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ORIGINAL PAPER

Escherichia coli persistence kinetics in dairy manure at moderate, mesophilic, and thermophilic temperatures under aerobic and anaerobic environments

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Abstract To assess *Escherichia coli* (*E. coli*) persistence in dairy manure, bench scale experiments were conducted under aerobic and anaerobic environments. The changes in *E. coli* levels in dairy manure were assessed at moderate (25 °C), mesophilic (37 °C), and thermophilic (52.5 °C) temperatures. The inactivation of *E. coli* at moderate, mesophilic, and thermophilic temperatures were described by linear regression equations. Subsequently, double-exponential kinetic models were developed to describe the *E. coli* decay curves under aerobic and anaerobic environments. The kinetics models were used to estimate *E. coli* log reductions at various temperatures. Results showed that the double-exponential kinetic models performed well while calculating *E. coli* reductions in dairy manure over the incubation period. In addition, we evaluated digestate to compare the changes in total solids and volatile solids, total organic carbon, total nitrogen, pH, and oxygen reduction potential levels in aerobic and anaerobic conditions under various temperatures. We anticipate that the results presented here will be useful for enhancing the understanding of pathogen reduction in anaerobic and aerobic processes during dairy manure treatment.

Keywords Aerobic · Anaerobic · Dairy manure · *E. coli* · Inactivation

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Introduction

Animal waste-borne pathogens, which can be a potential source of environmental contamination, pose a risk to human and animal health [1]. The U.S. has approximately, 238,000 confined animal feeding operations (CAFOs), which generate more than 317 million gallons of manure annually [2]. Although animal manure is a great source of soil nutrients, untreated manure with animal waste pathogens can pose risk to human and animal health. For example, Toth et al. [3] conducted a survey of five animal-borne pathogens in 13 dairy operations in south east and south-central Pennsylvania and 12 of the operations were found to be pathogen-positive.

The unsafe disposal of animal waste can potentially elevate the water-borne pathogen levels in ambient water bodies. In the U.S., more than 480,000 km of rivers and streams and 2 million ha of lakes are contaminated [4]. One of the major causes of contamination is elevated levels of water-borne pathogens. According to the U.S. Environmental Protection Agency (USEPA), water-borne pathogens cause more than 900,000 illnesses and 900 deaths each year in the U.S. [5].

Controlling water-borne pathogens requires improving existing animal manure disposal practices. For instance, applying manure into cropland as a fertilizer is a common method of animal waste disposal. However, pathogens such as *E. coli* O157:H7, *Salmonella enterica*, *Cryptosporidium* spp., *Campylobacter jejuni, Mycobacterium avium* ssp., and *Listeria monocytogenes* present in animal waste can survive in cropland (receiving animal manure as a fertilizer) and are likely to be transported from the land receiving manure to ambient waters [6–10]. Several of the above pathogens are prone to cause various diseases in humans and animals [11].

Increasing concerns over water and food safety require developing and identifying safe manure disposal techniques. The existing technologies such as anaerobic digestion, aerobic digestion, and composting are often used in manure treatment [12]. However, improvements in understanding of the efficiencies of anaerobic, aerobic, and composting processes is needed to control pathogens present in the animal waste. In animal waste treatment, anaerobic digestion has been found to be a sustainable practice for treating manure and protecting the environment [13]; moreover, the effectiveness of anaerobic digestion processes in pathogen reduction is a matter of renewed interest. Changes in pathogen/pathogen indicator levels during anaerobic digestion under various temperatures (thermophilic and mesophilic) are well-reported by a few studies indicating that pathogens may survive anaerobic processes [14, 15]. Previous studies showed the persistence of bacteria including antimicrobial-resistant bacteria in influent as well as effluent of wastewaters [13, 14]. Therefore, the improvement in anaerobic processes (i.e., identifying the optimum temperature and incubation period) which eliminates animal pathogens is needed. Enteric viruses and *Salmonella* spp. persist in anaerobic digestate. For example, Viancelli et al. [16] evaluated swine manure treatment systems (anaerobic and aerobic) and reported that aerobic treatment can be more effective than anaerobic digestion for inactivating pathogens such as total coliforms, *E. coli*, and *Salmonella*, spp. circovirus, and paravovirus. The performance of both anaerobic and aerobic processes depends on factors such as temperature, antibiosis, redox potential, and nutrients [17]. Composting is another common process used for treating the animal waste. The composting efficiency for controlling pathogens depends on various factors such as pH, C/N ratio, and moisture. The use of composting for elimination of pathogens completely, however, can be debatable [18–26].

Though the existing studies found that anaerobic and aerobic digestion processes can reduce pathogens levels considerably, very few comparative studies are available evaluating pathogen inactivation in both anaerobic and aerobic environments [27, 28, 17]. The removal of pathogens in anaerobic and aerobic digestion is controlled by many factors including temperature and retention periods (i.e., incubation days). Identifying the optimum temperature and incubation periods for pathogen reductions in animal waste are needed. In addition, an understanding of the efficiencies of anaerobic and aerobic processes for pathogen control under various environmental conditions can help in developing farm-scale animal waste management practices, which are likely to be safe practices for animal waste treatment.

Further, development of simple inactivation/persistence kinetic models can be beneficial to estimate the pathogen removal under various environmental conditions and can be useful in identifying/optimizing the controlling parameters such as temperature and incubation periods for enhancing animal waste treatment. Therefore, here we assessed pathogen indicator, *E. coli* inactivation under aerobic and anaerobic conditions at various temperatures, and developed the *E. coli* persistence kinetic models for estimating *E. coli* inactivation in anaerobic and aerobic processes for dairy manure treatment. The objectives of the study proposed here were to: (1) compare the performance of aerobic and anaerobic digestion processes on *E. coli* removal; (2) assess the impacts of temperature on *E. coli* inactivation; (3) assess the impacts of anaerobic and aerobic processes on total solids (TS), volatile solids (VS), pH, C/N, and C/P changes; and (4) develop the *E. coli* persistence kinetic models which are capable of calculating *E. coli* removal in aerobic and anaerobic digestion processes.

Materials and methods

The feedstock was prepared by collecting fresh dairy manure from Iowa State University's research dairy facility 24 h prior to the experiment. The fresh manure of 0.50 kg was mixed with 1,500 ml of distilled water to prepare the manure slurry. The slurry was sieved through a 850 μm opening (USA standard testing sieve, No 20, Fisher Scientific Company) for separating the fibers and large solid particles from the feedstock. The filtrate (i.e., slurry passed through 850 μm sieve) was transferred into six anaerobic and six aerobic batch reactors. The anaerobic reactors were 250 ml serum bottles (Scientific Instrument Services, NJ, USA), while aerobic reactors were 250 ml flasks. Each reactor was provided with 150 ml of feedstock. To create anaerobic environment, reactors were sealed with a rubber septum (Sigma-Aldrich, sleeve stopper, MW 09194, St. Louis, MO, USA). In aerobic conditions, the mouth of the flask was open. To ensure anaerobic conditions within the reactors, the air above the feedstock was removed. To provide the variable heating to reactors, aerobic and anaerobic reactors were placed in an orbital water bath shaker (New Brunswick Scientific, Classic Series C7, 400768741, Edison, New Jersey, USA). During each experiment, the water bath shaker speed was maintained at 150 rpm.

Six sets of experiments: three under anaerobic conditions and three under aerobic conditions were performed. Three anaerobic experiments include anaerobic incubation of feedstock at moderate (25 °C), mesophilic (37 °C) and thermophilic (52.5 °C) temperatures. Similarly, three aerobic incubations were performed at moderate, mesophilic, and thermophilic temperatures. To test the incubated

feedstock for *E. coli* levels, approximately, 5 ml of incubated slurry of each reactor (anaerobic and aerobic) was collected using a 35 ml gas tight glass syringe (Micro-Mate, Popper & Sons Inc, New Hyde Park, NY, USA). The *E. coli* concentrations in the incubated slurry were determined by membrane filtration technique (US EPA, method 1603) and enumerated on modified mTEC agar (Difco™, Modified mTEC agar, Becton, Dickinson and Company, Sparks, MD, USA) [29]. The liquid samples collected from the reactors were stored at 4 °C, and analyzed for *E. coli* concentrations within 24 h of collection. The slurry was serially diluted, and the diluted samples were filtered through membrane filters (white, grid marked, 47 mm diameter, with 0.45 µm pore size) (Millipore, FOEA 22910, HAWG047S, France). Subsequently, the filters were placed on petri dishes containing modified mTEC agar. All analyses were performed in triplicate. Petri dishes with filters were incubated at 44.5 ± 0.2 °C for 24 h, which resulted in the growth of the red or magenta *E. coli* colonies on filters. The colonies were counted using hand held electronic colony counter (Scienceware/Bel-Art Products). The total nitrogen (TN), total orthophosphate, and total carbon concentrations of the slurries were determined using a C:N analyzer [30]. The TS and VS were estimated using standard methods [29]. Oxygen reduction potential (ORP) and pH were measured using WTW portable meters 3,110 with pH and ORP sensors.

Results and discussion

E. coli reductions

Figure 1a shows comparative *E. coli* reductions under aerobic and anaerobic digestions at moderate temperature (25 °C). For aerobic digestion, the 15 day incubation resulted in *E. coli* reductions from log 7.8 to 3.2; in anaerobic digestion, 35 days incubation was required for *E. coli* reduction from log 7 to 3. In anaerobic digestion, *E. coli* levels of log 2 were still present at the end of the 60 day incubation period. In aerobic digestion, *E. coli* levels were <log 1 within 19 days of incubation. Liner regression line fitting between incubation days and *E. coli* reduction (log values) is shown in Fig. 1a. The liner fit yielded R^2 of 0.81 and 0.82 for anaerobic and aerobic digestions, respectively. While studying the pathogen survival during livestock manure storage and following land application, Nicholson et al. [31] reported the survival of *E. coli O157*, *Salmonella,* and *Campylobacter* in stored slurries and dirty water for up to 3 months (in normal temperature). Listeria survival was expected to be greater than 3 months. The survival of pathogens was considerable shorter (<30 days) in manure heaps when an inside

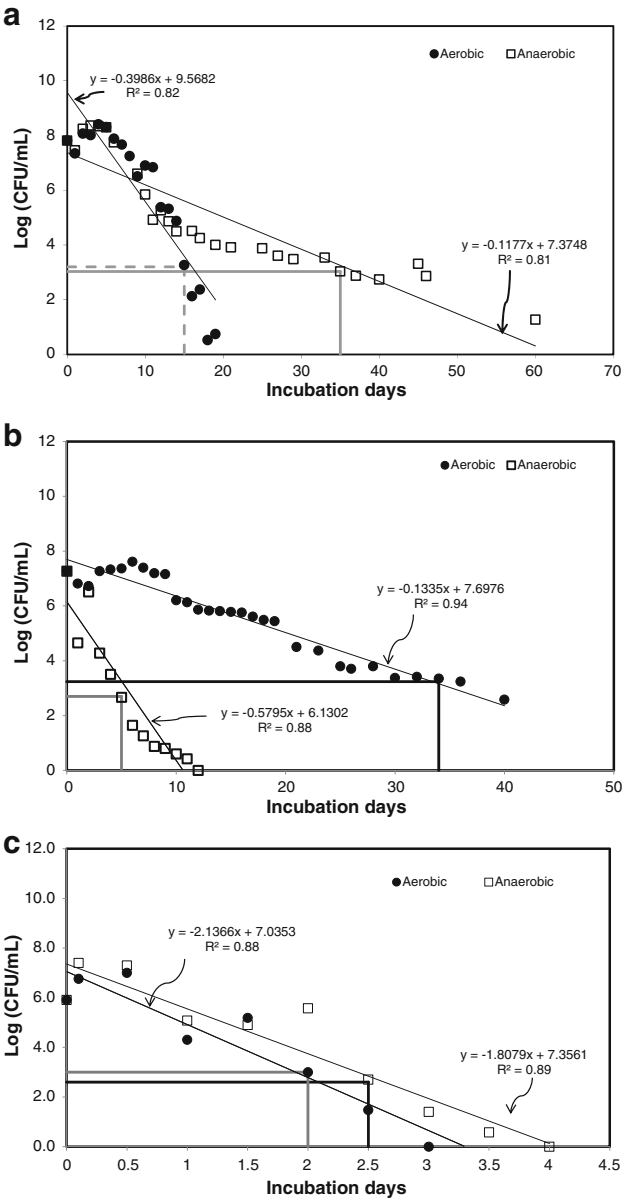

Fig. 1 **a** Comparative *E. coli* inactivations in aerobic and anaerobic digestions (anaerobic digestion data source: Pandey and Soupir [15]) at moderate temperature (25 °C). **b** Comparative *E. coli* inactivations in aerobic and anaerobic digestions at mesophilic temperature (37 °C). **c** Comparative *E. coli* inactivations in aerobic and anaerobic digestions at thermophilic temperature (52.5 °C)

temperature was increased to thermophilic conditions (greater than 55 °C).

E. coli reductions at mesophilic temperature is presented in Fig. 1b. The figure showed comparative *E. coli* reductions under aerobic and anaerobic digestions in mesophilic temperature (37 °C). Similar to moderate temperature, *E. coli* reductions in the aerobic reactors were faster than the anaerobic reactors. For example, *E. coli* reduction from log 7.2 to log 3.3 was observed in/during 34 days of

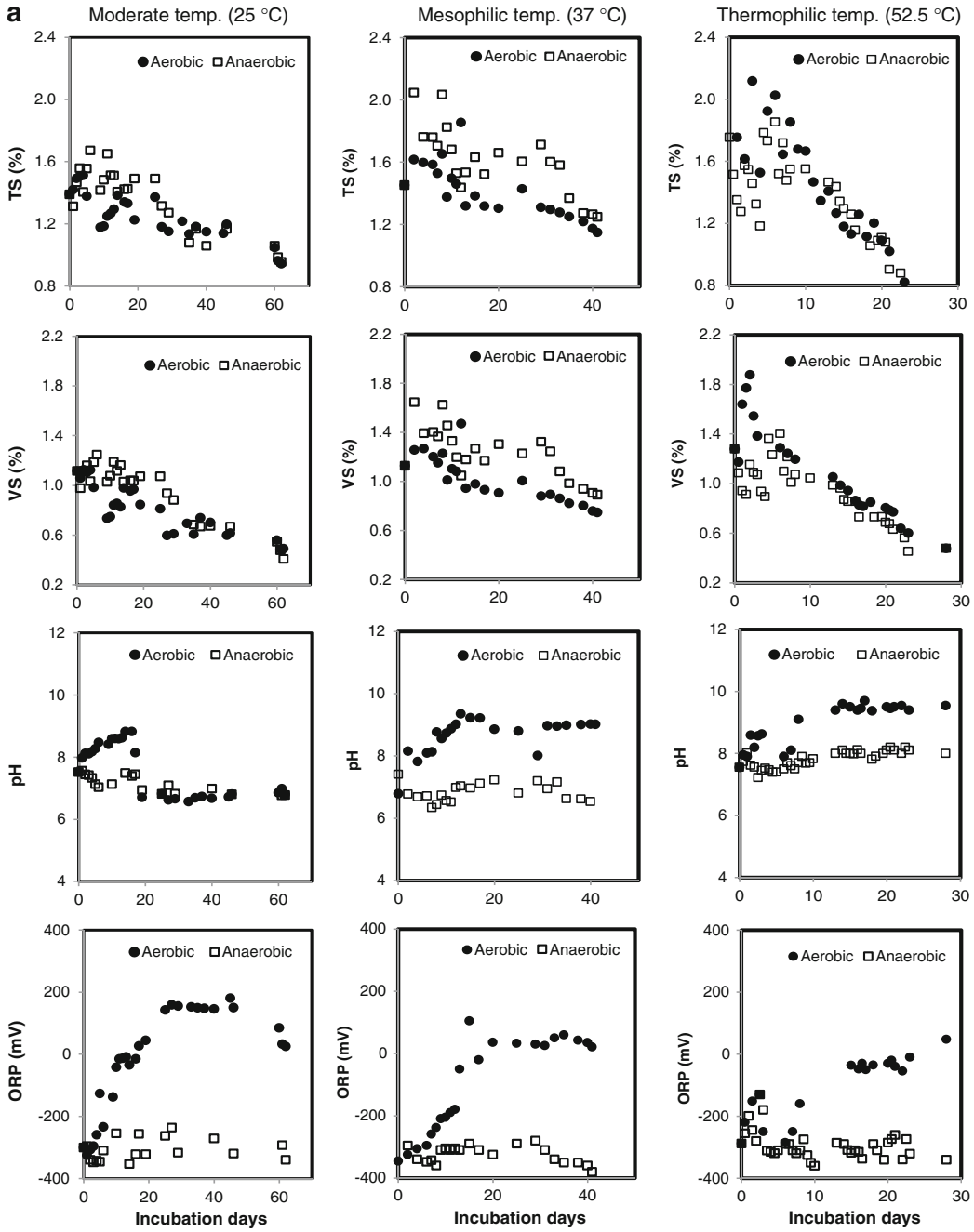

Fig. 2 **a** Comparative analysis of TS, VS, pH and ORP in aerobic and anaerobic digestions. **b** Comparative analysis of TOC/TN, and TOC/PO_4 in aerobic and anaerobic digestions

incubation in anaerobic digestion, while in aerobic digestion within 5 days of incubation, *E. coli* levels decreased from log 7.2 to log 2.7 (Fig. 1b). The linear regression correlations between incubation days and log *E. coli* levels in anaerobic and aerobic inactivations were performed; R^2 values of mesophilic temperature were slightly better than the moderate temperature. Linear regression trend line fittings in anaerobic and aerobic digestions resulted R^2 values of 0.94 and 0.88, respectively. Kearney et al. [9] evaluated the effect of anaerobic digestion on survival of pathogenic bacteria (*E. coli*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and *L. monocytogenes*) in beef slurry and reported the increased declinations in viable numbers with increased temperatures. The declination of pathogens was rapid in the beginning of mesophilic anaerobic digestion processes, followed by slow declination. The experiment

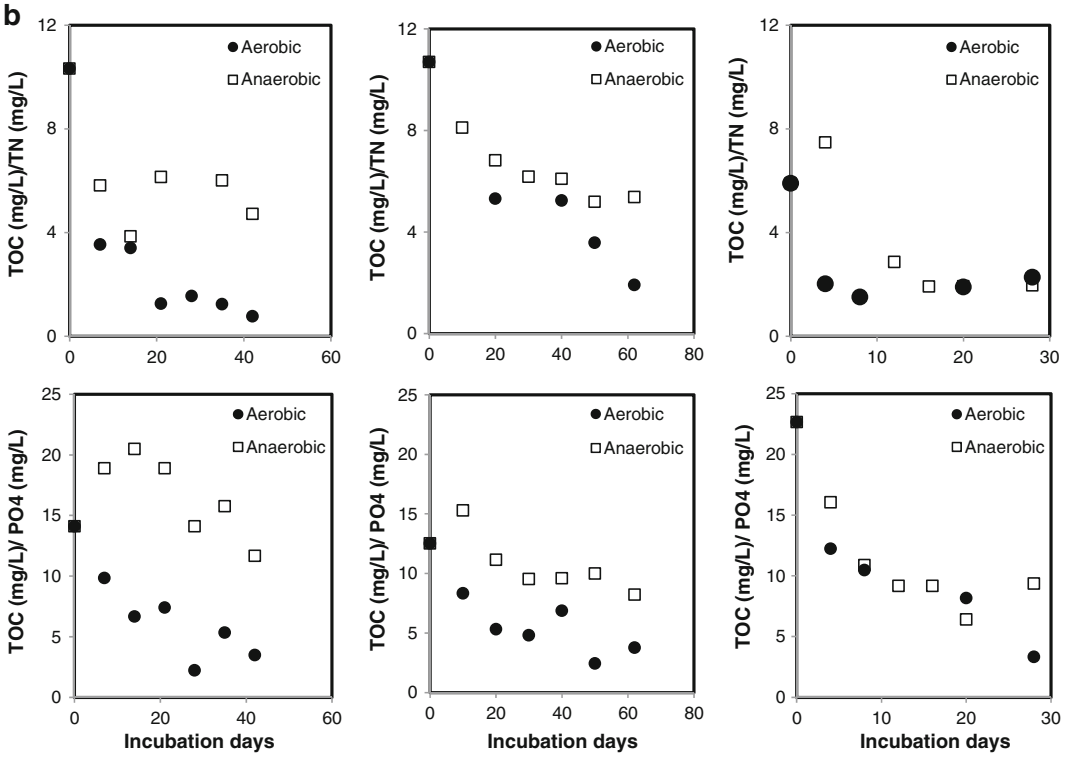

Fig. 2 continued

also indicated relatively slow declination in *E. coli* levels between 15 and 35 days of anaerobic incubation. In aerobic condition the declination was quicker than anaerobic conditions. Kearney et al. [9] noted that pathogen (*L. monocytogenes*) reduction was faster in batch digestions compared to semi-continuous digestion (T_{90} value in batch reactor was 12 days, while in semi-continuous reactor it was 36 days). Our study was solely focused on comparing the *E. coli* inactivations in anaerobic and aerobic conditions under batch mode; however, we anticipate relatively longer pathogen survival in semi-continuous/continuous processes either in anaerobic or aerobic environments.

E. coli reductions in anaerobic and aerobic conditions under thermophilic temperatures is presented in Fig. 1c. The figure showed comparative *E. coli* reductions under aerobic and anaerobic digestions. At the thermophilic temperature, *E. coli* reduction was quicker than at moderate and mesophilic temperatures. At the thermophilic temperature, *E. coli* reduction in aerobic and anaerobic environments was similar. For instance, *E. coli* reduction from log 6 to 2.5 occurred within 3 days (60 h), while in aerobic digestion, a similar reduction occurred in ≈48 h. Liner regression line fittings are shown in Fig. 1c, which resulted in a R^2 value of 0.89 for anaerobic digestion, and 0.88 for aerobic digestion.

Changes in TS, VS, pH, ORP, C/N and C/P

The changes in TS, VS, pH, ORP, C/N (TOC/TN) and C/P (TOC/PO_4) are given in Fig. 2a, b. Figure 2a shows TS, VS, pH, and ORP reduction at moderate, mesophilic, and thermophilic temperatures. As shown in the figure, TS and VS reductions in anaerobic digestion was 31 and 63 %, while in aerobic digestion the TS and VS reduction were 32 and 56 %, respectively. The ORP value in anaerobic digestion varied from −300 to −340 mV, while in aerobic digestion, ORP value varied from −300 mv (at the beginning of experiment) to 25 mV. Table 1 summarizes the TS, VS, ORP, *E. coli counts*, C/N, and C/P changes in anaerobic and aerobic conditions. Overall, pH was increased in the anaerobic process; however, when pH was decreased to 5, biogas production ceased indicating inhibition of anaerobic processes. In a previous study, Prochazka et al. [32] studied pH and biogas production in batch mode anaerobic digestion of animal waste, and reported that higher buffer capacity of slurry leads to smaller changes in the pH (over time pH increased slightly in the anaerobic digestion process).

Figure 2b demonstrates C/N and C/P reductions in moderate, mesophilic, and thermophilic temperatures under anaerobic and aerobic digestions. At moderate temperature, C/N and C/P reductions in anaerobic digestion

Table 1 Summary of changes in *E. coli*, pH, TS, VS, C/N, and C/P under anaerobic and aerobic digestion processes

	Digestion processes					
	Anaerobic			Aerobic		
Temperature (°C)	25	37	52.5	25	37	52.5
Incubation days	62	41	28	62	41	28
Initial *E. coli* levels (log)	7.8	7.2	5.9	7.8	7.2	6.7
E. coli reductions (log)	3 (in ≈35 days)	3 (in ≈32 days)	3 (in ≈2.5 days)	3 (in 15 days)	3 (in 5 days)	3 (in ≈2 days)
TS reductions (%)	31	14	62	32	21	53
VS reductions (%)	63	21	63	52	34	63
pH changes	10 % (−)	11 % (−)	6 % (+)	10 % (−)	33 % (+)	26 % (+)
C/N reductions (%)	54	50	67	93	82	61
C/P reductions (%)	17	37	59	75	75	85

were 54 and 17 %, while in aerobic digestion the reductions were 93 and 75 %, respectively. In mesophilic temperature, C/N values in anaerobic and aerobic digestions were reduced by 50 and 82 %; while C/P values were reduced by 37 and 70 %, respectively. In thermophilic temperature, C/N and C/P values in anaerobic digestion were reduced by 67 and 59 %, while in aerobic digestion these reductions were 61 and 85 %, respectively. Results showed reduction in C/N in both anaerobic and aerobic processes over the time. Previous studies [33–35] showed that C/N ratio controls the bacterial dynamics in anaerobic process. For instance, higher C/N ratio reduces microbial population because of the limited availability of essential nutrient such as nitrogen at higher C/N ratio. A study by Viancelli [16] compared changes in chemical compositions and pathogen levels in two swine manure treatment systems (system with solid–liquid separation followed by anaerobic and aerobic digestions and system without solid–liquid separation followed by anaerobic and maturation lagoons). Authors reported that the total coliform levels were decreased from 5 log (in raw manure) to 3 log (in effluent). Similarly, *E. coli* levels were decreased from 4.7 to 2.3 log. To compare the correlations among parameters, Table 2 shows multivariate correlation analysis of physicochemical parameters in anaerobic and aerobic environment at various temperatures. Results indicated TS, VS, and VS/TS were strongly correlated, while the correlations among other parameters (i.e., ORP, and pH) were lower or weaker.

Persistence kinetics models for *E. coli* reductions in anaerobic and aerobic digestion

To develop kinetics models, first, we estimated the decay rate coefficients, *k,* for moderate, mesophilic, and thermophilic temperatures. The *k* values of moderate, mesophilic, and thermophilic temperatures in aerobic and anaerobic digestions are shown in Fig. 1a–c (i.e., slopes of the linear fits). Second, the *k* values for aerobic and anaerobic digestions at different temperatures were used to obtain the inactivation coefficients E_1 and E_2 for aerobic and anaerobic digestions. The E_1 and E_2 were estimated by plotting $1/T$ vs ln (k) (shown in Fig. 3). A similar approach i.e., exploiting the use of activation energy for developing the Arrhenius plots for the inactivation of *Ascaris suum* and vaccine strain poliovirus type 1 (PVS-1) is reported elsewhere [36]. The authors assessed kinetics in the inactivation of indicator pathogens during thermophilic anaerobic digestion. Results showed that the first-order-inactivation rate constants (*k*) followed Arrhenius relationships indicating that indicator pathogens were sensitive to temperature changes. Another study by Aitken et al. [37] also evaluated time–temperature relationships, while determining the kinetics of inactivation of indicator pathogens, and concluded that the effect of temperature was dominant in the inactivation of indicator microorganisms.

We estimated E_1 and E_2, the slopes of the linear fit between $1/T$ and ln (k) (natural log of k), which resulted R^2 values of 0.93 for aerobic digestion and 0.82 for anaerobic digestion (Fig. 3). As shown in the figure, the values of decay rate coefficients were greater at elevated temperatures (i.e., lower values of $1/T$). That means increased temperature elevates the inactivation of *E. coli*, which is clearly shown in Fig. 1a–c where the inactivation of *E. coli* was greater at thermophilic and mesophilic temperatures than at moderate temperature (25 °C).

We used inactivation coefficients (E), temperatures (T), and incubation days (t) to derive the following (Eqs. 1, 2) two double–exponential models for calculating *E. coli* reductions in aerobic and anaerobic digestions. Exponential and double exponential models have been explored

Table 2 Multivariate correlation analysis of physicochemical parameters under anaerobic and aerobic environments (correlations were estimated by restricted maximum likelihood (REML))

	Anaerobic					Aerobic				
	TS	VS	VS/TS	ORP	pH	TS	VS	VS/TS	ORP	pH
Moderate temperature (25 °C)										
TS		0.97	0.84	0.45	−0.19		0.94	0.82	0.58	−0.69
VS	0.97		0.94	0.58	−0.19	0.94		0.96	0.66	−0.81
VS/TS	0.84	0.94		0.67	−0.05	0.82	0.96		0.67	−0.63
ORP	0.45	0.58	0.67		−0.3	0.58	0.66	0.67		−0.63
pH	−0.19	−0.15	−0.05	−0.3		−0.69	−0.81	−0.8	−0.63	
Mesophilic temperature (37 °C)										
TS		0.98	0.74	0.31	−0.04		0.99	0.89	−0.74	−0.34
VS	0.98		0.84	0.34	−0.08	0.99		0.95	−0.8	−0.41
VS/TS	0.74	0.84		0.42	−0.1	0.89	0.95		−0.88	−0.53
ORP	0.31	0.34	0.42		0.48	−0.74	−0.8	−0.88		−0.68
pH	−0.04	−0.08	−0.1	−0.48		−0.34	−0.41	−0.53	0.68	
Thermophilic temperature (52.5 °C)										
TS		0.97	−0.15	0.14	−0.65		0.89	0.4	−0.84	−0.81
VS	0.97		0.06	0.15	−0.74	0.89		0.76	−0.76	−0.75
VS/TS	−0.15	0.06		−0.01	−0.29	0.4	0.76		−0.38	−0.38
ORP	0.14	0.15	−0.01		−0.25	−0.84	−0.76	−0.38		0.92
pH	−0.65	−0.73	−0.29	−0.25		−0.81	−0.75	−0.38	0.92	

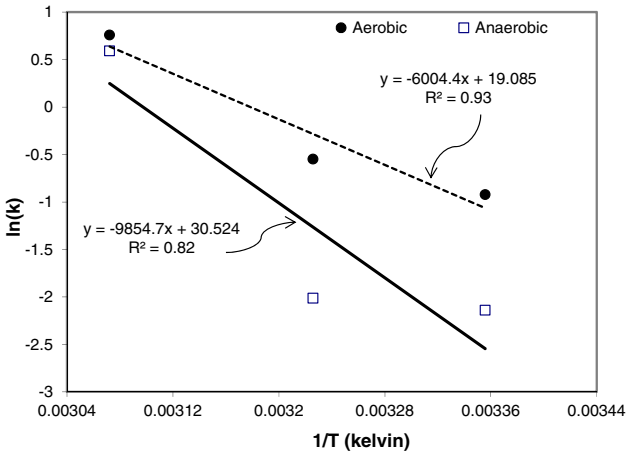

Fig. 3 Rate coefficients under aerobic and anaerobic digestions

previously to estimate microbial population survival [38–41]. For example, Balaban et al. [38] used double-exponential model to estimate survival of bacteria in an antibiotic environment and Feng et al. [39] suggested that killing curve is well-described by double-exponential kinetics when antibiotics were added to a bacteria culture. Besides, Maisonneuve et al. [41] studied the bacterial persistence during exponential growth phase. In this study, we derived following two double-exponential kinetics models for calculating bacteria survival in aerobic and anaerobic environments.

$$c = C_{\text{oaer}} \cdot \text{EXP}\left\{-\left(C_1 \cdot e^{-E_1/T}\right) \cdot t\right\} \quad (1)$$

$$c = C_{\text{oana}} \cdot \text{EXP}\left\{-\left(C_2 \cdot e^{-E_2/T}\right) \cdot t\right\} \quad (2)$$

where c is *E. coli* level at any given time and temperature, c_{oaer} and c_{oana} are initial *E. coli* levels in aerobic and anaerobic digestions, C_1 and C_2 are constants for aerobic and anaerobic digestions, E_1 and E_2 are inactivation coefficients for aerobic and anaerobic digestions. The E_1 and E_2 were 6,004.4 and 9,854.7 (shown in Fig. 3). The T is temperature in kelvin, and t is incubation period (days). The C_1 and C_2 values were 3.64×10^7 and 7.50×10^{12}, which were obtained by calibrating the model (i.e., obtaining the optimum R^2 values while comparing measured and predicted *E. coli* reductions). It is important to note that the above kinetics models (double-exponential functions) uses negative exponential function, which means if temperature is high then the function values (i.e., $e^{-E2/T}$) will be larger, and subsequently C/C_{oana} or C/C_{oaer} will be smaller.

The predictions of *E. coli* (i.e., c of Eqs. 1, 2) for moderate (20–30 °C), mesophilic (30–40 °C), and thermophilic (50–60 °C) temperatures in anaerobic and aerobic digestions are shown in Fig. 4a–c, respectively. Results showed that at 20 °C (in aerobic digestion), *E. coli* levels were reduced from log 8 to 1 (i.e., 7 log reductions) in 45 days, while in anaerobic condition, 7 log reductions required more than 60 days of incubation period (Fig. 4a).

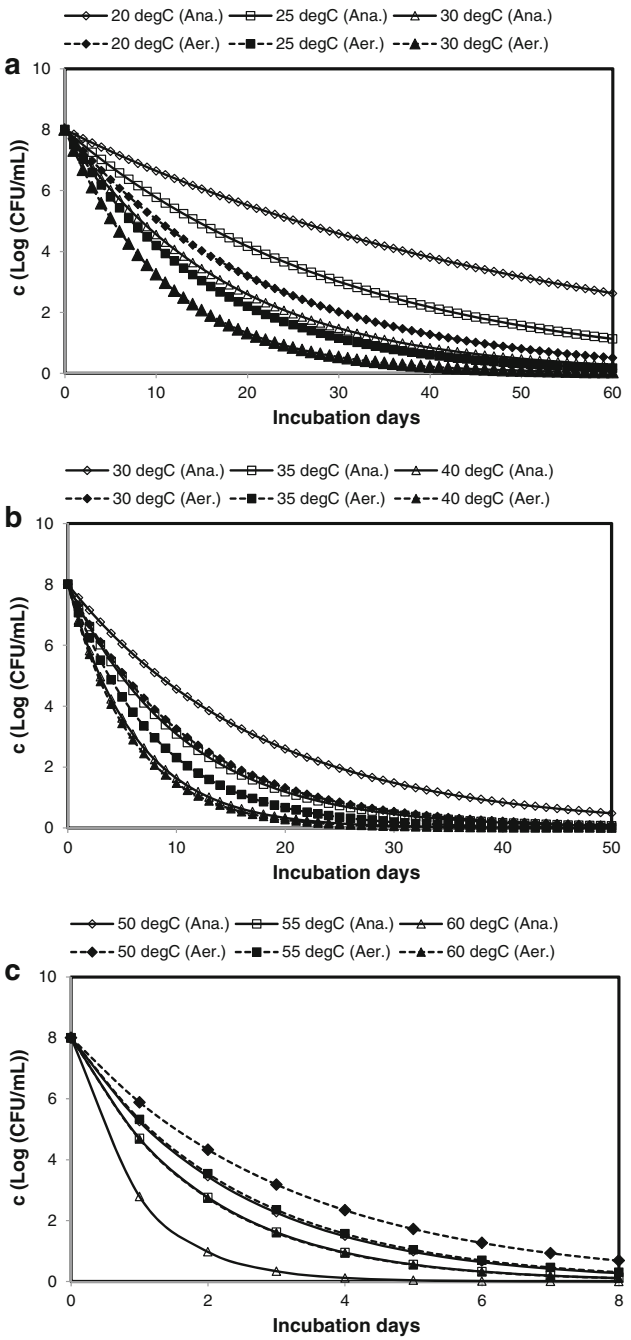

Fig. 4 Predicted *E. coli* inactivations at different temperatures under aerobic and anaerobic digestions: **a** moderate temperatures (20–30 °C); **b** mesophilic temperatures (30–40 °C); **c** thermophilic temperatures (50–60 °C)

To reduce *E. coli* from log 8 to 1 at 30 °C, aerobic digestion needed 30 days, while anaerobic digestion required 37 days of incubation. At increased temperature (i.e., 40 °C), 7 log reductions were obtained in 13 and 12 days in anaerobic and aerobic processes, respectively (Fig. 4b). At thermophilic temperature, 7 log reductions were obtained in between 4 and 5 days of incubation in both anaerobic and aerobic digestions (Fig. 4b).

Results obtained from the experiment demonstrate the impact of anaerobic and aerobic digestions on *E. coli*, a pathogen indicator, inactivation in dairy manure at moderate, mesophilic, and thermophilic temperatures. In addition, persistence kinetic models can be used to predict the *E. coli* inactivation processes at different temperature/environmental conditions. Aerobic digestion was more effective than anaerobic digestion for controlling or reducing pathogen levels in dairy manure under different temperatures (Fig. 4). While digestion of dairy manure in moderate and mesophilic temperatures is a common method [13, 15, 28], the mortality rate of *E. coli* in thermophilic digestions was considerably greater than at moderate and mesophilic temperatures indicating thermophilic anaerobic digestions can be a viable option for pathogen reduction in animal waste. We anticipate that the outcome of the study will be beneficial in improving the existing animal waste treatment processes (anaerobic and aerobic); however, further studies are required to understand the inactivation of other pathogens such as *Salmonella* spp., *E. coli* O157:H7, *Clostridium* spp., and *L. monocytogenes* in livestock manure. In addition, the current study was focused on identifying the relationships between *E. coli* inactivation, temperature and time. Future studies describing or determining the impacts of oxygen levels and digestate characteristics on *E. coli* reductions will enhance existing understanding of pathogen inactivation in anaerobic and aerobic digestions treating dairy manure. While temperature is known to be a dominant factor for controlling persistence of bacteria [36, 37], composition-dependent time–temperature relationships are needed [36].

Previous studies showed that the principal factors, which control the removal efficiency of pathogens by the two methods (i.e., aerobic and anaerobic) are temperature, degree of contact, presence of microorganisms, organic matter content, C: N ratio, pH, and retention periods (i.e., incubation days), and a very few studies are available indicating the best environmental conditions for optimum pathogen reductions [17, 27, 28]. To identify and implement suitable animal waste treatment practices (either anaerobic or aerobic or combination of both), it is required to improve the existing understanding of the controlling factors on pathogen removal efficiency.

With strict regulations on controlling human pathogens in municipal sludge, considerable work has been done on pathogen control in municipal waste [14, 36, 37, 42–44]. The focus on controlling pathogens in animal waste is thought to be lenient thus far, but further attention is required [15, 45] to improve water and food safety. Considerable levels of various pathogens (spore-forming and non-spore forming) in animal manure have been previously reported [46, 47], and the application of animal waste on

crop lands without pathogen inactivation can potentially elevate the risks to water and food security. Previous studies have reported the possibility of the transport of pathogens via runoff into streams at the watershed-scale [6–10, 48–50].

Previous studies have reported vegetative cells and spores of *C. botulinum* in soil receiving animal manure [46], which can tolerate thermophilic temperature conditions. Though the existing processes (i.e., anaerobic, aerobic, and composting) commonly used for animal manure treatment are known to lower the levels of pathogens such as *E. coli*, and *Salmonella* spp. [24, 51, 52], many other pathogens such as *Clostridium* spp. and BSE (bovine spongiform encephalopathy) prions may survive these processes even at elevated temperatures [45, 47, 53–55].

Optimizing the environmental factors such as temperature and incubation periods which controls the treatment processes is essential. The levels of pathogens in effluent of anaerobic and aerobic digesters treating animal waste varies considerably with incubation temperature and period [15, 24, 36, 37]. In addition to temperature and incubation period, studying the impacts C/N and C/P ratio on pathogen levels are significant. While studies on understanding the impacts of C/N and C/P ratio on waste degradation (in anaerobic, aerobic, and composting processes) are available, it is not well-reported how these ratios influence pathogen levels (*E. coli*, *Salmonella, Listeria* etc.).

In this study, we developed models for calculating *E. coli* inactivation in anaerobic and aerobic environments and results indicated that in mesophilic and moderate temperatures, an extended incubation period may be required to lower the *E. coli* levels to undetectable levels. The inactivation dynamics may vary for other pathogens such as *Salmonella* spp, *L. monocytogenes*, and *Clostridium* spp., therefore, further studies are required. While developing the persistence kinetic models, we included two factors (temperature and incubation periods), additional factors such as oxygen levels in digestate, and digestate characteristics can potentially influence pathogen inactivation in anaerobic and aerobic process.

It is also necessary to develop program standards for proposing the correct temperature and incubation period for treating animal waste using aerobic and anaerobic processes. Developing simple kinetics models capable of calculating pathogen inactivation in aerobic, anaerobic, and composting processes will certainly help in developing program standards. We anticipate that the study presented here will be useful in the development of program standards and in advancing the existing pathogen inactivation kinetics models. These models will be able to estimate the pathogen reductions in full- scale aerobic and anaerobic digesters treating dairy manure, and help improving existing treatment processes commonly applied for treating animal waste.

Conclusions

Elevated levels of pathogens in animal waste can potentially contaminate the environment. In this study, we have carried out a bench scale study for understanding pathogen inactivation in animal waste treatment methods. *E. coli*, pathogen indicator, inactivation was studied in aerobic and anaerobic environments under various temperatures to develop the persistence kinetics models for predicting dairy manure *E. coli* reductions in aerobic and anaerobic treatment processes. The aerobic and anaerobic batch mode experiments were performed at moderate (25 °C), mesophilic (35 °C), and thermophilic (52.5 °C) temperatures. The results showed that *E. coli* inactivation in the aerobic environment differs from the anaerobic environment, considerably, depending on the incubation temperature. We anticipate that the lab results combined with the modeling approach presented here will be useful for calculating potential pathogen levels, while treating animal waste using aerobic and anaerobic processes. These findings are useful for improving animal waste treatment processes.

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