Assessing dairy manure pathogen indicator inactivation under anaerobic and aerobic digestions in mesophilic temperature

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Abstract— **Fate and transport of pathogens of animal waste from farm land to ambient water is a serious issue. Controlling pathogens in ambient water requires mitigating pathogen transport from crop land, receiving animal waste as fertilizers, to surface and ground water. Treating manure prior to land application utilizing efficient pathogen treatment processes is an option. Here we have assessed the efficacies of animal waste treatment processes (anaerobic and aerobic digestions) on inactivation of dairy waste *E. coli*, a pathogen indicator, level. Anaerobic and aerobic digestions of animal waste were carried out in mesophilic temperature (37°C) under batch mode, and *E. coli* inactivation in digestate was assessed. Results show that about 40 days of incubation was required in anaerobic condition for *E. coli* reduction from seven orders of magnitude to two orders of magnitude, while in aerobic condition, *E. coli* levels were reduced from seven to one order of magnitude in 12 days of incubation. In addition, we have assessed total solid (TS) and volatile solid (VS) reductions in anaerobic and aerobic conditions. In 41 days of incubation, TS in anaerobic condition was reduced to 86% of the initial TS, while in aerobic condition it was reduced to 79% of the initial TS. After 41 days of incubation, the VS in anaerobic and aerobic conditions were 79 and 66% of the initial VS. In aerobic condition, pH was increased by 32% at the end incubation, while in anaerobic condition pH was reduced by 12%. We anticipate that the study presented here will be useful while assessing the anaerobic and aerobic reactor efficiencies on the inactivation of pathogen indicators in dairy manure.**

Keywords-pathogen indicator; dairy manure; inactivation; efficacies

I. INTRODUCTION

Dairy manure application into crop land as fertilizers is a common practice, however, elevated level of pathogens in manure can potentially cause risk to surface and ground water [1, 2, 3] as well as to human health. Previous studies have shown that manure contain numerous pathogenic organisms such as *Salmonella* species, *E. coli* O157:H7, *Campylobacter* species, *Salmonella* species *Yersinia* species, and *Cryptosporidium* species, which causes risk to human health [4, 5]. Controlling pathogen levels in manure prior to its application into crop land can be an option to reduce potential environmental and human health risks.

To assess the efficacies of animal waste treatment processes such as anaerobic and aerobic digestions on pathogen reductions, here we have carried out batch mode anaerobic and aerobic digestion tests treating dairy manure. The reduction in pathogen indicator (*E. coli*) levels was assessed. The primary objective of the study was to perform a comparative study to estimate the efficacies of *E. coli* inactivation in dairy manure under anaerobic and aerobic digestion processes. In addition, we have estimated TS, VS, and pH changes in both of the processes.

II. METHOD

To prepare the feedstock used in anaerobic and aerobic digestion processes, we collected fresh dairy manure from Iowa State University's dairy facility. Within 24 hours of manure collection, experiment was started. Feedstock was prepared by mixing 0.49 kg of fresh manure with 1,500 ml of distilled water. Large debris such as straw and large solid particles in manure was removed by sieving the feedstock with the screen of 850 µm openings. The screened feedstock of 150 mL was transferred into each reactor (serum bottle of 250 mL). Total of 12 reactors — 6 for anaerobic condition and 6 for aerobic condition were used for *E. coli* inactivation testing. To create anaerobic environment, anaerobic reactors were sealed with rubber septum. The water bath shaker (speed of 150 rpm) was used to control the reactors' temperatures (i.e., mesophilic temperature of 37°C) and provide mixing. A gas tight glass syringe was used to collect the digestate from the reactors. To enumerate *E. coli* cells in digestate, we used US EPA method 1603 using modified mTEC agar. The method for sample collection and the analysis of *E. coli*, TS, VS, and pH is described elsewhere [2].

III. RESULT AND DISCUSSION

Figure 1 shows *E. coli* reduction in anaerobic and aerobic processes. In anaerobic processes, over the 41 days of incubation, *E. coli* levels were reduced from seven orders of magnitude to two orders of magnitude. In aerobic environment, however, *E. coli* reduction was relatively faster. For example, within 12 days of incubation, *E. coli* levels were reduced from seven orders of magnitudes to less than an order of magnitude. In 12 days of incubation, *E. coli* in anaerobic environment were reduced from seven to five orders of magnitude only. In anaerobic condition, reduction of *E. coli* levels from seven to three orders of magnitude required approximately 40 days, however, aerobic environment required only 5 days of incubation (Figure 1). TS, VS, and pH reductions are shown in Figure 2.

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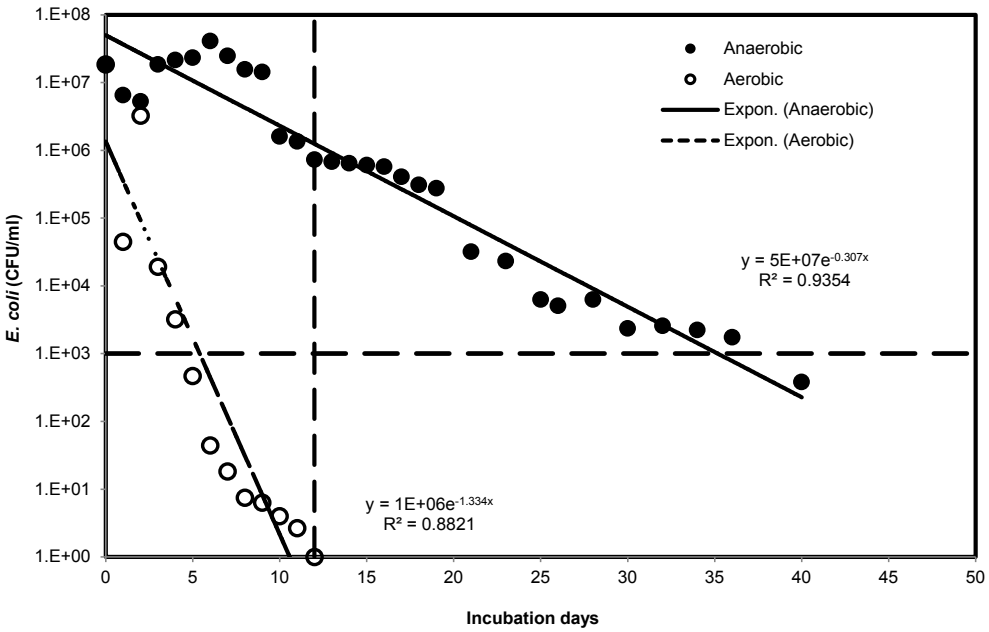

Figure 1. *E. coli* reduction in anaerobic and aerobic digestion processes treating dairy manure.

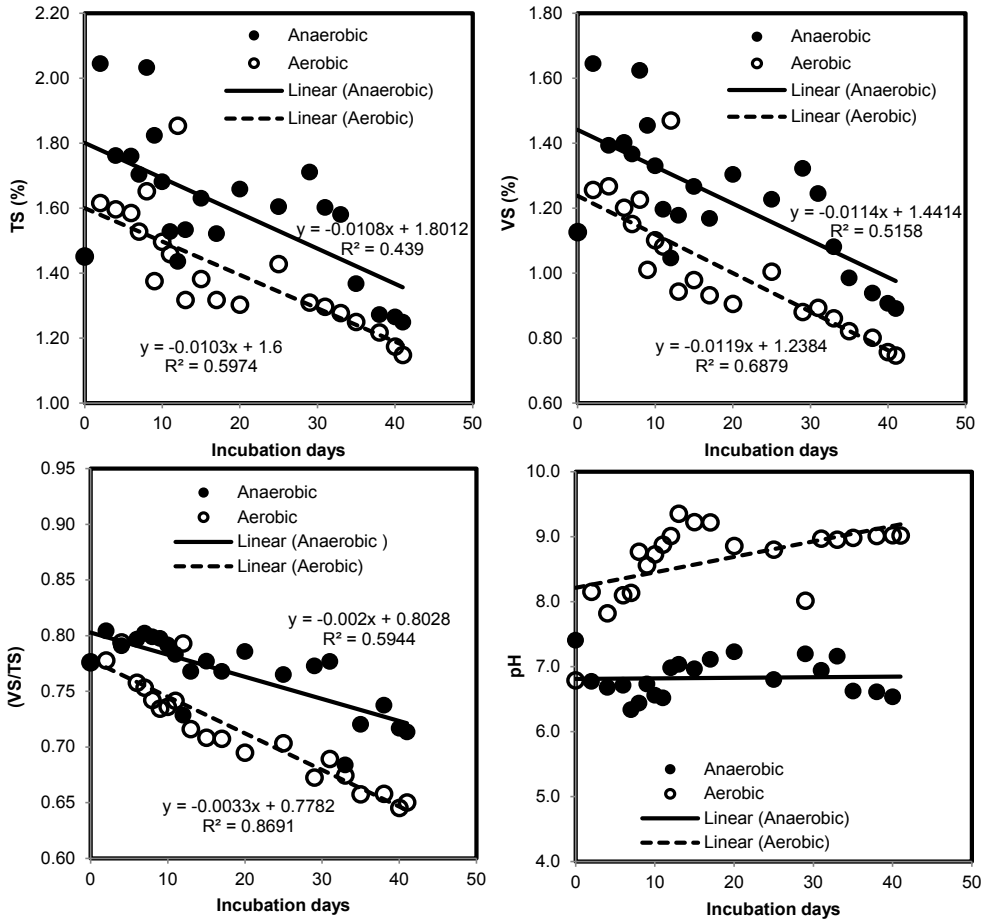

Figure 2. TS, VS, and pH reduction in anaerobic and aerobic processes treating dairy manure.

TABLE I. DIGESTATE PARAMETERS AFTER 41 DAYS OF INCUBATIONIN IN ANAEROBIC AND AEROBIC ENVIRONMENT

Parameter	Process		
	Anaerobic	***Aerobic***	***Aerobic/Anaerobic***
TS (%)	1.25	1.15	0.91
VS (%)	0.89	0.74	0.83
VS/TS	0.71	0.65	0.65
pH	6.5	9.0	1.38
E. coli (CFU/mL) (12 days incubation)	7.27E+05	1.E+0	1.38E-06

a. Initial TS, VS, pH, and *E. coli* levels were 1.45%, 1.12%, 7.4, and 1.85E+07 CFU/ml, respectively.

Table 1 shows final parameter (i.e., TS, VS, pH, and *E. coli* levels) in anaerobic and aerobic conditions. In anaerobic environment, TS and VS were 86 and 79% of the initial TS and VS, while in aerobic environment these values were 79 and 66%, respectively. Initial feedstock VS/TS ratio was 0.77; however, after 41 days of incubation, the ratio in the anaerobic condition was 0.71, while in the aerobic environment it was 0.65. Initial pH of feedstock was 7.4, which was slightly reduced in anaerobic environment (by 11%); however, under aerobic environment pH value was increased by 32% (Fig. 3).

Simple regression analyses were performed to estimate the relationships between incubation days and changes in *E. coli* levels in anaerobic and aerobic environment. Exponential regression between incubation days and *E. coli* levels yielded R^2 of 0.93 for anaerobic condition, while for aerobic condition R^2 was 0.88. We performed liner regressions for TS and VS changes, which are shown in Figure 2. Under anaerobic condition, R^2 value for TS and VS reductions were 0.43 and 0.51, while under aerobic condition R^2 values for TS and VS reductions were 0.59 and 0.68, respectively.

In summary, aerobic environment was more suitable for *E. coli* inactivation than the anaerobic environment. For example, in aerobic environment, *E. coli* level was not detectable after 12 days of incubation; however, in anaerobic environment, *E. coli* level was in five orders of magnitude. Even after 40 days of anaerobic incubation, digestate has shown *E. coli* levels in two orders of magnitude. Similarly, TS and VS reductions were slightly greater in aerobic condition than anaerobic condition. Extending this study further to assess the pathogen inactivation in dairy manure under field scale anaerobic and aerobic reactors is required. The study presented here was performed under controlled mesophilic temperature and mixing conditions. Understanding the impacts of temperature and mixing conditions on pathogen inactivation rates in anaerobic and aerobic treatment processes can yield crucial information required for improving manure management and application processes.

IV. CONCLUSIONS

To improve understanding of animal waste pathogen reduction in anaerobic and aerobic treatment processes, here we have performed batch mode test treating dairy manure in anaerobic and aerobic reactors. Results show that *E. coli* survival was prolonged in the anaerobic condition considerably compared with the aerobic condition. In the aerobic condition, *E. coli* levels were not detectable after 12 days of incubation, while in anaerobic condition, *E. coli* levels were in five orders of magnitude. After 40 days of incubation, *E. coli* levels in anaerobic reactors were in two orders of magnitude. While the results of batch mode tests presented here clearly indicate *E. coli* reductions considerably lower in anaerobic condition compared with aerobic condition, further field scale studies are required. Additional studies either pilot-scale or full-scale for testing pathogen inactivation will help in enhancing animal waste treatment processes. We anticipate that the result presented here will help in identifying the most efficient processes for pathogen reduction in dairy manure, which will be exploited for treating the manure prior to applying it into cropland, hence protecting human health as well as surface and ground water.

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