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Energy Production and Effluent Quality in Tubular Digesters Treating Livestock Waste in Rural Costa Rica

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by

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Abstract

Use of tubular anaerobic digesters to treat livestock waste in developing countries has energy, agricultural, health, social and environmental benefits. However, careful use of digester effluent as a soil amendment is required due to the potential presence of protozoan parasites *Cryptosporidium parvum* and *Giardia lamblia*. This research investigated the performance of four tubular digesters in the Monteverde region of Costa Rica. High (>75%) volatile solids and BOD_5 removal efficiencies were observed, which was attributed to the formation of a biologically active floccular sludge layer. Computational fluid dynamics (CFD) and bioprocess models were developed to evaluate the transport and transformation mechanisms in the digesters. The CFD model estimated a mean liquid hydraulic residence time (HRT) of 23 days and the bioprocess model estimated an average mean cell residence time (MCRT) of 115 days. *Cryptosporidium parvum* and *Giardia lamblia* inactivation studies were performed in the laboratory under conditions similar to the environmental conditions observed in the field tubular digesters. The environmental conditions included: ambient temperatures (21-24°C), neutral pH and total ammonia nitrogen (TAN) concentrations below 250 mg NH_4^+-N/L. Inactivation rate constants for *Cryptosporidium parvum* and *Giardia lamblia* were 0.056 and 0.726 day^{-1}, respectively. An (oo)cysts solid-liquid phase distribution study indicated that 70% of both (oo)cysts adhered to biosolids. A tubular digester model was used to estimate the concentration of viable (oo)cysts in the digester effluents. (Oo)cysts adhesion to solids, total solids concentration in the digester and HRT were the main factors contributing to the modeled effluent

concentration of viable (oo)cysts. Since the model predicted presence of viable (oo)cysts in the tubular digester effluent, a quantitative microbial risk assessment (QMRA) model was developed to estimate the risk of infection from exposure to raw livestock waste and tubular digester effluents in two rural communities in Costa Rica. The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was assessed for occupational and public exposure pathways; fomite and soil contamination and crop contamination from runoff. Results from the QMRA indicated that the concentration of (oo)cysts in the raw livestock waste, inactivation rates at the various exposure pathways and the treatment of livestock waste were the main contributing factors to the risk of infection. This research indicated that treatment of livestock waste in tubular digesters significantly decreased the risk of infection to below WHO's acceptable individual annual risk of infection (10^{-4}). This is the first study to combine mathematical modeling with field studies to determine the physical and biological processes in tubular digesters. This is also the first study to combine mathematical models with field and laboratory studies to determine the concentration of (oo)cysts in tubular digester effluents and to predict the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* if tubular digester effluent is used as a soil amendment.

Chapter 1:

Introduction

Small-scale anaerobic digesters treating livestock in the developing world are an attractive waste management technology. Biogas produced from anaerobic digestion can be used to heat water or buildings, generate electricity, which can be used on site, or provide hours of cooking in developing world applications (Lüer, 2010). There are three commonly used small-scale anaerobic digestion systems (fixed dome, floating drum and polyethylene tubular digesters), this research focused on tubular digesters because these systems do not require high levels of skilled labor to install, they are the easiest to operate, cost the least and can be operated at a variety of temperatures compared with the other digesters (Lüer, 2010).

Anaerobic digestion effluent contains primary nutrients (nitrogen and phosphorus) that can be used as a soil amendment to improve plant growth. Although economically and environmentally attractive, land application of anaerobic digestion effluent has potential health impacts due to transmission of pathogens to food and water. Protozoan parasites, *Cryptosporidium parvum* and *Giardia lamblia*, which are prevalent in livestock, were investigated in this study due to their high resistance to inactivation (Dufour et al., 2012). Apparently healthy livestock shed infective *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts that can be transmitted to humans through ingestion of soil, contaminated food and water (Erickson et al., 2006). Unfortunately, anaerobic digestion of wastewater does not effectively decrease the number of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts to

below the infective dose (Chauret et al., 1999), thus escalating the probability of a health effect (infection, illness or death). Since anaerobic digestion of livestock waste for energy production followed by land application of the effluent as a soil amendment is practical and feasible, quantitative microbial risk assessment (QMRA) can be a useful tool in evaluating the probability of infection to these protozoan parasites (Haas et al., 1999). QMRA allows for estimating the risk/probability of infection from exposure to a pathogen. By carrying out a risk assessment, management practices that reduce transmission can be put in place to justify application of anaerobic digestion effluent.

However, before carrying out a QMRA, the environmental conditions and the physical and biological processes in tubular digesters need to be understood. Exposing viable *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts to these factors influences the concentration of viable (oo)cysts in the tubular digester effluent. The concentration of viable (oo)cysts in the effluent concurrently affects the risk of infection to these parasites. The relationships between operating parameters, environmental conditions, physical and biological processes and the risk of infection are shown on Figure 1.1.

No prior studies have estimated the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts when effluents from small-scale tubular anaerobic digesters treating livestock waste are used as a soil amendment. The novelty of this research was the creation of tubular digester physical, biological, (oo)cysts inactivation and risk of infection models based on field and laboratory studies of small-scale tubular digesters treating livestock waste in the Monteverde region of Costa Rica.

The following research questions and objectives were used to guide this dissertation research. Each research question was addressed in the subsequent chapters:

The first research question (Chapter 2**)** was how does the design, operation and maintenance of tubular digesters affect their performance in bio-energy production and livestock waste management in the developing world?

- Provide a detailed literature review of the effect of substrate characteristics and operating parameters on the biochemical conditions in the tubular digesters.
- Summarize how these environmental conditions in the tubular digesters translate to energy, environmental, agricultural, social and public health benefits.
- Discuss policies promoting anaerobic digestion of livestock waste in developing countries.

The second research question (Chapter 3**)** was what are the environmental conditions and physical and biological processes occurring in tubular digesters treating livestock waste?

- Investigate the performance (biogas production and effluent quality) of and determine the environmental conditions in tubular digesters operated in a developing world setting.
- Perform a hydrodynamic study and develop a physical model to understand the mixing and transport mechanisms in the tubular digesters.
- Develop a bioprocess model to understand the biological mechanisms in the tubular digesters and determine how the physical and biological processes are related.

The third research question (Chapter 4**)** was how do the environmental conditions and the physical and biological processes in tubular digesters treating livestock waste affect the fate and viability of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts?

- Investigate the inactivation rates of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts under environmental conditions similar to those in field tubular digesters.

- Investigate adsorption of (oo)cysts to anaerobic digester biosolids under environmental conditions similar to those in field tubular digesters.
- Develop a model that combines the (oo)cysts' inactivation rates, adsorption of (oo)cysts to solids with the tubular digester physical processes to predict the concentration of viable (oo)cysts in the tubular digester effluent.

The fourth research question (Chapter 5**)** was how does use of tubular digesters to treat livestock waste lower the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts?

- Establish occupational and public exposure pathways for two rural communities generating livestock waste.
- Develop a QMRA model that uses the predicted concentration of viable (oo)cysts in the tubular digester effluent to estimate the risk of infection at the established exposure pathways.
- Compare the risk of infection between one community that uses tubular digesters to treat their livestock waste and another community that does not treat their livestock waste.

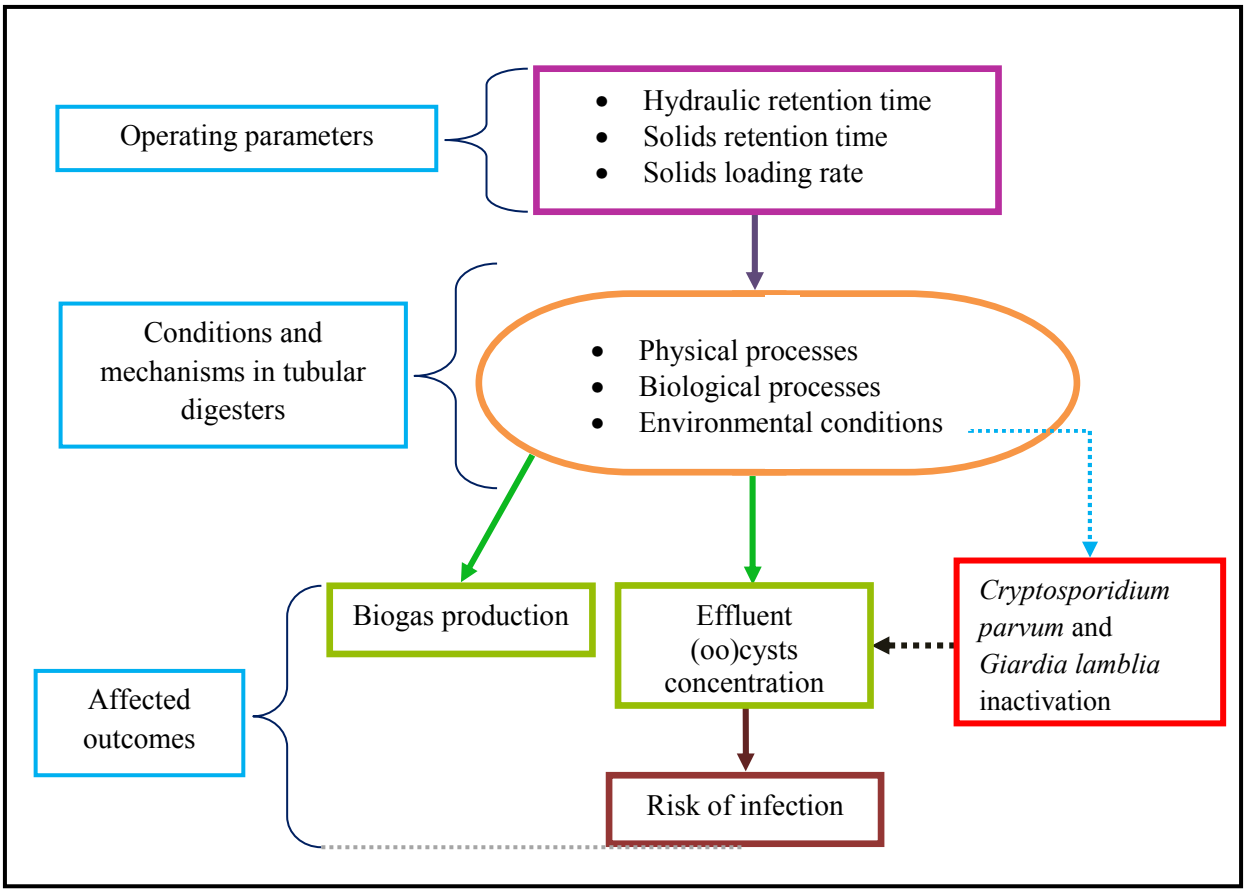


Figure 1.1: Relationship between operating parameters, environmental conditions, physical and biological processes, (oo)cysts inactivation and the risk of infection.

Chapter 2:

Literature Review[1]

2.1 Introduction

Anaerobic digestion of livestock waste is a waste management method that can improve the quality of life for those in the developing world. Biogas produced from small-scale anaerobic digesters is most often used as a cooking fuel, but can also be used to heat water or buildings or generate electricity for on-site use (Westerman et al., 2008; Ferrer et al., 2009; Lüer, 2010; Ocwieja, 2010). Anaerobic digesters can also be a useful tool to mitigate deforestation by using biogas as opposed to firewood. This also results in decreased public health concerns, especially for women and children who are disproportionally affected by indoor air pollution due to cultural and social roles. In addition, effluent from anaerobic digestion contains primary nutrients (nitrogen, phosphorus, potassium), that have agronomic benefits if used as a soil amendment to improve plant growth.

To achieve these energy, environmental, agronomic and public health benefits, it is critical to understand how small-scale anaerobic digesters treating livestock waste are operated in developing countries. There are three commonly used small-scale anaerobic systems: fixed dome, floating drum and polyethylene tubular digesters (Kossmann et al., 1999; Ocwieja, 2010; Kinyua, 2013). The choice of digester depends on cost and availability of construction material, temperature of the region, quantity of waste treated, operation and maintenance and skill level in

[1] This chapter is adapted from a manuscript under review at *Renewable and Sustainable Energy Reviews*. Kinyua, M.N., Rowse, L., Ergas, S.J. "Review of Small-Scale Tubular Anaerobic Digesters Treating Livestock Waste in the Developing World"

the community. This review will only summarize research on small-scale tubular digesters treating livestock waste because these systems do not require high levels of skilled labor to install, they are the easiest to operate, cost the least and can operate at a variety of temperatures compared with the other digesters (Lüer, 2010). Understanding the effects of design and substrate characteristics, operating parameters (organic loading rate [OLR], temperature and retention time) can assist in estimating the performance of tubular digesters and thus justify their benefits.

The aim of this review is to provide a detailed summary of the current and ongoing research on the design, operation, maintenance and performance of tubular digester treating livestock waste in the developing world. This review will focus on the effect of substrate characteristics and operating parameters on the biochemical conditions of the digesters and how these conditions translate to energy, environmental, agricultural, social and public health benefits and policies promoting anaerobic digestion of livestock waste. The link between these focus areas is demonstrated graphically in Figure 2.1. In addition to information from the literature, the authors' observations of design, operation and maintenance of tubular digesters treating livestock waste as well as discussions with farmers and development workers in Monteverde, Costa Rica will also be incorporated into this review.

2.2 Small-scale Tubular Anaerobic Digesters

The first plastic tubular digesters were installed in Colombia and Ethiopia in the 1980s by Botero and Preston (1987). After visiting the installed plastic tubular digesters in Colombia in 1992, a Vietnamese group designed a tubular digester using a polyethylene tube and PVC piping. This new design had a lower capital cost compared with using plastic bags. By 1995, more than 800 polyethylene tubular digesters were installed in Vietnam and 100 in Tanzania (An et al.,

1997). This type of digester is now commonly referred to as the Taiwanese-model, double tubular polyethylene bag digester. In this review, it will be referred to as simply a tubular digester.

The side view of a typical tubular digester is shown in Figure 2.2. The length of these digesters can vary from 8m to as long as 40m, with a circumference of 3.6-5m (Lansing et al., 2007; Eaton, 2009). Generally the total volume ranges from 2.4 to 12m^3, with approximately 75% of the total being working (liquid) volume (Martí-Herrero, 2011; Rajendran et al., 2012). The polyethylene tube is placed in a 2.0-5.0% slope deep trench (Fig. 2.2) to provide support for the weight of the slurry. Inlet and outlet pipes are installed at a 45° angle to maintain equal influent and effluent flows (An et al., 1997). In Costa Rica, a combined grit and flotation chamber is constructed upstream of the inlet section to remove large solid material, such as uneaten animal feed, sand and gravel, that are not biodegradable and can cause wear and tear on the system.

Roof shelters are often used to protect the polyethylene tube from UV radiation and to regulate the temperature inside the digester, due to ambient temperature fluctuations (An et al., 1997; Garfí et al., 2011). Commonly used roof shapes are gable, shed and dome. Depending on the amount of funds available, a polyethylene biogas storage bag can also be installed above the digester. Figure 2.2a is a photograph of a tubular digester with a biogas storage bag and Figure 2.2b is a tubular digester without a biogas storage bag. Both images are from digesters installed in the Monteverde region of Costa Rica and are used to treat swine and cow waste. The biogas pipe (Figure. 2.1) flows into a safety valve that keeps air from getting into the digester or biogas storage bag while providing an escape for excess biogas (An et al., 1997). Methane's (CH_4) greenhouse gas (GHG) potential is 21-24 times greater than carbon dioxide (CO_2), users are

therefore recommended to burn off excess biogas to avoid higher GHG emissions. Biogas contains CH_4, CO_2 and traces of H_2S. Combustion of H_2S forms sulfur dioxide, which can cause respiratory diseases such as emphysema and bronchitis (EPA, 2014). H_2S is also a corrosive gas that can reduce the life-span of metal cook stoves. Its rotten eggs smell is also not aesthetically pleasing to users of the biogas when cooking (Lüer, 2010). It is recommended to place iron steel wool inside the biogas pipe to scrub the hydrogen sulfide (H_2S) from the biogas, as shown in Equation 2.1 (Magomnang and Villanueva, 2014):

$$Fe_2O_3 + 3H_2S \rightarrow 2Fe_2S_3 + 6H_2O \quad \text{(Eq 2.1)}$$

Iron oxide reacts with H_2S to form iron sulfide that is not corrosive or toxic. However, scrubbing of the biogas can be dangerous if a leak in the biogas storage bag or biogas pipe is not easily detected due to lack of odor (Rowse, 2012).

2.3 Operation of Tubular Anaerobic Digesters

Anaerobic digestion is carried out by different groups of microbes in four main steps:

1. Fermentation - Fermenting bacteria hydrolyze complex insoluble molecules, such as proteins, carbohydrates, and lipids, into simpler soluble organic compounds.
2. Acidogenesis – Acidogens utilize the simple soluble organic compounds such amino acids, sugars, alcohols, and fatty acids to produce volatile fatty acids (VFA).
3. Acetogenesis – Acetogens utilize VFAs to produce acetate, H_2, and CO_2.
4. Methanogenesis – Methanogens produce CH_4 by consuming acetate, H_2, and CO_2

A syntrophic relationship between the different groups of microbes is vital for the anaerobic digestion process to be successful. For example, if the growth rate of fermenting bacteria is low, the rates of acidogenesis, acetogenesis and methanogenesis are decreased. In this scenario, hydrolysis is the rate limiting step leading to decreased biogas and CH_4 production.

Substrate characteristics (type of manure), operating parameters and chemical conditions in the digester affect the complex anaerobic microbial dynamics. Microbial dynamics in turn affect the overall performance of the system. Operating parameters include; temperature, retention time, and organic loading rate (OLR). Retention time includes hydraulic retention time (HRT) and solids retention time (SRT). Chemical conditions include; alkalinity, pH, total ammonia nitrogen (TAN) and VFA concentrations. Chemical conditions are controlled by the substrate characteristics and operating parameters. In industrial-scale anaerobic digestion systems, chemical conditions are often controlled by addition of acids or bases to control pH and alkalinity, providing heating and mixing or adjusting the OLR to control TAN or VFA inhibition (Gerardi, 2003). It is unlikely that households using small-scale tubular digesters can monitor and control the chemical conditions, therefore, only the substrate characteristics and operating parameters will be discussed in this review. A summary of the substrates and operating parameters used in countries in Africa, Asia and South and Central America are provided in Table 2.1.

2.3.1 Substrate Characteristics

Livestock manures commonly used as substrate for small-scale tubular digesters in the developing world include swine, cow, guinea pig, sheep, llama and buffalo (Table 2.1). The organic portion of livestock manure contains complex insoluble molecules, such as proteins, carbohydrates, and lipids that are converted to simpler soluble organic compounds. During hydrolysis, recalcitrant compounds in the substrates, such as cell walls, lignin and cellulose, are also degraded. However, fermenting bacteria degrade these compounds at a much slower rate because the extracellular enzymes that catalyze hydrolysis have a difficult time penetrating the lignocellulosic and hemicellulosic structures of these compounds (Converti et al., 1997).

The hydrolysis rate is affected by the fraction of recalcitrant compounds in the volatile solids (VS), therefore, it is important to know the concentration of these compounds based on the animal. The percent fraction of lignin, cellulose and hemicelluloses in the VS concentration of poultry, swine and cow manure is illustrated in Table 2.2 (USDA, 2008). Cow manure has the highest average concentration of recalcitrant compounds. Cows utilize a large fraction of the carbohydrates available in their food for energy; this leaves mostly recalcitrant compounds in the manure (Andrén et al., 1999). Therefore, depending on the concentration of recalcitrant compounds in the manure, hydrolysis can be the rate limiting step in anaerobic digestion. Hydrolysis rates have been shown to affect CH_4 production; when treating swine waste, hydrolysis rates ranging from 0.07-0.19gCOD/L-day were observed and CH_4 production decreased with decreasing hydrolysis rates (Kinyua et al., 2014).

To increase biogas production, different additives, such as human waste (Galvin, 2013), slaughter house waste (blood, rumen, meat) (Guyana Energy Agency, 2012; Vögeli et al., 2014), molasses (Guyana Energy Agency, 2012), fats, oils and grease (FOG) (Lansing et al., 2010), crop residues such as cassava peels (Adeyanju, 2008) and coffee hulls (Kivaisi, 2004) and domestic solid wastes (Vögeli et al., 2014) have been used. Addition of these energy rich organic wastes increases biogas production because they are readily biodegraded by anaerobic microbes. (Cirne et al., 2007). Careful addition of additives has to be considered (Cirne et al., 2007; Lansing et al., 2010). For example, FOG are mainly composed of long chain fatty acids (LCFA) that can substantially increase biogas production. However, LCFA can lead to decreased biogas production by (1) coating the methanogens cells' which reduces substrate uptake and biogas release and (2) causing sludge flotation and digester foaming leading to washout of anaerobic microbes (Long et al., 2012).

Livestock manures also differ in terms of nitrogen, phosphorus and potassium content. The nitrogen, phosphorus and potassium characteristics of manure excreted by swine, cows and poultry are illustrated in Table 2.2 (Choi, 2007; USDA, 2008). The term excreted refers to both the feces and urine before dilution or treatment. Compared with the other manure substrates, swine manure has the highest concentration of nitrogen. To promote growth and carcass leanness, pigs are fed a diet rich in protein (Kerr and Easter, 1995). Of this high nitrogen diet, 20% is excreted as feces and 50% as urine. The organic nitrogen fraction in the feces is slowly biodegradable, while the fraction in the urine is readily metabolized into TAN (Cahn et al., 1998). Ammonification occurs during anaerobic digestion, generating TAN (ionized ammonium (NH_4^+) + free ammonia (NH_3)) (Im and Gi, 2011; Tchobanoglous et al., 2003). An increase in temperature can increase the rate of ammonification due to increased microbial growth rates, as discussed below. Increased TAN concentrations may lead to decreased CH_4 yields because methanogens are susceptible to TAN inhibition especially when TAN is in the form of free ammonia. Free ammonia diffuses into the cellular membrane disrupting the microbial pH, energy requirements and enzyme kinetics (Wittmann et al., 1995). Although inhibitory TAN concentrations vary in the literature, generally maintaining TAN concentrations below 1.5g/L and pH between 6.8-7.4 will reduce TAN inhibition (Angelidaki and Ahring 1993; Troyer et al., 1997; Zhang et al., 1997; Hansen et al., 1998; Magbanua et al., 2001; Kaparaju and Rintala, 2005; Kinyua, 2013). TAN concentrations and pH values measured in tubular digesters in the developing world are shown in Table 2.1. Even though most digesters have pH values greater than 7.4, the digesters operate with TAN concentrations below 1.5g/L.

2.3.2 Temperature

Temperature is an important operating parameter because substrate utilization and microbial growth rates are affected by temperature. Microbes transport nutrients in and out of the cell through their cellular membrane. As temperatures decrease, the membrane becomes stiff, causing a decrease in the transport of nutrients (Nedwell, 1999). Anaerobic microbes are sensitive to changes in temperature as small as 1-3°C (Gerardi, 2003). A change in temperature affects the stability of fermenting bacteria. This change in stability may lead to decreased growth rates, pH changes and decreased CH_4 yields (Visser et al., 1993; Dohányos and Zábranská, 2001). Common anaerobic digestion temperature settings are psychrophilic (0-15°C), mesophilic (30-37°C) and thermophilic (50-60°C). However, anaerobic microbes can survive at temperatures between 0 and 82°C (Tchobanoglous et al., 2003). To achieve thermophilic temperatures, complex heat exchange systems are required and therefore have not been reported in small-scale tubular digester systems. Most tubular digesters in the developing world operate at ambient temperatures of 15-30°C (Table 2.1). This range is considered to be at the high and low extremes of psychrophilic and mesophilic temperatures respectively (Amani et al., 2010).

To maintain temperatures that are favorable to anaerobic microbes, tubular digesters were originally designed for use in tropical climates. However, through proper design, operation and maintenance, these systems have also been successfully used in high altitude temperate regions of South America (Alvarez and Lidén, 2009; Ferrer et al., 2011). As mentioned earlier, one such design is the use of greenhouse roofs. These roofs have been successful in maintaining favorable and stable temperature in tubular digesters even with fluctuations in the ambient temperature.

To establish stable temperatures while treating guinea pig manure using a tubular digester in the Andean Plateau of Peru, Garfí et al. (2011) investigated the effect of two roof shelters

(shed roof and dome roof) on biogas production. The digester with the shed roof shelter had significantly lower temperatures and biogas production however, the effluent characteristics were similar in both digesters. Although the authors did not specify why the shed roof had lower temperatures, it is likely that the dome roof has a greater surface area compared to the angled shed roof. Angling the shed roof towards or away from the sun may have also caused this difference.

2.3.3 Retention Time

HRT is the average time the liquid fraction of the waste is in the system and the mean cell residence time (MCRT), also known as SRT, is the average time the microorganisms are in the system. HRT (day) is approximated by the total volume of the digester (V; m^3) divided by the influent flow rate (Q; m^3/day):

$$\text{HRT} = \frac{V}{Q} \qquad \text{(Eq 2.2)}$$

SRT (day) is measured as the total biomass in the digester (X; kg/m^3) divided by the biomass wasted per day (X_{ef}; kg/m^3) (Tchobanoglous et al., 2003):

$$\text{SRT} = \frac{\text{Biomass in the digester}}{\text{Biomass wasted from the digester}} = \frac{V\,X}{Q\,X_{ef}} \qquad \text{(Eq 2.3)}$$

In systems with no solids separation and recycling, SRT is normally equal to the HRT. Although small-scale tubular digesters do not have solids separation or recycling, the SRT in these digesters may not be equal to the HRT. During field studies of tubular digesters in the Monteverde region of Costa Rica, we observed solids accumulation in the digesters, which led to SRTs greater than HRTs. For stable operation of the system, a HRT of at least twice the growth rate of the methanogens is required to avoid washout of this group of microorganisms. The design HRT is based on the growth rate of the methanogens because they are the slowest growing anaerobic microorganism (Shin et al., 2011).

Ambient temperatures and substrate characteristics also affect the required retention time in the digesters. In temperate climates, anaerobic microbes have slower growth rates. This decrease in growth rate requires an increase in digester volume or HRT to allow microbes to utilize substrates. For example, Ferrer et al. (2011) treated cow waste in cold climates and required a HRT of 60-90 days, while Usack et al. (2014), also treating cow waste in a tropical region needed a 3-4 times lower HRT.

Correct estimation of HRT is required because HRT is related to digester volume. Although a decreased digester volume may reduce capital cost, the effluent quality and biogas production may decrease due to insufficient time for microorganisms to degrade the VS. Often, HRT is calculated by dividing the total digester volume; which includes the liquid and gas volume, with the influent flow rate. This is a common error; the actual HRT should be calculated by dividing the working liquid volume by the inflow flow rate (Martí-Herrero, 2011). Even if the working volume is used to estimate the HRT, the total volume is calculated by multiplying the cross-sectional area with the length of the bag, assuming that the working volume will not change. This however is not accurate, as the working volume in tubular digesters changes with time due to settling and accumulation of solids (Lansing et al., 2008; Ferrer et al., 2011; Usack et al., 2014).

2.3.4 Organic Loading Rate

If VS are the 'fuel' (substrate) for anaerobic microorganisms, OLR (kgVS/m^3 digester volume/day) can be considered the digester's process capacity and is equal to influent VS concentration (VS_i; kgVS/m^3) divided by the HRT (day):

$$OLR = \frac{VS_i}{HRT} \qquad \text{(Eq 2.4)}$$

There are two considerations that must be addressed when determining a digester's OLR. First, as mentioned in the previous section, substrate characteristics, this affects the VS concentration and bioavailability. Second, the digester volume and influent and effluent flow rates. If a tubular digester is fed with an OLR greater than its capacity, acetogenic bacteria produce acetate faster than the methogens can utilize them, leading to decreased CH_4 yields. On the other hand, OLRs lower than the system's capacity does not provide anaerobic microbes enough substrate, leading to low biogas production (Kinyua et al., 2014).

Optimal OLRs when treating cow and swine manure anaerobically at mesophilic temperatures are 2.5-3.5kgVS/m^3-day and 3.0-3.5 kgVS/m^3-day respectively (Burton and Turner, 2003). However, it should be noted that these recommended values may not necessarily apply to small-scale tubular digesters. Most of these small-scale systems are operated at OLRs less than 2.0 kgVS/m^3-day (Table 2.1). This could be due to the operation temperatures lower than the mesophilic range (30-37°C) and the lack of mixing. Additionally, due to the simplicity of design it has been observed that loading rates greater than 2.8 kgVS/m^3-day are more difficult to manually mix in feed tanks and don't flow easily into the digester (An and Preston, 1999).

2.4 Tubular Digester Benefits

Use of small-scale tubular digesters has a number of benefits including: (1) energy benefits, anaerobic digestion is a net-energy producing process. (2) Agricultural benefits as the digester effluent is rich in nutrients and can be used as soil amendment. (3) Environmental benefits by decreasing deforestation and mitigating water pollution from livestock waste. (4) Public health benefits because biogas combustion results in very low air pollution compared to combustion of firewood and livestock waste. Lastly, (5) social benefits by helping address gender inequalities.

2.4.1 Energy Benefits

It was observed in Monteverde, Costa Rica that a frequent concern for a household or community with a tubular digester is how much biogas they will get. Although biogas production volume and rates are important, it is essential to compare the production rate with household energy requirements. The quality of biogas produced by small-scale tubular digesters will be considered in the form of heat energy. Electricity is a higher quality of energy compared to heat, but due to economic reasons, biogas from these systems is usually transferred to heat using biogas cook stoves (Bond and Templeton, 2011; Rutz et al., 2012). The biogas production rate is equal to the daily biogas volume produced (m^3_{biogas}/day) divided by the volume of the digester:

$$\text{Biogas Production Rate} = \frac{\text{Daily biogas volume produced}}{V} \quad \text{(Eq 2.5)}$$

Biogas yield (m^3_{biogas}/ $kgVS_{added}$) is dependent on the biogas production and OLR and expressed mathematically as:

$$\text{Biogas Yield} = \frac{Biogas\ Production\ Rate}{OLR} \quad \text{(Eq 2.6)}$$

Potential biogas and CH_4 yields produced for specific animals are summarized in Table 2.3. As mentioned previously, only a certain percentage of the influent VS are utilized in the anaerobic process depending on the animal. The volume of biogas and CH_4 produced per animal per day can be approximated assuming animal specific biogas yields from the literature (Burton and Turner, 2003). This potential CH_4 volume per animal per day can also be expressed as a heating value. Pure CH_4 has a heating energy value of 11.2-18.6MJ/m^3 CH_4 (Speight, 1994). Biogas from tubular digesters has CH_4 contents ranging from 21-76% (Table 2.1), resulting in potential heating energy values between 2.35 to 14.1MJ/m^3 CH_4. Potential heating energy production rates per day for various animal wastes are calculated in Table 2.3. The material of the cooking vessel and food being cooked affect the heating energy demands. To illustrate if the potential heating

energy production rates (Table 2.3) will meet a household's demands', the total heating energy required was calculated (Table 2.4). For this analysis it was assumed that water, rice, beans, potatoes and eggs, foods commonly eaten in the developing world, were boiled from 20 to 100°C in copper, aluminum, stainless steel, cast iron and clay vessels.

To better illustrate the heating energy benefits of the biogas produced by tubular digesters, an example of a household that typically cooks rice and beans and owns sows will be used. A 200 kg sow can produce an average of 8.1 kg of excreta per day containing 0.74 kg VS (USDA, 2008). If 49% of the VS are utilized and the average biogas yields for sow waste are 0.13-0.55 m^3biogas/kgVS (Burton and Turner, 2003), the volume of biogas produced per animal can be approximately 0.03-0.28m^3 biogas/day-animal. Assuming a CH_4 content of 60%, this 200kg sow can produce an average heating energy value of 1636 kJ/day. If a safety factor of 40% is included to account for the cook stove's combustion efficiency, the heating energy value would be 654kJ/animal-day. If a household wanted to boil 0.5kg of rice in a 0.5kg stainless steel cooking vessel in a 20°C room at sea level, the total heat energy required would be 355kJ. To boil 0.5kg of beans in the same stainless steel vessel, the heat energy required is 234kJ. The total heat energy required by this household to cook a meal of 0.5kg of rice and 0.5kg of beans is 589kJ. In this hypothetical example, the tubular digester is producing enough biogas to meet the household's cooking energy demands. This further justifies what was observed in households using biogas from tubular digesters in Monteverde, Costa Rica. Biogas produced from 2-4 pigs was enough for 3-4 hours of cooking for a household of 4-5 people. The households were also saving approximately $20 per month compared to other households that used propane for cooking.

2.4.2 Agricultural Benefits

Anaerobic digestion effluent contains primary nutrients, nitrogen and phosphorus, and secondary nutrients, potassium, calcium and magnesium, and micro-nutrients, zinc, copper and iron. The roles of nitrogen and phosphorus during crop production are illustrated in Table 2.5. Only the primary nutrients will be discussed in this review. Nutrient rich effluent can be used as a soil amendment to improve plant growth. This is an environmentally and economically attractive alternative compared to the use of mineral fertilizers, the production of which consumes about 1.2% of the world's energy and is responsible for about 1.2% of the world's GHG emissions (Kongshaug, 1998). Effluents from anaerobic digesters also contain more inorganic nutrients that are available to plants compared to raw waste due to mineralization of organics during digestion (Arthurson, 2009). Agricultural benefits therefore encompass agronomic benefits (increased crop yield due to anaerobic digester effluent application) and economic benefits (monetary gain/profit). It is important to understand the balance of benefits to the people using the small-scale tubular digesters, the soil and plants (Adeli et al., 2005; Chantigny et al., 2008).

2.4.2.1 Agronomic Benefits

Agronomic benefits mainly refer to the use of the effluent to improve the soil fertility leading to increased crop yields. Anaerobic effluents treating livestock waste not only provide nutrients to the soil but also organic matter. Addition of organic matter from the effluent strengthens soil structure by promoting aggregation of soil granules by soil microorganisms. Increase of organic carbon lowers the carbon nitrogen (C/N) ratio of the soil (Bronick and Lal, 2005). Application of stabilized manure has also been shown to increase soil's total porosity by about 24% compared to mineral fertilizers (Marinari et al., 2000). This is due to increased

microbial dehydrogenase activity (enzymatic removal of hydrogen from the soil), which is catalyzed by the organic matter in the effluent (Marinari et al., 2000). If applied before planting, effluents have been shown to assist in crop disease control. This phenomenon occurs when substrates, such as carbohydrates and lignin, in the effluent are utilized by the microbes in the soil. Through competition, antagonism and predation, biological disease control is achieved (Hoitink and Boehm, 1999).

Nitrification is a naturally occurring biological process that occurs when TAN is applied to land. During the nitrification process, hydrogen ions are formed that contribute to soil acidity. At low pH levels (<5.5), the solubility of toxins, such as aluminum (Al) and manganese (Mn) naturally found in clay soils, increases. Uptake of Al and Mn by plants results in root deterioration, discoloration, low yields and lack of growth. Soils therefore need buffering capacity to maintain the pH between 6.0 and 7.0. This buffering capacity can be provided by effluent with a neutral pH (Zhang and Raun, 2006). Garfí et al. (2011) found a significant difference in potato yields between soil fertilized with effluent from a tubular digester treating guinea pig waste and compost. The author noted a 27.5% increase in potato yield and attributed it to the nutrients present in the effluent. Chantigny et al. (2008) also credited micronutrient availability in increasing corn yield when comparing clay and loam soils fertilized with either mineral fertilizer or anaerobically digested swine effluent.

To use effluent as a soil amendment, the fate of the nutrients during anaerobic digestion (Masse et al., 2007) has to be evaluated. During anaerobic digestion, organic matter in the waste is degraded to produce CH_4 and CO_2, thus lowering the effluent carbon to nitrogen (C/N) ratio and solids concentration. Garfí et al. (2011) reported that when treating guinea pig waste in Peru, the C/N ratio decreased from 17.0 in raw waste to 2.9 in the effluent. This reduced C/N ratio is

favorable because it reduces the competition for nitrogen between soil microorganisms and plants. TAN produced during anaerobic digestion can either be utilized by microorganisms for growth, form precipitates such as struvite or ammonium carbonate, and/or volatilize (Möller and Müller, 2012). In Sudan, Mubarak et al. (2010) also concluded that mineralization of nitrogen in cow, pig, goat, pigeon and camel manure increased soil quality. Mineralization also assists in solids reduction; lower solids concentrations decrease effluent viscosity, which increases permeation of inorganic nitrogen into the soil for faster plant uptake (Masse et al., 2007).

Soils require both nitrogen and phosphorus, however, the natural supply of phosphorus for plants is usually low (Möller and Müller, 2012). Anaerobic digestion of livestock waste has been shown to increase the concentration of soluble phosphorus that can be easily taken up by plants, which increases the nitrogen to phosphorus (N/P) ratio (Masse et al., 2007). Mineralization of nitrogen, phosphorus and magnesium and increased pH during anaerobic digestion can lead to struvite precipitation (Equation 2.7), especially while treating swine waste, due to high concentrations of TAN and orthophosphates present (Loewenthal et al., 1995).

$$Mg^{2+} + NH_4^+ + H_2PO_4^- + 6H_2O \rightarrow MgNH_4PO_4.6H_2O + 2H^+ \quad \text{(Eq 2.7)}$$

Unfortunately, struvite precipitation during anaerobic digestion can be a problem due to fouling and clogging pipes (Marti et al., 2008), especially in tubular digesters that are already prone to solids accumulation and clogging due to their design.

2.4.2.2 Economic Benefits

More than 70% of people living in rural regions in the developing world depend on agriculture (FAO, 2011). Increased crop yield from using digester effluent may mean more crops available for sale. In Nepal for example, households observed an increase in crop production of up to 68% when digester effluent was used (Katuwal and Bohara, 2009). An economic analysis is important to link agronomic and economic benefits. Costs include fixed costs such the

construction of the digester, pipes, biogas stove and tractor depending on the farm size and operational costs include labor, maintenance, and reduced cost of fertilizer and cooking fuel (Park et al., 2010). Mineral fertilizer can cost about $76.3-313.7/ha which accounts for 39-85% of the cost depending on application rates and requirements. Digester effluent can cost about $29.8-85.3/ha also depending on the application rate. Digester construction, labor and repair costs account for most of the cost depending on type of manure and maintenance of the digester (Park et al., 2010). To reduce the labor cost for example, communities in Sudan constructed their own digesters (Omer and Fadalla, 2003). Since, effluents have higher crop yields and significantly lower total costs, the economic return is more attractive. There is little published literature on the economic benefits of tubular digesters therefore more research should be carried out in this topic.

2.4.3 Environmental Benefits

Environmental benefits that will be discussed in this section are reduction of deforestation and water pollution from livestock waste. It is important to understand how deforestation contributes to climate change and how tubular digester can be used to mitigate this issue. Understanding the link between mismanagement of livestock waste and water pollution is also critical in improving water sources for drinking, recreation, agriculture and fishing.

2.4.3.1 Deforestation

Deforestation is one of the causes of climate change. Deforestation accounts for about 20% of GHG emissions from biofuel production and from expansion of land for agriculture and shelter. Carbon that is stored in the trees' biomass is released during biomass combustion and wood degradation (Palmer and Engel 2009; Bellassen and Luyssaert, 2014). Trees are also beneficial because they absorb CO_2 from the atmosphere. The accumulation of GHG leads to

global temperature rise, which affects climate and hydrology. Decreased precipitation and water infiltration, results in soil erosion, which pollutes surface water, increases flooding, decreases biodiversity and soil quality, which lowers crop yield (Kaimowitz and Angelsen, 1998). Unfortunately, poor communities in the developing world are the most vulnerable to deforestation and climate change effects (Palmer and Engel 2009). For example in Haiti, population increase has lead to increased demand for firewood. This dependence on firewood has lead to severe deforestation leaving this country with only 1-2% of vegetation cover (ESMAP, 2007). Deforestation in Haiti's countryside has led to adverse effects. Water pollution from soil runoff and low groundwater recharge has led to water shortages (Wampler and Sisson, 2011).

Tubular digesters can assist in curbing deforestation and GHG impacts by providing a clean burning fuel. Installation of biogas systems has been shown to decrease dependence on firewood. In Nepal, Katuwal and Bohara (2009) observed a 53% decrease in firewood use after installing biogas systems. In Sub-Saharan Africa, use of biogas from anaerobic systems has been estimated to decrease deforestation by 26% by 2030 (Subedi et al., 2014). Although tubular digesters are beneficial in mitigating deforestation, a lack of knowledge and education on the link between climate change, deforestation and firewood use prevent further adaptation of the technology. This is because communities in the developing world consider firewood as a “free” fuel (Schlag and Zuzarte, 2008).

2.4.3.2 Water Pollution from Livestock Waste

Lack of proper livestock waste management leads to runoff of waste into water bodies and groundwater pollution. Untreated livestock waste contains a number of different pollutants of concern that are summarized in Table 2.6. Use of a tubular digester can mitigate some of these water pollutants of concern. During anaerobic digestion, the organic matter in the waste is

degraded to produce biogas that is a renewable energy source. The concentration of suspended solids is also decreased during the anaerobic digestion process. Lansing et al. (2008) treating swine waste in Costa Rica observed biochemical oxygen demand (BOD) and total suspended solids removals of 79 and 86%, respectively. Usack et al. (2014) treating dairy waste in Indonesia observed chemical oxygen demand (COD) and total solids removals of approximately 65 and 31%, respectively. These results show that the tubular digesters systems can be beneficial in reducing water pollution. Although the process of anaerobic digestion does not reduce the concentration of nitrogen and phosphorus, the waste is stabilized and can be used as a fertilizer, as mentioned previously (Marinari et al., 2000). However, farmers need to implement best management practices to control runoff. Some of these practices include: (1) a vegetation filter strip between the fields and water bodies, (2) a water and sediment drainage basin that receives agricultural runoff, (3) constructed wetlands and (4) duckweed and fish ponds (Miller et al., 2012).

According to WHO, *Cryptosporidium parvum, Giardia lamblia, Campylobacter jejuni, Salmonella* sp. and *E. coli O157* are the main zoonotic pathogens that cause illness to humans. Livestock waste contains high loads of these pathogens especially for protozoan parasites. There is limited research on the inactivation of these pathogens during livestock waste treatment in tubular digesters in the developing world. Garfi et al. (2011) measured total coliforms and *E.coli* while treating guinea-pig waste (Table 2.1). In this study, negligible removal of these pathogens was observed. Masse et al. (2011) investigated the fate of total and fecal coliforms, *E. coli, Salmonella, Campylobacter spp.,* and *Y. enterocolitica* in a 24°C farm scale sequencing batch anaerobic reactor operated at HRTs of 7 and 14 days. At both HRT values, a significant decrease of these pathogens was observed. Differences in results from Garfi et al. (2011) and Masse et al.

(2011) are unknown; indicating further research is needed on how the design, operation and maintenance of tubular digesters affect the fate of pathogens. Cote et al. (2006) treated swine waste in a 20°C anaerobic sequencing batch reactor with a 20 day SRT and observed level of *Salmonella, Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts below the detection limits in the effluent. This study did not explicitly indicate an inactivation mechanism; instead they mentioned that there was removal of pathogens which may be due to a physical removal process. Furthermore, the study did not analyze the viability of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts which is vital in determining their infectivity. Therefore, more research is needed to determine the viability of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts during livestock waste treatment in tubular digesters.

2.4.4 Social Benefits

Social benefits in this review are in regards to increasing gender equality. Due to cultural and social roles, women provide unpaid household labor by providing human energy in survival activities. Survival activities include; collection of firewood, water collection and food preparation. This human energy is undervalued when nations report their economic contributions. For example, in India, women spend about 9 hours per day on survival activities while men spend 5 hours per day (Cecelski, 2000). According to the International Labor Office firewood collection is the most time-consuming survival activity for women in rural villages in Peru, Ghana, Mozambique, India and Indonesia (Cecelski, 1987). In rural Nepal, women who utilize firewood as cooking fuel spent about 4 hours per day searching for firewood, usually over long distances (Katuwal and Bohara, 2009). Once anaerobic digestion systems were installed, 33% of the women spent their time participating in social and community activities. This saved the women up to 3 hours each day, time that was otherwise spent searching for firewood

(Gautam et al., 2009; Katuwal and Bohara, 2009). In same study, the 111,000 anaerobic digester systems installed translated to 35,000 woman hours per year (Gautam et al., 2009). Tubular digesters can reduce the need to spend hours per day searching for firewood. Household members, particularly women and girls, can use that time saved in income generating activities. Some of these activities reported include beer brewing in Burkina Faso and Tanzania, bakeries in Kenya and Peru, shea butter production in the Sahel region of Africa, soap making in Bangladesh, tea shops in Nepal and pottery making (Cecelski, 2000). The health of women and girls is also improved. This will be discussed in the section below. Therefore, tubular digesters can assist in empowering women by decreasing gender inequality. Incidence of disease and decreased time demands for women are positive outcomes; however, according to Cecelski (2000), seeking integrated approaches and various solutions is important in empowering women with respect to providing renewable energy choices for cooking.

2.4.5 Public Health Benefits

Millions of children die in the developing world due to exposure to indoor air pollution. Over reliance on firewood, coal and animal waste as a source of fuel has lead to deteriorating indoor air quality from the production of particulate matter, carbon monoxide, sulfur dioxide, oxides of nitrogen and many other harmful byproducts from combustion of biomass. In Sub-Saharan Africa, firewood is the predominant fuel source, especially in the rural places. In Tanzania, Uganda, Senegal, Zambia, Malawi, and Kenya, 96, 91, 89, 88, 96 and 88%, of rural households respectively, rely on firewood as their main fuel source (Schlag and Zuzarte, 2008). Women and children under the age of 5 are especially affected by indoor air pollution (WHO, 2007a). Indoor air pollution leads to acute lower and upper respiratory infections, chronic pulmonary disease, leading to lung fibroids and bronchiectasis, asthma, infant mortality, low

birth weight and eye infections (Ritz and Yu, 1999; Ezzati and Kammen, 2001; WHO, 2007a). Bonte (1974) showed that there were more deaths and hospitalizations of people in Kenya with respiratory complications compared to those caused by malaria. However, very little attention has been paid on how to deal with the causes of respiratory diseases in the developing world (Ezzati and Kammen, 2002).

Programs targeted at reducing or curbing the sources of indoor air pollution have shown to be successful at reducing the frequency of respiratory diseases in Brazil (Ribeiro and Cardoso, 2003). Such programs rely heavily on improving energy sources. As of 2002, 81% of the population in Nepal used solid biofuels, leading to 4820 deaths due to acute lower respiratory infections (ALRI) for children under the age of 5 and 2680 deaths due to chronic obstructive pulmonary disease (COPD) for people older than 30 years (WHO, 2007b). To assist with this situation, the Biogas Support Program (BSP-Nepal) organization was formed in 2003. Biogas systems were installed and the public health impacts of biogas systems were analyzed using the Biogas User Survey 2007/2008. Eye infections, respiratory disease, coughing and headaches were decreased in women, men and children who used biogas. The health improvements were especially significant for women, who reported a 40% reduction in eye infections and headaches and a 25% reduction in respiratory disease and coughing (Katuwal and Bohara, 2009).

2.5 Policies Promoting Anaerobic Digestion of Livestock Waste

Due to the benefits outlined above, small-scale tubular digesters have been adopted in many developing countries. However, financial obstacles, such as lack of capital and credit have prevented widespread adoption of the technology (Laichena and Wafula, 1997). Lack of local demand for biogas as an energy source due to other cheaper energy sources also decreases adoption of tubular digesters. In spite of India's large livestock waste production, there is a lack

of demand for its collection and transformation to energy (UNEP, 2010). Therefore, to overcome these obstacles developing country communities, national and local governments, funding agencies and development organizations need to provide funding and technical assistance for technology transfer to be successful. This includes:

1. Promotion of biogas as a renewable energy source on a country level,
2. Encouraging country-driven efforts and access to funding,
3. A shift from project-driven to programmatic-driven approaches and
4. Providing activities that are relevant to climate change and the millennium development goals (UNEP, 2010).

To promote the market for biogas as a renewable energy source, nation specific programs and policies can be developed. These programs may include, cash grants, subsidies or loans. In developed countries, such as the United States, the federal government provides stimulus packages that encourage farmers to produce energy from their livestock waste, such as the American Recovery and Reinvestment Act of 2009. These kinds of policies have also been used in developing countries to promote biogas technologies.

In the People's Republic of China (PRC) an increase in population led to increased livestock production, which resulted in increased water pollution from disposal of untreated livestock waste. In 2006, the PRC passed the Renewable Energy Law to promote national biogas development, which encouraged the Efficient Utilization of Agricultural Wastes project. The main goals of this project were to improve the lives of those living in rural households, to improve the environment and to promote sustainable agriculture. The government of PRC received a loan from the Asian Development Bank (ADB) and a grant from the Global Environment Facility to provide $77.3 million. The 2 year project targeted 4 rural disadvantaged

provinces (Henan, Hubei, Jiangxi, and Shanxi). The project led to construction of biogas systems that produced 600-87,600m^3 of biogas per year and served more than 19,000 households. Funds for implementing the digesters were dispersed from the Ministry of Finance to the provincial, municipal, township finance offices and finally to the village economic cooperatives that loaned the money to farmers. The loans had approximately a 10 year repayment plan with an average of 7.2% interest rate based on a 15 year life of the digester. The project led to an average of $1,390 annual increase in household income, an 88% reduction in combustion of biomass for fuel, a 64% reduction of time spent by women to cook and a 75% increase in health in households that used the biogas systems. Future recommendations for this type of project included, increased monitoring of digester effluent to reduce runoff, monitoring rural economic growth to further justify impact on quality of life for users and developing revolving funds so that ADB and the government of PRC can increase the project's impact (ADB, 2010). This project in the PRC demonstrates how promotion of biogas on a country level through policies, activities relevant to millennium development goals and a programmatic approach can be successful.

Collaborations between development banks, governments and micro-finance cooperatives can improve the environment and quality of life for their citizens through implementation of digesters. In Nepal, government subsidies lowered the capital cost of implementing digesters, leading to the government surpassing their goal of 800 digesters per year to 6,824 digesters in 1992-1994 (Katuwal and Bohara, 2009). While in Cambodia, the National Biogas Programme (NBP), part of Cambodia's Ministry of Agriculture, began collaboration with the Netherlands Development Finance Company in 2007 to provide households with loans to build digesters. The loans are for 2 years with a 1.2% interest rate per month. As of 2013, approximately 19,200 digesters had been installed (NBP, 2014).

Unfortunately, government policies, regulations and collaboration with organizations do not necessarily lead to successful implementation of biogas technologies. For example, the Haitian government has set environmental regulations to improve the country's environment. The Haitian Environment Ministry is collaborating with the United States Senate Committee on Foreign Relations to finance Waste to Energy (WTE) projects (Booth et al., 2010). However, the widespread lack of education on the dangers of deforestation, corruption, political instability, lack of knowledge of the law, lack of accountability by the public sector and poor infrastructure make financing WTE projects difficult. The National Renewable Energy Laboratory (NREL) is also working in Haiti on the implementation of biogas systems in the country; however, the process has been slow (Booth et al., 2010). This scenario is similar in other developing countries. More education and regular monitoring of environmental regulations and laws is needed to make financing of these projects easier by public and private sectors.

2.6 Conclusions and Recommendations

Tubular digesters are an efficient livestock waste treatment technology that generates biogas and a nutrient rich effluent. For a tubular digester to meet a household's or communities' cooking energy needs, the substrate characteristics and operating parameters have to be understood for proper design of the system. Good performance of the tubular digester can then lead to several energy, environmental, public health, social and agricultural benefits. For communities to enjoy these benefits, several developing countries have implemented policies that educate and curb financial obstacles that limit widespread adoption of anaerobic digestion technologies of livestock waste. These policies can be used as examples for other developing countries governments seeking to improve the quality of life for their citizens.

Table 2.1: Summary of substrate characteristics, operating parameters and performance of tubular digesters installed in different regions of the developing world to treat livestock waste.

Region	Country	Type of Manure	TAN concentration	pH	Organic Loading Rate	Retention Time	Temperature	Biogas Yield	CH_4 content	Reference
			g NH_4^+-N/L		kg VS/ m^3-day	Day	°C	m^3 biogas/kg VS_{added}	%	
Africa	Egypt	Water Buffalo	n/a	n/a	1.12 - 2.13	38, 58 and 95	17 - 22	0.04 - 0.16	43 - 66	Hamad et al., 1982
	Tanzania	Cow	n/a	n/a	0.33 - 3.93	19 - 23	n/a	0.07 - 0.32	62 - 76	Cortsen et al., 1995
Asia	Vietnam	Swine	n/a	6.5 - 6.6	0.66 - 2.66	30	25 - 27	0.30 - 0.36	54 - 56	An and Preston, 1999
	Cambodia	Swine	0.34 - 0.88	6.8 - 7.1	1.84	10 - 30	26 - 31	0.29 - 0.56	n/a	Thy et al., 2003
	Indonesia	Cow	n/a	7.3 - 7.7	2.00	21	27	0.14 - 0.15[c]	59	Usack et al., 2014
Central and South America	Bolivia	Cow, llama and sheep	n/a	74 - 7.8	0.50 - 8.00	10, 20 and 30	18 and 25	0.013 - 0.19	21 - 61	Alvarez and Lidén, 2009
	Costa Rica	Swine and grease	1.14 - 1.39	6.9 - 7.2	0.50 - 1.41[b]	40	22 - 26	n/a	63 - 70	Lansing et al., 2010
	Peru	Cow	0.14 - 1.11[a]	7.7 - 8.3	0.67 - 1.00	60 - 90	20 - 25	0.35	63 - 67	Ferrer et al., 2011
	Peru	Guinea Pig	0.20 - 0.21	7.2 - 8.8	0.60	75	23 - 30	0.058 - 0.061	61 -73	Garfi et al., 2011

[a] mg /g; [b] kg COD/m^3-day; [c] m^3 CH_4/kg VS_{added}; n/a stands for not available

Table 2.2: Nutrient content and recalcitrant compounds in livestock manure (Müller, 1980; Choi, 2007; USDA, 2008).

Parameter	Unit	Swine	Cow	Poultry
Lignin	% VS	2.2 - 16	7.9 - 10	3.4 - 5.2
Cellulose	% VS	15 - 20	17 - 25	11 - 15
Hemicellulose	% VS	20	22	11 - 17
Nitrogen	kg/day-animal	0.037 - 0.95	0.22 - 0.33	0.002 - 0.01
Phosphorus	kg/day-animal	0.024 - 0.25	0.08 - 0.14	0.001 - 0.37
Potassium	kg/day-animal	0.028 - 0.26	0.12 - 0.19	0.001 - 0.46

Table 2.3: Calculated average potential biogas and heat energy produced per animal calculated depending on manure type.

Type of manure	Unit	Swine		Cow		Poultry	Horse
		Sow	Boar	Dairy	Beef		
Weight of excrete [a]	kg/animal-day	8.10	3.80	59.0	39.0	0.086	25.4
VS in excrete [a]	kg/animal-day	0.74	0.34	5.18	4.05	0.02	3.02
VS reduction at 20°C [b]	%	49	49	31	41	56	31
Potential biogas yield [c]	m^3 biogas/kg VS	0.34	0.35	0.25	0.092	0.43	0.28
Potential biogas volume produced	m^3biogas/animal-day	0.15	0.06	0.52	0.16	0.004	0.26
Potential methane volume produced	m^3 CH_4/animal-day	0.09	0.03	0.31	0.10	0.002	0.15
Heat energy production rate	MJ/animal-day	1.6	0.62	5.5	1.7	0.042	2.7

[a] USDA (2008), [b] Fulhage et al. (1993), [c] Burton and Turner (2003)

Table 2.4: Calculated total heat energy requirements to boil 0.5 kg of different foods in different vessel material.

Vessel material		Aluminum	Copper	Stainless steel	Clay pot	Cast iron
Vessel material specific heat capacity [a]	kJ/kg-°C	0.90	0.85	0.50	0.94	0.46
Heat energy required to increase vessel temperature per degree	kJ/°C	0.45	0.43	0.25	0.47	0.23
Type of food		Water	Beans	Rice	Potatoes	Eggs
Food specific heat capacity [a]	kJ/kg-°C	4.18	1.17	4.18	3.43	3.18
Heat energy required to increase water temperature per degree	kJ/°C	2.09	2.09	2.09	2.09	2.09
Heat energy required to increase food temperature per degree	kJ/°C		0.59	2.09	1.72	1.59
Total heat energy required	kJ/°C	2.54	3.10	4.43	4.27	3.91
Total heat energy required to heat whole mass from 20 to 100°C	kJ	203	248	355	342	313

Heat energy required to increase item temperature per degree = mass of item x specific heat capacity ; [a] "Specific heats"

Table 2.5: Roles of primary nutrients during plant growth.

Nutrient	Roles derived from Tucker (1999)
Nitrogen	• Involved in photosynthesis. • Promotes plant growth as a component of cell division. • Increases quality and size of plants. • Assists in plant's protein synthesis through amino acids.
Phosphorus	• Involved in protein synthesis • Promotes germination, blooming and budding. • Assists in seed development. • Makes plants less vulnerable to cold temperatures.

Table 2.6: Summary of pollutants found in raw livestock waste and their environmental impacts.

Pollutant	Environmental Impacts	References
Biodegradable organic matter	Degradation of biodegradable organic matter decreases DO levels in water bodies which affect aquatic life.	
Nutrients Nitrogen Phosphorus	High concentrations of nutrients promote excessive growth of plants and algae. Decomposition of dead algae decreases DO levels. This process is called eutrophication.	Chislock et al., 2013
Suspended solids	Increase water turbidity, decreasing light penetration which affects aquatic plants photosynthesis process. Suspended solids also accumulate in fish gills affecting their growth rates and health.	Au et al., 2004
Pathogens *Cryptosporidium parvum, Giardia lamblia, Campylobacter jejuni, Salmonella* sp. and *E. coli* O157 H7	Human exposure to pathogens in water bodies that are used for drinking water, recreation and fishing.	Dufour et al., 2012
Air pollutants VOC, GHGs, odorous gases and particulate matter	Livestock waste decomposition forms air pollutants that cause respiratory illness such as asthma, smog formation, psychological stress from continued exposure and climate change.	EPA, 2011
Trace metals	Copper, zinc and boron can negatively affect the environment from accumulation in water bodies.	Kinyua, 2013
Chemicals of concern	Antibiotics and pharmaceuticals in water bodies affect aquatic life	

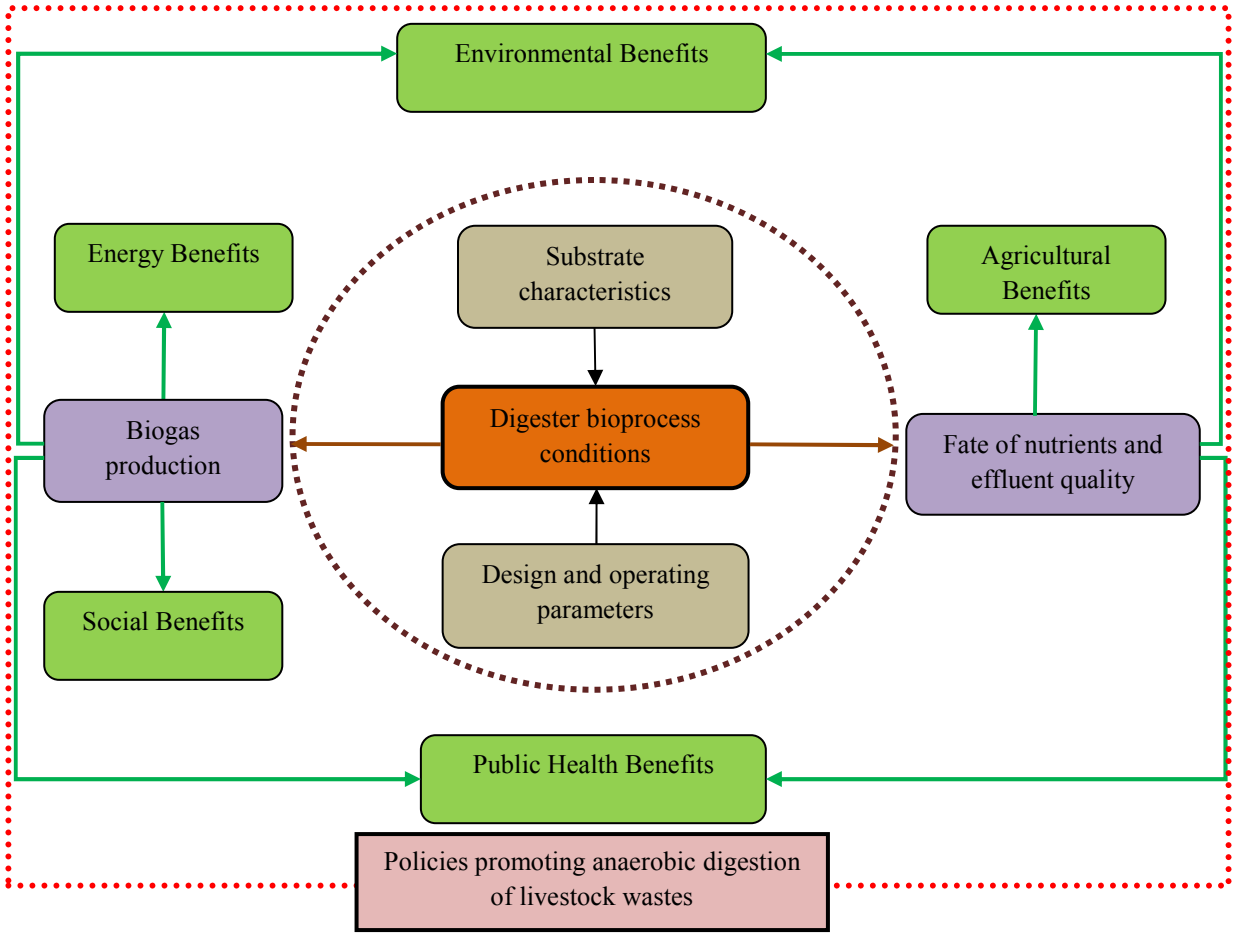


Figure 2.1: Schematic illustrating link between digester operation, benefits and policies.

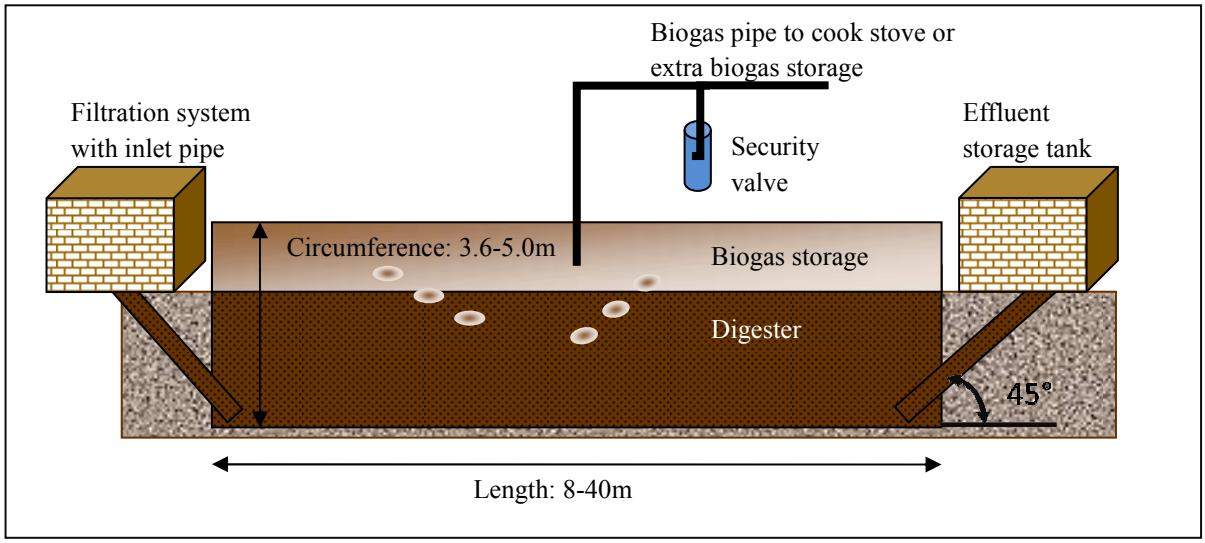


Figure 2.2: Schematic of a small-scale tubular digester used in the developing world.

Figure 2.3: Images of tubular digesters with and without a biogas storage bag treating swine waste in Costa Rica

Chapter 3:

Physical and Biological Processes in Tubular Digesters[2]

3.1 Introduction

Anaerobic digestion of livestock waste is a waste management technology that can improve the quality of life for those in the developing world. Biogas produced from small-scale anaerobic digesters is most often used as a cooking fuel, but can also be used to heat water or buildings or generate electricity for on-site use (Ferrer et al., 2011). Lack of proper livestock waste management leads to runoff of pollutants into surface waters and contamination of groundwater. Anaerobic digesters can assist in reducing water pollution by decreasing the concentration of organic matter in the waste. In addition, these systems can be a useful tool to mitigate deforestation when biogas is used as a cooking fuel rather than firewood. This also results in decreased public health concerns, especially for women and children who are disproportionally affected by indoor air pollution due to cultural and social roles (Ferrer et al., 2011). The effluent from anaerobic digestion also contains primary nutrients (nitrogen, phosphorus) that can be used as a soil amendment to improve crop yields (Ferrer et al., 2011).

Polyethylene tubular anaerobic digesters are commonly used in small-scale applications to recover energy from livestock waste in the developing world. These systems do not require a high level of skilled labor to install; they are easy to operate, low in cost and can operate under a range of temperature conditions. Several studies have been carried out investigating the effect of

[2] This chapter is adapted from a manuscript under review at *Applied Energy*: Kinyua, M.N., Zhang, J., Céspedes, F.C., Tejada-Martinez, A., Ergas, S.J, "Physical and biological process modeling of tubular digesters treating swine waste in rural Costa Rica"

influent waste characteristics and reactor design and operation on tubular digester performance (Lansing et al., 2010; Ferrer et al., 2011). However, no prior studies exist that combine mathematical modeling analyzing the physical (transport and mixing) and biological processes with field studies of tubular anaerobic digester performance.

Mathematical models have been used to provide insights into operating hydraulic residence time (HRT) and mean cell residence time (MCRT) in anaerobic digesters (Wu, 2012). Control of HRT and MCRT are needed to avoid wash-out of soluble substrates and slow growing microorganisms, respectively (Kinyua et al., 2014). Mixing mechanisms in digesters that can affect HRT include formation of dead zones, internal recirculation, and short-circuiting/channeling (substrate by-passing treatment) (Levenspiel, 1999). In bioreactors without solids separation and recycling, MCRT is normally considered to be equal to the HRT; however, even without solids separation or recycling, the MCRT in tubular digesters may be longer than the HRT due to solids settling and accumulation (Lansing et al., 2010; Ferrer et al., 2011; Usack et al., 2014). Tubular digesters are fed with a high solids concentration, which favors collisions between particles to form flocs that are denser than water. Over time a floccular sludge layer forms, similar to processes occurring in anaerobic baffled and upflow anaerobic sludge blanket (UASB) reactors (Angelidaki et al., 2002; Montteran et al., 2013). Hydrolysis of particulates into soluble substrates and formation of organic acids (intermediate substrates for methanogens) by acidogens are often considered the rate limiting steps during anaerobic digestion and are affected by the MCRT (Kinyua et al., 2014). As the MCRT increases, hydrolyzing and acidogenic bacterial growth rates increase because they have adequate time to utilize solid and soluble substrates to form acetate and hydrogen (H_2), which are utilized by the methanogens (Kinyua et al., 2014).

Computational fluid dynamic (CFD) modeling is a useful tool that can produce residence time distribution (RTD) data for estimating the mean liquid HRT in a digester. CFD models have been successfully applied to contactors used for water and wastewater treatment (Zhang et al. 2013). These models can also provide information on the physical mechanisms within the digester (Wu, 2012). Furthermore, CFD models can be used to inform simplified transport models, such as plug flow, completely mixed, tanks in series and or dispersion (Méndez-Romero et al., 2011). Several studies have been carried out using CFD to evaluate the effect of transport and mixing mechanisms on biogas production in completely mixed anaerobic digesters; however, more research is needed to understand the physical mechanisms contributing to tubular digester performance (Wu, 2012).

While CFD modeling is useful for evaluating the physical processes and liquid HRT in anaerobic digesters, these models do not evaluate the biological processes occurring in the digesters. The anaerobic digestion model 1 (ADM1) is a steady-state bioprocess model that incorporates hydrolysis and acidogenesis with influent and effluent characteristics to estimate volatile solids (VS) removal and methane (CH_4) production (Batstone et al., 2002). The ADM1 model is complex, involving 19 process kinetics and 24 biochemical components to determine digester performance. In addition, ADM1 models the reactor as a completely stirred tank reactor (CSTR), which may not be appropriate for modeling the performance of tubular digesters with floccular sludge accumulation. To overcome this limitation, Bernard et al. (2001) and Elmitwalli (2013) developed simple models for anaerobic floccular sludge layer reactors. The models incorporate acidogenesis and methanogenesis and have been applied to full-scale UASB reactors (Elmitwalli, 2013).

This study investigated the performance (biogas production and effluent quality) of a tubular digester treating livestock waste in the Monteverde region of Costa Rica. A tracer study was performed on this digester to gain insight into transport and mixing mechanisms in the system. Data from the tracer study was used to calibrate a CFD model, which was used to estimate the operating HRT and visualize transport and mixing mechanisms in the digester. A simplified bioprocess model, incorporating conversion of soluble substrates to CH_4 and biomass decay, was used to estimate the active biomass concentration in the digester. The active biomass concentration was used to estimate the operating MCRT. Results from the physical and biological processes models provided a supporting framework on the relationship between the transportation and transformation mechanisms and how these mechanisms affect biogas production and effluent quality in tubular digesters.

3.2 Materials and Methods

3.2.1 Site Description and Tubular Digesters

San Luis de Monteverde is a rural mountain community located on the Pacific slope of the Tilarán mountain chain in northwest region of Costa Rica with an altitude range of 600 to 1200 m (~0.85atm) above sea level. San Luis de Monteverde has a population of approximately 500 people. The main economic activities in San Luis de Monteverde are small-scale production and sale of farm products, including coffee, vegetables, fruit, beans, corn, pork, beef, chicken and eggs; rural tourism is also significant. Households in San Luis de Monteverde have access to potable water provided by a locally administrated aqueduct project. Domestic wastewater is normally treated using septic systems, while livestock wastewater remains largely untreated causing contamination of the watershed. To improve livestock waste management, community

leaders have obtained the support of local and international collaborators, such as the University of Georgia and the Monteverde Institute, to install tubular digesters in the San Luis valley.

Eight farmers in San Luis de Monteverde use Taiwanese-model tubular polypropylene bag digesters with PVC piping to treat livestock waste and produce biogas for cooking. The digesters have a 9-12 m^3 working volume with a 4 m^3 biogas storage bag. These household digesters typically treat swine waste from 4-10 pigs and provide fuel for approximately 4 hours of cooking per day, which meets the needs of an average family of 5 people. The operating parameters for the digester that was analyzed in this study, over a 5 week field-study are shown in Table 3.1.

3.2.2 Tracer Study

A tracer study was performed to understand the digester's transport and mixing behavior. It should be noted that this is the first study to perform a tracer study on a tubular digester to understand these system's hydrodynamic mechanisms. Fertilizer grade potassium chloride (KCl) (Mezcla Distribuido, Limón Costa Rica) was used as a tracer due to its ease of use and lack of hazardous waste produced, as the effluent from the digester is used to fertilize cattle pastureland. A calibration curve was developed to allow effluent tracer conductivity measurements to be reported in terms of KCl concentration (g KCl/L). The target influent concentration was 8.5 g KCl/L. This concentration was selected to ensure that the effluent conductivity was significantly higher than the measured background conductivity and that the chloride concentration did not inhibit methanogenesis (Serrano et al., 2014). Note that biogas production in the digester remained stable during the tracer study, as will be discussed later. A mass of 4.75 kg of KCl was added to the 572 L of influent, mixed thoroughly and quickly poured into the digester through

the influent pipe. Samples were collected from the outlet pipe every other day for 32 days and conductivity was measured as described below.

3.2.3 Analytical Methods

All laboratory analyses were carried out at the University of Georgia Costa Rica campus (San Luis, Puntarenas, Costa Rica). Influent and effluent samples were collected and analyzed weekly from the digester for 5 weeks. Standard Methods (APHA, 2012) were used to measure BOD_5 (5210), VS, and total solids (TS) (2540 G). Hach high range TNT test kits and a Hach DR 890 portable colorimeter (Loveland, CO) were used to measure total ammonia nitrogen (TAN) (TNT 832), total nitrogen (TN) (TNT 827) and total phosphorus (TP) (TNT 845) concentrations. pH was measured using Oakton portable waterproof pHTestr 10 (Vernon Hills, IL). COD measurements could not be carried out during the field-study because there were no facilities to dispose the hazardous waste generated. Biogas volume in the tubular digester was measured using a wet tip gas meter (Wayne, PA). CH_4 content of the biogas was measured using RKI Eagle 72-5335RK-05 portable gas detector equipped with CH_4 and CO_2 sensors. Digester temperature was measured using a Thomas traceable digital thermometer (Philadelphia, PA). Conductivity was measured using a Fisher scientific traceable conductivity meter (Hanover Park, IL) with a conductivity detection limit of 19.99mS/m. Method detection limits (MDL) were (mg/L): TAN (2), TN (5) and TP (1.5). A sample of floccular sludge from the tubular digester was collected from the outlet pipe (Figure 3.1a). A 0.25 L container was securely taped to a 1.5 m tube and slowly introduced into the tubular digester from the outlet pipe. A sample volume of 5.0 L was collected and analyzed based on standard methods (APHA, 2012) for total suspended solids (TSS) (2540 D), volatile suspended solids (VSS) (2540 E) and sludge volume index (SVI) (2710 D).

3.2.4 Data Analysis

Oracle Crystal Ball (Redwood City, CA) was used for Monte Carlo simulation. A Monte Carlo uncertainty analysis was performed by running 1,000 trials with selected parameter values within the range previously reported in the literature (Table 3.2) to determine which model inputs affected the MCRT value. All the kinetic inputs were considered uncertain inputs with triangular distribution. Triangular distribution was used due to lack of sufficient kinetic input data to fit a uniform distribution. Crystal Ball calculates the sensitivity by ranking what inputs significantly correlate with the output while the simulation is running. If an input and output have a high correlation percent, it means that the input will have a significant impact on the output. The percentage is the rank of correlations normalized to 100%. A positive contribution means that the input will increase the output and a negative contribution means the input will decrease the output.

3.3 Model Development

3.3.1 Physical Processes Model

3.3.1.1 Computational Fluid Dynamics: Governing Equations for Flow and Tracer Transport

In this study, the unsteady Reynolds-averaged Navier-Stokes equations (RANS) simulation technique was employed for flow and tracer transport simulation in the tubular digester. In the present unsteady RANS simulation, the governing equations for flow and tracer transport were solved simultaneously at each time step. Governing equations for the flow consisted of the Reynolds-averaged continuity equation and incompressible Navier-Stokes equations:

$$\frac{\partial \langle u_i \rangle}{\partial x_i} = 0 \qquad \text{(Eq. 3.1)}$$

$$\frac{\partial\langle u_i\rangle}{\partial t} + \langle u_j\rangle\frac{\partial\langle u_i\rangle}{\partial x_j} = -\frac{1}{\rho}\frac{\partial\langle p\rangle}{\partial x_i} + \nu\frac{\partial^2\langle u_i\rangle}{\partial x_j^2} - \frac{1}{\rho}\frac{\partial\langle u_i' u_j'\rangle}{\partial x_j} \quad \text{(Eq. 3.2)}$$

where a bracket denotes Reynolds-averaging, vector $\langle u_i\rangle$ is the Reynolds-averaged velocity, vector x_i is position, t is time, $\langle p\rangle$ is Reynolds-averaged pressure, ρ is density, and ν is kinematic viscosity.

The Reynolds stress tensor $\langle u_i' u_j'\rangle$ (expressed in terms of velocity fluctuation u_i') was closed using an eddy viscosity model in which:

$$\langle u_i' u_j'\rangle = -\mu_t\frac{\partial\langle u_i\rangle}{\partial x_j} \quad \text{(Eq. 3.3)}$$

and the eddy viscosity is:

$$\mu_t = C_\mu\frac{k^2}{\varepsilon} \quad \text{(Eq. 3.4)}$$

where k is the turbulent kinetic energy and ε is the turbulent kinetic energy dissipation rate. Transport equations for k and ε were specified via the standard k-ε model equipped with standard wall functions (Wilcox, 1994). Model coefficient, C_μ was taken as $C_\mu = 0.09$, its standard value. Tracer transport was governed by the Reynolds-averaged advection-diffusion equation:

$$\frac{\partial\langle C\rangle}{\partial t} + \langle u_j\rangle\frac{\partial\langle C\rangle}{\partial x_j} = -\frac{\partial\langle u_i' C'\rangle}{\partial x_j} \quad \text{(Eq. 3.5)}$$

where $\langle C\rangle$ is the ensemble-averaged tracer concentration, C' denotes tracer fluctuation and turbulent flux. $\langle u_j' C'\rangle$ was modeled as:

$$-\langle u_j' C'\rangle = D_t\frac{\partial\langle C\rangle}{\partial x_j} \quad \text{(Eq. 3.6)}$$

Note that $\langle C\rangle$ is time-dependent due to transient boundary conditions, which are described below. The eddy (turbulent) diffusivity was taken as $D_t = \frac{\mu_t}{\rho\cdot Sc_t}$ where the eddy viscosity μ_t, was

computed via the k-ε model and the turbulent Schmidt number, Sc_t, was taken as 0.7 (Launder, 1978). Usually the turbulent diffusivity is much greater than molecular diffusivity in convection-dominated flows. This was confirmed in the present simulations; therefore, molecular diffusion term was neglected in the tracer transport equation (Eq. 3.5).

It should be noted that in the present CFD simulation, the tubular digester's flexible walls, solids accumulation and biogas bubble generation were not considered. Although incorporating those features into the CFD model may make the CFD simulation more accurate and detailed, this additional complexity had no significant impacts on the primary function of the CFD model for this study, which was to validate the two assumptions that were used to develop the reduced order model (Section 3.3.1.3).

3.3.1.2 Numerical Set-Up and Tools

Digester 1 is 8m long with a 1.59m-internal diameter; other digester dimensions and components are shown in Figure 3.1a. The computational domain is shown in Figure 3.1b. The normal operating procedure was that each day swine waste was mixed with water to obtain a slurry with an average TS concentration of 6.35g/L. The slurry was fed into the digester for approximately 15 minutes at a rate of approximately 2,280 L/h. The Reynolds number was estimated to be 7,964 at the inlet zone based on inlet diameter (0.1 m) and influent velocity (0.078 m/s), and 674 in the main digester based on the diameter of the digester (1.59 m) and bulk velocity (4×10^{-4} m/s). The numerical digester was run to simulate the first day of the physical tracer study. During the 15 minutes of feeding (flow-through) at the beginning of the cycle, the inlet of the digester was assigned a constant influent velocity. A pressure outlet boundary condition is applied to the outlet of the digester. The other boundaries employed no-slip wall boundary conditions. After the 15-minute flow-through period ended, the flow into the digester

was shut down and thus the digester behaved as a batch-reactor until the next feeding period. During this stage the boundary conditions for the inlet and outlet changed to no-slip wall boundary conditions since the system was assumed to be closed. The other boundary conditions did not change.

In order to calculate the mean liquid HRT, a tracer pulse with a concentration 8.3 g KCl/L was injected into the digester during the first feeding 15-minute flow through period of the CFD simulation. This was calculated by dividing the total tracer mass (4.75 kg) by the influent flow (572L) during the 15 minute flow-through period. An unstructured grid, which was generated using Gambit (Fluent, Inc. 2004), was employed for the physical processes model simulations. The total number of grid cells was 85,134. This mesh is shown over half of the domain in Figure 3.1b. All simulations were conducted using OpenFOAM (OpenCFD Ltd., 2012), a collection of C++ libraries for solving continuum mechanics problems using the well-known finite volume method.

3.3.1.3 Reduced Order Model

A general mass balance equation for the system during the 15 minute flow-through period was written as:

$$V\frac{dC_d}{dt} = QC_{in} - QC_{out} \qquad \text{(Eq. 3.7)}$$

where C_d is the tracer concentration in the digester, C_{in} is the influent tracer concentration, C_{out} is the effluent tracer concentration, V is the volume of the digester, Q is flow rate and t is time. Based on the operating conditions, two assumptions were made. The first assumption was that the batch reactor period was sufficient to mix the tracer in the digester. Thus C_d was uniform everywhere in the digesters at the end of the batch reactor period or at the beginning of the next flow-through period

$$C_{out}^{n} = C_{d}^{n} \quad \text{(Eq. 3.8)}$$

where n indicates current day and $(n-1)$ indicates the previous day.

The second assumption was that during the 15 minute flow through period, the impact of the inflow on the effluent concentration was negligible. That is $C_{out}^{n} = C_{d}^{n}$ was valid for all the flow-through periods. The two assumptions were subsequently validated via CFD simulations. By applying the two assumptions, Equation 3.7 was integrated and expressed as:

$$C_{d}^{n} = C_{d}^{n-1} + \frac{TQ}{V}\left(C_{in}^{(n-1)} - C_{d}^{(n-1)}\right) \quad \text{(Eq. 3.9)}$$

with conditions:

$$C_{d}^{0} = 0 \quad \text{(Eq. 3.10)}$$

$$C_{in}^{n} = \begin{cases} C_0 & n = 0 \\ 0 & n \geq 1 \end{cases} \quad \text{(Eq. 3.11)}$$

where C_0 is the initial tracer concentration in the digester and T is the 15 minute flow-through period.

3.3.2 Bioprocess Model

Since granular and floccular sludge reactors, such as anaerobic baffled and UASB reactors, have been used to treat high strength swine waste (Angelidaki et al., 2002; Montteran et al., 2013), the bioprocess model was based on a sludge layer reactor model developed by Elmitwalli (2013). Given that the active biomass concentrations inside the tubular digester (X_m) and in the effluent (X_{me}) were unknown, the bioprocess model was used to calculate these values, which were used to estimate the MCRT according to the following:

$$MCRT = \frac{VX_m}{QX_{me}} \quad \text{(Eq. 3.12)}$$

The full bioprocess model formulation is presented in Elmitwalli (2013). Mass balances on X_m and daily methane production rate (ṁ) were sufficient to estimate the MCRT for this study, so only these equations are presented here. A steady-state mass balance on X_m in the digester yields:

$$0 = QX_{mi} - QX_{me} + Y_m \frac{K_m S_b X_m}{K_S + S_b} V - K_d X_m V \qquad \text{(Eq. 3.13)}$$

where X_{mi} is the active biomass in the influent (assumed to be negligible), S_b is the soluble biodegradable substrate concentration (assumed to be equal to the effluent BOD_5 concentration), K_S is the biomass half saturation constant and K_m is the Monod maximum substrate utilization rate. To verify that the estimated concentration of biomass (Xm) in the tubular digester was accurate, a steady state mass balance on volatile solids (Xb) was developed. The derivation and verification of the VS mass balance is summarized in Appendix B. A steady-state mass balance on CH_4 in the system yields:

$$0 = -\dot{m} + (1 - Y_m) \frac{K_m S_b X_m}{K_S + S_b} V \qquad \text{(Eq. 3.14)}$$

where ṁ is the daily methane production rate. Note that the daily methane production rate was expressed as g COD/day by adjusting the measured methane production rates (Table 3.2) to standard temperature and pressure (STP) and assuming 4 g COD/g CH_4. The kinetic constants that were applied to this model were obtained from studies treating swine waste at psychrophilic temperatures (Table 3.2).

3.4 Results and Discussion

3.4.1 Overall Tubular Digester Performance

Average performance of the tubular digester over the 5-week field study period is shown in Table 3.3. The TSS and VSS concentrations retrieved from the sludge settleability analysis were 33.2 gTSS/L and 0.40 gVSS/L respectively and sludge had a SVI of 1.51 mL/g TSS. From

these results, three main conclusions can be drawn. First, the results from the settleability analysis indicate that the sludge retrieved from the outlet pipe of the tubular digester had good settling characteristics. Sludge with a SVI below 10 mL/g TSS is considered to have good settling properties, which is characteristic of granular and floccular sludge flocs (Angenent et al, 2002). Having biologically active floccular sludge with a low SVI has shown to improve CH_4 production in an anaerobic sequencing batch reactor treating swine waste (Angenent et al, 2002). Immobilization of methanogens and other anaerobic digestion microorganisms onto inert material and other cells to form granules is beneficial because this promotes diversity in the microbial community. This is due to metabolic interactions within the granule that promote faster substrate utilization. Formation of granules also promotes a more flexible and stable sludge that can withstand temperature and loading rate fluctuations (Bialek et al., 2012). Since good CH_4 production was observed in the tubular digester, the low VSS concentration from the solids settleability test indicated that the microbial composition of the sludge changes diagonally within the tubular digester. This observation was in good agreement with Montteran et al. (2013), who showed diagonal distinction in the microbial composition of granules from an anaerobic baffled reactor treating swine waste. Each diagonal compartment contained separate microbial groups representing each anaerobic digestion phase (fermentation, acidogenesis, acetogenesis and methanogenesis). Due to the design of the tubular digester, it was not feasible to collect samples other than from the outlet pipe. The low VSS to TSS ratio indicated that the sludge taken from the outlet pipe was mainly inert material. This was likely influenced by the MCRT. Longer MCRT allow for greater degradation of the organic matter in the influent. Results from the biological model used to estimate the MCRT are discussed later.

The second main conclusion is that the digester had high VS and BOD_5 removal efficiencies (Table 3.3). The ability for anaerobic digestion to decrease the organic matter concentration in waste is one of its attractive benefits. The VS and BOD_5 removal efficiencies were greater than 75%. This is greater than previously reported values for complete mix digesters treating swine waste at mesophilic and thermophilic temperatures (Hill et al., 1986; Kinyua et al., 2014). These high organic matter removal rates provided further evidence of solids accumulation and formation of a biologically active floccular sludge layer.

The third main conclusion is that high TN and TP percent removals were observed; 83.6 and 91.6% respectively. Since there is no removal mechanism for TN and TP during anaerobic digestion, other processes were investigated to better understand TN and TP removal mechanisms. During anaerobic digestion, nitrogen, phosphorus and metal ions present in the organic matter are released as particulate organic matter is degraded. Swine waste contains high concentrations of magnesium (Mg), TN and TP, which may lead to the formation of struvite ($MgNH_4PO_4.6H_2O$), which can attach to solids within the reactor. Struvite precipitates can be an operational problem because they cause pumps to foul and pipe blockage (Martí et al., 2008). Struvite precipitation during anaerobic digestion is affected by pH, rate of mixing, temperature, solids settleability and ion molar ratios (Martí et al., 2008). To assess whether conditions in the tubular digester favored struvite precipitation, the thermodynamic solubility product of $[Mg^{2+}]$ $[NH_4^+]$ $[PO_4^{3-}]$ was analyzed (Rahaman et al., 2010). Influent Mg concentration was assumed to be 0.21g/L based on an average value from prior studies of swine waste characteristics (see Lin, 2012 for review). A thermodynamic solubility product (log K_{sp}) of $10^{-11.4}$ was estimated, which was within the struvite solubility product of $10^{-9.41} – 10^{-14.1}$ at 20-25°C and a pH of 6.45 – 8.97 (Rahaman et al., 2010). Thermodynamic solubility products within this range indicate struvite

precipitation within the digester was favored. Tubular digesters may require periodical desludging every 5-10 years due to the solids accumulation (Vögeli et al., 2014) and the sludge can be expected to have a high TP concentration.

3.4.2 CFD and Reduced Order Model

Unsteady RANS simulations were conducted for the first day after releasing the tracer for the 15 minute flow-through period and the subsequent 23 hours and 45 minutes batch-reactor period. For the 15 minute flow-through period, there was flow spanning the entire digester from the inlet through the outlet. Following the coordinate system established in Figure 3.1b, speed contours on the middle x-z plane and three y-z planes (at x = 0, 4, and 8 m respectively) for 1, 5, 10 and 15 minutes are shown in Figure 3.2 a-d respectively. From Figure 3.2, it was observed that the flow developed during the first 5 minutes and unsteady flow behavior was observed at the tail of the high-speed jet (red color) after 5 minutes (Figure 3.2 b, c and d). Areas within the digester with short circuiting and dead zones were also observed and are reflected in the tracer concentration contours shown in Figure 3.2 e and f. From Figure 3.2f, it can be seen that the tracer moves quickly through the digester due to the short-circuiting pathway but does not reach the digester outlet by the end of the 15 minute flow-through period. The simulation predicted an outlet tracer concentration less than 1E-30 g KCl/L at the end of the 15 minute flow-through period. This demonstrated that the influent flow had negligible effect on the effluent concentration and serves to validate the second assumption in the reduced order model (Figure 3.3a-d).

During the 23 hours and 45 minutes batch-reactor period there is no flow at the inlet or outlet. The speed contours in the middle x-z plane and three y-z planes (x=0, 4, and 8 m) at 15 and 30 minutes and 1, 3, 6 and 24 hours after batch-mode initiation are shown in Figure 3.4a-f.

From Figure 3.4, it was observed that although there was no flow through the inlet or outlet, flow continued within the digester. The tracer continued to move towards the outlet of the digester due to inertia, resulting in tracer transport during the first hour after the system was closed (Figure 3.4a, b, and c). The flow gradually slowed down due to viscosity, after which the main tracer transport mechanism was by diffusion. The tracer concentration contour had insignificant change after one hour because transport via diffusion is slower than that by advection (Figure 3.4d, e, and f).

At the end of the 23 hours and 45 minutes batch-reactor period, the tracer concentration was evenly distributed in the digester except for the area near the inlet. The effluent tracer concentration from the experimental data was 0.51 g KCl/L, which was 27% higher than the reduced-order model prediction (0.4 g KCl/L). In anaerobic digesters, biogas bubbles generated provide some mixing in the system. However, mixing from biogas bubbles was not considered during the unsteady RANS simulations. If it was considered, the tracer in the digester would be closer to completely mixed. Thus, the CFD simulation for the tracer transport during the 23 hours and 45 minutes batch-reactor period validated the first assumption that the system functions as a CSTR during this period. In addition, the experimental RTD curve (Figure 3.5a) is characteristic of a CSTR mixing mechanism (Levenspiel, 1999). After validating the first and second assumptions through the unsteady RANS simulation, the RTD was predicted using the reduced order model. The model RTD was compared to the experimental data in Figure 3.5a and b. From Figure 3.5b the predicted RTD was in good agreement with the experimental RTD, with a coefficient of determination, denoted R^2, of 0.874. The mean liquid HRT predicted from the reduced-order model was 22.8 days. For the physical model simulations, the field data was not monitored long enough for the mean liquid HRT estimation. In principle the CFD simulations

could have been used to obtain the mean liquid HRT, but this would require running the simulation for much longer than 24 hours, thereby making the simulation very expensive. For computational efficiency the CFD simulations were used to inform the reduced order model which made it less expensive to run for HRT calculations.

3.4.3 Bioprocess Model

Results for range of X_m, X_{me}, MCRT and CH_4 activity values are shown in Table 3.4. An average MCRT of 115 days was determined with a median of 110 days, minimum of 52 days, maximum of 265 days and a standard deviation of 33.6. It should be noted that the modeled X_{me} concentration was within the range of the measured effluent VS concentrations (Table 3.3). A Monte Carlo sensitivity analyses (as described in Section 3.2.4) was carried out to determine which bioprocess kinetic inputs significantly influenced the MCRT and the probability distribution of the MCRT (Figure B.1). The outcomes from the sensitivity analyses are graphically represented in Figure 3.6. From these results, two main conclusions can be drawn. First, the performance and MCRT values obtained from this study were similar to other studies using granular sludge layer reactors to treat wastewater at psychrophilic temperatures. Lo et al. (1994) and Lim and Fox (2011) both reported organic matter removal rates of approximately 90% when treating swine waste in UASB reactors at psychrophilic temperatures. Uemura and Harada (2000) obtained MCRTs ranging from 110 to 117 days and CH_4 activity of 0.03-0.09 g CH_4-COD/g VS $_{added}$-day while treating domestic wastewater in a UASB at 13 to 25°C. These results are similar to the results from the tubular digesters in this study (Table 3.4) and also in agreement with Henze et al. (2008), who recommended an MCRT of 60-140 days while treating domestic wastewater at 15-25°C in a granular sludge layer reactor. Although the recommended

values are for domestic wastewater, they are a useful starting point due to limited literature on MCRT in tubular and floccular sludge layer reactors treating swine waste.

Second, the MCRT value was most sensitive to K_m, K_d and Ks and least sensitive to Y_m (Figure 3.6). K_m represents the maximum rate at which the active biomass can hydrolyze the particulate substrates. An increase in K_m indicates that a shorter MCRT would be needed for the observed biogas production rate. K_d is that rate at which the active biomass undergoes endogenous decay, resulting in a decrease in the active biomass in the digester. K_S is the substrate concentration at which the anaerobic processes occur at half the biomass maximum growth rate and is an indicator of the active biomass affinity for substrate (Metcalf and Eddy, 2003). A decrease in K_S indicates an increase the microbes' affinity for the substrate, thus indicating that a shorter MCRT is sufficient for hydrolysis and conversion the particulate and soluble substrates to CH_4.

In tubular digesters, the physical and biological processes are interconnected. A sufficient mean liquid HRT is needed to allow for the solubilization of complex organic matter in the influent and to provide adequate contact time between the active floccular sludge layer and the dissolved substrates for efficient biogas production. A decrease in HRT reduces the contact time between the active floccular sludge layer and the dissolved substrates resulting in decreased biogas production. From the influent and effluent characteristics, sludge settleability and CH_4 production rates observed in the tubular digester, a mean liquid HRT of 23 days combined with a MCRT of 115 days indicates a robust syntrophic relationship between the physical and biological processes. This good relationship led to low organic matter in the effluent and sufficient biogas production to meet households' energy demands. Although the effluent quality in the tubular digester was good in terms of organic matter, more research is needed to determine

how the physical and biological processes affect other effluent characteristics such as pathogen concentrations and how these models can be used to predict pathogen removal efficiencies. This is an important public health task because effluents from tubular digesters are often used as a soil amendment to reduce cost of purchasing mineral fertilizers.

3.5 Conclusions

The low effluent organic matter concentrations were attributed to the formation of a biologically active floccular sludge layer, resulting in good biogas production to meet households' energy demands. CFD modeling indicated the system functioning as a CSTR. The mean liquid HRT was estimated at 22.8 days. The bioprocess model predicted an average MCRT of 115 days. The model can be a useful tool to design tubular anaerobic digesters but further work is needed to validate the model.

Table 3.1: Tubular digester operating parameters (n=5).

Parameter	Unit	Tubular Digester
Working volume (V)	L_d	12000
Temperature	°C	20.7 ± 0.48
Influent flow (Q)	L/day	543 ± 10.0
OLR	g VS $_{added}$ /L_d-day	0.26 ± 0.10

Table 3.2: Kinetic constants applied in the bioprocess model.

Parameter	Unit	Minimum	Maximum	Reference
K_d	day^{-1}	0.006	0.04	Massé and Droste, 2000
Y_m	g biomass COD/g COD utilized	0.23	0.25	Massé and Droste, 2000
K_m	g COD utilized/ g biomass COD-day	2.00	8.00	Vavilin, 1997
K_S	g/L	0.15	1.81	Vavilin et al., 1997; Massé and Droste, 2000

Table 3.3: Average tubular digester influent and effluent characteristics (n=5).

Parameter	Unit	Influent	Effluent
BOD_5	g/L	5.09 ± 0.30	0.030 ± 0.015
TS	g TS/L	6.35 ± 2.87	0.77 ± 0.25
VS	g VS/L	5.17 ± 2.44	0.58 ± 0.24
TAN	mg NH_4^+-N/L	140 ± 49.0	52.8 ± 4.66
TN	mg N/L	300 ± 23.6	49.3 ± 5.12
TP	mg PO_4/L	402 ± 126	33.9 ± 8.91
pH		7.08 ± 0.62	7.04 ± 0.14
Methane content (S_{CH4})	%	71.0 ± 10.0	
CH_4 production rate (ṁ)	m^3 CH_4/day	2.01 ± 0.87	

Table 3.4: Bioprocess model results and calculated CH_4 activity.

Parameter	Unit	Value
X_m	g COD/L	2.00 - 4.94
X_{me}	g COD/L	0.34 - 1.03
MCRT	day	51 - 259
CH_4 activity	g CH_4-COD/g VS $_{added}$-day	0.056 - 0.086

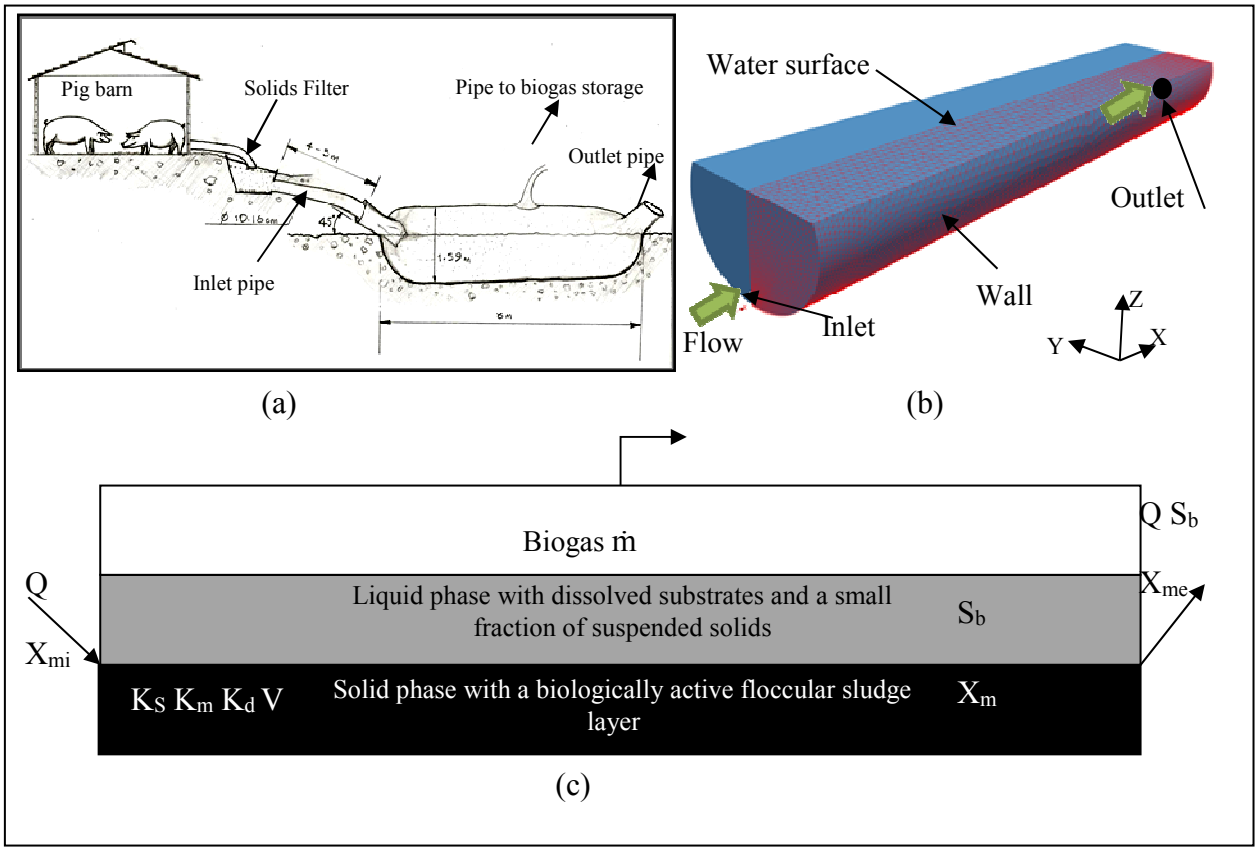


Figure 3.1: (a) Schematic, (b) CFD domain and grid of the tubular digester, (c) tubular digester phases.

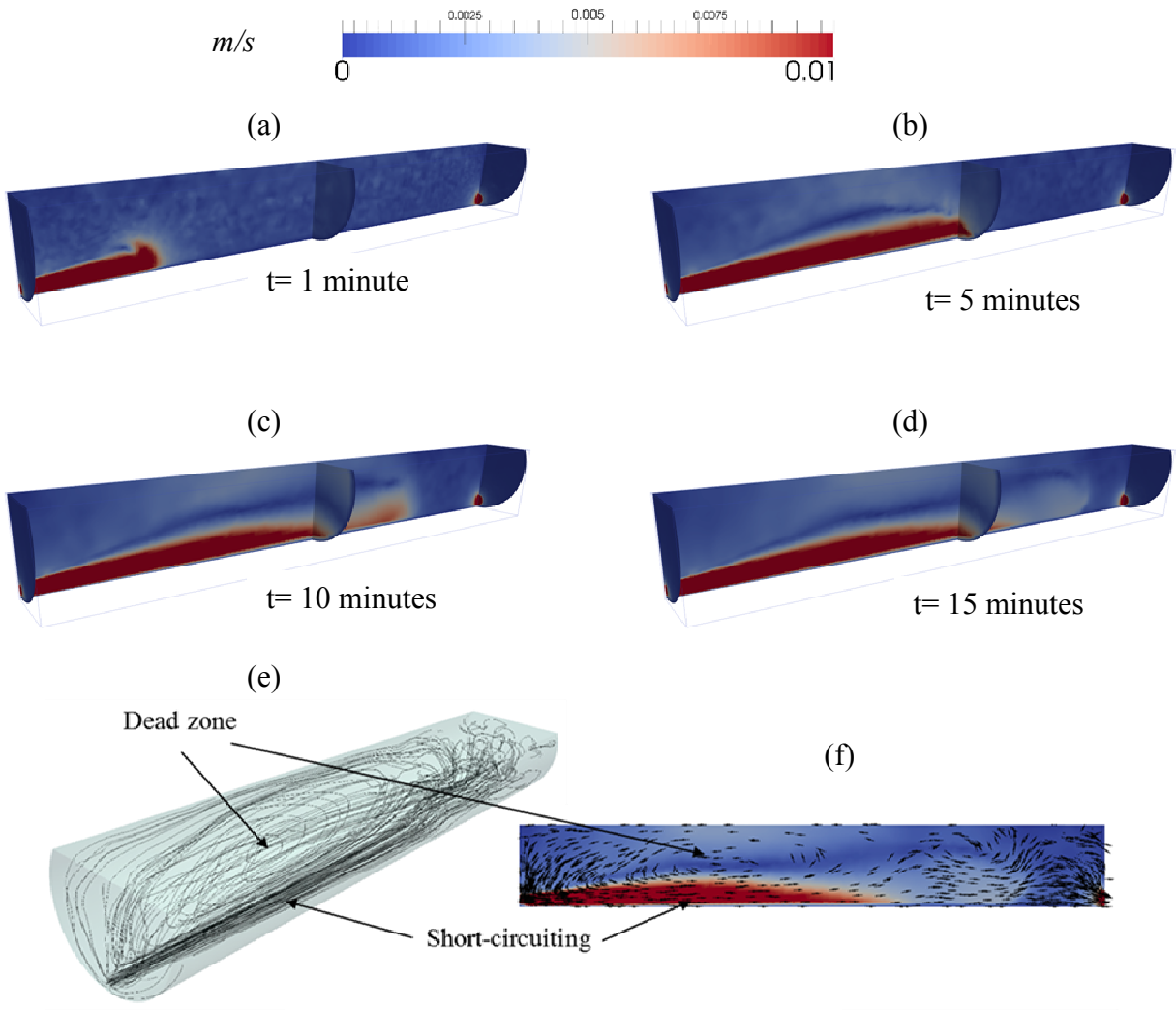


Figure 3.2: (a-d) Contour of speed (flow pattern) at various times on middle x-z plane and three y-z planes (x=0, 4, and 8 m), (e) streamline of flow in the digester (15 minutes) and (f) velocity vector distribution on middle x-z plane (15 minutes)

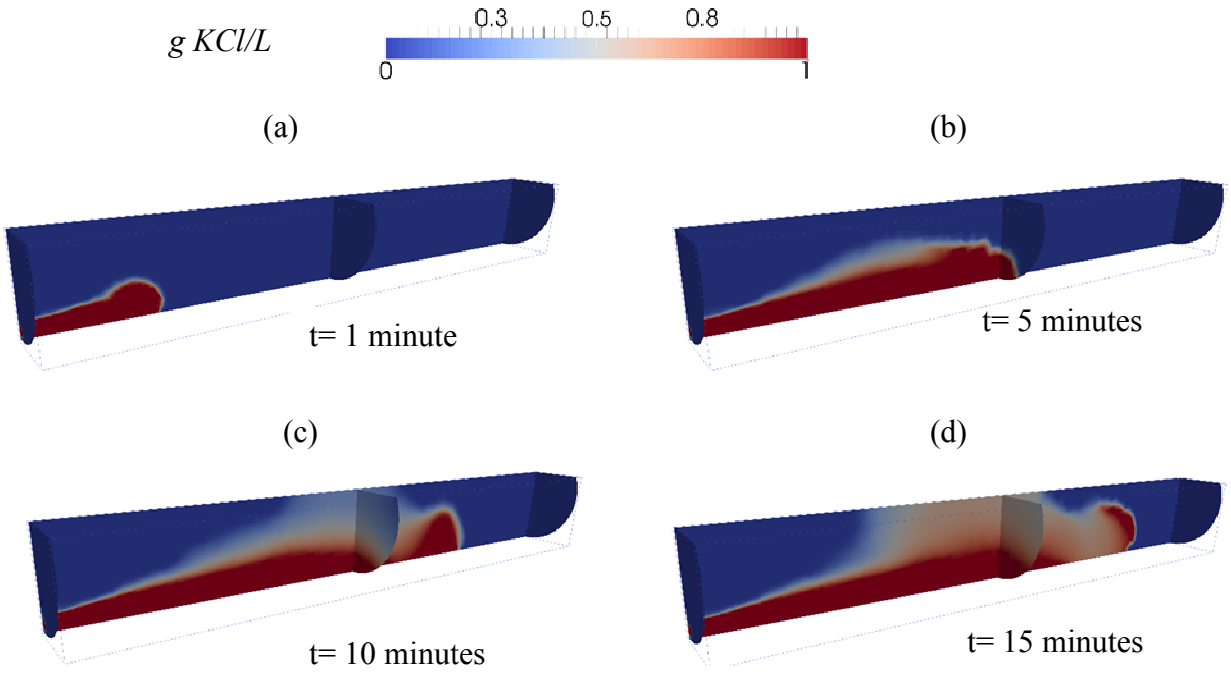


Figure 3.3: Contour of tracer concentration (tracer distribution) at various times on middle x-z plane and three y-z planes (x=0, 4, and 8 m)

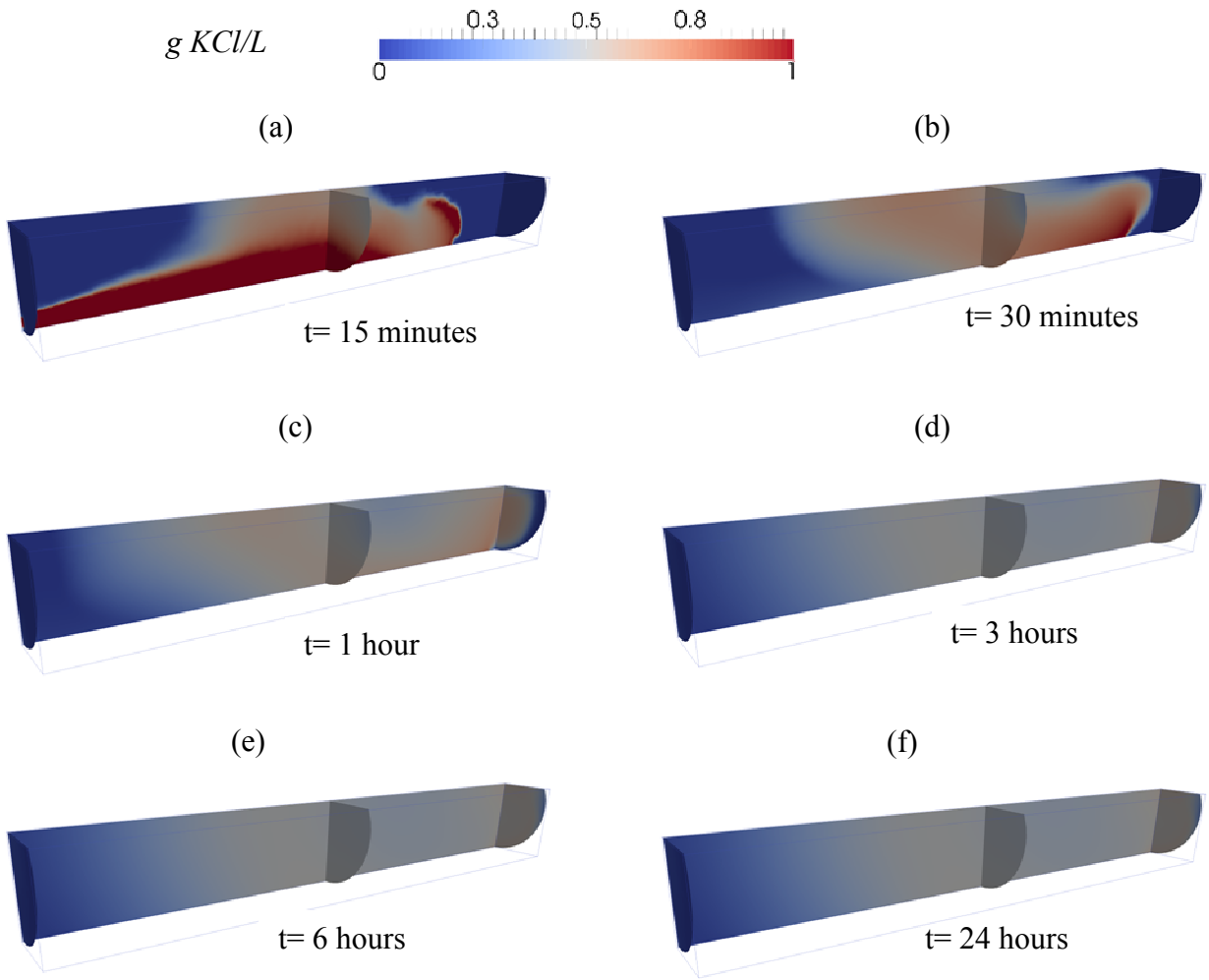


Figure 3.4: Contour of tracer concentration (tracer distribution) at various times on middle x-z plane and three y-z planes (x=0, 4, and 8 m)

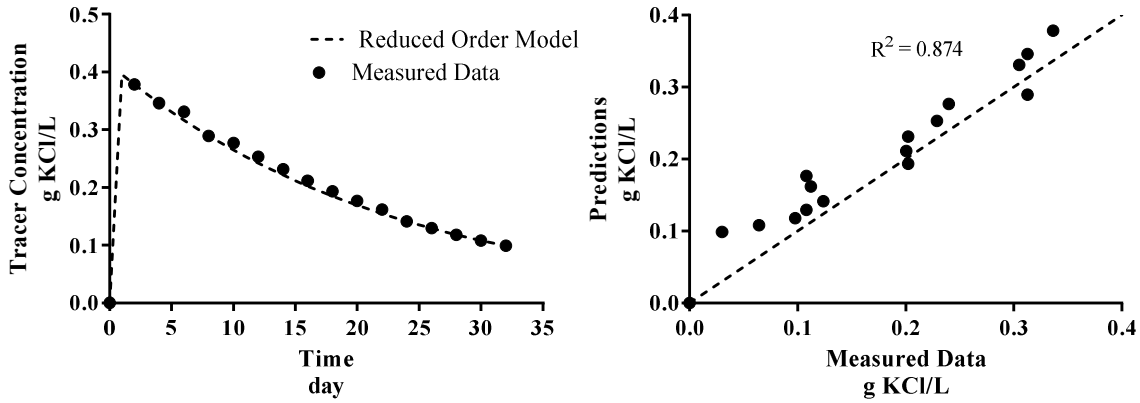


Figure 3.5: Comparison of predicted RTD from measured data (a) concentration versus time; (b) predictions versus measured data.

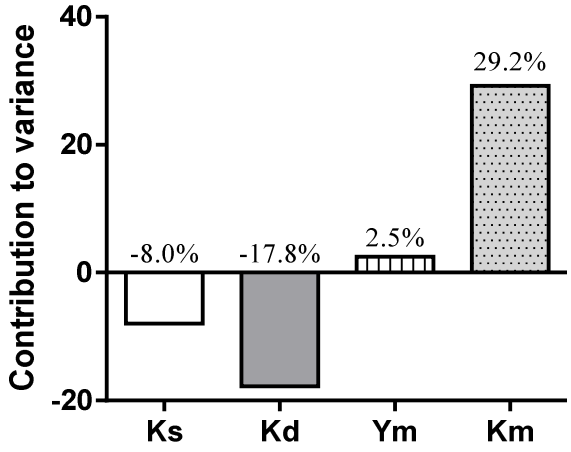


Figure 3.6: MCRT variables sensitivity analysis

Chapter 4:

Fate and Viability of *Cryptosporidium parvum* and *Giardia lamblia* in Tubular Digesters[3]

4.1 Introduction

Tubular digesters are small-scale anaerobic digestion systems that are used for bio-energy production and livestock waste management, primarily in Asia, Africa and Latin America. 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 generate electricity for on-site use (Chapter 2). These systems can be a useful tool to mitigate deforestation when biogas is used as a cooking fuel an alternative to firewood. This also results in decreased public health concerns, especially for women and children who are disproportionally affected by indoor air pollution caused by burning wood or dung for cooking due to their cultural and social roles. Anaerobic digesters can also 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) that can be used as a soil amendment to improve crop yields (Chapter 2).

In many developing countries, poor livestock waste management 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 due to transfer of pathogens from soil to food crops or water bodies through runoff events (Erickson et al., 2006). According to the World

[3] This chapter is adapted from a manuscript under review at *Environmental Science and Technology*. Kinyua, M.N., Trimmer, J., Izurieta, R., Ergas, S.J. "Viability and Fate of *Cryptosporidium parvum* and *Giardia lamblia* in Small-Scale Tubular Anaerobic Digesters in Rural Costa Rica"

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, 2012). *Cryptosporidium parvum* accounts for 23.7% of all worldwide waterborne outbreaks annually, while *Giardia lamblia* infects approximately 2.8 billion people worldwide annually (Dufour et al., 2012). The low infectivity 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 (Erickson et al., 2006). Young, old and immune-compromised individuals are particularly susceptible to disease from infection with these protozoan parasites (Haas et al., 1999).

Treatment of livestock waste using tubular anaerobic digesters has the potential to mitigate exposure to these pathogens. However, there is limited information about the fate of C*ryptosporidium* sp. and *Giardia* sp. (oo)cysts during anaerobic digestion. Several studies have investigated the effect of various environmental factors on the susceptibility of these protozoan parasites to inactivation including: UV radiation (Betancourt and Rose, 2004), moisture content (Van Herk et al., 2004), volatile fatty acids (VFA), temperature, pH and free ammonia. Of particular importance in tubular digesters are VFA, temperature, pH and free ammonia (NH_3). Jenkins et al. (2002) and Olson et al., (1999) reported an increase in (oo)cyst inactivation rate as the temperature increased from 4 to 25°C in soil and water. VFA influence pathogen inactivation rates by acidifying pathogens' cells by decreasing the pH (Medhat and Stafford, 1989). Free ammonia in solution is in equilibrium with ionized ammonium (NH_4^+). Concentrations of free ammonia increase with increasing total ammonia nitrogen (TAN) concentrations, pH and temperature. 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. As pH increases, oocysts wall increases

in permeability, leading to free ammonia easily penetrating into the *Cryptosporidium* sp. oocysts. Once inside the (oo)cysts, the free ammonia disrupts the cell chemistry and structure by denaturation of proteins, making the cells vulnerable to inactivation (Kidd, 2011).

Only a few studies on the fate of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts during anaerobic digestion have been published previously (Chauret et al., 1999; Cote et al., 2006; Kato et al., 2010). Chauret et al. (1999) and Kato et al. (2010) studied the inactivation of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts in mesophilic (36°C) anaerobic digesters. Less than 0.15 log removal/day was observed in both studies while 1.0 log removal/day was observed within one hour at thermophilic temperatures (47-55°C) (Kato et al., 2010). 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 VFA and temperature on inactivation of the protozoa *Entamoeba histolytica* (a protozoan 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 1.5-3.0 g acetate/L. Maximum log removals were 0.5 and 4.0 at 37°C and 55°C respectively. It should be noted that although high VFA concentrations have been shown to cause inactivation of (oo)cysts, VFA concentrations greater than 600 mg acetate/L have been shown to be inhibitory to methanogenesis (Wang et al., 1999). Cote et al. (2006) treated swine waste in a 20°C anaerobic sequencing batch reactor with a 20 day SRT. 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 0.40-23.2 g/L.

Tubular digesters are being actively promoted as a waste management and bio-energy production alternative in developing countries where there is a high prevalence of giardiasis and cryptosporidiosis. It is therefore critical to understand the effect of environmental and operating conditions in tubular digesters on the viability and fate of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts. This study investigated the environmental conditions in tubular digesters treating swine waste in the Monteverde region of Costa Rica. A kinetic study was performed in the laboratory to investigate the kinetics of (oo)cysts inactivation under similar environmental conditions (pH, temperature and TAN) as those observed in the field. Distribution of (oo)cysts in the solid and liquid phases was also assessed. The results were combined with mathematical models of tubular anaerobic digesters to estimate the concentration of viable (oo)cysts in the tubular digester effluents. These results can be used to provide guidance on proper design and operation of tubular digesters to minimize exposure to zoonotic pathogens.

4.2 Materials and Methods

4.2.1 Site Description and Tubular Digesters

San Luis de Monteverde (N 10' 16.973" W 84' 47.882") is a rural mountain community located in the northwest region of Costa Rica. San Luis de Monteverde has a population of approximately 500 people. The main economic activities in San Luis de Monteverde are small-scale farming and eco-tourism. Installation of tubular digesters in the San Luis was promoted by local educational and non-governmental organizations for energy production and to reduce livestock waste pollution of the watershed. Farmers in San Luis de Monteverde use Taiwanese-model tubular anaerobic digesters to treat livestock waste and produce biogas for cooking. These systems typically treat swine waste from 4-10 pigs and provide fuel for approximately 4 hours of cooking per day, which can meet the needs of an average family of 5 people. Operating

parameters for four tubular digesters treating livestock waste in San Luis de Monteverde that were studied in this research are summarized in Table 4.1. In addition, all of the farmers who participated in this study were interviewed to gain insight into their livestock and tubular digester management practices. Interviews were conducted in Spanish at the field sites. A list of interview questions and answers is provided in Appendix C.

4.2.2 Inactivation Kinetics

Three bench-scale reactors were set-up at the University of South Florida (USF) Environmental Engineering laboratory (Tampa, FL), to determine the inactivation kinetics of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts under similar environmental conditions as measured in the field-scale tubular digesters treating swine waste. The bench-scale reactors were set up in 1.0 L glass bottles with a working volume of 0.9 L. Reactor 1 was a bench-scale anaerobic digester and is described in Section 4.2.3. Reactor 2 contained phosphate-buffered saline (PBS) solution with added acetate (300 mg/L) and 80 mL of a 3 g NH_4Cl/L stock solution, resulting in a TAN concentration of 240 mg NH_4^+-N /L. PBS solution contained 0.0027 M KCl and 0.137 M NaCl at a pH of 7.4. Reactor 3 was a control reactor and contained only PBS solution. The purpose for Reactor 2 was to determine if VFA and TAN were the main contributors to (oo)cysts inactivation during anaerobic digestion. Each of the three reactors contained two 10 mL Float-A-Lyzers (Spectrum Labs, Houston, TX). Float-A-Lyzers are a dialysis device with a synthetic membrane that allows solutes, such as TAN and VFA, to diffuse from areas of high concentration to low concentration across a semi-permeable membrane until equilibrium is reached. The porous membrane allowed solutes with a molecular weight of up to 1000 kg/mole to pass through while retaining the 4 by 6µm and 12 by 5µm *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts, respectively. Each Float-A-Lyzer initially contained 2.5 x

10^4 viable (oo)cysts/mL of *Cryptosporidium parvum* and *Giardia lamblia* suspended in PBS buffer solution. Viable *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts were obtained from Waterborne Inc. (New Orleans, LA). Reactors were purged with nitrogen for 1 minute each time they were opened for sample collection to maintain anaerobic conditions. Reactors were incubated at 21°C in a Hach Model 205 compact incubator (Loveland, CO) for 24 days. Samples were analyzed for (oo)cysts viability as described below. The inactivation kinetics study was carried out in duplicate, with each study period for 24 days. This study period was based on a physical model and tracer study that showed a liquid hydraulic retention time (HRT) of approximately 23 days for a tubular digester treating livestock waste in Monteverde (Chapter 3).

4.2.3 Bench-Scale Anaerobic Digester

The bench-scale anaerobic digester (Reactor 1) was initially inoculated with seed sludge from anaerobic digesters treating swine waste at the USF Environmental Engineering laboratory (Manser et al., 2015). Influent was prepared by blending swine waste with tap water to achieve a target volatile solids (VS) concentration of 20 g/L, similar to that found in the field tubular digester's influent. Tap water was used for this study because farmers in San Luis de Monteverde used chlorinated tap water to prepare influent for the tubular digesters. Swine waste was collected weekly from Twenty Four Rivers Farm in Plant City, FL. Reactor 1 was operated at a 24 day HRT. This digester was managed in a semi-continuous mode (fed once a week) and remained unmixed.

4.2.4 Batch (Oo)cysts Adsorption Study

In Chapter 3 it was observed that solids accumulation and settling in tubular digesters lead to the formation of a biological active floccular sludge layer with a long solids retention time (SRT). To account for this settling and accumulation of solids in the tubular digesters,

adsorption of (oo)cysts onto the solids was examined to determine the distribution of (oo)cysts in the solid and liquid phases. Microcosms were set-up in 50 mL bottles. 50 mL of effluent from Reactor 1 was collected and centrifuged for 10 minutes at 3500 rpm using a Thermo scientific CL2 centrifuge (West Palm Beach, FL). 15 g of solids from the dewatered sludge was placed into a 50 mL bottle. To this, 30 mL of supernatant was added. This mixture was spiked with 10^6 non-viable Cryptosporidium parvum and Giardia lamblia. Microcosms were initially purged with nitrogen gas for 1 minute, thoroughly mixed for 15 minutes and incubated at 21°C in a Gyromax 727 orbital shaker incubator (Lafayette, CA) for 24 hours. Mixing was turned off to allow for settling of solids to simulate conditions in the field tubular digesters. Liquid and solid samples were analyzed for (oo)cysts concentration as described below. The batch (oo)cysts adsorption study was carried out in triplicate, each study period was 24 hours.

4.2.5 *Cryptosporidium parvum* and *Giardia lamblia* Detection and Enumeration

For the field study, (oo)cysts presence in raw swine manure from farms using tubular digesters were analyzed using a commercially available rapid immune-assay, ImmunoCard STAT! *Crypto/Giardia* from Meridian Bioscience Inc. (Cincinnati, Ohio). Since the ImmunoCard STAT! *Crypto/Giardia* assay is designed for use in human fecal samples, a sensitivity analysis for swine manure was performed. Three grams of swine manure that had tested negative for the presence of (oo)cysts were spiked with 6000 non-viable (oo)cysts and assessed according to manufacturer's instructions. It was noted that for swine manure with spiked (oo)cysts, 20 minutes was required to reveal a positive *Cryptosporidium parvum* and *Giardia lamblia* test result compared with the 10 minutes recommended by the manufacturer. This may have been due to higher solids content in livestock feces compared with human waste.

For the batch adsorption study, liquid and solid phase samples were extracted and processed using the $ZnSO_4$ density gradient method described in Kuczynka and Shelton (1999). A direct immunofluorescence protocol from Waterborne Inc. (New Orleans, LA) using fluorescein isothiocyanate (FITC) labeled mouse monoclonal antibody reagent, was used for *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts detection. Three aliquots were withdrawn from each phase and the concentration of (oo)cysts/g TS or mL in each phase was calculated as the average number of (oo)cysts counted per g TS or mL (mass or volume of sample analyzed) from each of the three aliquots divided by recovery rate. The recovery rates in both phases were assumed to be the same. The fraction of (oo)cysts in the liquid phase was calculated as: Total number of (oo)cysts enumerated in the liquid phase x 30 mL/ Total number of (oo)cysts enumerated in both phases. The distribution coefficient (K_d), which represents the partitioning of (oo)cysts in the solid and liquid phases ((oo)cysts/L/ (oo)cysts/g TS) was calculated as the concentration of (oo)cysts in the solid phase divided by the concentration of (oo)cysts in the liquid phase.

For the inactivation study, the viability of (oo)cysts during the inactivation kinetic study was determined through an exclusion/inclusion dye permeability assay described in Jenkins et al. (1997). Aliquots were stained with 4', 6-diamidino-2-phenylindole (DAPI), propidium iodide (PI) and *Cryptosporidium parvum* and *Giardia lamblia* specific FITC labeled mouse monoclonal antibodies. Viable (oo)cysts and reagents were purchased from Waterborne, Inc. (New Orleans, LA). The percent of viable (oo)cysts was calculated as those with blue DAPI staining but lacked the red PI staining: Percent nonviable (%) = 100 x Number with PI stain/(Total number - Empty (oo)cysts). The percent of viable (oo)cysts was calculated as the average number of (oo)cysts counted in 2 wells.

For microscopy, samples were examined using a Nikon Eclipse E200-LED (Melville, NY) equipped with a QBC ParaLens Advance LED fluorescent microscope attachment (Port Matilda, PA) with an excitation wavelength of 410-480 nm and an emission wavelength of above 520 nm. For DAPI, a Leica DM 2000 fluorescent microscope (Allendale, NJ) with a UV filter (excitation wavelength of 340-380nm) was used. All samples were enumerated at 100x magnification using oil immersion lenses.

4.2.6 Analytical Methods

During the 5-week field study, all laboratory analyses were carried out at the University of Georgia Costa Rica campus (San Luis, Puntarenas, Costa Rica). Influent and effluent samples were collected and analyzed weekly from all four digesters for 5 weeks for conductivity, pH, BOD_5, VS, TS, TN, TAN, and TP concentrations and CH_4 content as described in Chapter 3. *E.coli* (indicator organism) influent and effluent concentrations were measured using 3M Petrifilm *E. coli*/Coliform Count Plates (St. Paul, Minnesota). COD and VFA measurements could not be carried out during the field-study because there were no facilities to dispose of the hazardous waste generated. Biogas production was only measured in Digester 1 using a wet tip gas meter (Wayne, PA). Floccular sludge from Digester 3 and 4 was also analyzed for SVI, TSS and VSS as described in Chapter 3. Floccular sludge characteristics from Digester 3 and 4 are summarized in Appendix C. During the 7-week bench-scale reactor and inactivation study, all laboratory analyses were carried out at USF Environmental Engineering laboratory (Tampa, FL). Influent and effluent samples were collected and analyzed weekly from the bench-scale anaerobic reactor for 7 weeks. Centrate from the bench-scale anaerobic reactor was obtained by centrifuging influent and effluent samples for 10 minutes at 3500 rpm using a Thermo scientific CL2 centrifuge (West Palm Beach, FL). Conductivity, pH, BOD_5, VS, TS, TN, TAN, TP and *E.*

coli concentrations and CH_4 content were analyzed as described above. *Standard Methods* (APHA, 2012) were used to measure soluble COD (5200B). Hach high range TNT test kits and a Hach DR 2800 spectrophotometer (Loveland, CO) were used to measure VFA (TNT 872). Biogas volume from Reactor 1 was collected in a 0.75 L SamplePro FlexFilm Air Sample Bag from SKC Inc (Eighty Four, PA) and measured by water displacement. Method detection limits (MDL) were (mg/L): COD (30) and VFA (50).

4.2.7 Inactivation and Effluent Concentration Modeling

(Oo)cysts inactivation model for first order kinetics have been used (Jenkins et al., 1998) to model the inactivation rate of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts as:

$$C_L = C_V e^{(-kt)} \qquad \text{(Eq. 4.1)}$$

where C_L is the percent viable (oo)cysts measured at time t (%),C_V is the percent of viable (oo)cysts at time t = 0 days (%), k is the inactivation rate constant (day^{-1}) and t is time (day). Even though Reactor 1 was intermittently fed (fed once a week), the (oo)cysts were held in the Float-A-Lyzers devices, and therefore the system was modeled as a batch reactor to estimate the (oo)cysts inactivation rates.

In Chapter 3, physical and biological process models were developed and calibrated to understand the transformation and transport mechanisms in tubular digesters. The biological process model developed, indicated that solids accumulation and settling in the tubular digester led to the formation of a biologically active floccular sludge layer, with a SRT of more than 100 days. A tracer study was carried out on Digester 1 and incorporated into a computational fluid dynamics (CFD) model to understand hydrodynamic mechanisms in the liquid phase. The liquid phase of Digester 1 behaved similar to a completely stirred tank reactor (CSTR). By applying the CSTR model to all of the digesters studied, HRTs ranging from 23 to 180 days were

calculated, as shown in Table 4.1. An overall mass balance on the number of (oo)cysts in a tubular digester was written as:

$$\frac{dN}{dt} = QC_{0(t)} - QC_t - kN \qquad \text{(Eq. 4.2)}$$

where N is the total number of (oo)cysts in the digester, C_t is the (oo)cysts concentration in the liquid phase of the digester ((oo)cysts/L), $C_{0\,(t)}$ is the concentration of (oo)cysts in the influent ((oo)cysts/L), V is the tubular digester working volume (L), Q is the flow rate (L/day) and k is the inactivation rate constant (day^{-1}). Note that due to the low solids concentrations observed in the digester's effluents, the concentration of (oo)cysts in the effluent was assumed to be the same as the concentration of (oo)cysts in the liquid phase of the digester. Due to solids accumulation and settling in the tubular digesters, adsorption of (oo)cysts to the solid phase was also considered as a concentration-altering mechanism in addition to inactivation. Therefore, the total number of (oo)cysts in the digester can be described by:

$$N = N_{(oo)cysts\ in\ liquid} + N_{(oo)cysts\ in\ solid} \qquad \text{(Eq. 4.3)}$$

where $N_{(oo)cysts\ in\ liquid} = C_t\,V$ and $N_{(oo)cysts\ in\ solid} = STS_F V$. S is the number of (oo)cysts adsorbed on the solids per g TS of the floccular sludge layer ((oo)cysts/g TS) and was considered to be a linear function of the concentration of (oo)cysts in the liquid:

$$S = K_d C_t \qquad \text{(Eq. 4.4)}$$

where K_d is the distribution coefficient, representing partitioning of (oo)cysts between the solid and liquid phases (L/ g TS). Values for K_d were determined from laboratory studies, as described in Section 4.2.4. TS_F is the total solids concentration in the digesters' floccular sludge layer. Values for TS_F were determined for each digester as the TSS concentrations (values are reported in Chapter 3 and Appendix C). Substitution into Equation 4.3 yields the following expression for the number of (oo)cysts in the digester:

$$N = C_t\, V(1 + K_d TS_F) \qquad \text{(Eq. 4.5)}$$

Equation 4.2 can then be re-written as:

$$\frac{dC_t\, V(1 + K_d TS_F)}{dt} = QC_{0(t)} - QC_t - kC_t\, V(1 + K_d TS_F) \qquad \text{(Eq. 4.6)}$$

Assuming the term $V(1 + K_d TS_F)$ is constant over time, Equation 4.6 can be expressed as:

$$\frac{dC_t}{dt} = \frac{1}{\tau R} C_{0(t)} - \frac{1}{\tau R} C_t - kC_t \qquad \text{(Eq. 4.7)}$$

where τ is equal to V/Q (the HRT) and the retardation factor, R is equal to $(1 + K_d TS_F)$.

Pigs have been shown to shed viable (oo)cysts for 10-28 days following a second-order polynomial distribution (Nydam et al., 2001; Guselle et al., 2003). To accommodate this change of influent (oo)cysts concentration with time and to simplify the tubular digester (oo)cysts model, a finite difference approximation was used for the differential term in Equation 4.7:

$$\frac{dC_t}{dt} \cong \frac{C_{t+\Delta t} - C_t}{\Delta t} \qquad \text{(Eq. 4.8)}$$

The overall mass balance on the (oo)cysts in the tubular digester was re-written as:

$$\frac{C_{t+\Delta t} - C_t}{\Delta t} = \frac{1}{\tau R} C_{0(t)} - \frac{1}{\tau R} C_t - kC_t \qquad \text{(Eq. 4.9)}$$

The concentration of (oo)cysts in the tubular digester effluent was expressed as:

$$C_{t+\Delta t} = C_t \left(1 - \frac{\Delta t}{\tau R} - k\Delta t\right) + \frac{\Delta t}{\tau R} C_{0(t)} \qquad \text{(Eq. 4.10)}$$

Due to the sporadic shedding pattern of pigs, the initial condition was for the period of time before pigs shed (oo)cysts was $t = 0\ C_t = 0$. The influent concentration was assumed to follow the following:

$$\begin{gathered} t < 1\,d\ \ C_0 = 0 \\ 1 \geq t \geq 19\,d\ \ C_0 = at^2 + bt + c \\ t > 19\,d\ \ C_0 = 0 \end{gathered} \qquad \text{(Eq. 4.11)}$$

The first and third equations describe the periods before and after the period of infection. During the period of time when pigs are shedding (oo)cysts the influent concentration assumed to follow a second order polynomial based on prior studies (Nydam et al., 2001; Yui et al., 2014). A maximum of 19 days of shedding has been reported for pigs less than 6 months old. The concentration of (oo)cysts at day 1 of shedding was assumed to be <1000/g TS and the concentration of (oo)cysts at day 19 was <100/g TS. It was also assumed that maximum concentration of (oo)cysts shed by the pigs peaked at day 9 (Nydam et al., 2001; Yui et al., 2014). Values for $C_{0\,(t)}$ during the shedding period were multiplied by the influent TS concentration from each digester to convert the units from (oo)cysts/ g TS to (oo)cysts /L. The polynomial extrapolation of data for pigs (oo)cysts shedding for 19 days and expression of $C_{0\,(t)} = f\,(t)$ are provided in Appendix C as Figure C.1.

4.2.8 Data Analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) test using GraphPad Prism version 6.0 for Windows 8 (San Diego, California). *p* values less than 0.05 were considered statistically significant and values less than 0.0001 were considered extremely significant. Nonlinear regression analysis of one-phase exponential decay of the observed inactivation data was performed to determine the inactivation rate constant (k) within a 95% confidence interval using GraphPad Prism. Oracle Crystal Ball (Redwood City, CA) was used for Monte Carlo simulation. A Monte Carlo uncertainty analysis was performed by running 1,000 trials with selected parameter values within the range from experimental data to determine which model inputs affected the (oo)cysts effluent concentration. The inactivation rate constant and retardation factor were considered uncertain inputs with uniform distribution. Crystal Ball calculates the sensitivity by ranking what inputs significantly correlate with the output while the

simulation is running. If an input and output have a high correlation percent, it means that the input will have a significant impact on the output. The percentage is the rank of correlations normalized to 100%. A positive contribution means that the input will increase the output and a negative contribution means the input will decrease the output.

4.3 Results and Discussion

4.3.1 Field and Bench-Scale Anaerobic Digester Performance

Average influent and effluent characteristics from Reactor 1, which was operated for a period of 7 weeks, and the field study of four tubular digesters conducted over 5 weeks in Costa Rica are shown in Table 4.3. Influent soluble COD and VFA concentrations in Reactor 1 were 1.46 ± 0.28 g COD/L and 306 ± 66.3 mg COD/L respectively. Effluent soluble COD and VFA concentrations in Reactor 1 were 1.08 ± 0.16 g COD/L and 310 ± 44.1 mg COD/L respectively. These results indicate three important points.

First, with regard to the reduction of organic matter and solids, on average, the four tubular digesters exhibited BOD_5 and VS removal efficiencies of approximately 98% and 87%, respectively, while removal efficiencies in Reactor 1 were significantly lower. Process modeling in Chapter 3 revealed that solids settle and accumulate in these tubular digesters over time, resulting in the formation of a biologically active sludge layer with a SRT that is higher than the mean liquid HRT. Due to the combined effects of the accumulation of solids and the conversion of organic substrate to biogas, tubular digesters can achieve higher removal of solids and associated organic matter than other types of digesters. Lansing et al. (2010) found that reported VS removal efficiencies among completely-mixed and non-mixed baffled digesters averaged approximately 50%, similar to the removal observed in this study's Reactor 1. The reduction of

TP observed in the tubular digesters also provides evidence for solids accumulation, as a significant fraction of TP is associated with the solids (Lansing et al., 2010; Chapter 3).

The second important point was that the removal of *E. coli* was higher in the tubular digesters than in Reactor 1. The tubular digesters exhibited *E. coli* reductions of between 1.0 and 1.8 $\log_{10}$ while a 0.4 $\log_{10}$ removal was observed in Reactor 1. In other studies investigating tubular digesters in Costa Rica, *E. coli* removal efficiencies similar to those observed in this study were reported (Lansing et al., 2010). Solids settling and accumulation may play a significant role in reducing effluent *E. coli* concentrations. *E. coli* has been shown to be closely associated with the solids fraction (Lansing et al., 2010), causing them to remain in the digester for a period longer than the HRT. This extended period inside the tubular digester could contribute to a greater level of inactivation.

Lastly, CH_4 content of the biogas was similar in Reactor 1 and in the tubular digesters. In the tubular digesters, CH_4 content ranged from 60% to 71%, while the CH_4 yield measured in Digester 1 averaged 0.204 L CH_4 / g VS. The CH_4 content and CH_4 yield of Reactor 1 averaged 65% and 0.161 L CH_4 / g VS, respectively. These results are similar to those reported in previous studies. Tubular digesters in Peru, operated between 20°C and 25°C, exhibited CH_4 contents between 60% and 67%, and the average CH_4 yield was calculated to be 0.22 L CH_4 / g VS (Ferrer et al., 2011). Lansing et al. (2010) co-digesting swine waste with used cooking grease in tubular digesters at an average temperature of 25.5°C, reported similar CH_4 content, between 63% and 70%, with a CH_4 yield of 0.31 L CH_4 / g VS, which was higher than this study. The greater level of biogas production and CH_4 yield observed by Lansing et al. (2010) was likely attributable to the fact that swine manure was being co-digested with used cooking grease, a carbon-rich material known to have a positive effect on biogas production (Cirne et al., 2007).

4.3.2 Inactivation of *Cryptosporidium parvum* and *Giardia lamblia*

Inactivation kinetics for *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts in the three bench-scale reactors are shown in Figure 4.1. A fit of the inactivation model to the observed data of Reactor 1 is also illustrated in Figure 4.1. The log-removal rates for each of the 3 reactors are shown in Table 4.2. The inactivation rate constants for *Cryptosporidium parvum* and *Giardia lamblia* were 0.056 ± 0.013 and 0.726 ± 0.064 day^{-1}, respectively, in Reactor 1. From these results, two conclusions were drawn.

First, there were no significant differences between log removal in Reactors 1 and 2 for either *Giardia lamblia* (p= 0.78) or *Cryptosporidium parvum* (p=0.29) (Table 4.2), however, inactivation rates were significantly lower in Reactor 3 (PBS control). These results indicate that at 20°C and neutral pH, the presence of TAN and VFA significantly increased the inactivation rate of the (oo)cysts when compared with the control reactor. Several studies have demonstrated that exposing (oo)cysts to free ammonia can increase the rate of inactivation (Jenkins et al., 1998; Reinoso et al., 2007). Free ammonia that is highly soluble penetrates the (oo)cysts walls denaturing cell proteins by disrupting the intramolecular hydrogen bonds (Kidd, 2011). This process causes inactivation of the parasites. Reinoso et al. (2007) observed a 0.39 and 0.83 log removals when they exposed *Cryptosporidium parvum* oocysts to 5 and 50 mg NH_3/L respectively for 4 days at 25°C. In this study, the free ammonia concentrations in Reactors 1 and 2 were below 0.005 mg NH_3/L. Although the free ammonia concentration was significantly lower than in previous studies, the longer exposure time (24 days) may have increased the rate of inactivation. In addition, the presence of VFA may have also contributed to the inactivation. The presence of short chain VFA such as acetate and propionate, commonly present during anaerobic digestion, have been shown to cause inactivation of bacteria such as *Salmonella* sp. and parasites

such as *Ascaris* sp. (Butkus et al., 2011; Chen et al., 2012). This inactivation is attributed to diffusion of VFA molecules across the pathogen membrane causing acidification of the cell (Butkus et al., 2011). The change of pH in the cell also causes protein denaturation and DNA damage (Kidd, 2011). Although VFAs could not be measured during the field study, prior studies of tubular digesters treating livestock waste have reported VFA concentrations of 300-600 mg/L which were similar to values in Reactors 1 and 2 (Usack et al., 2014). Inactivation due to TAN and VFA were not investigated independently since the objective of this study was to create environmental conditions similar to those observed in the field tubular digesters.

The second conclusion was that the rate of *Giardia lamblia* inactivation was significantly higher than the rate of *Cryptosporidium parvum* inactivation. This difference in inactivation is most likely due to the physicochemical characteristics of the *Giardia lamblia* cysts and the *Cryptosporidium parvum* oocysts. A *Giardia lamblia* cyst wall is comprised of a 300-500 nm thick wall with 2 layers (Dumétre et al., 2011). The outer layer is made from a matrix of cysteine-rich proteins and the inner layer is a membranous wall. A *Cryptosporidium parvum* oocyst wall is 50-80 nm thick with 3 layers; an outer layer that is comprised of a glucose-rich glycocalyx carbohydrate membrane, a central layer formed from lipids and proteins and an inner wall made from a matrix of lectin proteins (Dumétre et al., 2011). Although the *Giardia lamblia* cyst wall is thicker, the multilayer composition and rigidity of the *Cryptosporidium parvum* oocyst wall layers adds complexity (Samuelson et al., 2013). This complexity may lead to slower inactivation of *Cryptosporidium* sp. compared to *Giardia* sp. This trend of *Giardia* sp. inactivation being greater than *Cryptosporidium* sp. under the same environmental conditions has also been noted in other studies (Olson et al., 1999). Based on the first order decay kinetics model used to estimate the inactivation rate constant, exposing *Cryptosporidium parvum* oocysts

to the same environmental conditions for 65 days would be required to achieve a similar log removal as that observed for *Giardia lamblia* in Reactor 1.

4.3.3 Phase Distribution

Results from the phase distribution study for *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts are shown in Figure 4.2. The concentration of (oo)cysts in the liquid phase was $1.49x10^7 \pm 1.25x10^5$ and $1.33x10^7 \pm 7.50x10^5$ (oo)cysts/L of *Cryptosporidium parvum* and *Giardia lamblia* respectively. The concentration of (oo)cysts in the solid phase was 35,125 ± 125 and 36,750 ± 750 (oo)cysts/ g TS of *Cryptosporidium parvum* and *Giardia lamblia* respectively. Distribution coefficients were 0.0024 ± 0.00066 L/g TS and 0.0028 ± 0.00090 L/g TS for *Cryptosporidium parvum* and *Giardia lamblia* respectively. These results indicate that both parasites have a high attraction to the biosolids during anaerobic digestion. Although the difference in the fraction of both parasites in either of the phases was not significantly different (p=0.08), the interactions between the (oo)cysts and solids, leading to their adhesion to solids are different. Zeta potential and ionic strength of the solution that the (oo)cysts are in affects the surface charge and repulsion or attraction of (oo)cysts to solids. Zeta potential, which refers to the electrostatic repulsion force between particles with the same charge, decreases with increasing ionic strength (Hsu and Huang, 2002). As the zeta potential decreases, the (oo)cysts become more negatively charged at neutral pH. This makes the (oo)cysts behave like colloidal particles adhering to the solids (Hsu and Huang, 2002). Since *Cryptosporidium* sp. oocysts tend to be more negatively charged than *Giardia* sp. cysts, the surface charge on *Cryptosporidium* sp. oocysts is the main contributing factor to their adsorption to solids. It was observed that conductivity was greater in the effluent compared to the influent in Reactor 1 and in the four tubular digesters (Table 4.3). Based on this, it is likely that the *Cryptosporidium* sp. oocysts in

the tubular digester would be associated with the solid phase. Although, the surface charge of *Giardia* sp. is also negative, the surface hydrophobic nature of these cysts plays a more significant role in their adhesion to solids (Dai et al., 2004). This is due to the physicochemical characteristics of *Giardia* sp. cysts' outer wall. As discussed previously, the outer wall of *Giardia* sp. cysts is composed of cysteine-rich proteins that are hydrophobic in nature (Dai et al., 2004; Dumétre et al., 2011). It should noted that a preliminary study on (oo)cysts phase distribution over a longer period of time (21 days) was carried out to determine if the (oo)cysts phase distribution changed with time. The preliminary results are shown in Appendix C and indicate the (oo)cysts did re-suspended in the liquid phase over time. Therefore further research is needed to determine the mechanisms affecting phase distribution over longer periods of time.

4.3.4 Concentration of (Oo)cysts in Tubular Digester Effluent

During the field-study period, feces from 22 pigs (8 suckling pigs, 11 growing pigs aged 1 to 6 months, 2 sows and 1 boar) were sampled for 2 weeks for the presence of *Cryptosporidium parvum* or *Giardia lamblia*. None of the feces samples produced positive results for either *Cryptosporidium parvum* or *Giardia lamblia*. Although the pigs were not shedding viable (oo)cysts at the time of this study, *Cryptosporidium* sp. and *Giardia* sp. is a public health concern for the Monteverde community (Peña, personal communication, July, 2014). Since pigs less than 6 months old have been shown to shed more *Cryptosporidium* sp. oocysts compared to older pigs (Yui et al., 2014) and most of the farmers in San Luis de Monteverde using tubular digesters generally have pigs less than 4 months old, the maximum concentration of (oo)cysts in the influent was taken from the literature as $C_{0\,(t)}$ = 90,000 oocysts/g TS and 54,000 cysts/g TS (Yui et al., 2014). These values represent the average concentration of (oo)cysts measured from 334 pigs less than a month to 6 months old(Yui et al.,

2014). Values for $C_{0\,(t)}$ were multiplied by the influent TS concentration from each digester to convert the units from (oo)cysts/ g TS to (oo)cysts /L. Effluent (oo)cysts concentrations were estimated based on the assumptions described earlier.

Modeled effluent concentrations of viable *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts for the Digester 1, 3 and 4 are shown in Figure 4.3. Digester 2 was not included in the model because TS_F concentrations were not evaluated for this digester. The model results indicate that more than 3 log removal was predicted in all the digesters by 75 days after an outbreak of infection for *Cryptosporidium parvum*. More than a 4 log removal was predicted in the 3 digesters by day 20 for *Giardia lamblia*. This significant difference in log removal between the two parasites can be attributed to the differences in the inactivation rates (Section 4.3.2). A Monte Carlo sensitivity analyses (as described in Section 4.2.8) was carried out to determine which tubular digester model inputs significantly influenced the (oo)cysts effluent concentration. For *Cryptosporidium parvum*, in the three digesters, the sensitivity analyses indicated that the retardation factor, R, had a -96.5 ± 2.78% contribution to variance while the inactivation rate constant, *k*, had a -3.53 ± 2.4% contribution to variance. For *Giardia lamblia*, in the three digesters, the sensitivity analyses indicated that the retardation factor, R, had a -96.6 ± 2.01% contribution to variance while the inactivation rate constant, k, had a -3.43 ± 0.69% contribution to variance. These results indicate that the retardation factor that is a function of HRT, TS_F and K_d was the main contributing factor to the (oo)cysts effluent concentration. The inactivation of (oo)cysts in the tubular digesters is enhanced due to adhesion of (oo)cysts to solids and retardation of transport through the digester. Since solids accumulation in the tubular digesters has its benefits, further research is needed to assess the fate of (oo)cysts during required maintenance of the tubular digesters. If periodic desludging of the tubular digester is required,

best management practices need to be utilized to ensure safe handling and disposal of the biosolids.

The TS_F concentration in the digesters may have been influenced by the TS loading rate. The digester's TS loading rates were 0.28, 0.069 and 0.36 g TS/L-day for Digesters 1, 3, and 4 respectively. Digester 4 with the highest TS loading rate also had the highest TS_F concentration (Table C.2). An increase in TS loading rate may encourage increased solids accumulation forming a dense floccular sludge layer in the digester which may promote further adhesion of (oo)cysts to the solids. Increasing the HRT also decreases the (oo)cysts effluent concentration as the pathogens are exposed to inactivating environmental conditions for a longer period of time. These results show that a combination of a high TS_F and HRT affects the concentration of (oo)cysts in the effluent. Farmers can be encouraged to decrease the amount of water used to clean their pig barns to increase their HRT. Although the HRT, TS_F and TS loading rates between the tubular digesters were significantly different, all the farmers reported having enough biogas for 4-7 hours per day which was adequate to meet their daily household cooking needs. Therefore, increasing the HRT may decrease the concentration of viable (oo)cysts in the effluent and not affect the biogas production.

Lastly, although the swine manure analyzed in this study did not produce positive results for either *Cryptosporidium parvum* or *Giardia lamblia*, the model predicted a significant decrease in the concentration of (oo)cysts in the effluent compared to the concentration of (oo)cysts in the raw swine waste during an infection outbreak. Apart from biogas production, the reduction in pathogen concentration is an important environmental and public health benefit to communities with livestock using tubular digesters. The use of tubular digesters has gained interest among dairy farmers in rural Costa Rica. Among livestock, cattle are the main reservoir

for *Cryptosporidium* sp. (Dufour et al., 2012), thus assessing how the environmental conditions and physical processes in tubular digesters affect the fate and viability of these protozoan parasites is crucial. In addition, some of the farmers in San Luis de Monteverde using tubular digesters co-digest cattle and swine manure. In San Luis de Monteverde and elsewhere in the developing world, farmers are often encouraged to use the digester effluent as a soil amendment to increase crop yields and decrease the cost associated with purchasing mineral fertilizers (Chapter 2). Therefore, further research is needed to assess the risk of infection when farmers with cattle and/or swine handle the tubular digester effluent to apply to crops and when humans eat crops fertilized with this effluent.

4.4 Conclusions

The low effluent organic matter and *E. coli* concentrations observed in this study were attributed to the solids settling and accumulation in the tubular digester leading to the formation of an active floccular sludge layer. *Cryptosporidium parvum* and *Giardia lamblia* inactivation studies indicated that at neutral pH, a temperature of 21°C, VFA concentration of about 300 mg/L and TAN concentration of about 240 mg NH_4^+-N/L led to about 1.56 $\log_{10}$ removal for *Giardia lamblia* and less than 1.00 $\log_{10}$ removal for *Cryptosporidium parvum* over 24 days. A tubular digester model to estimate the concentration of viable (oo)cysts in the tubular digesters effluents indicated that tubular digester TS loading rate, concentration of TS in the floccular sludge layer and HRT were the main contributing factors to the concentration of viable (oo)cysts in the tubular digesters effluent.

Table 4.1: Average operating parameters for the bench-scale anaerobic reactor (Reactor 1) (n=7) and four tubular digesters (n=5).

Parameter	Unit	Reactor 1	Digester 1	Digester 2	Digester 3	Digester 4
Working volume	L	0.90	12000	9000	9000	9000
Temperature	°C	21 ± 0.10	20.7 ± 0.48	20.9 ± 0.00	21.5 ± 0.00	23.8 ± 0.75
Influent flow	L/day	0.048 ± 0	543 ± 10	90 ± 30	50 ± 4	164 ± 30
HRT	day	24	23	100	180	55

Table 4.2: 24 day $\log_{10}$ removal rates (n=2).

Reactor	Unit	*Cryptosporidium parvum*	*Giardia lamblia*
Reactor 1	$\log_{10}$/24 days	0.55 ± 0.098	1.56 ± 0.240
Reactor 2	$\log_{10}$/24 days	0.73 ± 0.150	1.34 ± 0.035
Reactor 3	$\log_{10}$/24 days	0.31 ± 0.050	0.62 ± 0.024

Table 4.3: Average influent and effluent characteristics from the bench-scale anaerobic reactor (Reactor 1) (n=7) and four tubular digesters (n=5).

Parameter	Unit	Reactor 1		Digester 1		Digester 2		Digester 3		Digester 4	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
BOD_5	g/L	0.927 ± 0.14	0.567 ± 0.098	5.09 ± 0.30	0.030 ± 0.015	2.99 ± 1.26	0.126 ± 0.023	12.4 ± 2.45	0.155 ± 0.008	15.0 ± 1.5	0.074 ± 0.03
TS	g TS/L	32.1 ± 3.88	18.9 ± 2.60	6.35 ± 2.87	0.77 ± 0.25	19.6 ± 5.96	4.22 ± 3.60	12.4 ± 5.88	4.49 ± 1.39	19.6 ± 2.72	4.99 ± 0.53
VS	g VS/L	20.0 ± 1.32	12.2 ± 1.03	5.17 ± 2.44	0.58 ± 0.24	15.4 ± 5.36	0.81 ± 0.01	9.75 ± 5.08	2.26 ± 1.65	15.9 ± 1.22	2.26 ± 0.44
TAN	mg NH_4^+-N/L	25.4 ± 6.96	176 ± 56.5	140 ± 49.0	52.8 ± 4.66	224 ± 79.2	254 ± 16.9	193 ± 99.8	195 ± 7.54	180 ± 56.6	165 ± 52.8
Soluble TN	mg N/L	298 ± 30.7	219 ± 81.1	300 ± 23.6	49.3 ± 5.12	319 ± 120	227 ± 12.5	350 ± 75.0	186 ± 1.00	513 ± 112	303 ± 27.5
Soluble TP	mg PO_4/L	154 ± 67.0	521 ± 31.3	402 ± 126	33.9 ± 8.91	573 ± 72.0	228 ± 28.3	333 ± 131	25.0 ± 0.00	503 ± 81.0	139 ± 0.00
pH		7.48 ± 0.09	7.06 ± 0.057	7.08 ± 0.62	7.04 ± 0.14	7.96 ± 0.57	7.74 ± 0.19	6.57 ± 1.18	7.50 ± 0.14	5.83 ± 0.12	7.67 ± 0.09
E. coli	10^4 CFU/100mL	478 ± 0.00	188 ± 1.30	68.5 ± 1.30	6.40 ± 0.00	22.5 ± 21.0	2.00 ± 1.30	79.0 ± 32.9	21.1 ± 1.18	89.0 ± 34.3	3.13 ± 2.35
Conductivity	µS/m	188 ± 6.33	271 ± 100	198 ± 0.0	1339 ± 22.3	3923 ± 1753	5201 ± 1341	4499 ± 1200	5109 ± 461	6537 ± 896	7906 ± 543
Methane content	%	65 ± 0.0		71.0 ± 10		71.0 ± 10		60.8 ± 6.20		65.0 ± 5.40	
CH_4 yield	L CH_4/g VS added	0.161 ± 0.018		0.204 ± 0.099		n/m		n/m		n/m	

n/m; not measured

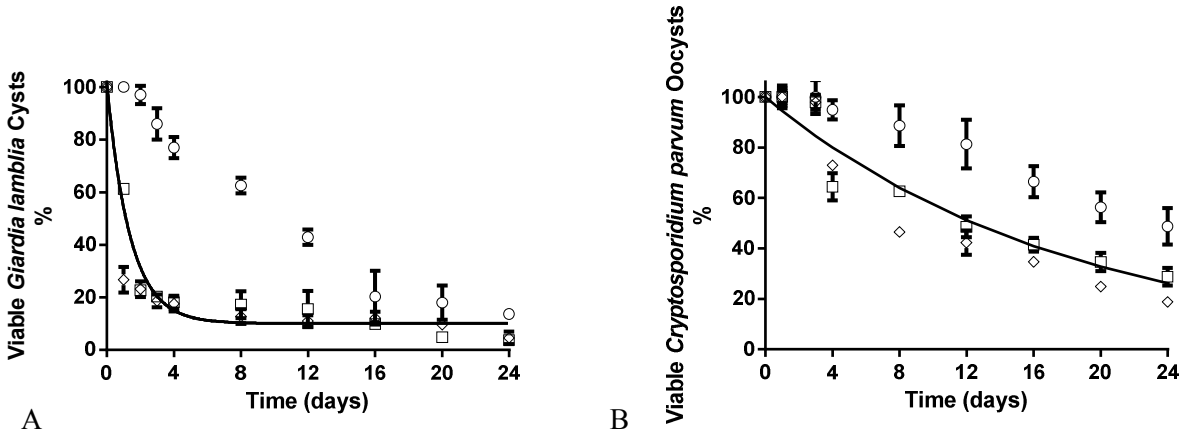


Figure 4.1: Inactivation kinetics of *Giardia lamblia* cysts (A) and *Cryptosporidium parvum* oocysts (B). Each data point represents the mean ± standard deviation of four replicates, with 150 cysts enumerated per replicate. Reactor 1 (□); Reactor 2 (◊); Reactor 3 (○); Model (—).

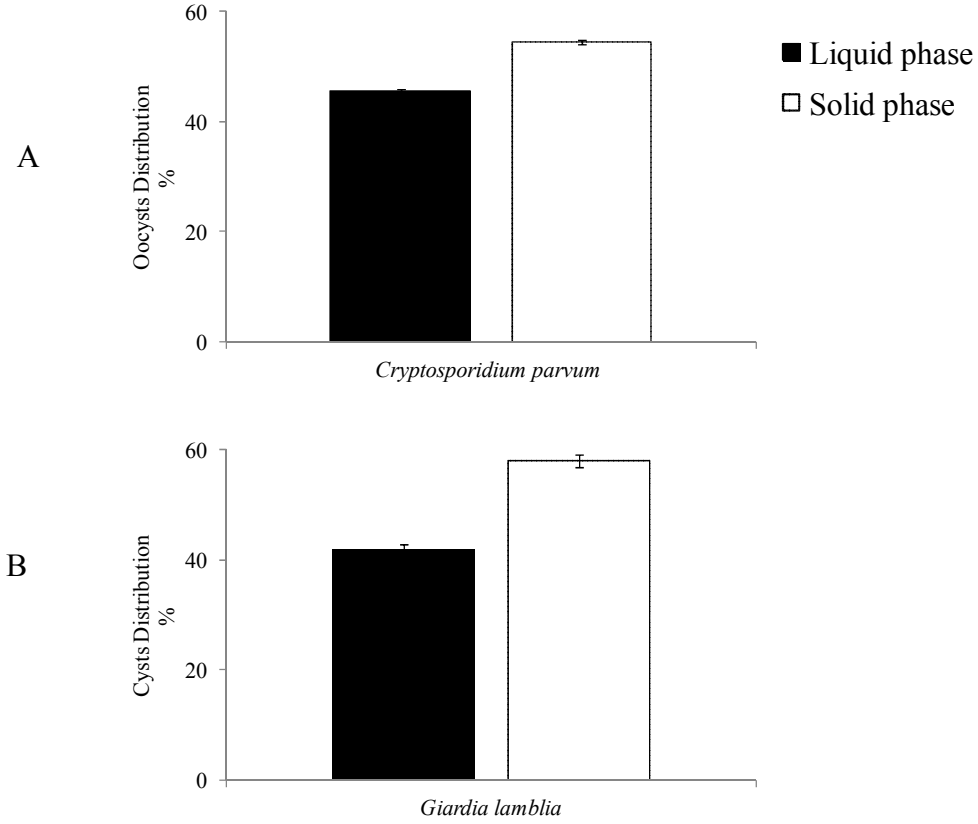
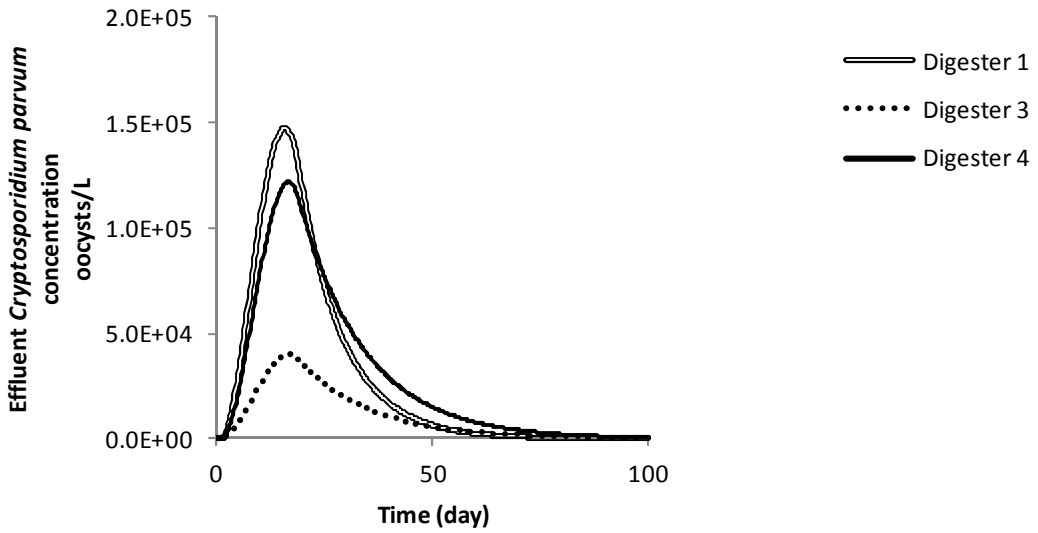


Figure 4.2: (Oo)cysts phase distribution for *Cryptosporidium parvum* oocysts (A) and *Giardia lamblia* cysts (B). Error bars represent standard deviation.

A



B

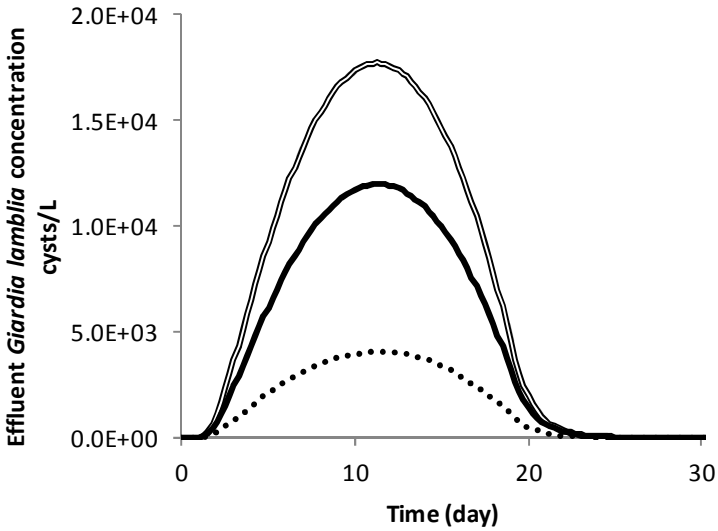


Figure 4.3: Modeled effluent concentrations of viable *Cryptosporidium parvum* oocysts (A) and *Giardia lamblia* cysts (B) in the three tubular digesters.

Chapter 5:

Risk of infection from *Cryptosporidium parvum* and *Giardia lamblia*[4]

5.1 Introduction

Around the world, food-borne and water-borne outbreaks have been caused by pathogens from livestock wastes. Runoff from land applied livestock waste has been the main contributing factor to these outbreaks (Brooks et al., 2012; Dufour et al., 2012). Presence of pathogens such as *Cryptosporidium* sp., *Giardia lamblia, Ascaris lumbricoides, Entamoeba histolytica, E. coli* and fecal coliforms in raw vegetables sold in open markets in Costa Rica, Egypt and Nigeria has been attributed to use of irrigation water contaminated by livestock waste (Monge and Chinchilla, 1996; Damen et al., 2007; Eraky et al., 2014). Although there are a variety of zoonotic pathogens that cause illness to humans, two protozoan parasites, *Cryptosporidium parvum* and *Giardia lamblia*, and three bacteria, *Campylobacter jejuni*, *Salmonella* sp. and *E. coli* O157:H7 are the main zoonotic pathogens of concern according to the World Health Organization (WHO) (Dufour, 2012). The reasons for this include; (1) disease from these pathogens occur in healthy humans and can result in serious illness and/or death, (2) these pathogens are distributed globally, (3) they are resistant to commonly used water and wastewater treatment technologies, such as chlorination, (4) the livestock genotypes are closely related to human genotypes, and (5) water transmission is the main route of exposure (Dufour, 2012).

[4] This chapter is adapted from a manuscript under preparation by Kinyua, M.N., Wald, I., Céspedes, F.C., Ergas, S.J. "Does the use of tubular digesters to treat livestock waste lower the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia*?"

Livestock waste can contain high loads of these pathogens, in particular the protozoan parasites, *Cryptosporidium parvum* and *Giardia lamblia*. *Cryptosporidium parvum* accounts for 23.7% of all worldwide waterborne outbreaks annually while *Giardia lamblia* infects about 2.8 billion people worldwide annually (Dufour et al., 2012). The low infectivity of the protozoan parasites also increases their associated public health concern. One *Giardia lamblia* cyst or approximately nine *Cryptosporidium parvum* oocysts have been shown to cause illness in humans (Erickson et al., 2006). Young, old and immune-compromised individuals are particularly susceptible to disease from infection with these pathogens (Haas et al., 1999).

Although policies and regulations management of livestock waste may be in place in developing countries, successful enforcement is often lacking due to lack of commitment by local and national authorities, lack of infrastructure for regular monitoring and lack of education on the negative environmental and public health consequences from mismanagement of livestock waste (Chapter 2). In countries where national and local commitment is evident, small-scale anaerobic digesters are promoted for livestock waste management and energy production. In addition to environmental and energy benefits, the use of small-scale anaerobic digestion systems to treat livestock waste in the developing countries has several social, public health and agricultural benefits as described in Chapter 2. However, the use of anaerobic digestion effluents to achieve agricultural benefits still needs monitoring due to the presence of pathogens in the effluent. Anaerobic digestion operating parameters such as retention times and temperature and chemical conditions such as pH, free ammonia and volatile fatty acids (VFA) concentrations have been shown to inactivate pathogens during treatment of livestock waste (Chen et al., 2012; Chapter 4, Manser et al., 2015). Unfortunately, exposing *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts to potentially inactivating conditions does not guarantee absence of viable

(oo)cysts in the anaerobic digestion effluents (Chauret et al., 1999; Chapter 4). In developed countries, operating parameters and chemical conditions can be monitored and controlled to promote greater inactivation of pathogens. In small-scale anaerobic digesters used in the developing world operating parameters, such as the retention time, can be adjusted to promote greater pathogen inactivation. However, determining the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia,* associated with handling raw livestock waste and anaerobic digestion effluents, is still critical in an effort to empower communities in the developing world by protecting their health.

The risk of infection can be determined through the use of quantitative microbial risk assessment (QMRA). QMRA is a useful tool that is used to estimate the risk of a health effect to humans from exposure to pathogens. Health effects include infection, illness and or death. By carrying out a QMRA, management practices that reduce exposure can be put in place. A risk-based management strategy is more attractive than a treatment technology based management strategy due to its versatility, depending on the region, location, culture, socio-economic status and other community dependent variables (Razzolini et al., 2011). The QMRA process involves four steps:

- Step 1: Pathogen identification - This step involves identifying pathogens of concern for the target population and describing the symptoms and rates of infections from the pathogen. For this study *Cryptosporidium parvum* and *Giardia lamblia* were chosen for the reasons described earlier.
- Step 2: Exposure assessment - This step involves estimating the concentration of pathogens at different exposure pathways and the frequency of exposure such as daily water intake. Examples of agriculturally relevant exposure pathways include farmers

handling raw manure, farmers handling anaerobic digester effluent and farmers tending to soil fertilized with raw or treated livestock waste (Mara and Horan, 2003; Brooks et al., 2012).

- Step 3: Dose response - This step involves the use of epidemiological studies to determine the capacity of the pathogens identified in step 1 to cause a health effect to humans. During the epidemiological studies, human volunteers ingest the pathogen of concern at varied doses. Dose is the number of pathogens ingested per kg of body weight. Dose response curves are then generated based on health outcomes. A dose response curve is a mathematical relationship between the concentrations of a pathogen a human ingests (dose) and the overall health effect (response) to the pathogen. These curves also generate the minimum infective dose (ID_{50}) and dose response parameters (Haas et al., 1999).
- Step 4: Risk characterization - This step involves integrating data from steps 1, 2, and 3 into a mathematical model to calculate the risk of a health effect. Due to variability (mean, minimum, maximum and standard deviation) and uncertainty of the inputs from steps 1 to 3, a sensitivity analysis is conducted. This is carried out using a Monte Carlo simulation to better understand how pathway specific inputs from steps 1 to 3 affect the final risk estimated (Haas et al., 1999).

There are two types of QMRA models that are used to assess the probability of a health effect; individual-based and population-based risk models (Cooper et al., 2012). The individual-based risk model estimates the probability of infection after a single exposure event. Recurring exposure events are considered as independent single exposure events. Individual immunity to the pathogens decreases the probability of a health effect and secondary transmission increases

the probability of a health effect. Influences from individual immunity and secondary transmission are assumed to be negligible as they cancel each other out (Cooper et al., 2012). In a population-based risk model, only a fraction of the population that is susceptible to infection and illness is considered. This fraction of the population is determined based on immunity, duration of infection and the number of infected people with whom the susceptible population comes into contact (Cooper et al., 2012). Due to the complexity of the population-based risk model, the individual-based risk model was used for this study.

Several studies have evaluated the concentration of *Cryptosporidium parvum* oocysts, and *Giardia lamblia* cysts and the risk of infection associated with exposure at various pathways to these pathogens in raw livestock waste, raw domestic wastewater and class B biosolids (Heitman et al., 2002; Hutchison et al., 2004; Brooks et al., 2012a; Harder et al., 2014). Class B biosolids are domestic wastewater sludge which is treated through, anaerobic digestion (35-60°C), aerobic digestion, composting, air drying or lime stabilization (USEPA, 2003). Class B biosolids produced from an anaerobic digestion system at municipal wastewater treatment facilities differ from effluents from small-scale anaerobic digestion systems treating livestock waste due to digestion treatment temperatures.

The acceptable individual annual risk of infection from *Cryptosporidium parvum* and or *Giardia lamblia* according WHO and USEPA is 10^{-4}. This means for every 100,000 people, only 10 people can get infected in a year (USEPA, 2001). Brooks et al. (2012) investigated the risk of infection from occupational exposure to soil contaminated with *Cryptosporidium parvum* from raw cattle waste and class B biosolids. This study found that over the course of 30 days the risk of infection was greater during exposure to soils fertilized with raw cattle waste (3×10^{-4}) compared to soil fertilized with class B biosolids (1×10^{-5}). This can be attributed to the lower

concentration of *Cryptosporidium parvum* in the class B biosolids. Another study investigated the risk of infection from *Giardia* sp., and *Cryptosporidium* sp. from consumption of crops irrigated with wastewater that had undergone tertiary treatment. The annual risk of infection from *Giardia* sp., and *Cryptosporidium* sp. was 8.54×10^{-5} and 2.04×10^{-4} respectively (Cooper et al., 2012). These studies indicate that the treatment of domestic wastewater reduces the risk of infection at various exposure pathways. However, there are no prior studies that have investigated how the treatment of livestock waste through small-scale anaerobic digestion systems used in developing countries influences the risk of infection from *Giardia lamblia* and *Cryptosporidium parvum* when the effluent from the systems is used as a soil amendment. Assessing the health risk through a QMRA, to provide a guideline for safe reuse of livestock waste for agricultural purposes is critical. This study investigated the concentration of *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts in dairy cattle and swine waste in two communities in rural Costa Rica. Based on the pathogen concentrations in the raw livestock waste and modeled (oo)cysts effluent concentrations from small-scale tubular anaerobic digester systems (Chapter 4), a QMRA was carried out to determine the risk of infection from exposure to these pathogens at different exposure pathways. The exposure pathways assessed were fomite contamination, soil contamination and crop contamination from runoff. The influence of using small-scale tubular anaerobic digestion systems on the risk of infection was also analyzed.

5.2 Materials and Methods

5.2.1 Site Description and Tubular Digesters

This study was carried in two rural communities located on the Pacific Slope of the Tilarán Mountain Range of Costa Rica. The first community is San Luis de Monteverde (N 10' 16.973" W 84' 47.882") located in the province of Puntarenas, with an altitude range of up to

1200 m above sea level. San Luis de Monteverde has a population of approximately 500 people. The main economic activities in San Luis de Monteverde are small-scale farming and eco-tourism. Households in San Luis de Monteverde typically have about 10 cows and 4-10 pigs. Eight households in San Luis de Monteverde installed tubular anaerobic digesters to promote energy production and reduce livestock waste pollution. Three of the households with tubular digesters co-digest swine and cattle waste, four treat only swine waste and one treats only cattle waste. The biogas produced is sufficient to meet household daily energy demands for cooking for an average family of five people (Chapter 3).

The second community is La Florida (N 10° 23' 45.33" W 84° 54' 10.2492") located in the province of Guanacaste, with an altitude range of up to 900 m above sea level. La Florida has a population of approximately 150 people. La Florida is located close to the continental divide where the climate and presence of rich volcanic soils, make it ideal for tropical dairy farming. The predominant dairy farming system produces high quality varieties of grass and cow breeds. Most dairy farms in La Florida are family owned and operated. The dairy farms have about 26-80 cows and most of the milk produced in La Florida is sold to Costa Rica's largest dairy cooperative Dos Pinos. Dos Pinos collects the milk from the dairy farmers and processes it to various dairy products that are sold in the domestic and international markets. To meet the milk quality demands set by the dairy cooperatives that buy the milk, the farmers spend about 51% of their annual budget importing cattle feed to sustain their productivity and 15% of their annual budget on electricity for milking and cooling purposes. There are about 25 dairy farms in La Florida. The cows are free range and waste from the cows is only collected when milking and disposed of through land application on the cattle pastureland. Only one farm treats their waste through composting. In an effort to reduce electrical costs and reduce the environmental burden

of dairy farming, the farmers of La Florida are interested in installing tubular anaerobic digestion systems to treat their livestock waste. In addition interviews were conducted in Spanish at the field sites. A list of interview questions and answers is provided in Appendix D.

5.2.2 Sample Collection

In the community of San Luis de Monteverde, feces from 4 farms using tubular digesters were sampled from 22 pigs (8 suckling pigs, 11 growing pigs aged 1 to 6 months, 2 sows and 1 boar). In the community of La Florida, feces from 8 farms were sampled from 326 cattle at various age groups (234 dairy cattle actively producing milk, 24 pregnant cows, 68 calves and 2 bulls). These analyses were carried out in January and February 2015. Samples were analyzed within 24 hours of collection for the presence of *Cryptosporidium* sp. and *Giardia* sp. as described below. In addition, all of the farmers who participated in this study were interviewed to gain insight into their livestock and tubular digester management practices. Interviews were conducted in Spanish at the field sites. A list of interview questions is provided in Appendix B and C.

5.2.3 QMRA Model Development

5.2.3.1 Pathogen Identification

During the field study, all pathogen identification analyses were carried out at the University of Georgia Costa Rica campus (San Luis, Puntarenas, Costa Rica). Before pathogen analyses were carried out for each sample, 15-20 g of raw feces were mixed with 20 mL of deionized water and passed through a 152 mm sieve to remove debris and produce sieved manure slurry. A commercially available rapid immune-assay, ImmunoCard STAT! from Meridian Bioscience Inc. (Cincinnati, Ohio) and a fecal floatation method were used to detect *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts in the dairy and swine manures. The ImmunoCard

STAT! Crypto/Giardia assay detects and distinguishes between *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts antigens in feces using a non-enzymatic rapid immunoassay format. Since the ImmunoCard Stat! assay is designed for use in human fecal samples, a sensitivity analysis to livestock feces was performed. Three grams of cow and swine feces that had tested negative for the presence of (oo)cysts was spiked with 6000 non-viable (oo)cysts each and assessed according to manufacturer's instructions. It was noted that for livestock manure with spiked (oo)cysts, 20 minutes versus the 10 minutes recommended by the manufacturer was required to reveal a positive *Cryptosporidium parvum* and *Giardia lamblia* test result possibly due to higher solids content in livestock feces compared to human feces. For the fecal floatation method, approximately 3 g of the sieved manure slurry was thoroughly mixed with 5 mL of a $ZnSO_4$ solution in a 10 mL test tube. An additional 5 mL of the $ZnSO_4$ solution was slowly added to the 10 mL test tube to get a positive meniscus. A coverslip was applied to the top for 15 minutes. The coverslip was then placed on a microscope slide and examined by light microscopy at 100x magnification for the presence of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts using a Fisher Scientific Micromaster microscope (Hanover Park, IL). Samples were analyzed in triplicate. The $ZnSO_4$ solution was prepared by mixing 386 g of $ZnSO_4$ in 1000 mL of deionized water. Non-viable (oo)cysts were purchased from Waterborne Inc. (New Orleans, LA).

5.2.3.2 Dose Response

Epidemiological studies are required to determine the capacity of *Cryptosporidium parvum* and *Giardia lamblia* to harm human beings. Prior epidemiological studies on healthy human volunteers have estimated a dose response parameter of a single agent causing infection (r) from exposure to *Cryptosporidium parvum* and *Giardia lamblia* as 5.72×10^{-2}, 1.99×10^{-2} and 1.5×10^{-2} respectively (Rendtorff, 1954; Messner et al., 2001).

5.2.3.3 Exposure Assessment

Mathematical equations for exposure assessment were derived from Brooks et al. (2012) and values for model inputs are summarized in Table 5.1. The pathways that were considered in this study are fomite and soil contamination and crop contamination from runoff.

Fomite contamination model: Fomite contamination was considered when the farmers are handling raw manure to dispose of it in the cattle pastureland or when preparing tubular digester influent. Fomite contamination was calculated assuming that a fraction of raw manure was transferred to a fomite such as the handle of the shovel or bucket. During a single event exposure, with no decay, the fomite pathogen concentration was:

$$C_f = C_{rm} \times F_{rm} \quad \text{(Eq. 5.1)}$$

where C_f is the fomite pathogen concentration ((oo)cysts/fomite); C_{rm} is the pathogen concentration in the raw cattle and swine waste ((oo)cysts/g TS); and F_{rm} is the amount of raw waste transferred to a fomite (g/fomite). Fomite pathogen concentration accounting for inactivation over time was calculated as:

$$C_f = C_{rm} \times F_{rm} \times (1/10^{K_f}) \quad \text{(Eq. 5.2)}$$

where K_f is (oo)cysts inactivation rates on a fomite (log removal/day). Fomite log removal rates were linearly extrapolated from day 0 to day 5.

Soil contamination model: Soil contamination resulted from land application of tubular digester effluent in the soil. To calculate the pathogen concentrations in the soil, inactivation rates of *Cryptosporidium parvum* and *Giardia lamblia* in the soil were considered:

$$C_s = C_{de} \times D_s \times (1/10^{K_s}) \times (1000 \text{ g/kg}) \quad \text{(Eq. 5.3)}$$

where C_s is the soil pathogen concentration ((oo)cysts/kg soil); C_{de} is the concentration of pathogens in the digester effluent ((oo)cysts/L); D_s is the soil dilution ratio (L of digester

effluent/ g of soil); and K_s is the (oo)cysts inactivation rates in the soil at 25°C (log removal/day). Soil log inactivation rates were linearly extrapolated from day 0 to day 120. It should be noted that only soil contamination from the use of tubular digester effluent was considered. After performing a participatory observation on livestock waste disposal methods and interviews with dairy farmers in the La Florida community, it was concluded that there was negligible exposure to farmers from soil contamination as they disposed of the manure in the cattle pastureland and did not tend to this soil.

Of the eight farmers using tubular digesters in San Luis de Monteverde, only one farmer used their digester effluent to fertilize crops eaten raw such as tomatoes and lettuce. All other farmers with digesters used the effluent to fertilize corn, beans, fruit trees and root crops that were later cooked. Therefore, direct crop contamination from use of raw livestock waste and tubular digester effluent was not assessed for this study.

Crop contamination from runoff model: Only indirect crop contamination from runoff for low hanging foods eaten raw (lettuce, cabbage and cilantro) was considered. To determine the concentration of pathogens on foods eaten raw, the concentration of pathogens in the raw cattle waste and tubular digester effluent deposited on a field was first estimated:

$$C_p = C_{rm} \times A_{rm} \times (10^6 \text{ g/Mg}) \text{ or } C_{de} \times A_{de} \qquad \text{(Eq. 5.4)}$$

where C_p is the concentration of (oo)cysts per hectare ((oo)cysts/ha); A_{rm} is the application rate of raw cattle waste (Mg/ha); and A_{de} is the application rate of tubular digester effluent (L/ha). A_{de} was calculated as the tubular digester effluent flow rate divided by the area where the farmers apply their effluent. The concentration of (oo)cysts on crops contaminated by runoff was expressed as:

$$C_c = C_p \times F_r \times T_r \times D_r \times (1/10^{Kw}) \times T_c \times (1/10^{Kc}) \times T_w \qquad \text{(Eq. 5.5)}$$

where C_c is the concentration of (oo)cysts on crops contaminated by runoff ((oo)cysts/kg); F_r is the fraction of (oo)cysts available for runoff; T_r is the percentage of (oo)cysts once applied to the soil will be transferred to the runoff water; D_r is the (oo)cysts dilution ratio in the runoff water; K_w is the inactivation rate of (oo)cysts in water at 25°C (log removal/day); T_c is the percent attachment of (oo)cysts from the runoff water to crops; K_c is the inactivation rate of (oo)cysts on crops at 22°C (log removal/day); and T_w is the percentage of soil particles that remain on the crops after washing. There is limited research on the percent attachment of *Cryptosporidium* sp. and *Giardia* sp. in runoff water to crops. However, various studies have recovered (oo)cysts from leafy crops irrigated with wastewater (Monge and Chinchilla, 1996; Amoros et al., 2010; Rzeżutka et al., 2010). T_c values for this study were assumed to be the same as the recovery rates obtained by Monge and Chinchilla (1996) who investigated the presence of *Cryptosporidium* sp. and *Giardia* sp. in lettuce and cilantro leaves sold in markets in Costa Rica. Water and crop log inactivation rates were linearly extrapolated from day 0 to day 14.

5.2.3.4 Risk Characterization

Risk characterization combines the exposure assessment data with the dose response data to determine the risk of infection to the pathogens at the different exposure pathways. First, the concentration of (oo)cysts ingested (dose) is estimated:

$$d = C_{ep} \times C_i \text{ or}$$
$$d = C_{ep} \times C_i \times T_h \times T_m \quad \text{(Eq. 5.6)}$$

where d is the dose ((oo)cysts/dose); C_{ep} is the concentration of (oo)cysts at each exposure pathway; C_i is the amount of soil ingested during occupational activities (0.48 kg soil/day) or leafy crops consumed per day (0.292 kg leafy crops/day); T_h is the transfer of (oo)cysts from fomite to hand; and T_m is the transfer of (oo)cysts from hand to mouth. Protozoan parasites

follow an exponential distribution dose-response model and the probability of infection during a one-time exposure to *Cryptosporidium parvum* or *Giardia lamblia* is expressed as:

$$P_i = 1\text{-}\exp(-rd) \qquad \text{(Eq. 5.7)}$$

where P_i is the one-time pathogen exposure probability of infection (Haas et al., 1999).

5.2.3.5 Data Analysis

Oracle Crystal Ball (Redwood City, CA) was used for Monte Carlo simulation. A Monte Carlo uncertainty analysis was performed by running 10,000 trials with varying contamination model inputs to determine how model inputs affected the risk of infection. All the kinetic inputs were considered uncertain inputs with normal distributions.

5.3 Results and Discussion

5.3.1 Pathogen Concentrations

During the three week field study, none of the feces sampled produced positive results for either *Cryptosporidium parvum* or *Giardia lamblia*. Although the pigs and dairy cattle were not shedding viable (oo)cysts at the time of this study, giardiasis and cryptosporidiosis is a public health concern for communities in Costa Rica (Monge et al., 1996). *Cryptosporidium* sp. and *Giardia* sp. shedding in pigs and adult cattle is sporadic, with younger animals shedding the highest concentration of (oo)cysts. Therefore, determining the risk of infection from these protozoan parasites is necessarily to determine the appropriate risk management strategies to reduce the health burdens associated with giardiasis and cryptosporidiosis. Average concentrations of viable *Cryptosporidium* sp. and *Giardia* sp. in raw dairy cattle and swine waste from the literature were used for this study and are summarized in Table 5.2. The highest viable (oo)cysts effluent concentrations modeled in Chapter 4 for four tubular digesters are summarized

in Table 5.3. Modeled viable (oo)cysts effluent concentrations varied between the three tubular digesters due to differences in the digesters operating parameters.

5.3.2 Fomite Contamination

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for occupational based exposure for an adult handling a wooden fomite for periods of 1 to 5 days. Inactivation of (oo)cysts on a wooden fomite was used because farmers in these two communities used shovels with wooden handles to prepare swine waste slurry for the tubular digesters and when disposing of raw cattle waste. The risk of infection from occupational exposure to a contaminated wooden fomite is shown in Table 5.4. For this exposure scenario 0.1 g of raw cattle or swine waste was assumed to be transferred to the wooden fomite, based on USEPA occupational transfer (Brooks et al., 2012). Occupational exposure to wooden fomites contaminated with raw cattle waste presented a greater risk compared to raw swine waste due to the higher concentration (3-5 orders of magnitude) of (oo)cysts in the raw cattle waste. Inactivation of (oo)cysts was the main factor contributing to the risk of infection from exposure to contaminated fomites. Inactivation of (oo)cysts on fomites is affected by surface characteristics (porous or nonporous) and environmental conditions such as temperature, relative humidity and exposure to UV radiation (Bowman, 2009). Anderson (1986) investigated the inactivation of *Cryptosporidium* sp. on a wooden surface at ambient temperature and reported a 4 log removal in 3 days. Other studies have investigated inactivation of *Cryptosporidium* sp. oocysts on dry metal surgical blades and dry glass surfaces and reported higher inactivation rates compared to wooden surfaces. This difference in inactivation could be due to cracks and crevices on wooden surfaces that may protect the oocysts from inactivation (Barbee, 1999; Robertson, 1992). It should be noted that the inactivation rate of *Giardia lamblia* on wooden fomites was

assumed to be similar to the inactivation rate of *Cryptosporidium* sp. on a wooden surface due to lack of literature on *Giardia* sp. inactivation on fomites. This assumption may have overestimated the risk of infection from *Giardia lamblia* as the inactivation of *Giardia lamblia* has been shown to be greater than *Cryptosporidium* sp. when the (oo)cysts are exposed to similar environmental conditions (Olson et al., 1999; Chapter 4). More research is required to investigate inactivation of *Giardia lamblia* on fomites. Such a study would provide more accurate data for communities at risk of infection from these parasites. Additionally, risk management strategies, such as personal hand hygiene and placing shovels in the sun for parasite inactivation through UV radiation, can be encouraged to lower the risk of infection from occupational exposure to contaminated fomites.

5.3.3 Soil Contamination

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for occupational based exposure for an adult tending to the soil after application of the tubular digester effluent for periods of 1 to 120 days. The risk of infection from (oo)cysts from using tubular digester effluents as a soil amendment are summarized on Table 5.5. A Monte Carlo simulation was performed to determine how model inputs influenced the risk of infection. Three conclusions were drawn from these results. First, the risk of infection from *Giardia lamblia* was significantly different from the risk of infection from *Cryptosporidium parvum* for the same time periods. Although the soil inactivation rates between the two parasites were not significantly different, the tubular digester effluent (oo)cysts concentrations differed significantly (Table 5.2) due to the differences in operating parameters between the four digesters and (oo)cysts inactivation rates during digestion (Chapter 4).

Second, the risk of infection during occupational exposure to contaminated soil was higher in this study than reported risk of infection in other studies. Brooks et al. (2012) investigated the risk of infection to *Cryptosporidium parvum* from use of class B biosolids on soils. By day 7, the risk of infection was lower than 1×10^{-4}, the acceptable risk of infection according to WHO, while for this study the risk of infection was 1. The difference in the risk of infection is mainly due to the concentration of viable (oo)cysts in the tubular digester effluent compared to class B biosolids. Tubular digesters used in this study had a temperature of approximately 21°C, resulting in lower (oo)cysts inactivation rates (Chapter 4) compared to anaerobic digesters producing class B biosolids operated at mesophilic (30-37°C) and thermophilic (50-60°C) temperatures. At 21°C, log removal rates of 0.065 and 0.023 log removal/day were observed for *Giardia lamblia* and *Cryptosporidium parvum* respectively (Chapter 4). At 36°C, 0.15 log removal/day was observed for *Cryptosporidium parvum*, 3 log removal for *Giardia lamblia* and 1.0 log removal/day was observed at thermophilic temperatures (47-55°C) (Gale, 2005; Kato et al., 2010).

The third main point was that for both *Cryptosporidium parvum* and *Giardia lamblia*, the (oo)cysts inactivation rates in the soil had the greatest contribution to the risk of infection, at about 95% for all tubular digester effluents. All other soil contamination model inputs (C_{de}, D_s, r and C_i) each had less than 1.5% contribution. Inactivation of (oo)cysts in the soil is affected by environmental conditions, such as temperature and moisture content. As the temperature of the soil increases, the inactivation of (oo)cysts would also increase leading to a lower risk of infection. The (oo)cysts inactivation rates in soil were reported at 25°C (Hu et al., 1996; Olson et al., 1999). Moisture content of the soil also influences the (oo)cysts inactivation rates in soil. As moisture content increases (oo)cysts inactivation rates decrease (Barwick et al., 2003). Moisture

contents less than 1% result in desiccation/drying of pathogen membranes which causes inactivation (Cotruvo, 2004). Soil moisture content can be increased by rainfall events. The Monteverde region of Costa Rica, where this study was performed, has a mean annual temperature of 18.8°C with a mean annual precipitation of 2519 mm. This region also houses the Monteverde Cloud Forest where the cloud cover leads to soil moisture contents of about 70% during the rainy season and 20-40% during the dry season (Nadkarni and Wheelwright, 2000). Although soil moisture content was not incorporated in the soil contamination model, the high soil moisture content in Monteverde may decrease (oo)cysts inactivation rates in soil, thus increasing the risk of infection.

5.3.4 Crop Contamination from Runoff

The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was estimated for consumption of crops contaminated with (oo)cysts in runoff water. The crop contamination from runoff model was based on leafy crops eaten raw due to dietary habits of households in La Florida and San Luis de Monteverde based on interviews. There is lacking literature on the inactivation of *Giardia* sp. on crops, therefore, the crop inactivation rate of *Giardia lamblia* was assumed to be similar to that of *Cryptosporidium parvum*. Results from the crop contamination from runoff model are summarized in Table 5.6. From these results, 2 main conclusions were noted. First, several assumptions were made for the crop contamination from runoff model. To determine how these assumptions on model inputs affected the risk of infection, a Monte Carlo simulation was performed. The (oo)cysts inactivation rates on leafy crops (> 93%) had the greatest contribution to the risk of infection for both tubular digester effluents and raw cattle waste. (Oo)cysts inactivation rates in water contributed to the risk of infection by less than 3.5%. *Cryptosporidium parvum* oocysts survival on crops has been shown to depend on the type

of leaf, for example iceberg lettuce leaves versus parsley leaves, when the crops are stored at the same temperature (Warnes and Keevil, 2003). Oocysts survive longer in crinkly textured and larger leafed crops, as the contours in the leaves provide the oocysts protection from desiccation. In smaller leaved crops such as cilantro and parsley, the crop's shorter shelf life promotes desiccation as the crop dries up (Warnes and Keevil, 2003).

Second, it was noted that a one-time runoff event resulted in risks of infection greater than 10^{-2} from both parasites originating from tubular digester effluents and raw cattle waste within the first 3 days. However by day 7 after a runoff event, the risk of infection had decreased significantly when tubular digester effluent was land applied. These results indicate that if leafy crops are harvested 7 days after a runoff event in San Luis de Monteverde where farmers use tubular digester effluent as a soil amendment, the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* is significantly less compared to harvesting leafy crops in La Florida where cattle waste remains untreated. This indicates that the use of tubular digesters does significantly reduce the risk of infection and illness from either giardiasis or cryptosporidiosis.

5.3.5 Risk Simulation

Results from this risk assessment indicate that occupation exposure (fomite and soil contamination) resulted in higher risks than indirect exposure (crop contamination from runoff). Although dairy farmers in La Florida do not tend to the soil after applying raw cattle waste, a worst-case scenario risk of infection from exposure to soil contaminated with raw cattle waste (data not shown) was estimated. The risk of infection to both (oo)cysts was significantly higher due to higher concentrations of (oo)cysts in cattle waste compared to swine waste. While the cattle waste analyzed in this study was negative for the presence of *Cryptosporidium* sp. and *Giardia* sp., cows are the main reservoir for these parasites (Dufour et al., 2012). This study is a

good starting point to understand and predict the risk of infection of *Cryptosporidium parvum*, *Giardia lamblia* and other pathogens of concern especially for communities in the developing world and how tubular digesters can assist in reducing direct and indirect risks of infection. The exposure pathways and risks estimated in this study did not account for variability such as soil moisture content, wildlife contribution to (oo)cysts loads, continuous rainfall events and other environmental inactivation mechanisms such as UV radiation on soil. In addition, several assumptions were made to estimate the risk of infection due to lacking literature indicating more research is needed on these two parasites to provide accurate predictions especially for communities in the developing world.

5.4 Conclusions

This study investigated the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from exposure to raw livestock waste and modeled (oo)cysts effluent concentrations at different exposure pathways. The higher concentrations of (oo)cysts in the cattle waste compared to the swine waste lead to the risk of infection during occupational exposure to contaminated wooden fomites to be greater when handling raw cattle waste. The risk of infection from *Cryptosporidium parvum* during occupational exposure to contaminated soil from tubular digester effluents was higher than from exposure to *Giardia lamblia* due to higher inactivation of *Giardia lamblia* during anaerobic digestion. The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from consumption of leafy crops contaminated with runoff water in San Luis de Monteverde where tubular digesters was significantly lower than the risk of infection in La Florida where cattle waste was untreated.

Table 5.1: Exposure assessment model inputs.

Parameter	Unit	*Cryptosporidium* sp.	*Giardia* sp.	References
A_{rm}	MgTS/ha	6.57		Gale et al., 2005
D_r		0.045		Brooks et al., 2012
D_s		0.00175		Gale et al., 2005
F_r		0.1		Brooks et al., 2012
F_{rm}		0.1		Gale et al., 2005
K_c	log_{10}/day	1.33		Warnes and Keevil, 2003
K_f	log_{10}/day	1.33		Anderson, 1986
K_s	log_{10}/day	0.052 ± 0.0045	0.057± 0.024	Hu et al., 1996; Olson et al., 1999; Hutchison et al., 2002
K_w	log_{10}/day	0.048	0.127	Olson et al., 1999
T_c	%	0.043 ± 0.032		Monge and Chinchilla, 1996
T_h	%	0.43		Brooks et al., 2012
T_m	%	0.36		Brooks et al., 2012
T_r*	%	9.00 ± 2.85		Trask et al., 2004
T_w	%	0.1		Gale et al., 2005

* The Monteverde region receives approximately 160 mm rainfall/hour. T_r was calculated assuming a 2.2-5.2% transfer per 63.5 mm of rainfall/hour.

Table 5.2: Average *Cryptosporidium* sp. and *Giardia* sp. concentrations from literature in the raw cattle and swine manure.

Pathogen	*Cryptosporidium* sp. oocysts/ g TS	*Giardia* sp. cysts/ g TS
Raw dairy cattle manure (C_{rm})[a]	$3.89 \times 10^{10} \pm 1.94 \times 10^{10}$	$3.80 \times 10^{7} \pm 1.90 \times 10^{7}$
Raw swine manure (C_{rm})[b]	$9.00 \times 10^{4} \pm 4.15 \times 10^{4}$	$5.40 \times 10^{4} \pm 2.66 \times 10^{4}$

[a]Nydam et al., 2001; Hutchison et al., 2004; Maddox-Hyttel et al., 2006; [b]Yui et al., 2014

Table 5.3: Modeled highest concentration of viable *Cryptosporidium parvum* and *Giardia lamblia* in four tubular digester effluents (Chapter 4).

Digester	*Giardia lamblia* cysts/L	*Cryptosporidium parvum* oocysts/L
Digester 1	1.77E+04	1.47E+05
Digester 3	4.06E+03	3.97E+04
Digester 4	1.20E+04	1.21E+05

Table 5.4: Risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* during occupational exposure to contaminated wooden fomites. Day represents number of days after contamination of fomite.

Giardia lamblia				
Day	**1**	**2**	**3**	**5**
Raw swine waste	5.38E-01	3.53E-02	1.67E-03	3.60E-06
Raw cattle waste	1.00E+00	1.00E+00	6.91E-01	2.53E-03
Cryptosporidium parvum				
Day	**1**	**2**	**3**	**5**
Raw swine waste	9.75E-01	1.58E-01	7.96E-03	1.72E-05
Raw cattle waste	1.00E+00	1.00E+00	1.00E+00	9.99E-01

Table 5.5: Risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* during occupational exposure to contaminated soil from tubular digester effluents. Day represents number of days after application of digester effluent on soil.

Giardia lamblia								
Day	**1**	**7**	**14**	**21**	**28**	**60**	**90**	**120**
Digester 1	1.00E+00	1.00E+00	1.00E+00	1.00E+00	9.99E-01	1.06E-01	2.19E-03	4.28E-05
Digester 3	1.00E+00	1.00E+00	1.00E+00	9.87E-01	8.21E-01	2.55E-02	5.03E-04	9.8E-06
Digester 4	1.00E+00	1.00E+00	1.00E+00	1.00E+00	9.94E-01	7.33E-02	1.48E-03	2.89E-05
Cryptosporidium parvum								
Day	**1**	**7**	**14**	**21**	**28**	**60**	**90**	**120**
Digester 1	1.00E+00	1.00E+00	1.00E+00	1.00E+00	9.84E-01	8.64E-02	2.48E-03	6.85E-05
Digester 3	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	7.65E-01	3.91E-02	1.10E-03
Digester 4	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	9.88E-01	1.14E-01	3.34E-03

Table 5.6: Risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* from consumption of leafy crops contaminated with runoff water. Day represents number of days after rainfall event.

Giardia lamblia					
Day	**1**	**3**	**7**	**11**	**14**
Digester 1	1.00E+00	8.50E-02	1.32E-07	1.96E-13	0.00E+00
Digester 3	1.00E+00	6.15E-02	9.44E-08	1.40E-13	0.00E+00
Digester 4	1.00E+00	4.25E-01	8.22E-07	1.22E-12	0.00E+00
Raw cattle waste	1.00E+00	1.00E+00	7.61E-01	2.13E-06	9.05E-11
Cryptosporidium parvum					
Day	**1**	**3**	**7**	**11**	**14**
Digester 1	1.00E+00	3.18E-02	9.94E-08	3.06E-13	0.00E+00
Digester 3	1.00E+00	8.97E-02	2.89E-07	8.89E-13	0.00E+00
Digester 4	1.00E+00	6.09E-01	2.89E-06	8.89E-12	0.00E+00
Raw cattle waste	1.00E+00	1.00E+00	1.00E+00	4.52E-02	3.40E-06

Chapter 6:

Conclusions

Tubular anaerobic digesters are being promoted in developing countries to treat livestock waste while generating biogas and recovering nutrients for use as fertilizers. For a tubular digester to meet a household's or communities' cooking energy needs, the substrate characteristics and operating parameters have to be understood for proper system design. Good performance of the tubular digester can then lead to a number of energy, environmental, public health, social and agricultural benefits. Unfortunately, the use of tubular digester effluents as a soil amendment may result in transmission of zoonotic pathogens if they are not sufficiently inactivated during digestion. This dissertation sought to understand (1) the environmental conditions and physical and biological processes occurring in tubular digesters treating livestock waste; (2) how the environmental conditions and the physical and biological processes in the tubular digesters affect the fate and viability of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts; and (3) whether the use of tubular digesters to treat livestock waste lowers the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts. This dissertation contains guidelines for designing and operating tubular digesters that can be used by engineers, development workers, public health workers and policy makers seeking to improve the quality of life for citizens in developing countries.

Field studies were carried out at small-scale farms in the Monteverde region of Costa Rica that used tubular digesters to treat swine waste and produce biogas. Significantly high removal efficiencies of BOD_5, VS, and *E. coli* and adequate biogas production rates were

observed in the tubular digesters. This was attributed to the formation of a biologically active floccular sludge layer, which resulted in separation of hydraulic residence time (HRT) and mean cell residence time (MCRT). Computational fluid dynamic (CFD) and bioprocess models were developed for one of the tubular digesters to evaluate the transport and transformation mechanisms in the digester. A reduced order model was validated through CFD modeling and field tracer study data to estimate a mean liquid HRT of 23 days. The reduced order model also validated the assumptions that the liquid phase of the tubular digester functioned as a CSTR, with turbulence as the main transport mechanism. The bioprocess model was based on a simplified floccular sludge layer reactor model and was used to estimate an average MCRT of 115 days. A mean liquid HRT of 23 days combined with a MCRT of more than 100 days indicated a robust syntrophic relationship between the physical and biological processes. This good relationship led to low effluent organic matter concentrations and sufficient biogas production to meet households' energy demands.

Environmental conditions of four tubular digesters were investigated. Ambient temperatures (21-24°C), neutral pH and total ammonia nitrogen (TAN) concentrations below 250 mg NH_4^+-N/L were observed in the tubular digesters. Laboratory (oo)cysts inactivation studies were performed under similar conditions. Inactivation rate constants for *Cryptosporidium parvum* and *Giardia lamblia* (oo)cysts were 0.056 and 0.726 day^{-1}, respectively. These values were similar to inactivation rates observed in a reactor with PBS solution and similar TAN and VFA concentrations but were significantly higher than in a control reactor with only PBS solution. Due to the substantial settling and accumulation of solids that was occurring in the tubular digesters, it was important to determine the fraction of (oo)cysts that adhered to the digester biosolids or remained suspended in the liquid. An (oo)cysts solid-liquid phase

distribution study indicated that 60% of both (oo)cysts adhered to biosolids. A simplified tubular digester model was used to estimate the concentration of viable (oo)cysts in the digester effluents. (Oo)cysts adhesion to solids, total solids concentration in the digester and HRT were the main factors contributing to the modeled effluent concentration of viable (oo)cysts.

A QMRA model was developed to investigate the risk of infection from exposure to raw livestock waste and tubular digester effluent. This model was developed for two rural communities in Costa Rica. One community used tubular digesters to treat their swine waste while the other community was comprised of dairy farmers who did not treat their cattle waste. The risk of infection from *Cryptosporidium parvum* and *Giardia lamblia* was assessed for direct and indirect exposure pathways, fomite and soil contamination and crop contamination from runoff. There were three main contributing factors to the risk of infection from both parasites at the different exposure pathways. The first was the differences in the concentration of (oo)cysts in the raw cattle and swine waste. At the three exposure pathways, the risks related to cattle waste were greater than swine waste due to the higher concentration of (oo)cysts in the raw cattle waste compared to swine waste. Animal-specific risk management guidelines should be developed to reduce exposure to wastes from animals with high pathogen shedding rates. Second, the inactivation rates at the various exposure pathways were the main contributing factor to the risk of infection. The risk of infection at all exposure pathways decreased with increasing inactivation rates. In addition, *Cryptosporidium parvum* posed a greater risk than *Giardia lamblia* in all exposure pathways due to livestock shedding high loads of *Cryptosporidium parvum* oocysts and oocysts' lower inactivation rates during anaerobic digestion compared to *Giardia lamblia* cysts. Lastly, it was noted that in the community using tubular digesters to treat livestock waste, the risk of infection from exposure to contaminated soil and crops was significantly lower compared

to the community where livestock waste was applied to soil untreated. This indicates that treatment of livestock manure in small-scale tubular digesters has the potential to significantly decrease the risk of infection below the World Health Organization's acceptable individual annual risk of infection (10^{-4}).

This dissertation provides information on how the use of tubular digesters to treat livestock waste can lower the risk of infection from pathogens. The physical, biological and QMRA models developed for this research were simplified to model the dynamic and complex mechanisms in tubular digesters. This encourages their use by engineers, development workers and public health workers to predict biogas production, (oo)cysts inactivation, (oo)cysts concentrations in tubular digester effluents, and the risk of infection from *Cryptosporidium parvum* and *Giardia lamblia*. However, additional research is recommended to verify inactivation of (oo)cysts at different exposure pathways and to validate the biological model used to estimate the MCRT. This research will also aid communities in developing countries that are greatly affected by lack of education, lack of resources, lack of clean water and waste management systems. Impoverished communities need to be empowered (especially women and girls) by providing them with a well designed technology that provides energy, environmental, social, economic, public health and agricultural benefits. As a result of this type of research and technology, women and girls are enabled to pursue other necessities such as farming, entrepreneurship, or an education. The benefit of empowering a woman is she empowers her household which trickles "up" to empowered nations.

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Appendices

Appendix A Bioavailability of Organic Carbon in Anaerobically Digested Swine Waste[5]

A.1 Introduction

Increasing demand for meat worldwide has led to the construction of large concentrated animal feeding operations (CAFOs) for livestock. Pigs make up 40% of the world's meat demand, and swine waste presents a number of problems for CAFOs (Choi, 2007). Untreated swine waste contains organic matter, nutrients such as nitrogen and phosphorus, suspended solids, pathogens, odorous volatile compounds, trace elements, and other chemicals of concern (Bernet and Beline, 2009; Choi, 2007). Land application is a common method for disposing of CAFO waste in the United States (US) and European Union (EU) (Bernet and Beline, 2009); however, waste produced in CAFOs often exceeds the amount that can be used directly on the land without causing a strain to the environment. Anaerobic lagoons are also commonly used for swine waste treatment. Although these systems are inexpensive, they have high land requirements and contribute to greenhouse gases (GHG) emissions and eutrophication of receiving waters (Moser, 1998).

In an effort to reduce GHG emissions and improve waste management, the USEPA requires that CAFOs limit land application of waste and the use of uncovered and unlined anaerobic lagoons (USEPA, 2008). Therefore, farmers are seeking alternative waste treatment technologies such as anaerobic digestion. One major advantage of using anaerobic digestion is that the biogas produced is captured and can either be utilized to produce green renewable energy for use on farms to heat water or buildings, or to generate electricity, which can be used on site or sold to power companies (Westerman, 2008). Although anaerobic digestion is a potential solution to land application and anaerobic lagoons, centrate from anaerobic digestion is

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rich in organic nitrogen and ammonia that can cause eutrophication in receiving waters. Therefore, further treatment for nitrogen removal is becoming increasingly common.

Biological nitrogen removal (BNR) systems, similar to those that have been applied to treatment of municipal wastewater, can be used to remove nitrogen from the centrate produced by anaerobic digestion of livestock wastes (Park et al., 2007; Bortone et al., 2009; Rajagopal et al., 2011). During the denitrification step of BNR processes, organic carbon is needed as an electron donor and carbon source for denitrifying bacteria. Centrate from anaerobically digested swine waste contains organic carbon that has the potential to serve as an internal organic carbon source for denitrification. Indeed, several prior studies have been conducted on the use of centrate from anaerobically digested swine waste as a carbon source for denitrification (Font et al., 1997; Boursier et al., 2005; Obaja et al., 2005; Park et al., 2009; Rajagopal et al., 2011).

The issue with using the organic carbon in anaerobic digester centrate as an internal organic carbon source for denitrification is that much of this organic carbon is not readily biodegradable, and hence limits the rate and extent of denitrification. Boursier et al. (2005) and Obaja et al. (2005) found that centrate from anaerobic digestion of swine waste did not adequately support denitrification because the centrate did not supply sufficient readily biodegradable organic carbon. However, Obaja et al. (2005) and Park et al. (2009) were able to achieve better than 90% removal of nitrogen by supplying additional volatile fatty acids (VFAs) as a source of readily biodegradable organic carbon. The adequacy of the supply of readily biodegradable organic carbon can be quantified by the ratio of biodegradable COD to the concentration of nitrate (as nitrogen). Boursier et al. (2005) suggested that denitrification requires a ratio of at least 5.0 g COD / g N, which is higher than the COD/N ratio of 2.86 that can be calculated based on stoichiometry relationships.

The bioavailability of organic carbon in the centrate for denitrification depends on the operational parameters used in the anaerobic digestion system. Specifically, solids retention time (SRT) is one of the main parameters that affect the biodegradable COD to N ratio. Kuo et al. (1996) found that with increasing SRT, more readily biodegradable COD was produced in the form of VFAs. Similarly, De Lucas et al. (2000) found that increasing SRT in an anaerobic digester treating synthetic wastewater increased the readily biodegradable COD fraction in the centrate by 37.5%. The authors attributed this to the microorganisms having more time to hydrolyze slowly biodegradable COD to readily biodegradable COD. Boursier et al. (2005) suggested that operating an anaerobic digester at an SRT of 40–60 days would provide a suitable ratio of biodegradable COD to N. Thus, based on previous literature, it can by hypothesized that increasing SRT in the digester leads to an increase in the ratio of biodegradable COD to N, and thereby improves the centrate's ability to serve as a substrate for denitrification.

However, lengthening the SRT will affect other aspects of a digester's performance. The SRT that is best for providing readily biodegradable COD might not be best for removing solids or producing methane (CH_4), for example. To the best of our knowledge, there has been no previous study that has examined the simultaneous effects of SRT on both digester performance and production of readily biodegradable COD.

Therefore, the objective of this paper is to quantify the effects of SRT on biogas production, CH_4 yield, removal of VS, concentration of readily biodegradable COD, and subsequent rates of denitrification during the anaerobic digestion of swine manure. The rationale is that quantifying these simultaneous effects may enable us to identify a favorable SRT at which centrate can be used as a substrate for denitrification, while still providing desired removal of VS and production of valuable CH_4.

A.2 Methods and Materials

A.2.1 Anaerobic Digesters

Bench-scale anaerobic digesters were initially inoculated with seed sludge from an anaerobic digester treating food waste in the laboratory of Dr. Ann Wilkie in the Department of Soil and Water Science at the University of Florida in Gainesville, FL. Digester influent was prepared by blending swine waste with groundwater to achieve a target VS concentration of 51 g/L. Swine waste was collected weekly from Twenty Four Rivers Farm in Plant City, FL. Groundwater used for this study contained micronutrients such as calcium, magnesium and iron that are beneficial for anaerobic microorganisms' growth (Gerardi, 2003). During start-up, varying influent VS concentrations were tested to determine efficient biogas production without total ammonia nitrogen (TAN) inhibition. It was found that 51 g VS/L produced stable digester performance and this value is also in the range used by other authors (Choi, 2007). Characteristics of the influent swine waste are shown in Table A.1.

Bench-scale anaerobic digesters were constructed using 2-L glass bottles equipped with rubber stoppers and tubing for gas release. A working volume of 1.5L was maintained in each reactor. The digesters were operated at 14, 21, 28 and 42 day SRTs, resulting in organic loading rates (OLR) of 3.6, 2.4, 1.8 and 1.2 (kg VS)/(m^3.d), respectively. Digesters were managed in semi-continuous mode (fed three times per week), continuously mixed and incubated at 35 °C using a Gyromax 727 orbital shaker incubator (Lafayette, CA). Reactor pH was maintained between 7.0-7.4 by addition of 3.0 N NaOH as needed. Influent and effluent samples were collected weekly and measurements of total nitrogen (TN), total phosphorus (TP), COD, VFA, alkalinity, TAN, VS, and total solids (TS) were performed as described below. Results shown in

Table A.1 and A.2 represent averages of weekly sample analysis during the 12 weeks of operation.

A.2.2 Respirometry

The respirometric assessment of readily biodegradable COD concentrations in the anaerobically digested centrate was performed using a pulse flow (PF-8000) respirometer system from Respirometer Systems and Applications (RSA) LLC (Springdale, AK). The oxygen uptake rate (OUR) test procedure, which measures the oxygen used by microorganisms for respiration is described in detail in Kinyua (2013). Briefly, the laboratory set-up consisted of 0.15 L test vessels operated in batch mode. Each test vessel was seeded with mixed liquor volatile suspended solids (MLVSS) from the *Hillsborough County* Northwest *Regional Water Reclamation Facility (NWRWRF)* in Odessa, FL to achieve a TS concentration of 3.0±0.5 g/L. *NWRWRF* employs a 5-stage Bardenpho process for single sludge BNR. Each test vessel also received centrate from the bench scale anaerobic digesters to reach a food-to-microorganisms (F/M) ratio of 0.67 mg centrate COD/mg biomass VSS. The respirometer was used to measure the oxygen uptake rate (OUR) over time in units of (mg O_2)/(L·hr) in each test vessel for at least 14 hours. Based on the shape of the curves, the measured OUR over the first 4 hours of the test was then integrated to calculate the concentration of oxygen consumed during that 4-hour period. This calculated concentration was used as one estimate of readily biodegradable COD in the centrate.

A.2.3 Denitrification Kinetics

The effect of anaerobic digester SRT on the rate of denitrification was investigated using a microcosm study. Microcosms were set up in duplicate in 100 mL glass serum bottles. Centrate from the bench scale anaerobic digesters and MLVSS from the NWRWRF were added to the

microcosms to achieve a F/M ratio of 1.0 mg centrate COD/mg biomass VSS. Microcosms were initially purged with nitrogen gas for 10 minutes, and then spiked with a stock NO_3^- solution to achieve a NO_3^--N concentration of 1 g/L. Nitrification inhibitor (Formula 2533, Hach company, Loveland, CO), was added to the microcosms at a concentration of 0.05 mg/L to prevent interference from nitrification. Microcosms were incubated at room temperature (20 °C) on a VWR OS-500 shaker table for 8 hours. Samples were collected hourly and analyzed for NO_3^--N concentrations as described below.

A.2.4 Analytical Methods

Centrate was obtained by centrifuging effluent samples from the bench-scale digesters for 10 minutes at 3500 rpm using a Thermo scientific CL2 centrifuge (West Palm Beach, FL). *Standard Methods* (APHA, 2012) were used to measure TN (4500- NO_3^- E and 4500-P E), TP (4500-P J), COD (5200 B), alkalinity (2320 B), NO_3^--N (4500- NO_3^--B), VS, and TS (2540 G). The TAN and VFA testing methods were adapted from literature as described by Kinyua (2013). The TAN testing method was adapted from Willis et al. (1996), with modification of color reagent storage time. VFA concentrations were measured using the method described by Montgomery et al. (1962), with modification of spectophotometer wavelength to 500nm. Method detection limits (MDL) were (mg/L): 0.7 for TN, 0.04 for TP, 30 for COD, 0.7 for TAN and 14 for VFA.

Biogas volume was measured using wet tip gas meters (Wayne, PA). CH_4 content of the biogas was measured using a Gow Mac Instrument Co. gas chromatograph (GC) (Bethlehem, PA) equipped an 8’ x 1/8” stainless steel Molesieve column and a thermal conductivity detector. Helium was used as the carrier gas at a flow rate of 30mL/min. Injector temperature during analysis was set at 120° C, while the detector and column temperatures were both set at 80° C.

The current was maintained at 80mA. The GC was calibrated using mixtures of CH_4 and CO_2 with known composition.

A.2.5 Data Analysis

The steady state anaerobic digestion model 1 (ADM1) described by Sötemann et al. (2005) was used to estimate the rate of hydrolysis of the swine waste solids in anaerobic digestion. The ADM1 model, which is based on Monod kinetics simplifies the dynamic and complex relationships of microorganisms during anaerobic digestion by using hydrolysis as the rate-limiting step. This allowed for better understanding of the effect of SRT on hydrolysis rates in the digesters. Statistical analysis was performed using one-way analysis of variance (ANOVA) test using GraphPad Prism version 6.0 for Windows 7 (GraphPad Software, San Diego California USA, www.graphpad.com). *p* values less than 0.05 were considered statistically significant and values less than 0.0001 were considered extremely significant.

A.3 Results and Discussion

A.3.1 Overall Anaerobic Digesters Performance

Average performance over the 12-week study period for the four reactors operating at different SRTs and OLRs is shown in Table A.2. The % VS removal, CH_4 yield, CH_4 content, and CH_4 production of the digesters are compared in Figure A.1. Overall hydrolysis rates, which were calculated using ADM1, are compared in Figure A.2. From these results, three main conclusions can be drawn. First, the performance of all four digesters was very good compared to prior results in the published literature for anaerobic digestion of swine manure. Kaparaju and Rintala (2005), and Ndegwa et al. (2005) observed soluble COD removal in the range of 49-73% while this study achieved approximately 71- 75% removal in all digesters (Table A.1 and A. 2).

VS removal for this study was about 51-60% for all digesters (Table A.1 and A.2), while other authors only reported 33-52% removal (Kaparaju and Rintala, 2005; Sanchez et al., 1995). The good performance observed may have been due to an absence of free ammonia (FA) inhibition in the digesters. TAN concentrations were below the typical range (1.24-1.70 g NH_4^+-N/L) for anaerobic digestion of swine waste (Choi, 2007; Nuchdang and Phalakornkule, 2012) and below 1.7 g NH_4^+-N/L, which has been observed to be an inhibitory concentration for anaerobic digestion (Gerardi, 2003). The relatively low TAN concentration in the influent (Table A.1) may have been due to swine farm management practices. Since only the feces portion of the swine waste was collected, it is likely that some of the urine, which contains most of the TN in swine waste, drained into the soil in the pig barns. At 35°C and the average pH values of the digesters (Table A.1), calculated FA concentrations for all four digesters were 3.3, 4.2, 12.3 and 11.0 mg N/L for the 14-, 21-, 28- and 42- day SRT digesters respectively. These values were lower than those reported to be inhibitory in prior literature (Angelidaki and Ahring 1993; Hansen et al., 1998).

Moreover, even though the VFA concentrations for the 14-, 21- and 42-day digesters were higher than values (0.1-0.4 g COD/L) previously reported to be inhibitory to methanogenesis (Ndegwa et al., 2005), biogas production and CH_4 yield were consistently high in the digesters. It appears that there was enough alkalinity to provide buffering capacity, and the pH did not decrease significantly despite the production of VFAs. pH and alkalinity values during the 12 weeks of operation were within the range favorable to methanogens (Gerardi, 2003). The VFA-to-alkalinity ratios for the 14-, 21-, 28- and 42- day SRT digesters were 0.3, 0.2, 0.1 and 0.2, respectively. The recommended VFA-to-alkalinity ratio is 0.1-0.2, with a ratio

greater than 0.5 causing complete system failures (Gerardi, 2003). All but the 14-day SRT digester were within this recommended VFA-to-alkalinity ratio.

The second main conclusion is that, the digester operated at a 21-day SRT had the best overall performance. For some performance metrics, such as % VS removal, total COD removal, and soluble COD removal, the differences between the reactors were not meaningful at a statistically significant level. However, CH_4 yields between the four digesters were significantly different (p =0.0003) based on the highest and lowest yields, with the digester operated at a 21-day SRT having the highest average CH_4 yield (Figure A.1). The 21-day SRT digester also had the highest % CH_4 content in the biogas and CH_4 production (Figure A.1).

Third, the SRT of the digesters affected CH_4 yield. The digester with the lowest SRT had the lowest CH_4 yield despite having a high rate of hydrolysis (Figure A.2) and a VS removal similar to that of the other digesters. This indicates that microorganisms in the 14-day SRT digester had time to metabolize the solid substrates into organic acids but did not have adequate time to convert the organic acids into CH_4. High variability in CH_4 yield and production was also observed in the 14-day SRT digester, as shown by the error bars in Figure A.1. This may have been due to having an SRT too close to the minimum SRT required to prevent washout of slow growing methanogens and/or its high VFA-to-alkalinity ratio. In addition, the digester with the longest SRT had the lowest CH_4 production rate, most likely due to the low OLR and slower hydrolysis rate compared to the other digesters (Figure A.2) however, this was not statistically significant.

A.3.2 Bioavailability of COD in Centrate

A key objective of this paper is to quantify the effect of digester SRT on the ability of the digester centrate to serve as an internal organic carbon source for denitrification. To make this

assessment, three types of analyses were performed: respirometric measurements of readily biodegradable COD (which can serve as a suitable substrate for denitrification) in the centrate, microcosm denitrification tests using centrate as an electron donor, and measurement of the concentrations of volatile fatty acids (VFAs) in the centrate.

A.3.2.1 Respirometric Measurement of Readily Biodegradable COD

An example of an OUR curve produced during the respirometry test is shown in Figure A.3. A period of 4 hours was chosen to calculate the readily biodegradable COD because the first 1-4 hours have been found to be the time during which aerobic microorganisms have sufficient substrate for growth (Ekama et al., 1986). The average readily biodegradable COD concentrations for the 4 reactors were 1.9, 1.7, 1.8 and 1.9 g COD/L for the 14-day, 21-day, 28-day, and 42-day SRT digesters, respectively.

A.3.2.2 Denitrification Microcosm

Results of the microcosm tests are shown in Figure A.4. Nitrate removal appears to have occurred in three phases, which is a trend previously observed when a complex organic carbon source is used for denitrification (Henze et al., 1999; Sage et al., 2006). In the first phase of denitrification, readily biodegradable COD in the centrate is utilized, followed by slowly biodegradable COD in the second phase. In the third phase, endogenous organic carbon is utilized (Henze et al., 1999; Sage et al., 2006; Fernández-Nava et al., 2010). The highest denitrification rate was observed during the first 3–4 hours, as shown in Figure A.4, suggesting that readily biodegradable COD is consumed in about 3–4 hours. This supports the choice made in the analysis of OUR tests (Section A.2.1, above) to integrate the OUR over the first four hours.

Phase 1denitrification rates using centrate from the four digesters were significantly different (p <0.0001), and occurred at rates in the range of 47–56 (mg NO_3^--N)/(g VSS·hr) (Table A.3 and Figure A.4). The centrate from the 21-day-SRT digester had the highest denitrification rate. Maximum denitrification rates in this study were higher than those previously reported using centrate from anaerobic digestion; Bickers and van Oostrom, (2000) achieved 2.8–10.5(mg NO_3^--N)/(g VSS·hr), Obaja et al. (2005) reported 31.1–40.1 (mg NO_3^--N)/(g VSS·hr) and Fernández-Nava et al. (2010) had 41.6–46.8 (mg NO_3^--N)/(g VSS·hr).

A.3.2.3 Concentration of Volatile Fatty Acids

As shown in Table A.2, the average concentrations of VFAs in the centrate of the four digesters were 1.4, 1.1, 0.8, and 1.0 g COD/L. Concentrations of VFAs were measured as g acetate/L; however, concentrations are reported as g COD/L based on the theoretical oxygen demand of acetate for comparison to results above. For all four digesters, the concentration of VFAs was lower than the estimated concentrations of readily biodegradable COD reported above; the differences were statistically significant at a 95% confidence level. The wet chemistry method used to analyze the VFAs in the effluent is based on measuring the short-chain fatty acids such as acetate and propionate. However, the centrate may have also contained branched-chain fatty acids, such as iso-butyric and iso-valeric acids, which are produced during the anaerobic digestion of swine waste. These branched-chain fatty acids may have contributed to the readily biodegradable COD concentration, but were not detected by the analytical method used in this study (Wang et al., 1999).

A.3.3 COD Fractionation

The fractionation of COD in the four digesters is shown graphically in Figure A.5. Approximately 50-80% of the readily biodegradable COD was in the form of short-chain VFAs.

Approximating that denitrification requires 2.86 g of COD per g of NO_3^--N, it was possible to compute the concentrations of readily biodegradable COD in the centrate based on the concentration of NO_3^--N consumed during the first four hours of the tests (Figure A.4). The calculated readily biodegradable COD concentrations were 1.7, 1.8, 1.7 and 1.7 g COD/L for the 14-, 21-, 28- and 42-day-SRT digesters, respectively. These estimates agree well with the estimates made using respirometry, as shown in Figure A.5. The good agreement lends confidence that either method is suitable for estimating concentrations of readily biodegradable COD.

In addition to readily biodegradable COD, soluble COD consists of slowly biodegradable COD and soluble microbial products (SMP). Concentrations of SMP have been shown to increase with increasing SRT, as microorganisms have more time to convert the inert portion of COD to soluble products (Kuo et al., 1996). There are contradicting views on the biodegradability of SMP. Kuo et al. (1996) defined SMP as partially biodegradable, while Duran and Speece (1999) defined SMP as effluent organics that cannot be biologically transformed. For this study, SMPs were considered as part of the total slowly biodegradable COD, they neither need to go through hydrolysis to become readily biodegradable, nor are they easily utilized by microorganisms. By subtracting the average of the readily biodegradable COD from the respirometer and the microcosm study from the total soluble COD, total slowly biodegradable COD concentrations were estimated as 1.3, 0.8, 0.9 and 1.0 g COD/L for the 14, 21, 28 and 42 day digester, respectively. The results are consistent with the results of Boursier et al. (2005), who also found that anaerobically digested swine waste had a large fraction of slowly biodegradable COD.

A.3.4 Effect of SRT on Readily Biodegradable COD and Denitrification

Estimates of the concentration of readily biodegradable COD were similar for all four digesters. Based on respirometry, the concentration of readily biodegradable COD was estimated to range from 1.7–1.9 g COD/L. Based on microcosm tests, the concentration was estimated to range from 1.7–1.8 g COD/L. For this study, two phenomena were initially expected; first, at low SRT a higher readily biodegradable COD was expected due to a higher hydrolysis rate (Figure A.2); which favors the production of VFAs without providing sufficient time for methanogenesis. Second, a high SRT would allow more time for hydrolysis of VS, i.e., for the conversion of complex VS into a readily biodegradable form. However, surprisingly, the data did not support these assumptions. Concentrations of readily biodegradable COD, VFAs and soluble COD were similar for all four digesters, as can be seen in Figure A.5.

Previous researchers have suggested that a suitable denitrification potential, which is the ratio of biodegradable COD to N is 5 g COD/N. In this study, the denitrification potential for centrate from each digester was calculated by dividing the average readily biodegradable COD concentrations from the microcosm and respirometry tests by the average TAN concentration from each digester (Table A.3). TAN concentrations were used assuming that all the TAN would be converted to NO_3^- during the BNR process. Contrary to other authors (Boursier et al., 2005) even a biodegradable COD/N ratio (denitrification potential) below 5 gCOD/gN in reactors operated with a 28- and 42-day SRT did not affect denitrification rates. These results further proved that the centrate from these digesters could be apposite for denitrification and the biodegradable COD was sufficient for microorganisms' biosynthesis and endogenous respiration. These results also disproved our earlier hypothesis that increasing SRT in the digesters would improve the centrate's ability to serve as a substrate for denitrification.

Although using centrate from the 21-day-SRT digester produced the highest denitrification rate, the greatest overall NO_3^- removal efficiency after eight hours (>90%) was observed when centrate from the digester operated at a 42-day SRT was used in the microcosm studies (Table A.3). Denitrifying microcosm results with centrates from digesters operated at 14, 21, and 28 days indicate that supplemental carbon would be required to meet the target TN removal efficiency of 90% suggested for BNR of swine waste (Obaja et al., 2005). Therefore operation at an SRT of 42-days may be an attractive option if compliance with TN limits without supplemental carbon addition is a priority.

A.4 Conclusions

Excellent performance was observed for all four digesters, with VS removals greater than 60%, CH_4 yield between 0.1-0.3 m^3CH_4/kg VS added and CH_4 production rates between 0.3-0.8 m^3CH_4/m^3 reactor-day. The digester operated at a 21-day SRT had the highest average CH_4 yield and net energy production and would be favorable for COD removal and energy production. A 42-day SRT would be recommended if the priority is for a low effluent TN concentration without supplemental carbon addition.

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Table A.1: Characteristics of influent swine waste. Table indicates arithmetic average and standard deviation of data collected weekly over a 12-week period (n=12).

Parameter	Unit	Measured value
TS	g/L	75.8 ± 15
VS	g/L	50.8 ± 11
Alkalinity	g $CaCO_3$/L	2.9 ± 0.6
TAN	g NH_4^+-N /L	0.2 ± 0.1
Soluble TN	g N/L	1.3 ± 0.2
Soluble TP	mg P/L	506 ± 109
Soluble COD	g COD/L	10.6 ± 3.4
Total COD	g COD/L	56.8 ± 8.8
VFA*	g COD/L	3.7 ± 0.9
pH		7.6 ± 0.4

Table A.2: Average and standard deviations of performance data for four anaerobic digesters operated at varying OLR and SRT for 12 weeks (n=12).

Parameter	Unit	14-day		21-day		28-day		42-day	
OLR	kg VS/m^3-d	3.60		2.40		1.80		1.20	
TS	g/L	40.7	± 4.4	42.6	± 4.9	41.5	± 5.2	50.6	± 6.9
VS	g/L	24.9	± 2.4	25.1	± 2.2	24.1	± 2.7	28.1	± 3.3
Alkalinity	g $CaCO_3$/L	5.1	± 1.6	4.8	± 0.9	5.3	± 0.9	5.7	± 0.8
TAN	g NH_4^+-N /L	0.3	± 0.1	0.3	± 0.1	0.7	± 0.1	0.5	± 0.1
Soluble TN	g N/L	1.6	± 0.4	1.4	± 0.5	1.5	± 0.5	1.6	± 0.6
Soluble TP	mg P/L	340	± 90	222	± 59	177	± 44	124	± 25
Soluble COD	g COD/L	3.1	± 0.5	2.6	± 0.4	2.7	± 0.3	2.8	± 0.5
Total COD	g COD/L	39.1	± 3.9	38.8	± 4.3	37.6	± 4.5	41.3	± 2.9
VFA*	g COD/L	1.4	± 0.1	1.1	± 0.1	0.8	± 0.1	1.0	± 0.1
pH		7.0	± 0.1	7.1	± 0.1	7.2	± 0.1	7.3	± 0.1

Table A.3: Denitrification potential, denitrification rates and NO_3^--N concentrations in effluent during 8 hour microcosm test.

Digester	Denitrification potential	Denitrification rate			Effluent NO_3^--N
		Phase 1	Phase 2	Phase 3	
Day	**g COD/g N**	**mg NO_3^--N / g VSS-hr**			**mg NO_3^-N/L**
14	5.6	49.4	17.6	2.3	129.2
21	6.2	56.3	27.0	5.0	123.0
28	2.5	47.2	25.2	1.6	112.7
42	3.6	48.9	11.1	3.9	79.7

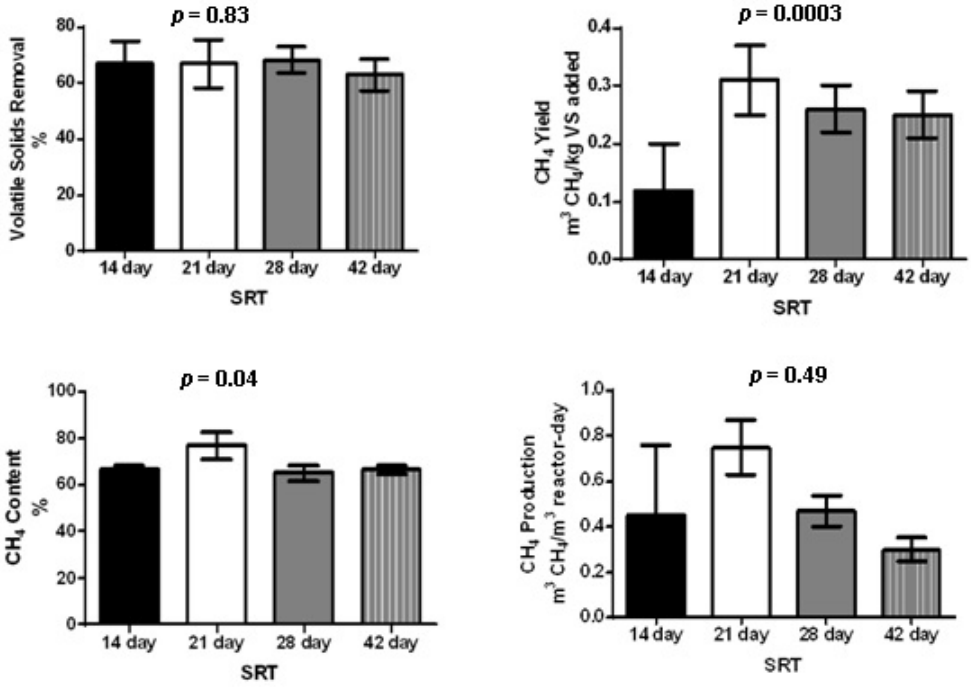

Figure A.1: Average performance data and *p* values for four 1.5 L reactors operated at varying OLR and SRT for 12 weeks.

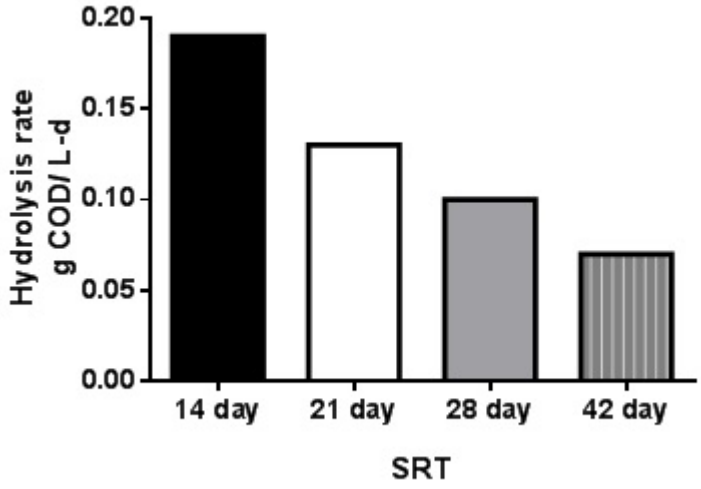

Figure A.2: Overall hydrolysis rates of swine waste solids in AD for four 1.5 L reactors operated at varying OLR and SRT for 12 weeks.

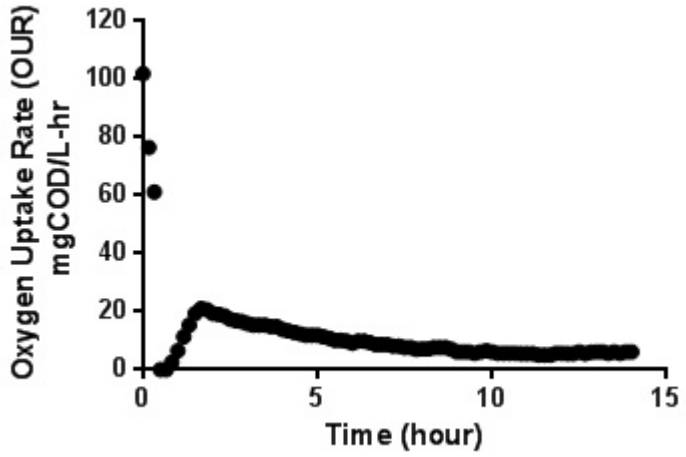

Figure A.3: Example of OUR curve during respirometer tests using the centrate from the 28 day SRT digester and MLVSS from NWRWRF.

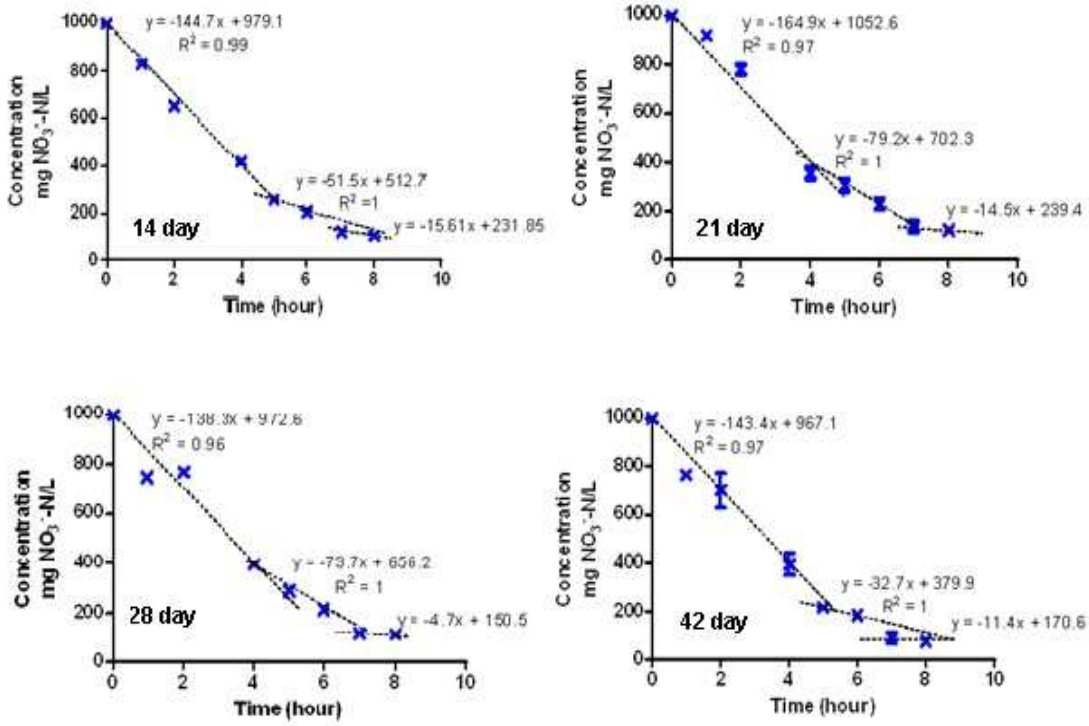

Figure A.4: Denitrification rate profiles for three phases using varying SRT digesters' centrate as carbon source.

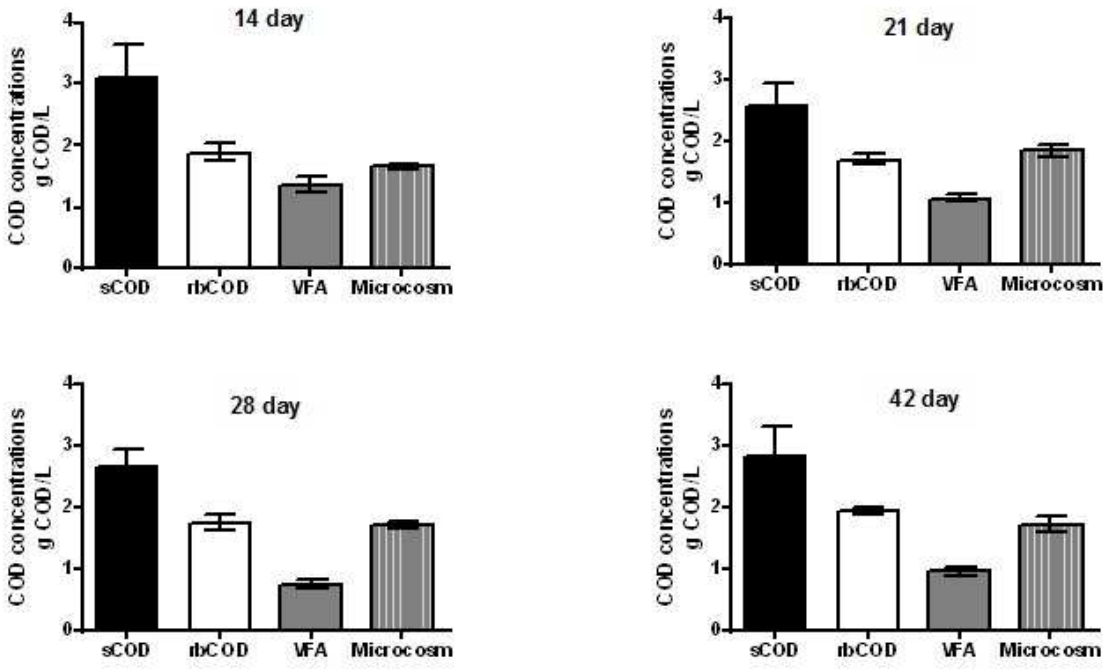

Figure A.5: Different COD fractions from varying SRT digesters' centrates. sCOD = soluble COD, rbCOD = readily biodegradable COD and microcosm = theoretical biodegradable COD from microcosm study

Appendix B Chapter 3 Supplementary Information

B.1 Volatile Solids Mass Balance

To verify that the estimated concentration of biomass (X_m) in the tubular digester was accurate, a steady state mass balance on volatile solids (X_b) was developed:

$$0 = \frac{dX_b}{dt}V = QX_{bi} - QX_{be} - K_H X_b V + K_d X_m V \qquad \text{(Eq. B.1)}$$

where X_{bi} is the influent VS concentration (3.64-7.98 g COD/L), X_{be} is the effluent VS concentration (0.43-1.38 g COD/L) and K_H is the kinetic hydrolysis rate (0.06-0.25/day). X_{bi} and X_{be} values were calculated based on the influent and effluent VS concentrations assuming 1.42 g COD/g VS. X_b was estimated at 2.05-2.55 g COD/L. The VS mass balance verified that the X_m value estimated by the biomass in the digester mass balance was accurate because the VS mass balance came to zero.

B.2 MCRT Frequency Distribution

The MCRT frequency distribution at a 95 percentile was estimated after a 1000 Monte Carlo trial run using Oracle Crystal Ball. This distribution is illustrated below.

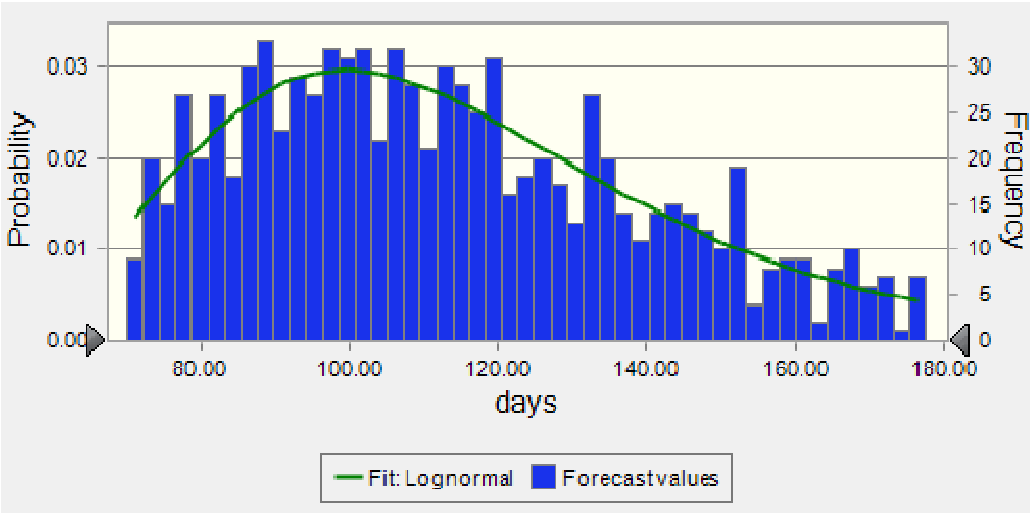

Figure B.1: MCRT frequency distribution from the Monte Carlo simulation. Mean = 115 days, Median = 110 days, Minimum = 52 days, Maximum = 265 days, Standard Deviation = 33.56.

Appendix C Chapter 4 Supplementary Information

C.1 Interview Questions and Answers for San Luis de Monteverde Farmers

Table C.1: Interview questions and answers for four San Luis de Monteverde farmers using tubular digesters.

How did you manage your livestock waste before you got a tubular digester?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
No treatment, went into pasture land	No treatment, went into pasture land	No treatment, went into pasture land	No treatment, went into pasture land
How many hours of cooking do you get each day with the biogas?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Not used for cooking	2-4 hours for 3 people	3 hours for 4 people	9 hours for 6 people
What kind of animals do you have and what are they for?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Pigs to provide pork for the university	Pigs for sale of piglets	Pigs for biogas production, cows for milk	Pigs for pork sale
How many of each do you have and what type; female, male, lactating, piglets?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
4 pigs (1-4 months old)	14 pigs 1 boar 3 sows 10 piglets	4 pigs (1-3 months old) 3 female 1 male 15 cows	10 pigs (1-6 months old) 6 female 4 male
How long do you spend cleaning the pig barn?			

Table C.1 (Continued)

Farmer 1	Farmer 2	Farmer 3	Farmer 4
15 minutes	10-15 minutes	30 minutes	1 hour
Does anyone else help with the maintenance of the biodigesters? If children, how old are the children?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Only one male farmer cleans the barn	Only one male farmer cleans the barn	Wife and one son clean the barn	Only one male farmer cleans the barn
Do you give the animals any de-wormers? If yes, could you please tell me, or can I see the de-wormer you use.			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
n/a	Yes, gives dewormers (decomax) every 4-5 months	Yes, gives dewormers (oxitetraciclina) when pigs have symptoms such as vomiting	Yes, gives dewormers when pigs show symptoms such as diarrhea
How many times in a day are you tending to the pigs beside the time spent cleaning the barn?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Cleans once a day in the morning	Cleans once a day in the morning	Cleans once a day in the morning	Mostly cleans once a day but some days may also clean in the afternoon
What do you do with the effluent? What crops are the farmers fertilizing with effluent?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Flows into the cattle pasture land	Uses effluent on root crops, corn and banana trees	Uses effluent on fruit trees and cattle pasture land	Uses effluent on root crops, corn and fruit trees
How many times a week do you spend tending to your crops fertilized with the tubular digester effluent?			

Table C.1 (Continued)

Farmer 1	Farmer 2	Farmer 3	Farmer 4
None	After planting corn doesn't tend to the soil very often	n/a	Depends on the season, during the dry season, husband and wife tend to the soil almost everyday
Do you wash your of hands after work on the pig barns and tending to crops?			
Farmer 1	Farmer 2	Farmer 3	Farmer 4
Yes because they milk the cows after cleaning pig barn	Sometimes	Sometimes	Yes, prefers to use gloves

C.2 Floccular Sludge Characteristics

In addition to Digester 1, floccular sludge from Digesters 3 and 4 was also analyzed for TSS, VSS and SVI. Table C.2 is summarizes results from these two digesters.

Table C.2: TSS, VSS and SVI from Digesters 3 and 4.

Parameter	Unit	Digester 3	Digester 4
SVI	mL / g TSS	10.9	2.36
TSS	g TSS/L	88.0	420
VSS	g VSS/L	0.001	0.02

C.3 Tubular Digester Model

A polynomial distribution of (oo)cysts was extrapolated to estimate the concentration of (oo)cysts in the tubular digester influent (oo)cysts/g TS. Figure C.1 illustrates the polynomial extrapolation of data for pigs (oo)cysts shedding for 19 days used to estimate values for $C_{0\,(t)}$ for each digester.

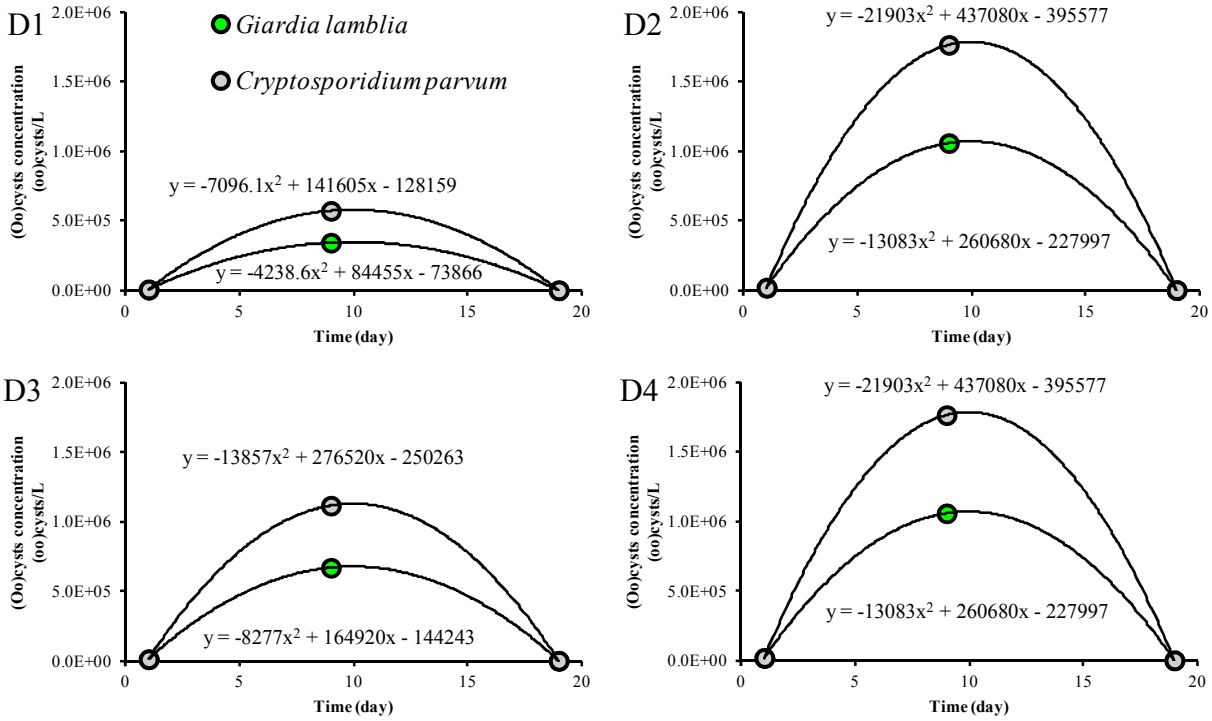

Figure C.1: Polynomial extrapolation of data for pigs (oo)cysts shedding for 19 days.

C.4 Partitioning of *C. parvum* and *G. lamblia* during Anaerobic Digestion

Over the course of three weeks, the partitioning of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts in anaerobic digesters fed with swine manure was studied. Six 50 mL microcosms were prepared using effluent from Reactor 1 (Chapter 4). The microcosms were mixed by inverting at least ten times each day to simulate daily feeding and completely mixed conditions. On each sampling day, three samples from the liquid supernatant and three samples from the settled sludge in one of the microcosms were collected, and (oo)cysts were enumerated

to determine partitioning. It should be noted that these partitioning results assume that the recovery rate in the solid phase is the same as that in the liquid phase.

Averages and standard deviations of partitioning results for each sampling day are shown in Table C.3. Results for each sampling day varied considerably, as shown by the large standard deviations, however, the partitioning of both types of (oo)cysts was similar. Despite the significant variations on each sampling day, a pattern did appear to be present over time. On day 0, most (oo)cysts were present in the solid phase, while the percentage of (oo)cysts in the liquid phase increased on the following day, this could be attributed to mixing. On subsequent sampling days, the percentage of (oo)cysts in the solid phase slowly increased but did not return to the high level observed on day 0. Again, this difference with the initial measurement may be due to daily mixing, while the slow increase in the solid phase could suggest that the (oo)cysts become more strongly associated with the solids over time. The changes over time can be seen in Figure C.2, in which linear regression lines show the trends from Day 1 to Day 21. The results from Day 3 (highlighted in Table C.3) are not included in Figure C.2, since these results are drastically different from the pattern exhibited on other days. Day 3 appears to be an outlier.

Table C.3: Partitioning of *C. parvum* and *G. lamblia* (oo)cysts over time n=3.

	***Cryptosporidium parvum* oocysts**		***Giardia lamblia* cysts**	
Day	Liquid Phase	Solid Phase	Liquid Phase	Solid Phase
0	29 ± 24%	71 ± 18%	28 ± 26%	72 ± 23%
1	46 ± 33%	54 ± 15%	56 ± 41%	44 ± 18%
3	12 ± 3%	88 ± 41%	11 ± 0%	89 ± 47%
7	39 ± 13%	61 ± 10%	39 ± 15%	61 ± 19%
14	39 ± 11%	61 ± 24%	40 ± 13%	60 ± 32%
21	35 ± 23%	65 ± 18%	35 ± 26%	65 ± 19%

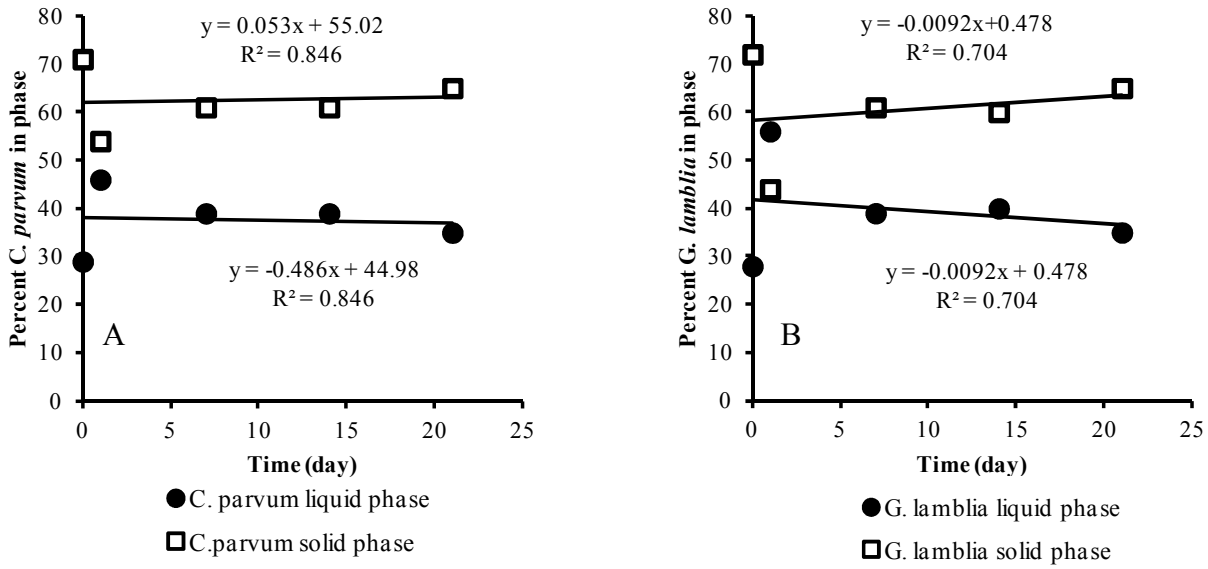

Figure C.2: Average partitioning in liquid and solid phases over time. (A) Partitioning of *Cryptosporidium parvum* oocysts; (B) partitioning of *Giardia lamblia* cysts.

Appendix D Interview Questions and Answers for La Florida Farmers

Table D.1: Interview questions and answers for dairy farmers in La Florida

What kind of livestock do you have and what are they for?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Dairy cows							
How many of each? What type, male, lactating, calves?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Females = 20 Males = 1 Calves = 5	Females = 25 Males = 0 Calves = 4	Females = 25 Males = 1 Calves = 0	Females = 45 (10 pregnant) Males = 1 Calves = 28-30	30 total	Females = 40 Males = 0 Calves = 6	Females = 32 Males = 0 Calves = 20	35 total
Do you keep the animals in a barn or are they free range							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
All the cattle are free range, the cows are only in the barn twice a day for milking							
How do you maintain your cattle manure?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Spreads manure on cattle pasture land as fertilizer. Does not handle the manure, washes it out of the barn after milking		Composts manure that is collected after milking cows	Puts manure in a wheel barrow after milking cows and disposes of it in the pasture land or Washes the manure from the barn after milking, manure drains into the cattle pasture land				

Table D.1 (Continued)

Do you ever come into contact with the manure after washing the barn or tending to soil?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Doesn't use manure on any crops and doesn't tend to soil where the cows graze							
Do you use any energy source on your dairy farm?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Uses electricity for milking and cooling the milk							
How much do you spend on electricity each month?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
₡ 85,000 colones = $160	₡ 70,000 colones = $132	₡ 70,000-85,000 colones = $132-160	₡ 120,000 colones = $226	n/a	₡ 137,000 colones = $258	n/a	n/a
What energy source do you use at home for cooking?							
Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	Farmer 6	Farmer 7	Farmer 8
Households use propane gas for cooking, costs about $20-40 a month							

Appendix E List of Acronyms

ADB	Asian Development Bank
ADM1	Anaerobic Digestion Model 1
ALRI	Acute Lower Respiratory Infections
BOD_5	Five day Biochemical Oxygen Demand
C/N	Carbon Nitrogen ratio
CFD	Computational Fluid Dynamic
COD	Chemical Oxygen Demand
COPD	Chronic Obstructive Pulmonary Disease
CSTR	Completely Stirred Tank Reactor
DAPI	4', 6-diamidino-2-phenylindole
F/M	Food-to-Microorganism
FITC	Fluorescein Isothiocyanate
FOG	Fats, Oils and Grease
GHG	Greenhouse Gases
HRT	Hydraulic Retention Time
LCFA	Long Chain Fatty Acids
LPG	Liquefied Petroleum Gas
MCRT	Mean Cell Retention Time
N/P	Nitrogen Phosphorus ratio
NGO	Non-Governmental Organization
NREL	National Renewable Energy Laboratory
OLR	Organic Loading Rate
PBS	Phosphate-Buffered Saline
PI	Propidium Iodide
PRC	People's Republic of China
QMRA	Quantitative Microbial Risk Assessment
RANS	Reynolds-Averaged Navier-Stokes
RTD	Residence Time Distribution
SRT	Solids Retention Time
STP	Standard Temperature Pressure
SVI	Sludge Volume Index
TAN	Total Ammonia Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VOC	Volatile Organic Compounds
VS	Volatile Solids
WHO	World Health Organization
WTE	Waste to Energy

Appendix F List of Equation Nomenclature

F.1 List of Equation Nomenclature used in Chapter 3

μ_t	Eddy viscosity	$N \cdot s/m^2$
C	Ensemble-averaged tracer concentration	g KCl/L
C'	Tracer fluctuation and flux	g KCl/L
C_d	Tracer concentration in the digester	g KCl/L
C_{in}	Influent tracer concentration	g KCl/L
C_o	Initial tracer concentration in the digester	g KCl/L
C_{out}	Effluent tracer concentration	g KCl/L
D_t	Eddy (turbulent) diffusivity	m^2/s
k	Turbulent kinetic energy	J/Kg or m^2/s^2
K_d	Biomass cell decay coefficient	day^{-1}
K_m	Maximum specific substrate utilization rate	g COD utilized/ g biomass COD-day
K_S	Biomass half saturation constant	g COD/L
$\dot{m}$	Daily methane production rate	g COD/day
n	Current day	day
p	Reynolds-averaged pressure	Pa
Q	Flow	L/day
S_b	Soluble substrate concentration	g COD/L
S_{CH4}	Methane content	%
Sc_t,	Schmidt number	dimensionless
t	Time	day or hour
T	Flow-through period	minute
u_i	Reynolds-averaged velocity	m/s
v	Kinematic viscosity	m^2/s
V	Volume	L
x_i	Position	m
X_m	Active digester biomass concentration	g COD/L
X_{me}	Active biomass in effluent concentration	g COD/L
X_{mi}	Active biomass in influent concentration	g COD/L
Y_m	Biomass yield coefficient	g biomass COD/g COD utilized
ε	Turbulent kinetic energy dissipation rate	m^2/s^3
ρ	Density	Kg/m^3

F.2 List of Equation Nomenclature used in Chapter 4

$C_{0(t)}$	(Oo)cysts concentration in the influent	(oo)cysts/L
C_L	Percent viable (oo)cysts measured at time t	%
C_t	(Oo)cysts concentration in the digester	(oo)cysts/L
$C_{t+\Delta t}$	Concentration of (oo)cysts in the tubular digester effluent	(oo)cysts/L
C_V	Percent of viable (oo)cysts at time t=0 days	%

k	Inactivation rate constant	day^{-1}
K_d	Distribution coefficient	L/g TS
N	Total number of (oo)cysts in the digester	(oo)cysts
Q	Flow rate	L/day
S	Degree of adsorption of (oo)cysts to the solids	(oo)cysts/g TS
t	Time	day
TS_F	TS concentration in the digesters' floccular sludge layer	g TS/L
V	Tubular digester working volume	L

F.3 List of Equation Nomenclature used in Chapter 5

A_{de}	Application rate of tubular digester effluent	L/ha
A_{rm}	Application rate of raw cattle waste	Mg/ha
C_c	(Oo)cysts concentration on contaminated leafy crops	(oo)cysts/kg food
C_{de}	(Oo)cysts concentration in tubular digester effluent	(oo)cysts/L
C_{ep}	(Oo)cysts concentration at each exposure pathway	(oo)cysts/exposure pathway
C_f	Fomite (oo)cysts concentration	(oo)cysts/fomite
C_i	Concentration of soil ingested during occupational activities or leafy crops consumed per day	g soil/day or g leafy crops/day
C_p	(Oo)cysts concentration per hectare	(oo)cysts/ha
C_{rm}	(Oo)cysts concentration in the raw cattle and swine waste	(oo)cysts/g TS
C_s	(Oo)cysts concentration in soil	(oo)cysts/kg soil
d	Dose	(oo)cysts/dose
D_r	(Oo)cysts dilution ratio in the runoff water	
D_s	Soil dilution ratio	L of digester effluent/ g of soil
F_r	Fraction of (oo)cysts available for runoff	
F_{rm}	Raw waste transferred to a fomite	g/fomite
K_c	(Oo)cysts inactivation rates on leafy crops	log removal/day
K_f	(Oo)cysts inactivation rates on a fomite	log removal/day
K_s	(Oo)cysts inactivation rates in soil	log removal/day
K_w	(Oo)cysts inactivation rates in water	log removal/day
r	Probability of a single agent causing infection	
T_c	Percent attachment of (oo)cysts from the water to crops	%
T_h	Percent transfer of (oo)cysts from fomite to hand	%
T_m	Percent transfer of (oo)cysts from hand to mouth	%
T_r	Percentage of (oo)cysts once applied to the soil will be transferred to the runoff water	%
T_w	Percentage of soil particles that remain on the crops after washing	%

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