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Viability and fate of *Cryptosporidium parvum* and *Giardia lamblia* in tubular anaerobic digesters

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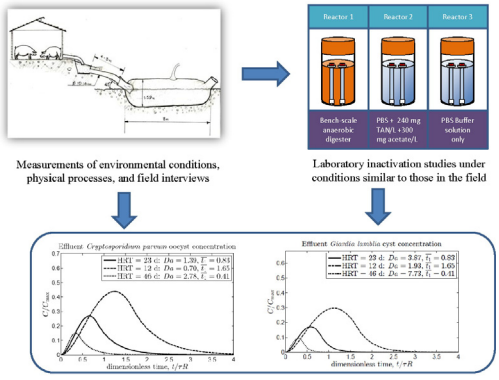
HIGHLIGHTS

- Environmental conditions in tubular digesters treating swine waste were measured.
- Parasites' inactivation rates were studied based on observed environmental conditions.
- Inactivation rates for C. parvum were lower than for G. lamblia.
- A mathematical model estimated the (oo)cyst concentrations in digester effluents.

GRAPHICAL ABSTRACT



Mathematical modeling to predict effluent concentration of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts in tubular digesters

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ABSTRACT

In many developing countries where pathogenic diseases of animal waste origin, such as giardiasis and cryptosporidiosis, are often prevalent, facilities are limited to treat livestock waste. However, household-scale anaerobic digesters are currently being promoted for bioenergy production from livestock manure. Since the effluent is often used as a fertilizer for food crops, it is critical to understand the effect of environmental conditions within household-scale digesters on the viability of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts. In this study, key environmental parameters affecting (oo)cyst inactivation were measured in four tubular anaerobic digesters, which are a type of household-scale digester promoted for treatment of swine waste in rural Costa Rica. Interviews and participant observations were used to understand digester operation and maintenance procedures. Ambient temperatures (21–24 °C), near-neutral pH, total ammonia nitrogen (TAN) concentrations < 250 mg/L and hydraulic retention times (HRTs) between 23 and 180 days were observed. Laboratory (oo)cysts inactivation studies were performed in bench-scale digesters, which were maintained under conditions similar to those observed in the field. Apparent first-order inactivation rate coefficients for *Giardia lamblia* and *Cryptosporidium parvum* were 0.155 ± 0.041 and 0.054 ± 0.006 day^{-1}, respectively. Temperature

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and volatile fatty acids were the main factors contributing to *Cryptosporidium parvum* and *Giardia lamblia* inactivation. A mathematical model was developed that predicts the concentration of (oo)cysts in the liquid effluent of tubular digesters like those observed in Costa Rica. A mathematical model was developed that predicts the concentration of (oo)cysts in the liquid effluent of tubular digesters like those observed in Costa Rica. Two dimensionless groups can be used to predict the performance of the digesters for inactivating pathogens; both dimensionless groups depend upon the average HRT in the digester. This is the first study to combine mathematical modeling with qualitative analysis, field and laboratory studies to predict the concentrations of (oo)cysts in tubular digester effluents.

1. Introduction

In many developing countries, poor management of livestock waste leads to human exposure to zoonotic pathogens. Exposure to pathogens in raw livestock manure occurs when farmers handle manure and apply it to soil and when pathogens are transferred from soil to food crops or to water bodies through runoff events (Erickson and Ortega, 2006). In Costa Rica, Egypt, and Nigeria, for example, the presence of pathogens such as *Cryptosporidium* sp., *Giardia lamblia*, *Ascaris lumbricoides*, *Entamoeba histolytica*, *Escherichia coli* and fecal coliforms in raw vegetables sold in open-air markets has been attributed to use of irrigation water contaminated by livestock waste (Monge and Chinchilla, 1996; Damen et al., 2007; Eraky et al., 2014). According to the World Health Organization (WHO), *Cryptosporidium parvum*, *Giardia lamblia*, *Campylobacter jejuni*, *Salmonella* sp. and *E. coli O157*:*H7* are the main zoonotic pathogens present in livestock waste that cause illness to humans (Dufour et al., 2012). In particular, *Cryptosporidium parvum* accounts for 23.7% of all worldwide waterborne disease outbreaks annually, while *Giardia lamblia* infects approximately 4% (0.28 billion people) worldwide annually (Dufour et al., 2012). The low infective dose of these protozoan parasites increases the associated public health risk. One *Giardia lamblia* cyst or approximately nine *Cryptosporidium parvum* oocysts have been shown to cause illness in humans, especially young, old and immunocompromised individuals (Erickson and Ortega, 2006; Haas et al., 1999). Symptoms from giardiasis and cryptosporidiosis include diarrhea or gastroenteritis, and these diseases can be fatal for immunocompromised individuals (Dufour et al., 2012). Gao et al. (2015) carried out a disease burden analysis for infections from *Cryptosporidium* sp. and *Giardia* sp. originating from livestock waste for communities in China and Ghana. This study found that infections from these parasites increased morbidity, mortality and disability burden.

There are three types of small-scale anaerobic digestion systems commonly used in the developing world: fixed dome, floating drum and tubular. Unlike the floating drum and fixed dome digesters, tubular digesters do not require a high level of skilled labor to install, they are the easiest to operate, cost the least and can operate at a variety of temperatures. Therefore, tubular digesters are being promoted in Asia, Africa and Latin America. However, these digesters are easily damaged and have a shorter life span; 10 years compared to 15–20 years for the other two digester systems. Tubular digesters can improve the quality of life for those in the developing world by producing biogas that is most often used as a cooking fuel, but can also be used to heat water or buildings or to generate electricity for on-site use (Kinyua et al., 2016b). When biogas is used as a cooking fuel in place of firewood, these systems can be a useful tool to mitigate deforestation. Using biogas for cooking also results in decreased respiratory health concerns, especially for women and children who, due to their cultural and social roles, are disproportionally affected by indoor air pollution caused by burning wood or dung. Additionally, anaerobic digesters can assist in reducing water pollution by stabilizing dissolved and particulate organic matter in the waste. The treated effluent from anaerobic digestion contains primary nutrients (nitrogen, phosphorus) and is promoted for its use as a soil amendment to improve crop yields (Lansing et al., 2010). However, if pathogens in the livestock waste are not sufficiently inactivated during anaerobic digestion, then use of the effluent as a soil amendment may lead to public health concerns from human consumption of contaminated crops. Although prior studies have investigated *E. coli* inactivation during anaerobic digestion at moderate, mesophilic and thermophilic temperatures (Pandey et al., 2015) and *Ascaris ovum* (Manser et al., 2015), there is limited information on the fate of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts during anaerobic digestion of livestock wastes. Therefore, it is critical to understand the fate of pathogens during anaerobic digestion and to ensure that efforts to recover energy and nutrients from livestock waste do not lead to public health concerns from human consumption of contaminated crops.

Prior studies on the fate of *Cryptosporidium* sp. and *Giardia* sp. have investigated the susceptibility of these protozoan parasites to inactivation in the environment (water and soil) as a function of various factors, including: UV radiation (Betancourt and Rose, 2004), moisture content (Van Herk et al., 2004), concentrations of volatile fatty acids (VFA), temperature, pH, and exposure to free ammonia (NH_3). Of particular importance in tubular digesters are VFAs, temperature, pH, and free ammonia. Jenkins et al. (2002) and Olson et al. (1999) reported an increase in (oo) cyst inactivation rate as the temperature increased from 4 °C to 25 °C in soil and water. Jenkins et al. (1998) reported that free ammonia concentrations between 0.12 and 2.52 g NH_3/L and pH levels above 9 inactivated oocysts in water. Concentrations of free ammonia in solution increase with increasing total ammonia nitrogen (TAN) concentrations, pH, and temperature. As temperature increases, oocyst walls increase in permeability, allowing free ammonia to more easily penetrate into *Cryptosporidium* sp. oocysts. Once inside the (oo)cysts, free ammonia disrupts cell chemistry and structure through protein denaturation, making the cells vulnerable to inactivation (Kidd, 2011).

Chauret et al. (1999) and Kato et al. (2003) studied the inactivation of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts in mesophilic (36 °C) anaerobic digesters. <30% removal per day (0.15 log removal/day) was observed in both studies, while 90% removal (1.0 log removal) was observed within one hour at thermophilic temperatures (47–55 °C) (Kato et al., 2003). The authors attributed this reduction to the increased temperature damaging the oocyst walls and DNA, resulting in non-infective *Cryptosporidium* sp. sporozites. Medhat and Stafford (1989) investigated the effect of VFAs and temperature on inactivation of the protozoan *Entamoeba histolytica* (a parasite closely related to *Giardia* sp.) during mesophilic (37 °C) and thermophilic (55 °C) digestion of swine waste at a solids retention time (SRT) of 10 days. VFA concentrations were maintained between 1.5 and 3.0 g acetate/L. *Maximum* log removals were 0.5 and 4.0 at 37 °C and 55 °C, respectively. VFAs influence pathogen inactivation rates by decreasing pH, which acidifies pathogens' cells (Medhat and Stafford, 1989). It should be noted that although high VFA concentrations have been shown to cause inactivation of (oo)cysts, VFA concentrations > 600 mg acetate/L have been shown to inhibit methanogenesis (Wang et al., 1999), so high concentration of VFAs should be avoided in anaerobic digestion. Cote et al. (2006) treated swine waste in a 20 °C anaerobic sequencing batch reactor with a 20-day SRT and analyzed the concentration of oocysts in the influent and effluent. The level of oocysts in the effluent was below detection limits. Although the authors did not explicitly indicate the inactivation mechanism, VFA concentrations in reactors where (oo)cysts inactivation was observed were between 0.40 and 23.2 g acetate/L. Although a few studies have investigated the fate of *Cryptosporidium* sp. and *Giardia* sp. (oo)