagoon systems on pathogen inactivation. The inactivation of *Salmonella* and *E. coli* was studied for more than 70 days. The change in pH, total solids, total carbon, and total nitrogen during manure treatment was determined. The results showed that the lagoon system with air injection to be more effective in pathogen removal compared to the lagoon system with no air injection, anaerobic digestion, and mesophilic composting. Both types of lagoon systems (with and without air injection) were found to be more effective in eliminating pathogens than anaerobic digester systems. Mesophilic manure pile extended the survival of both *Salmonella* and *E. coli*. In the anaerobic system, *E. coli* was detected beyond 60 days of incubation, while in the aerobic system; it was eliminated in 30–35 days. In anaerobic system, *Salmonella* reached to non-detectable level in 30–35 days, while in aerobic system it took 4–12 days. In the mesophilic manure pile, however, *E. coli* survival was extended beyond 78 days. Less than one log reduction was obtained in 78 days of mesophilic composting. *Salmonella* survival was also extended in mesophilic composting pile.

1. Introduction

Controlling the elevated pathogen/pathogen indicator levels in animal manure is a serious issue, which can potentially pose a risk to public and environmental health (USEPA, 2015). In the USA, more than 238,000 confined animal feeding operations generate greater than 317 million gallons of manure, which is commonly used as fertilizer to increase the nutrient levels in crop land (Armstrong et al., 2010; Pandey et al., 2015). Previous studies have reported pathogen presence in animal waste (McLaughlin et al., 2009; Toth et al., 2013). For example, in more than 81–90% of lagoons in Mid-South USA were found to be positive for various pathogens including *Salmonella, E. coli* and *Campylobacter* (McLaughlin et al., 2009). Another study conducted a survey to enumerate pathogens in dairy operation and reported pathogens in 12 out of 13 surveyed dairy operations in south east and south-central Pennsylvania (Toth et al., 2013)

To treat the manure prior to land application, various manure treatment methods including anaerobic digestion, lagoon system, and composting are commonly used (Cole, 2015; Grewal et al., 2006; Pandey et al., 2015; Ravva et al., 2006; Ravva and Sarreal, 2014; Rumburg et al., 2004). Previous studies reported certain degree of effectiveness of all of these methods in eliminating pathogens; however, additional understanding is still needed to improve the existing manure management practices to control the influx of manure borne pathogens into environment and crop land for minimizing the food and public health risks.

While aerobic processes are known to be more effective in eliminating pathogens compared to anaerobic digestion (Grewal et al., 2006; Pandey et al., 2015), treatment of manure using anaerobic digestion has a potential to produce biogas, which can be used as renewable energy (Belle et al., 2015; Saady and Massé, 2015; Witarsa and Lansing, 2015). In addition to anaerobic digestion and lagoon systems, composting is also used to treat dairy, pig, and chicken manure (Erickson et al., 2014a; Jiang et al., 2015;

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http://dx.doi.org/10.1016/j.ecoleng.2016.10.047
0925-8574/

Zhou et al., 2015). It has been shown that proteins degradation by ammonification in composting process increases temperature, pH and ammonia levels, which lead to hygienisation (Heinonen-Tanski et al., 2006).While composting process produces elevated temperature (>55 °C), which is detrimental to pathogens (Grewal et al., 2006; Pandey et al., 2016a) the attainment of this higher temperature for extended period of time is often challenging (Pandey et al., 2016b). In low temperature composting, pathogen survival is likely extended. The composting piles with sub-composting temperature (<50 °C) or with mesophilic temperature are often reported describing the potential challenges in temperature enhancement (Erickson et al., 2014b; Grewal et al., 2006; Turner, 2002; Pandey et al., 2016b). In general, the mesophilic temperature for extended period of time is common in anaerobic digesters, lagoon system, and composting pile treating dairy manure. How these processes influence *Salmonella* and *E. coli* levels in flushed dairy manure at mesophilic temperature condition is not well understood. Further, how the injection of air into lagoon systems affect the inactivation of pathogens in flushed manure is not clearly understood.

The goal of this study is to understand the pathogen inactivation in anaerobic digestion, lagoon system, and composting pile in mesophilic temperature condition. The objectives are to: (1) assess *Salmonella* and *E. coli* inactivation in anaerobic digestion; (2) evaluate *Salmonella* and *E. coli* inactivation in lagoon system with and without air injection; and (3) determine *Salmonella* and *E. coli* inactivation in mesophilic manure pile. Additionally, we evaluated the changes in pH, total solids (TS), total carbon, and total nitrogen (TN) during anaerobic, lagoon, and mesophilic composting processes.

2. Methods

2.1. Experiment setup

Flushed manure used in this study was collected from a dairy farm in Tulare County, California, Davis. The farm houses between 2000 and 3000 dairy cows. More than 30 gallons of flushed manure was collected at the inlet to lagoon system one day prior to starting the experiment and was transported to School of Veterinary Medicine Extension, University of California, Davis for executing the pathogen challenge study in flushed manure using aerobic, lagoon, composting, and anaerobic systems. The experiment setup is shown in Fig. 1. Three 22 gallon bins (75cm × 45cm × 20 cm) were connected with a closed loop heating system (Pandey et al., 2016a) to maintain the mesophilic temperature (36 ± 2 °C). Two bins (each 22 gallons) were converted into a representative of lagoon system (i.e., top open), and one bin was used for creating a manure pile (Fig. 1), which has an open top. One lagoon system received air injection, while other does not receive air injection. The air was injected for 15 min with 12 h interval. A programmable timer (Prime, Cutnstk624) was used to control on/off of the aerator. To inject the air, a piston pump, ¼ HP, 115 VAC (Welch 2546B-01A) was used. The lagoon system, which did not receive air injection, was named AerD1, and the lagoon system which received air injection was named as AerD2. The bin which was used for creating mesophilic manure pile was named as MMP. Two additional 5 gallon bins were transformed into anaerobic digesters shown in Fig. 1. Anaerobic environment in both of the bins were created, and placed in mesophilic incubators (36 ± 2 °C). The one anaerobic reactor received flushed manure with 2.5% total solid (TS) and was named as AnaD1, and another anaerobic digester, which received flushed manure with the TS of 1% was named as AnaD2. Each anaerobic reactor received 4 gallons of inoculated flushed manure. Each of AerD1 and AerD2 received 8 gallons of inoculated flushed manure. The solid manure was collected from the same dairy farm to create mesophilic manure pile (i.e., MMP). In flushed manure system, solid is separated from flushed manure using a mechanical liquid solid separator prior to disposing flushed manure into lagoon system. All AnaD1, AnaD2, AerD1, AerD2, and MMP were maintained at 36 ± 2 °C temperature conditions during the whole experiment.

2.2. Pathogen inoculation, sampling procedure, and sample testing

To inoculate *Salmonella* into manure, pure strains of *Salmonella typhimurium LT2* (ATCC # 700720) was grown overnight in Luria-Bertani (LB) broth (Difco LB Broth Miller; Becton, Dickinson and Company, Sparks, MD, USA). A bench top incubator shaker (MaxQ 4000, Thermo Scientific, Ohio, USA) was used at 100 rpm and 37 °C for 24 h for growing *Salmonella*. Quality control was ensured using a negative control in the respective growth media. Subsequently the growth of pathogen (300 mL) was dissolved in each bin and mixed homogeneously to distribute *Salmonella* uniformly in the feedstock.

In addition to *Salmonella* measurement, we also enumerated *E. coli* levels. While *Salmonella* was inoculated, *E. coli* was naturally present in the dairy manure; therefore, *E. coli* inoculation was not needed. This naturally occurring *E. coli* in manure was measured throughout the digestion period to understand *E. coli* inactivation. MacConckey II agar with sorbitol (BBL, Becton, Dickinson and Company, Sparks, MD, USA), a selective and differential media for the detection of sorbitol-nonfermenting *E. coli* was used. When plated on MacConckey II agar with sorbitol, manure sample with *E. coli* resulted in pink colonies, which were enumerated as *E. coli* colonies. To enumerate *Salmonella,* Xylose Lysine Desoxycholate (XLD) agar (Difco, Becton, Dickinson and Company, Sparks, MD, USA) was used. Colonies with red-yellow with black centers in the agar plates were enumerated as *Salmonella*. The anaerobically and aerobically digested manure samples were collected from anaerobic and aerobic chambers, respectively. To test pathogens, initial feedstock was collected on day 0 from anaerobic and aerobic chambers. Subsequently, digested samples were collected on day 1, 5, 9, 12, 15, 19, 22, 26, 33, 40, 48, 56, 61 and 77. To test the pathogens, a composite sample collection and testing strategy was adopted. As an example, during each sample collection, firstly the mixing was performed inside the digesters. Secondly, samples were collected three times consecutively from three different locations in aerobic chambers. In anaerobic chambers, three consecutive samples (within 2–3 min) were collected from anaerobic digester effluent ports. To create the composite samples, all these three consecutive samples from each chamber were mixed thoroughly. Subsequently the composite samples were stored in 4 °C till processing. Bacterial analysis (i.e., *E. coli* and *Salmonella*) was performed within 24 h of sample collection. To enumerate *Salmonella* and *E. coli*, the serial dilutions of the samples were created using phosphate buffer solution (PBS). Several levels [higher (10^{-7}) and lower (10^{-3}–10°)] of dilution was plated in agar plates. Subsequently, pathogen growth in agar plates was enumerated, and pathogen levels in per unit mass (g/ml) of original sample were calculated. All the samples were plated in duplicates. Bacteriological Analytical Manual (BAM) culture procedure was followed for pathogen testing (USFDA, 2014). While MacConckey agar plates were used for *E. coli* testing, *Salmonella* was enumerated using XLD. MacConckey plates were incubated at 37 °C, and XLD plates were incubated at 35 °C for 24 h to promote the growth of pathogens in agar plates. The growth of the respective pathogens was enumerated using a colony counter. As this study was mainly focused on understanding pathogen inactivation, the limited observations were made for estimating total nitrogen, pH, and carbon content. Dissolved oxygen concentration, which may have changed over the time during aeration, was not monitored considering that aeration was executed for limited time. The total nitrogen (TN), pH, and total carbon were determined using standard method (APHA, 1998). All these parameters were measured in