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Anaerobic digestion (AD) is an attractive technology that can be used to recover energy (in form of methane in biogas) and nutrients from waste poultry blood. One challenge with treating high-strength high-protein wastes is the production of total ammonia nitrogen (TAN) during decomposition. The TAN severely inhibits methane producing bacteria and results in accumulation of fatty acids, which destabilizes the digester and causes AD process failure. This dissertation proposed and examined several ways to manage these drawbacks, namely, use of biocarriers, two-stage process, and struvite precipitation. Results show that a single-stage anaerobic digester filled with biochar has

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Report Title

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Anaerobic digestion (AD) is an attractive technology that can be used to recover energy (in form of methane in biogas) and nutrients from waste poultry blood. One challenge with treating high-strength high-protein wastes is the production of total ammonia nitrogen (TAN) during decomposition. The TAN severely inhibits methane producing bacteria and results in accumulation of fatty acids, which destabilizes the digester and causes AD process failure. This dissertation proposed and examined several ways to manage these drawbacks, namely, use of biocarriers, two-stage process, and struvite precipitation. Results show that a single-stage anaerobic digester filled with biochar has methane yield of 82 mL g-1 CODadded, and maximum volumetric biogas production of 0.64 L L-1d-1, when operated at 35oC and OLR of 4.7 g COD L-1 d-1. Two-stage anaerobic digesters (127-L) were tested for treating poultry blood wastes over a 400-d period. Digesters (operated at 26oC) had higher methane yield (189 mL g-1 CODadded, at OLR of 0.4 g COD L-1 d-1) than results from the literature on tests on similar feedstocks using single-stage digesters. The use of bamboo biocarriers improved the performance of two-stage digesters resulting in methane production of 361 mL g-1 CODadded, at OLR of 0.4 g COD L-1 d-1. Struvite precipitation (SP) removed more than 70% of TAN from effluents of the acidogenic
digesters and showed an improved methane yield in subsequent biochemical methane potential (BMP) tests. The best performing reagent combination for SP was found to be Mg(OH)2 and H3PO4, which resulted in 74.1% nitrogen recovery and subsequently 29.4% increase in methane production (measured via a BMP). Results of this dissertation show that optimization methods reduce the impact of TAN inhibition of the AD process treating poultry blood wastes.

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by

SHUNLI WANG

(Under the Direction of Keshav C. Das)

ABSTRACT

Anaerobic digestion (AD) is an attractive technology that can be used to recover energy (in form of methane in biogas) and nutrients from waste poultry blood. One challenge with treating high-strength high-protein wastes is the production of total ammonia nitrogen (TAN) during decomposition. The TAN severely inhibits methane producing bacteria and results in accumulation of fatty acids, which destabilizes the digester and causes AD process failure. This dissertation proposed and examined several ways to manage these drawbacks, namely, use of biocarriers, two-stage process, and struvite precipitation. Results show that a single-stage anaerobic digester filled with biochar has methane yield of 82 mL g^{-1} COD_{added}, and maximum volumetric biogas production of 0.64 L $L^{-1}d^{-1}$, when operated at 35^{o}C and OLR of 4.7 g COD L^{-1} d^{-1}. Two-stage anaerobic digesters (127-L) were tested for treating poultry blood wastes over a 400-d period. Digesters (operated at 26^{o}C) had higher methane yield (189 mL g^{-1} COD_{added}, at OLR of 0.4 g COD L^{-1} d^{-1}) than results from the literature on tests on similar feedstocks using single-stage digesters. The use of bamboo biocarriers improved the performance of two-stage digesters resulting in methane production of 361 mL g^{-1} COD_{added}, at OLR of 0.4 g COD L^{-1} d^{-1}. Struvite precipitation (SP) removed more than 70% of TAN from effluents of the acidogenic

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INDEX WORDS: Anaerobic digestion, Poultry processing wastes, Blood wastes, Biocarriers, Two stage anaerobic digesters, Pilot digesters, Ammonia inhibition, Struvite precipitation, Bioenergy, Nutrients recovery

ANAEROBIC DIGESTION OF POULTRY PROCESSING WASTES FOR BIOENERGY AND NUTRIENTS RECOVERY

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DEDICATION

For my parents, Yuzhong Wang and Guilan Li.

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CHAPTER 1

INTRODUCTION

The U.S. poultry industry produced 17.4 million metric tons of ready-to-eat (RTE) broiler products and approximately 5.8 million metric tons of inedible byproducts in 2013 (USDA, 2014). Blood represents 7-11 % live weight of broiler depending on the broiler weight, and amounts to 1.6-2.6 million metric tons of blood discharged from US poultry processing industry in 2013 (Kiepper, 2007). Containing 13-15 % (wet basis) protein, blood is a strong contaminant which needs proper handling and treatment, and must be disposed in the shortest time possible to prevent environmental pollution and public health concerns. Rendering is a typical means to process and reuse blood as a protein supplement used in animal feeds or as a compounded fertilizer. However, larger amount of chemicals and energy input needed makes it expensive and not environmentally beneficial (Ockerman and Hansen, 2000).

Anaerobic digestion (AD) is an attractive alternative to treat organic wastes with benefits of bioenergy production, pollutants removal, greenhouse gases control, and pathogen reduction. This technology has been employed to treat different types of wastes including animal manures, waste activated sludge, crop residues, and slaughterhouse wastes (Wellinger et al., 2013). The protein content in blood provides sufficient nutrients and buffering condition to the microorganisms in AD and can result in high methane yield potential (Salminen and Rintala, 2002). However, high ammonia and fatty acids concentrations resulting from protein decomposition can cause inhibition of methanogeneisis, and subsequent instability and low efficiency of the AD process (Cuetos et al., 2013).

To improve the AD of poultry blood, optimization strategies were proposed and tested in this dissertation research. This dissertation has three parts. The first part involved the investigation of single-stage laboratory-scale anaerobic digesters filled with low-cost biocarriers. The second part involved the evaluation of extended-duration operations of two-stage pilot anaerobic digesters, and the third part covered the evaluation of struvite ($NH_4MgPO_4.6H_2O$) precipitation as a method of reducing ammonia toxicity in AD while recovering nitrogen.

Chapter 2 is a literature review that presents the status of poultry blood production and its disposal/treatment and introduces benefits, challenges and opportunities of AD of animal blood. Chapter 3 covers the design and testing of two semi-continuous laboratory-scale anaerobic digesters. The objectives were to investigate performance of AD treating blood co-digested with poultry processing wastewater (PPWW) using anaerobic filters filled with biocarriers of biochar and bamboo at different organic loading rates (OLRs). Evaluation of energy and nutrients recovery from AD was also conducted. Chapter 4 includes the design and testing of a two-stage pilot anaerobic digester treating blood and PPWW. The objectives were to evaluate performance of this digester and determine the optimal OLRs for each stage and the overall system using methane yield, COD removal and energy recovery as criteria. Chapter 5 covers the testing of five acidogenic digesters and three methanogenic digesters treating blood and PPWW. The objectives were to investigate the performance of low-cost biocarriers (biochar, bamboo and seashell) on methane yields and COD removal in the pilot digesters at different OLRs and identify optimal conditions for the digesters. Chapter 6 covers experimental validation of the strategy of using struvite precipitations (SP) in the acidogenic digester effluent to control ammonia and thus improve methanogenesis. Different sources of magnesium and phosphorus were evaluated for SP and a standard Biochemical Methane Potential (BMP) test was used to measure enhancements in

methanogenesis. The objectives were to measure SP effects on nitrogen recovery from the effluent of acidogenic digester, ammonia inhibition mitigation in the methanogenic digester, and pH adjustment of substrate required. Chapter 7 presents the conclusions of the dissertation.

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CHAPTER 2

LITERATURE REVIEW

Broiler Processing in the United States

In 2013, U.S. poultry producers processed 8.6 billion broilers with the average weight of 2.7 kg (5.9 lbs). Georgia accounted for 1.2 billion broilers making it the leading producer of broiler meat in the U.S. (USDA, 2014). Approximately 200,000 broilers are slaughtered every day in a typical processing plant and approximately 162 processing plants are in operation across the U.S. (Kiepper, 2007; USDA, 2015).

Broiler processing is generally defined as the steps to convert live broiler birds into the ready-to-cook whole carcasses or separate carcass parts (Northcutt, 2001). It can be divided into two processing treatments. In the first processing, the live birds are unloaded, stunned, killed, defeathered, eviscerated and chilled in sequence to obtain the whole carcasses (Sams, 2001a), and in the second processing, carcasses are cut into parts or deboned for products of added-value in the market (Sams, 2001b).

In broiler processing, approximately 75% of the live body weight of live birds is processed for ready-to-cook carcasses while the remaining 25% is inedible byproducts defined as offal, typically including feather, blood, intestine residues, etc. (Kiepper, 2007; USDA, 2014; Yoon et al., 2014).

Broiler Blood Collection

Blood is a body fluid in animals to carry oxygen and nutrients to and discharge carbon dioxide and other wastes from the body tissues (The Columbia Encyclopedia, 2014). The blood

content in broilers varies based on their body weight. Blood constitutes more than 11% of live body weight for 1.0 kg bird, and around 7% for 3.0 kg bird (Kiepper, 2007). Considering 8.6 billion broilers with the average weight of 2.7 kg were processed in the U.S. in 2013 (USDA, 2014), the maximum weight of broiler blood collected is estimated to be 1.8 million metric tons per year.

During broiler processing, blood is collected in the killing section. After the bird is stunned, it is transported to the killing section where blood vessels of its neck are cut by the cutting blade. The bird bleeds for 2-3 minutes to allow 30-50% of its blood to drain, eventually causing death. The blood still left in the bird's body is partially washed out into the wastewater in later processing (Kiepper, 2007; Sams, 2001a). In the U.S., this translates to total broiler blood weight that can be actually collected to be between 0.5 to 0.9 million metric tons per year, which is further treated or disposed.

Animal Blood Characteristics

Blood of farmed animals including cattle, pigs, and broiler chickens, are made of cells, water, enzymes, and other organic/inorganic compounds. Blood of slaughtered animals has a relatively constant composition with relatively minor differences, such as the shape of blood cells, which depend on animal species. Blood contains cells, representing 30-40% of total mass, and plasma, which represents up to 60% of total mass. Blood cells include three cellular fractions, red corpuscles, white corpuscles and platelets. These are dispersed in the plasma to provide different functions for the normal activities of animal tissues. Plasma is the liquid part remaining after removal of blood cells. It mainly contains proteins identified as albumin, globulins and fibrinogen (Bah et al., 2013).

Composition of blood is relatively similar in most farmed animals. Total solids (TS) range from 13.2 to 20% (db), and volatile solids (VS) between 93.9-95.9% (db). Protein contents in the blood are also high, representing more than 82.8 % (w/w, db). Chemical oxygen demand (COD) of fresh blood is 150-220 g L^{-1}. Lipid contents are typically 0.3 to 3.1% (w/w, db) (Table 2.1). Blood contains approximately 4.8% (w/w, db) of mineral components (Okanovic et al., 2009). Minerals in animal blood include phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Iron (Fe), Cobalt (Co), Sulfur (S), Cadmium (Cd), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn) (Table 2.2). Minerals in blood perform functions such as maintain the osmotic pressure balance, act as enzyme catalysts and regulate cell reproduction (Suttle, 2009).

Working under the coagulation system (Nature, 2015), blood is able to coagulate or clot when removed from blood vessels, which helps wound heal and defends against infection (Oladele and Samuel, 2014). Blood coagulation time for normal cats, dogs, turkeys, chickens and ducks are known to be 1.1, 1.2, 1.1, 2.9 and 6.6 minutes, respectively (Oladele and Samuel, 2014; See et al., 2009). Stratification of blood could occur after long storage times in the storage tank because heavier blood settles impacting composition differences that further affect downstream treatment and disposal.

The Disposal or Treatment of Animal Blood Byproducts

Because animal blood has high COD concentration (150-220 g L^{-1}, Table 2.1), direct discharge into the sewer system can increase the organic strength of wastewater by 35-50% leading to high processing cost (USEPA, 1973). In order to reduce loading to the sewer system and beneficially reuse blood, it is generally collected in a tunnel and drained into storage

containers, then transferred to further processing or disposal at regular intervals (Bah et al., 2013).

Animal blood can be used for edible or inedible purposes after collection. In the former, whole blood or blood extracts are directly consumed as an ingredient of human food, such as emulsifier and color additive; in the latter, it is converted to useful products used in different agricultural and industrial applications, such as blood meal and blood char (Ockerman and Hansen, 2000).

Edible Use of Animal Blood

Animal blood protein accounts for approximately 60% of total animal byproducts protein. In some European and Asian countries, blood is used to make traditional foods such as blood sausage, blood pudding, blood soup and other special foods. It is also commercially used as food emulsifier, stabilizer, clarifier, etc. and medically used as blood-clotting factors, fibrin product, plasminogen, etc. (Ockerman and Hansen, 2000; Ofori and Hsieh, 2011). Animal blood, however, is prohibited for human consumption by Kosher and Halal dietary laws followed by Jewish and Muslim populations, respectively. Human consumption of blood derivatives is also forbidden or seriously questioned in these two groups (Regenstein and Chaudry, 2001).

In the US, animal blood discharged from processing plant may be collected and used in products for human consumption under the Federal Meat Inspection Act (U.S. National Archives and Records Administration, 2014). In this Act, blood collected from livestock whose carcass is inspected and passed is acceptable for human consumption.

Beef cattle blood has been collected and used for human consumption for many years (Ockerman and Hansen, 2000). In this process, a small area of neck hide, approximately 10 x 15 cm area is first removed to reduce contamination. Subsequently, a sterilized hollow knife is

inserted into the artery in this area and blood is allowed to flow gravitationally through the knife into a container for about 6 minutes. Finally, the blood is inspected and approved before moving it to a storage cooler for the human consumption, or into a centrifuge to separate the lighter plasma and heavier blood cells for further reprocessing. Anticoagulants approved by law can be added to the blood by supplying it at the knifepoint to prevent clotting (Ockerman and Hansen, 2000; U.S. National Archives and Records Administration, 2014).

Inedible Use of Animal Blood

Animal bloods not permitted for human consumption, such as broiler blood, can be converted to other useful products or disposed of in different ways. Commonly used options include rendering, composting, land application, and anaerobic digestion (Salminen and Rintala, 2002). Other methods may be available but are not common for animal blood and will not be discussed. These methods are further described below.

Rendering

Rendering is a physical and chemical process to convert inedible byproducts of animal processing to stable and value-added products such as blood meal, bone meal, animal fats, etc. (Meeker and Hamilton, 2006). One primary objective of rendering is to obtain a stable, dry and pathogen free product. Blood meal, in its final form is a dark-brown granular solid, containing 8-12% moisture, 75-83% protein and 1.2-1.6% fat (Fernando, 1984; Ockerman and Hansen, 2000).

The most common approaches to blood rendering includes direct drying and drying after preliminary dewatering or coagulation. In direct drying, the whole blood is dried in a batch dryer to completely recover blood components. However, this process consumes a large amount of steam, requires a long drying time, and is difficult to manage as blood sticks to the dryer surfaces. This method is also not economical to treat a large quantity of blood that many times

may be diluted with wash water in the processing plant. Therefore, coagulating and dewatering blood before drying is a more commonly used approach. In this method, steam is added to whole blood to produce a coagulum with about 40% total solids. A decanter centrifuge is used to separate the solids, which is separately dried in ring or spray dryers. Lime is often added to raw blood to reduce rapid spoilage and reduce odor emitted from drying (Bureau et al., 1999; Fernando, 1984; Ockerman and Hansen, 2000). Figure 2.1 shows the diagram of a typical blood meal producing process.

Due to the high protein and mineral content of blood meals, it is often used as dietary supplements in animal feed. Blood meal can also be mixed with phosphates to make a compounded plant growth fertilizer. In addition, it can be further upgraded to other value-added products such as blood chars, blood foam compounds, etc. (Ockerman and Hansen, 2000).

Composting

Composting is a controlled aerobic biological process that accelerates the breakdown of raw organic wastes to produce biologically stable compost that can be used as soil amendment (Christian et al., 2009; Cooperband, 2002). The primary objective of composting is the safe and beneficial use of organic wastes and promotion of soil fertility (Hubbe et al., 2010). Under optimized composting conditions, volume and mass of the waste stream is reduced and the high temperatures resulting in compost piles reduce pathogens. The compost product is rich in active benign microorganisms and nutrients that are beneficial to plant and soil health.

Fleming and MacAlpine (2005) studied forced air composting of slaughterhouse blood with various amendments. They report that amending blood with high carbon amendments such as wood fiber and tree leaves, blood can be easily composted under optimal composting conditions. Amendment to blood ratios reported were 5.25 kg-blood/kg-tree leaves and 1.2 kg-

blood/kg-wood fiber. Pisa and Wuta (2013) studied the composting of different ratios of maize stover to chicken blood in a 72-day test and found treatments of 10% and 30% of maize stover performed better and had longer thermophilic conditions exceeding 8 days. The disadvantages of composting include the need for large land area, long durations, and the purchase of amendments rich in carbon.

Land Application

Land application of wastes is the process of spreading or injecting liquid or solid wastes on soil surface or into soil subsurface. Degradation of these wastes occurs over time and releases nutrients that are utilized by the vegetative cover over the area. Due to the high nutrient availability, animal blood is a potential candidate for land application. Cost of land application of blood wastes is about one third that of the dewatering process in rendering. However, land application requires large land area for treating the large amount of blood wastes discharged from a processing plant (Ockerman and Hansen, 2000). In Kosher meat processing, blood collected from broiler birds is mixed with sawdust and returned to the earth, through land application (Regenstein and Chaudry, 2001).

Anaerobic Digestion

Anaerobic digestion (AD) is a biological conversion of organic waste in the absence of oxygen in which complex organics are converted to simpler compounds and biogas (primarily consisting of methane and carbon dioxide). The AD process reduces organic strength of the waste while conserving nutrients (N and P) and producing methane, which can be used for energy applications. For the optimum functioning of AD, environmental conditions should be properly controlled to accommodate the complex microbial flora and maximize their metabolic conversion rates. The process has three main steps: hydrolysis, acidogenesis, and

methanogenesis. In hydrolysis, bacteria convert carbohydrates, proteins and lipids into smaller molecules such as sugars, amino acids and fatty acids. Hydrolysis could be the rate-limiting step when treating organics that degrade slowly, such as cellulose (Noike et al., 1985). Acidogenesis is dominated by a different set of organisms called acidogenic bacteria, which utilize products from hydrolysis and convert them to simpler organic acids, e.g. butyric acid, propionic acid, acetic acid, and carbon dioxide and hydrogen. In the third and final step, methanogenic bacteria convert the products of acidogenesis to methane and carbon dioxide that are the main components of biogas. Methanogenic bacteria are extreme anaerobes and can be divided into two groups, the aceticlasitc and the hydrogenotropic. Aceticlastic bacteria convert acetic acids into methane and carbon dioxide. These bacteria are slow growing, but produce approximately 70% of the total methane produced. Hydrogenotropic bacteria utilize carbon dioxide and hydrogen to generate methane and water. They grow faster and contribute to the remaining 30% of methane produced in typical digesters. Methanogenesis is the rate-limiting step in AD, primarily due to the slow reproduction rate of methanogenic bacteria (Monnet, 2003; Welllinger et al., 2013).

Several operating variables impacting the AD process can be controlled to maximize microbial conversion rates of organics. These include total solids (TS), temperature, pH, retention time, and nutrient balance. TS is expressed as percentage (%) for solid-based samples or mg L^{-1} for liquid-based samples. Based on TS of substrate, AD systems are classified as wet (less than 10% TS), semi-solid (10-20% of TS) or solid (more than 20%) (Karthikeyan and Visvanathan, 2013). Wet-state AD has been widely used for a long time, primary because low cost equipment such as pipes and pumps can be used to handle wastes with high moisture. However, because TS is low, digester volumes are high and significant internal mixing is required for the digester to operate at high organic loading rates (OLR). This leads to high initial

capital costs and ongoing maintenance costs. Pre-treatment of feedstock is also often required to remove materials that lead to floating layers in the digester and non-degradable heavy fractions which deposit at the bottom of the digester. These challenges can gradually result in either reducing the effective digester volume or interfering with proper mixing. Solid-state AD has been studied since 1980's using solid wastes treated anaerobically to stabilize it and produce biogas. In this system, solid wastes are evenly premixed with an inoculum and placed in batch or plug-flow digesters. Solid-state AD is preferred because digesters do not need mixing, materials do not need pre-treatment, and digester volumes are typically smaller. However, equipment for materials handling and treatment are typically more expensive than wet-state AD, and in some cases accumulation of inhibitors resulting from digesting undiluted feedstock could result in process instability and lower digester performance (Karthikeyan and Visvanathan, 2013; Monnet, 2003).

Temperature is an important variable impacting metabolic activities of microorganisms. Methanogenic bacteria are more sensitive to temperature, compared to microorganisms functioning in hydrolysis and acidogenesis (Banks and Heaven, 2013). In AD, there are three typical temperature ranges used. They are Psychrophiles (5-25^{o}C), Mesophiles (30-35^{o}C), and Thermophiles (50-60^{o}C) (Gerardi, 2003). To enhance microbial activity, most digesters are operated at the mesophilic or thermophilic conditions. Digesters operated at thermophilic conditions can have 25-50% higher microbial activities than that at mesophilic conditions (Banks and Heaven, 2013). Although thermophilic digesters can operate at lower hydraulic retention times (HRT) and have the benefit of destroying pathogens, they have poorer energy balance, higher ammonia inhibition, and higher digester instability caused by temperature fluctuations (Banks and Heaven, 2013; Gerardi, 2003). Therefore, most digesters in AD are typically

operated at mesophilic condition. Exceptions include digesters treating high temperature wastes such as effluents from breweries and alcohol distilling plants.

Microbial activity in AD is greatly affected by the pH of the substrate. The pH of successful digesters is typically maintained in the range of 6.5-8.2 (Speece, 1996). Gerardi (2003) reports good performance of methanogenic bacteria at pH 6.8-7.2, while other organisms that are less sensitive to pH are active at pH above 5.0. When processing high strength feedstocks, large amounts of volatile fatty acids (VFA) are produced through acidogenesis. In a healthy digester, these VFAs are consumed by methanogens, thus keeping pH in the optimum range and producing large amounts of high quality biogas. In an unhealthy digester, VFAs are not consumed quickly enough, thus accumulating and reducing pH. This further inhibits methanogens process and finally fails the AD. Methods to prevent this include the addition of alkali to stablilize the pH, or the use of two-stage digesters that restrict methanogens to a separate 2^{nd} stage digester thus preventing pH inhibition and providing more stability (Speece, 1996).

Retention time includes solid retention time (SRT) and hydraulic retention time (HRT). SRT is the time microorganisms are in the digester and HRT is the time substrates are in the digester. Since reproduction time of methanogenic bacteria is very high (3-30 days) relative to microorganisms in hydrolysis and acidogenesis (15-30 min), SRT impacts methanogenic bacteria more severely. Recommended SRT is typically 12 days for retaining active methanogenic bacteria in the digester. Addition of a fixed media (for bacteria to attach to and grow) or recycling microbial solids from the effluent can increase SRT. HRT impacts the conversion of organics to biogas and is chosen based on the conversion rates of specific substrates (Gerardi, 2003).

A balance of nutrients is required for optimal performance of digesters. Carbon and nitrogen are important nutrients for AD and the ratio of carbon to nitrogen (C/N) should be in the range of 20:1 to 30:1 (Monnet, 2003). Higher or lower C/N in substrate can cause inhibition of microbial metabolism or the presence of severe ammonia toxicities. Mixing different feedstocks is the most common method of obtaining an appropriate range of C/N.

Some inorganic and organic compounds in the feedstock can cause toxicity and inhibition of AD process. Common inorganic toxins and their toxic concentration levels include ammonia at 1500 mg L^{-1}, sulfide at 50 mg L^{-1}, sodium at 3500 mg L^{-1}, and magnesium at 1000 mg L^{-1}. Organic toxins and their toxic concentrations are alcohol at 100-200 mg L^{-1}, Benzidine at 5 mg L^{-1}, and methylene chloride at 100-500 mg L^{-1}. Additional information on AD toxins and their function are provided in Chen et al. (2008) and Gerardi (2003). Methods of managing toxins to protect the digester include removal of toxins from the feedstock, dilution to reduce toxin concentration, increase of microorganisms' resistance to toxin by adaptation, etc. Pretreatments applied to remove or eliminate high levels of toxins involve e.g. ammonia scrubbing and precipitating. Dilution of feedstocks with water or with other feedstocks can also reduce toxicity. Increasing the SRT by adding biofilm media or recycling sludge could enhance adaption of microorganisms to some toxins.

Anaerobic Digestion of Animal Blood

Animal blood contains high concentrations of degradable organics and is reported to have higher biogas (650 mL biogas g^{-1}VS) and methane (500 mL methane g^{-1} VS) yield potential relative to other common substrate (Steffen et al., 1998; Salminen and Rintala, 2002). Due to the significantly high protein content and low C/N ratio in animal blood wastes, it is beneficial to co-digest blood with other wastes having higher C/N. The two main digesters used to study AD of

blood wastes include batch and continuous digesters at the lab and pilot scales. These are described further below.

Bench-scale Batch Digester

Batch digesters are often used to test biochemical methane potential (BMP) of feedstocks. The BMP test is applied to measure the maximum methane production from potential feedstocks and identify operating conditions that maximize yield (Esposito et al., 2012). The size of digester used for this purpose is usually less than 2 L (Hejnfelt and Angelidaki, 2009; Lopez et al., 2006; Yoon et al., 2014). In a BMP test, the active inoculum and the substrate being tested are mixed at an optimal ratio of 0.3-1.0 g-VS substrate/g-VS inoculum (Cuetos et al., 2013; Yoon et al., 2014) or 1 g-substrate COD/ g-VS inoculum (Moody et al., 2009) and placed in a batch digester. The digester is sealed and purged using an inert gas such as nitrogen to ensure strict anaerobic conditions. The digester is maintained at mesophilic condition (35^{o}C) with continuous or semi-continuous stirring. The biogas volume and methane concentrations in the biogas are over a period of time, one day to one week, which depends on the level of microbial activity. Results are expressed as mL methane per gram organic matter added (expressed either as COD or VS). The test period varies depending on the anaerobic biodegradability of the feedstock. Generally the test period is 30-60 days for easily degraded feedstocks and longer for more recalcitrant feedstocks (Angelidaki et al., 2009; Moody et al., 2009; Speece, 1996).

Cuetos et al. (2013) reported the optimum ratio of maize residue to poultry blood to be 70:30 (w/w of VS) and its methane yield potential was 188 mL g^{-1} VS in a 100-mL batch digester operated at 34^{o}C for 30 days. Hejnfelt and Angelidaki (2009) determined the BMP of differently diluted pig blood in 0.5-2 L bottles operated at 55^{o}C for 30 days. Maximum methane

yield was found to be 490 mL g^{-1} VS in the 5% pig blood. The lowest methane yield was found to be approximate 50 mL g^{-1} VS in the 100% pig blood treatment. Marcos et al. (2010) studied anaerobic co-digestion of wastewater and blood in 2-L batch digesters. Comparing different organic loading obtained by dilution, it was found that treatments with loading of 0.2 g COD L^{-1} d^{-1} had higher COD removal (than 0.3-0.6 g COD L^{-1} d^{-1}) with methane yields of 314-224 mL g^{-1} COD. Yoon et al. (2014) tested the BMP of poultry blood using 160-mL serum bottles at 38^{o}C. The methane yield of blood was 250 mL g^{-1} VS. Within the studies reported here, blood BMP ranged from 188-490 mL g^{-1} VS, or 224-314 mL g^{-1} COD, depending on the test conditions such as temperature, inoculum volume and ammonia level. Hejnfelt and Angelidaki (2009) reported that high blood content added in the feedstock (20- 100%) could reduce methane yield potential, potentially due to inhibition of high VFA and ammonia resulting from protein degradation.

Continuous Lab-scale Digester

Popular continuous high-rate digesters include the continuously stirred tank reactor (CSTR), anaerobic filter (AF), upflow anaerobic sludge blanket (UASB), etc. The types of anaerobic digesters and their function in treating industrial wastewaters are described in Saleth and Mahmood (2004).

Few scientific publications report work on continuous anaerobic digesters of animal blood wastes. Banks and Wang (1999) used an 8-L two-phase anaerobic system to treat diluted mixtures of cattle blood and paunch contents at a ratio of 1 to 3 (w/w). They reported a methane yield of 270 mL g^{-1} TS and TS removal of 63% at a loading rate of 3.6 g TS $L^{-1}day^{-1}$. Cuetos et al. (2009) evaluated the co-digestion of poultry blood with the organic fraction of municipal solid waste (OFMSW) at a 1:2 (w/w) in a 3-L CSTR operated at 34^{o}C. At a HRT of 36 days and OLR of 2.0 g VS L^{-1} d^{-1} the specific methane yield was 200 mL g^{-1} VS. Cuetos et al. (2013)

evaluated a mixture of 60% maize residue and 40% poultry blood on a VS basis in a 3-L CSTR operated at OLR of 3.1 g VS L^{-1} d^{-1} and 34^{o}C. The biogas production was 2.7 L d^{-1} and the methane yield was 165 mL g^{-1}VS. In a study by Hansen and West (1992), a UASB digester was used to treat a mixture of 2% blood and 98% rendering condensate at 35^{o}C. A COD removal of 50% to 66% was observed at a HRT of 10 to 15.6 days (corresponding to OLR of 0.54 to 0.34 g L^{-1} day^{-1}, respectively). The biogas had a methane concentration exceeding 80% at all tested OLRs and the methane yield was 100-180 mL g^{-1} COD. Zhang and Banks (2012) co-digested the OFMSW and sheep blood in the 5-L CSTR digester operated at 2-4 g VS L^{-1} d^{-1} at 36^{o}C. The methane yield was 289 and 180 mL g^{-1}VS at OLR of 2 and 3 g VS L^{-1} d^{-1}, respectively. VFA concentrations increased with increasing OLR, suggesting an inhibition of methanogens at high OLR possibly due to ammonia inhibition.

Continuous Pilot Digester

Pilot scale digesters are used not only to confirm the scalability of lab-scale results, but also to evaluate other technologies which will be used in the industrial scale, and are usually not tested in the lab scale (Donati and Paludetto, 1997). The size of the pilot scale digester used in several studies reported in the literature range between 75 L and 10,000 L (Kaparaju et al., 2008; Gobema et al., 2010; Parawira et al., 2008). Lopez et al. (2006) treated the mixture of ruminal content and blood (10:1) in a 3.5-m^3 pilot digester at a HRT of 20 days and 37^{o}C. Daily biogas production in this digester was reported to be 3.5 m^3.

Opportunities and Challenges of Treating Animal Blood Wastes using Anaerobic Digestion

AD is one of best candidates to treat high strength animal blood wastes because it provides energy and nutrient recovery considering its characteristics of high moisture, high nutrients availability and buffering capacity, compared to other treatment alternatives. Previous

studies have shown that high levels of ammonia produced from blood protein decomposition can severely inhibit methane production and cause VFA accumulation leading to destabilization and ultimate failure of AD systems.

Several approaches can be used to reduce ammonia inhibition and balance nutrients in the AD process. These include co-digestion, biocarriers addition, ammonia scrubbing, etc. Research on pilot scale digesters treating such wastes are few and additional work is needed for understanding the variability in feedstock and digester performance and the potential of pilot digesters in providing quantitative data for industrial scale digester design and operation.

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Table 2.1 Farmed animal blood characteristics

Animal[a]	pH[b]	TS[b] (%)	VS[c] (%)	COD[b] (g L^{-1})	Protein[c] (%)	Lipid[c] (%)	Reference
Cattle	7.3	20	na	220	100	na	Ockerman and Hansen, 2000
Pig	na	17.9	93.9	na	88.6	0.3	Hejnfelt and Angelidaki, 2009
Broiler	na	13.2	95.5	na	89.7	3.1	Yoon et al., 2014
Broiler	na	19.2	na	na	82.8	1.4	Okanovic et al., 2009
Sheep	7.2	19.7	95.9	na	92.6	na	Zhang and Banks, 2012
Unknown	na	na	na	150	na	na	Hansen and West, 1992

[a] na, not available; [b] wet base; [c] dry base

Table 2.2 Micronutrients in the farmed animal blood

Animal[a]	P	K	Ca	Mg	Na	Fe	Co	S	Cd	Cr	Cu	Ni	Pb	Zn
Broiler[b]	118	92.7	44.9	5.4	148.3	48.0	0.01	na	na	na	0.1	1.0	na	1.7
Sheep[c]	164	731	na	na	na	na	na	na	0.20	<0.40	1.32	<1.0	<2.0	3.2
Unknown[d]	183	798	55	27	818	164	<0.02	300	0.05	0.3	0.7	<0.2	<0.6	1.3

[a]All micronutrients elements is in ppm on wet base and assume the bulk density of animal blood is 1 kg L^{-1}; na, not available
[b]Yoon et al., 2014; [c]Zhang and Banks, 2012; [d]Hansen and west, 1992

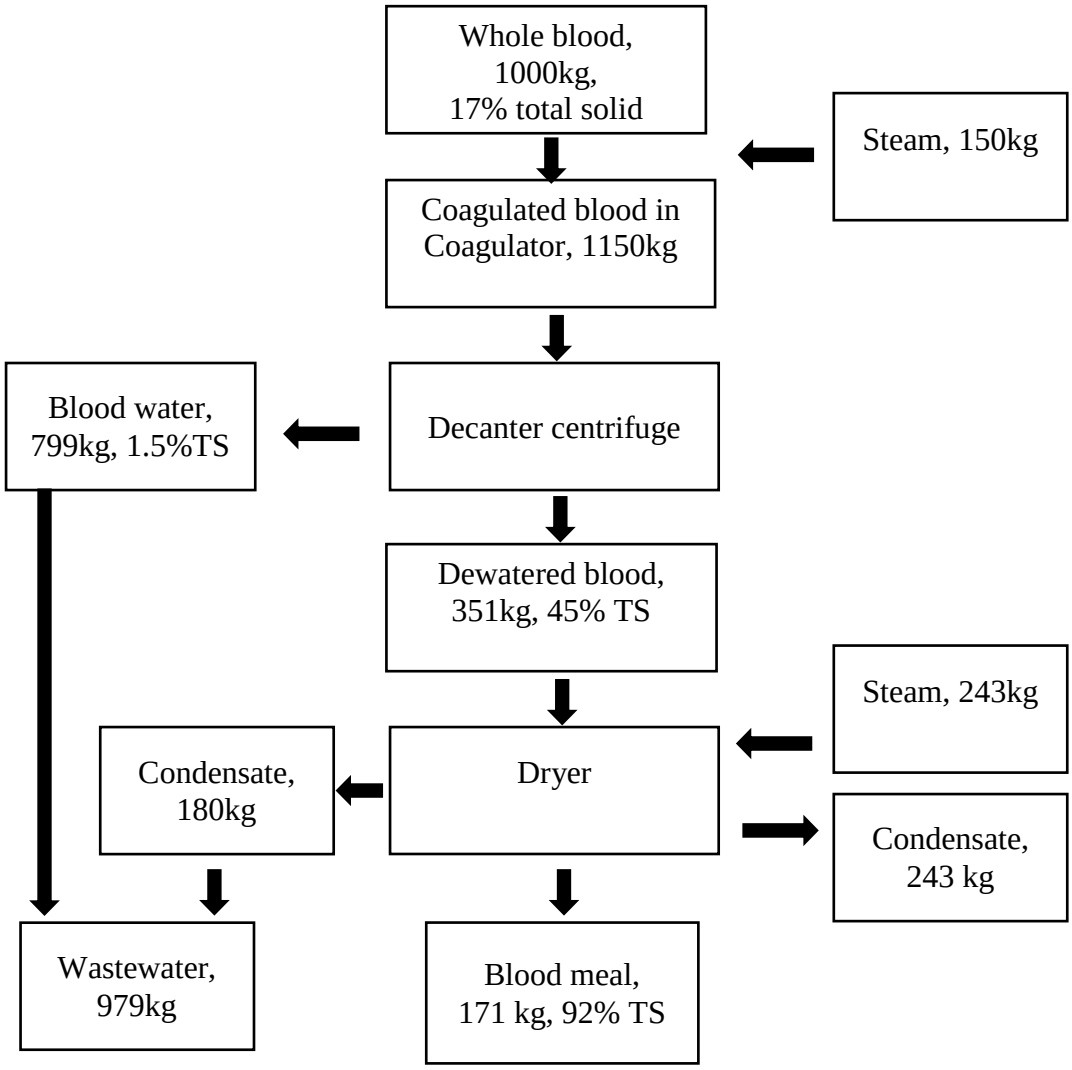

Figure 2.1 Blood meal production (adapted from Ockerman and Hansen, 2000)

CHAPTER 3

LABORATORY SCALE ANAEROBIC CO-DIGESTION OF POULTRY PROCESSING LIQUID WASTES USING LOW-COST BIOCARRIERS FOR ENERGY RECOVERY[1]

[1]Wang, S., B.H. Kiepper, G.L. Hawkins, and K.C. Das. To be summited to *Bioresource Technology*.

Abstract

Anaerobic co-digestion of poultry blood waste and poultry processing wastewaters were evaluated using semi-continuous mesophilic upflow anaerobic filters containing biochar granules or bamboo cylinders as biocarriers. Energy and nutrients recovery were estimated based on data collected. At an OLR of 4.7 g COD L^{-1} d^{-1}, the treatment with biochar granules had a methane yield of 331 mL g^{-1} $COD_{removed}$ and volumetric biogas production of 0.64 L $L^{-1}d^{-1}$. Ammonia and volatile fatty acids accumulation at maximum concentrations of 3,277 mg L^{-1} and 15,035mg L^{-1}, respectively, were observed. Estimated recovery of energy and nutrients from the processing of these wastes in a typical processing plant are 1.5 GJ d^{-1} and 252 kg-N d^{-1}, 3.0 kg-P d^{-1} and 3.7 kg-K d^{-1}.

Introduction

In 2013, the U.S. poultry industry produced 17.2 million metric tons of ready-to-eat (RTE) broiler products and approximately 6.1 million metric tons of inedible offal as well (USDA, 2014). A major component of offal is blood and approximately 1.8 million metric tons of blood per year was discharged into the wastewater treatment system or collected in blood storage tanks for final treatment elsewhere (Kiepper, 2007; Ockerman and Hansen, 2000). Rendering is typically used to process blood waste to recycle protein for use as animal feed or as compounded fertilizer. However, this may not always be an effective use as transportation energy use and costs are high and stabilizing is required due to the high moisture of blood wastes (80-82%) and the inherent instability of proteins (Ockerman and Hansen, 2000).

Anaerobic digestion (AD) is an attractive alternative to process offal and wastewater from poultry processing plants as it provides for energy and nutrients recovery, pollutant removal and pathogens reduction (Arvanitoyannis and Ladas, 2008; Salminen and Rintala, 2002). This

has been a popular method for treating wastewaters (including some limited inclusion of blood) within poultry industries for decades (Harper et al., 1990; Rajakumar et al., 2011). Animal blood waste is known to have a methane yield potential of 500 mL g^{-1} VS because of high concentrations of degradable components including 94.4% protein and 0.3% lipid on a volatile solids (VS) basis (Hejnfelt and Angelidaki, 2009; Salminen and Rintala, 2002).

Previous studies on AD of slaughterhouse blood waste have reported process instability caused by high total ammonia nitrogen (TAN) resulting from the breakdown of proteins (Cuetos et al., 2009; 2013). Inhibitory TAN levels depended on different parameters such as pH, inocula adaption, and temperature. TAN inhibition was observed generally at TAN concentrations of 3,000 mg L^{-1} and at TAN concentrations of 6,000 mg L^{-1} in systems that have been adapted to the presence of TAN. Different strategies have been used to control TAN inhibition including co-digestion to increase feedstock carbon to nitrogen ratio (C/N), microflora adaption, and feedstock dilution (Rajagopal et al., 2013). Among these, anaerobic co-digestion is widely used for improving nutrients balance, dilution of inhibitory components, and cost reduction (Nges et al., 2012). Cuetos et al. (2013) reported that co-digestion of poultry blood and maize residues alleviated ammonia inhibition and in mixes containing 70% maize residues maximum methane production was found to be 188 mL g^{-1}VS. Lopez et al. (2006) reported biogas production of 1.0 L L^{-1} d^{-1} with 43% VS removal during co-digestion of poultry blood with ruminal content on a 1:10 (dry weight basis). The total solids (TS) of co-substrates were 3-4 % and hydraulic retention time (HRT) was 20 days. Although improved methane production was shown, the high transportation cost of co-substrates limits adoption of this practice in large-scale (Salminen and Rintala, 2002).

Co-digestion of poultry blood and poultry processing wastewater (PPWW) can be a cost effective approach to AD as both substrates are available at a single location. The PPWW is produced from the scalding, bird washing, and offal transport out of processing areas, and contains high concentrations of lipids. It is produced in large quantities at the poultry processing plant (approximate 251 million metric tons in U.S. in 2013) (Kiepper et al., 2008; USDA, 2014). There were few studies on anaerobic co-digestion of animal blood waste and wastewater. Hansen and West (1992) conducted co-digestion of a mixture of blood and condensate discharge (1:49, w/w) at a rendering plant using a 7.8-L Upflow Anaerobic Sludge Blanket (UASB) operated at 35^{o}C. The system produced 0.06 to 0.11 L_{biogas} $L^{-1}_{digester}d^{-1}$ at an organic loading rate (OLR) of 0.34 to 1.01 g COD L^{-1} d^{-1}. Marcos et al. (2010) anaerobically treated a similar ratio of blood to wastewater (1:49, w/w) in a 2-L discontinuous digester (38^{o}C) at different loadings (0.17-0.56 g COD L^{-1} d^{-1}) and obtained the highest chemical oxygen demand (COD) removal of 56.9% at OLR of 0.17 g COD L^{-1} d^{-1}. Reported biogas productions from co-digestion of blood and wastewater were relatively low. In addition, longer HRTs required for treating mixtures with low blood content resulted in larger digester volumes, thus increasing capital costs.

Anaerobic filters (AF) which use packing media for the bacteria attachment are a popular digester choice for treating PPWW and has proven to be a feasible way to achieve high biogas productions and organic matter removal (Del Pozo et al., 2000; Rajakumar et al., 2011). One drawback of these systems has been the cost of packing media/biocarriers (Harper et al., 1990). Inexpensive biocarriers are needed to reduce capital and operating costs. Two candidates for low-cost biocarriers are Biochar and Bamboo cylinders. Biochar is a byproduct of pyrolysis, a way to produce liquid biofuels from biomass. Bamboo is a fast growing grass that is widely seen in the southeastern U.S. Both have been used as packing media in biological filters treating

wastewaters because of their relatively high specific surface area and their physical and biological robustness (Lehmann and Joseph, 2009; Regmi et al., 2012; Tritt, 1992). Specific surface areas of wood biochar and bamboo rings are in the ranges of 0.1 to 24 $m^2 g^{-1}$ and 85 $m^2 m^{-3}$, respectively (Spokas and Reicosky, 2009; Tritt, 1992).

The objectives of the work reported here were to: (1) measure performance of AFs using biochar and bamboo cylinders as biocarriers and treating a mixture of blood waste and PPWW at different OLRs, and (2) estimate energy and nutrients recovery from the proposed full-scale digester.

Materials and Methods

Experimental setup

Two identical 5.6-L glass columns (71-cm height and 10-cm diameter) were used in this study. Each digester used was filled to a 50% volume with either biochar granules or bamboo cylinders. The effective volume of digesters filled with biochar (BC digester) and bamboo (BB digester) were 3.8 L and 4.2 L, respectively. Digesters were maintained between 34-38^{o}C using a PID temperature controller connected to a thermocouple probe and heating blanket around the column. The headspace was connected to a tipping bucket gas flow meter (wettipgasmeter.com) to measure biogas production rate.

Biocarriers used in digesters

Biochar granules used here were the byproduct of pyrolysis of pine wood pellet (500^{o}C for 0.5-1 hour) and bamboo was naturally dried bamboo poles from the local private bamboo garden in Athens GA, USA that was cut to small cylinders. Biochar was used as received. Fifteen pieces of biochar were randomly picked from the biocarrier bulk and measured using a digital caliper (CEN-TECH, Virginia, USA). Bamboo was cut into 1.5 cm long pieces from

bamboo poles of 1.9 cm diameter using a table saw. Bulk density and porosity were measured following the modified procedure described by Akdeniz et al. (2011). Bulk density was measured by placing 1 L biocarrier in a 1-L glass breaker and calculating its weight. Porosity was measured by placing 300 mL biocarrier bulk in a 1-L plastic beaker, and adding and calculating the water volume required to fill the voids up to the 300 mL volume level. To remove any toxins present in biocarries, they were immersed in tap water for 24 hours and then dried at 105^{o}C for 12 hours before placing in the digesters.

Substrate and inoculum

Poultry blood and PPWW were collected once every two weeks from the top of a 17,000-L (4500-gallon) blood tanker and post-secondary screening wastewater pit, respectively, at a commercial poultry processing plant. Blood and PPWW were mixed in the ratio of 1:3 (v/v). In the first phase, the mixture was stored in a refrigerator (<4^{o}C) and then directly fed into the digester. In the second phase, in order to prevent digester clogging while operating at shorter HRTs, the mixture was filtered using a 1-mm mesh to remove feathers and debris and then stored in a freezer (-20^{o}C), and subsequently diluted prior to feeding the digester (Table 3.1). Inoculum used in these experiments was collected from a 30-L CSTR digester actively treating slaughterhouse wastewater at 35^{o}C for 6 months.

Experimental operation

Steps in the experimental protocol are presented in Table 3.1. Digesters were operated in two separate phases using feedstocks of different COD concentrations (Table 3.2). In the first phase, after adding inoculum, digesters were started with an OLR of 1.1 g COD L^{-1} d^{-1} (equivalent to HRT of 27.7 days). Subsequently, the OLR was increased stepwise to 1.8, 2.9 and 4.7 g COD L^{-1} d^{-1} (equivalent to HRTs of 13.7, 9.2 and 6.9 days, respectively) over a period of

105 days. The second phase started six months after the end of the first phase. During the six months between the two phases, feedstock (similar in characteristics as used in Phase 1) was fed to the digesters at an HRT of 13.8 days. In this phase, for reducing the TAN inhibition and preventing the clogging in digesters, the raw feedstock was filtered using 1-mm mesh and diluted to an approximate COD of 15,000 mg L^{-1} using dechlorinated tap water and fed into digesters at the OLRs of 3.9, 4.6 and 2.5 g COD L^{-1} d^{-1} corresponding to HRTs of 3.7, 3.0 and 6.0 days, respectively for a total of 95 days.

The feedstock was sampled once a week in both phases for chemical analysis. Effluents of digesters were sampled for chemical analysis twice a week during the first phase, and once a week in the second phase. Biogas production volume was measured daily in both phases, while methane concentration in the biogas was measured twice a week in the first phase, and once a week in the second phase.

Analytical methods

The pH was measured using an Accumet portable AP61 pH meter (Fisher Scientific, Hampton, New Hampshire). COD was measured in samples that were diluted 100-fold using the HACH method 8000 (HACH, Loveland, CO). TS, VS, total suspended solid (TSS) and volatile fatty acids (VFA) were analyzed following standard laboratory methods used in the wastewater industry (APHA, 1992). TS and VS were measured by drying 30-mL samples at 105^{o}C for 24 h and then burning at 500±50^{o}C for 1 h. TSS was measured by filtering 10 mL samples though a 1.6-µm filter and drying the filter with residue at 105^{o}C for 24 h. For VFA measurements, samples were centrifuged at 1000 rpm for 5 min and the supernatant was diluted 10-fold using deionized water and distilled at the rate of 3 mL min^{-1}. Total nitrogen (TN) and TAN were measured on a 100-fold diluted sample using HACH method 10072 and 10031, respectively

(HACH, Loveland, CO). Carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) compositions were measured using FLASH 2000 CHNS-O analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 1 to 3 mg oven dried samples (105^{o}C for 24 hours) were weighed in tin capsules and placed in the instrument that measured and reported total weight percent of each element using a combustion analysis. Biogas production volume was measured using a tipping bucket gas flow meter. In the first phase, methane concentrations were measured using a GC-TCD (HP 5890 Series II plus Gas Chromatograph). Column is stainless steel column (Alltech Porapak Q 6' × 1/8" ×0.85mm) with Porapak Q (100/120 mesh). The oven temperature was 90^{o}C and the temperatures of injector and detector were at 100 and 140 ^{o}C, respectively. Helium at a flow rate of 30 mL min^{-1} was the carrying gas. Biogas samples of 30-µL were taken from the headspace of each digester using a gastight syringe and tested in this GC.

In the second phase, methane concentrations were measured using GC-TCD (SRI 310C Gas Chromatograph). The GC had a stainless steel column (80/100 HayeSep D 6' ×1/8'') ; oven and detector temperatures of 40^{o}C and 380^{o}C, respectively; Carrier gas, fuel gas and oxidizing gas were helium (10 mL min^{-1}), hydrogen(25 mL min^{-1}) and air (250 mL min^{-1}), respectively. Biogas samples (0.1-mL) were taken from the headspace of each digester using a gastight syringe and tested in this GC.

Scanning electron microscope

The surface texture of biocarriers dramatically impacts the microorganisms' attachment and growth on biocarriers, and further the digester performance (Show and Tay, 1999). It can be identified using the scanning electron microscope (SEM). One piece of biochar was randomly collected from the storage of biochar; one piece of bamboo ring was randomly collected from the storage of bamboo rings, and carefully cut into 4-5 pieces using for the SEM analysis.

Biochar and bamboo samples was sputter-coated with gold in the vacuum (< 2 mbar) (SPI Module Sputter Coater, West Chester, PA) and examined using the Zeiss 1450EP variable pressure Scanning Electron Microscope (SEM).

Results and discussion

Feedstock characterization

The characteristics of feedstock are summarized in Table 3.2. Raw feedstock was the mixture of poultry blood and PPWW (1:3, v/v). Diluted feedstock was made by diluting filtered (using 1-mm mesh) raw feedstock 2-fold using dechlorinated water. At this dilution, the COD was reduced from 29,241 mg L^{-1} to 14,179 mg L^{-1}. Filtration using the 1-mm mesh removed partial TSS and decreased TSS of diluted feedstock expectedly to prevent the digester clogging at the short HRTs. Slaughterhouse blood waste is known to have protein contents in the range of 118,000-159,000 mg L^{-1} (Hejnfelt and Angelidaki, 2009; Yoon et al., 2014) and is a significant contributor to nitrogen in the feedstock mixture. The TN in the feedstock is hydrolyzed to TAN and can be inhibitory to the AD process when levels exceed 3,000 mg L^{-1} (Rajagopal et al., 2013). Diluting the feedstock potentially reduces TAN inhibition of methanogenesis and can improve digester performance.

Biogas production

In Phase 1 when un-diluted feedstock was used, average biogas production in the BC digester ranged from 0.08 to 0.58 L L^{-1} d^{-1} and contained 48-60% methane (Figure 3.1). In this phase, the OLRs were increased stepwise from 1.1 to 4.7 g COD L^{-1} d^{-1}. At steady-state (Table 3.3), biogas productions were 0.55 and 0.64 L L^{-1} d^{-1} for OLRs of 2.9 and 4.7 g COD L^{-1} d^{-1} (corresponding to 1.9-3.1 g VS L^{-1} d^{-1}), respectively. As compared to other types of digesters such as CSTR and UASB treating similar feedstock (Cuetos et al., 2009; Hansen and West,

1992), the BC digester in this study handled the higher OLRs, and correspondingly produced higher biogas production. Cuetos et al. (2009) reported AD of blood with the Organic Fraction Municipal Solid Waste (OFMSW) at an OLR of 1.5 g VS L^{-1} d^{-1} producing biogas at 0.5 L L^{-1} d^{-1} with 60% methane. Another study by Hansen and West (1992) treating blood and rendering condensate produced biogas at 0.11 L L^{-1} d^{-1} with 84% methane content at an OLR of 1.01 g COD L^{-1} d^{-1}. At OLRs of 2.9 and 4.7 g COD L^{-1} d^{-1}, methane yields were 221 and 331 mL g^{-1} $COD_{removed}$, respectively, comparable to the 240 mL g^{-1} $COD_{removed}$, reported by Hansen and West (1992).

In Phase 2 when diluted feedstock was used, biogas production at steady-state decreased from 0.64 to 0.46 L L^{-1} d^{-1} at OLRs 3.9 to 4.6 g COD L^{-1} d^{-1} with decreased methane content and yield. Diluted feedstock was expected to increase methane yield by alleviating TAN inhibition, however, the concomitant shorter HRTs of 3-3.7 days possibly resulted in instability of the methanogenic population and wash out of the active sludge resulting in lower methane yield.

The BB digester produced 0.07 to 0.23 L L^{-1} d^{-1} of biogas with 30-47% methane in it during Phase 1, which was significantly less than that from the BC digester at the higher OLRs. The low biogas quantity and quality suggested that methanogenesis was inhibited in this digester possibly by accumulation of intermediates such as TAN and VFA. Diluting the feedstock in Phase 2 did not result in recovery of the digester at higher OLRs of 3.9 and 4.6 g COD L^{-1} d^{-1} (Table 3.4). One reason could be that the shorter HRTs corresponding to higher OLRs did not give sufficient time for methanogens to grow and the population could have washed out of the digester. The methanogens generation time was 3-30 days (Gerardi, 2003) and if HRTs were much shorter, washout rate of active sludge including methanogens can be higher than its reproduction, which results in low biogas production and COD removal. This explanation agrees

with the results of Hansen and West (1992) that COD removal decreased from 71.6% to 28.0% when HRTs changed from 15.6 to 5.2 days.

COD Removal

In Phase 1, COD removal of both treatments decreased with stepwise increase of OLR (Figure 3.2). The BC digester had higher COD removals (22-69%) relative to the BB digester (0-59%). These results agreed with results reported by Hansen and West (1992) in that COD removal ranged between 28.0 and 71.6%, decreasing with increasing OLR. At higher OLR, higher VFA and TAN concentrations could inhibit methanogenesis and result in lower COD removal. In Phase 2, COD removals continued to remain low at levels of 20-41% for BC digester and 0-26% for the BB digester. Diluting the feedstock slightly improved the COD removal at higher OLRs.

TSS Change

At OLRs of 2.9 and 4.7 g COD L^{-1} d^{-1} TSS was reduced from influent values of 4,679 mg L^{-1} to effluent values of 355 and 808 mg L^{-1}, respectively, in the BC digester in Phase 1 (Table 3.2 and 3.3). Biochar is a porous adsorbent that is sometimes used for filtering pollutants and nutrient recovery from wastewater (Ghezzehei et al., 2014). At the beginning of the experiment, the microflora in the inoculum could have deposited in the porous biochar and adapted gradually to the digester environment. This microflora in the biochar is responsible for substrate hydrolysis and production of TAN and VFA. The BB digester showed lower TSS removal than the BC digester, possibly because of relatively low hydrolysis at high OLRs caused by differences in the biocarrier surface structure (Table 3.4). In Phase 2, TSS removal decreased in both digesters, although the feedstock was filtered and diluted (Table 3.2, 3.3 and 3.4). Lopez et al. (2006) reported the hydrolysis of blood was completed in five days. Therefore, particles in the blood

could be partially hydrolyzed at shorter HRTs of 3.0-3.7 days and the remaining particles would be discharged from the digesters, resulting in low TSS removal.

Intermediates

TAN and VFA are key intermediates from protein and lipid decomposition in AD of blood and PPWW. In Phase 1 of our experimental work, TAN and VFA gradually accumulated to levels around 3,110 mg L^{-1} and 15,237 mg L^{-1}, respectively (Figure 3.3). High TAN concentrations seem to have increased the buffering capacity of the substrate, thus maintaining a pH of 6.6-7.7 in both digesters. However, these high levels of TAN could have also inhibited methanogenesis, as it is known that inhibition occurs at TAN levels exceeding 1,500 mg L^{-1} (Rajagopal et al., 2013). Porous biochar could have served as a harbor for attachment of methanogens protecting it from the TAN inhibition and producing higher methane yields (Figure 3.1). These results agree with previous reports in the literature (e.g. Mumme et al., 2014). Diluting the feedstock reduced the TAN and VFA level to around 1,400 mg L^{-1} and 7,000 mg L^{-1}, respectively, which appear to have reduced TAN and VFA toxicity; however, the higher buffering capacity provided by TAN decreased at shorter HRTs, resulting in the low methane yield (Table 3.3 and 3.4).

COD removal kinetics

Kinetics in anaerobic digestion is studied to understand the organic matters degradation rates under the specific operational factors. The results of this study can be used to estimate and optimize the performance of different scale digesters operated at the same conditions (Debik and Coskun, 2009; Padilla-Gasca and López-López, 2010).

Since, the BC digester had superior performance as measured by methane yield and COD removal, the kinetics of COD reduction in the BC digester were modeled using modified Stover-

Kincannon model and Grau second-order model (Figure 3.4). Modified Stover-Kincannon model was derived from the Stover-Kincannon model used in modeling rotating biological contactor (RBC) systems (Kincannon and Stover, 1982). The modified model replaced surface area of rotating discs (which represent attached-biofilm activities in the RBC) with the volume of anaerobic filter. This modification is justified based on the fact that the suspended active sludge in an AF shows similar waste removal capability to active sludge attached to the fixed media (Tay et al., 1996; Yu et al., 1998).

At steady-state conditions, the substrate degradation rate in an AF can be expressed as

$$\frac{dS}{dt} = \frac{Q}{V}(S_i - S_e) \tag{1}$$

Where, Q is substrate flow rate (L d^{-1}), V is effective volume of AF (L), S_i and S_e are influent and effluents COD concentration (g L^{-1}). The dS/dt can also be defined by the following equation from the original Stover-Kincannon model

$$\frac{dS}{dt} = \frac{U_{max}(QS_i/A)}{K_B + (QS_i/A)} \tag{2}$$

Where, U_{max} is the maximum utilization rate constant (g L^{-1} d^{-1}), K_B is the saturation value constant (g L^{-1} d^{-1}), A is the surface area of the rotating disc.

After the modification of equation 2 using V replacing A, the modified equation is

$$\frac{dS}{dt} = \frac{U_{max}(QS_i/V)}{K_B + (QS_i/V)} \tag{3}$$

Combining equation 1 and 3, the modified Stover-Kincannon model is expressed as

$$\frac{V}{(S_i - S_e)Q} = \frac{K_B}{U_{max}}\frac{V}{QS_i} + \frac{1}{U_{max}} \tag{4}$$

The equation of Grau second-order model (Grau et al., 1975) was derived from the general differential equation of chemical reaction kinetics and expressed as

$$-\frac{dS}{dt} = k_S * X * (\frac{S}{S_0})^2 \quad (5)$$

After integration with boundary condition and linearization, equation 5 becomes

$$\frac{S_0 * \theta_H}{S_0 - S} = \theta_H - \frac{S_0}{K_S * X} \quad (6)$$

$$\frac{\theta_H}{E} = m + n * \theta_H \quad (7)$$

Where θ_H is HRT (day), E is removal rate, m and n are constants.

From the linear regression of the two models (Figure 3.4), R^2 of the Stover-Kincannon model was found to be more than 90% indicating a better fit over the two phases of experimental work. In the Phase 1, K_B and U_{max} were found to be 1.36 and 1.69 g L^{-1} d^{-1}, respectively, and in Phase 2, K_B and U_{max} were 102 and 33 g L^{-1} d^{-1}, respectively. The higher U_{max} in the second phase when diluted feedstock was used confirms that diluting the feed improved COD degradation. These constants were less than results of the study of Padilla-Gasca and López-López (2010) treating slaughterhouse wastewater under similar condition in an AF at 35^{o}C. Their results showed that K_B and U_{max} were 120.88 and 99.01 g $L^{-1}d^{-1}$, respectively. The COD removal in the study of Padilla-Gasca and López-López (2010) was over 83% at OLRs of 3.1-6.2 g COD L^{-1} d^{-1}, however, the result of this study was less than 80% at OLRs of 1.1-4.7 COD L^{-1} d^{-1}.Higher nitrogen and COD in the feedstock were considered as important factors resulting in lower value of U_{max}.

Characteristics of biocarriers and SEM of biocarrier surface texture

Biochar had diameter of 0.49±0.03 cm and length of 0.85±0.13 cm. The bulk density of biochar and bamboo was found to be 394.6±11.8 and 240.3±6.8 kg m^{-3}, respectively, and porosities were 62.5±0.2 and 75.0±2.3 %, respectively. Biochar and bamboo biocarriers had higher porosity (>62%) that potentially helped the microflora colonize them easily. Results of the SEM to describe surface textures of the two biocarriers show rougher surface texture on biochar which could allow the microflora to attach more readily and thus produce higher methane yield (Figure 3.5 a and b). Bamboo was found to have a relatively flat and smooth surface (Figure 3.5 b and c). These observations support our experimental results that show that the BC treatment had higher biogas production relative to BB.

Potential for energy and nutrient recovery from a full-scale AD system

Energy and nutrient recovery are important benefits relating to AD of slaughterhouse wastes (Yoon et al., 2014). Results from this semi-continuous lab-scale study were used to estimate the energy and nutrient recovery from a proposed full-scale anaerobic digester treating co-substrates of poultry blood waste and PPWW.

The parameters of a proposed full-scale AD system are shown in Table 3.5. In the U.S., a typical poultry processing plant slaughtering 200,000 birds per day discharges approximately 5,200-m^3 PPWW and collects 13.6-m^3 blood (40% of total blood) per day (Northcutt and Jones, 2004; Kiepper, 2007). Using the 1:3 (v/v) ratio of blood to PPWW, 54.4-m^3 d^{-1} of feedstock including blood waste can be processed in a digester. The effective volume of digester will be 381 m^3 when operated at a HRT of 7 days. After providing a 20% headspace and accounting for 50% effective volume of packing media (65% of porosity), total digester volume required would be 524 m^3.

The energy and nutrients recovery from such a system is presented in Table 3.6. Energy production was calculated from daily methane production and using a low heating value for methane as 35.8 kJ L^{-1}. Heat consumed for the digester maintenance includes energy needed for pre-heating feedstock from storage temperature (20^{o}C) to digester temperature (35^{o}C) and compensating for energy losses from the digester body and other mechanical energy requirements (substrate mixing in digester, water recirculating in the heat exchanger and feeding to digester). Feedstock storage temperature was estimated from annual average temperature in Athens GA, USA and blood and PPWW discharging temperature. Following the method used by Bouallagui et al. (2004), the methane energy production was calculated to be 5.2 GJ d^{-1} and the energy consumed for digester maintenance was 3.7 GJ d^{-1}, resulting in a net available energy of 1.5 GJ d^{-1} (416 kWh d^{-1}) for other uses.

Blood waste and PPWW had high nitrogen (N), phosphorus (P) and Potassium (K) contents, which were calculated as 4,623 mg L^{-1}, 55 mg L^{-1} and 69 mg L^{-1}, respectively from the results of literature (Kiepper, 2009; Yoon et al., 2014). These minerals are the main components of plant fertilizers and can be recovered from the effluent of the AD system, for example through land application for crop growth. Assuming no nutrient loss occurs during the stable operation of the digester, N, P and K recovered would be 252, 3.0 and 3.7 kg d^{-1}, respectively, from the proposed full-scale digester.

Conclusions

Anaerobic co-digestion of poultry blood waste and PPWW can be a feasible way for stabilizing the waste streams while providing positive energy and nutrients recovery. Biochar can be used in the digester as biocarrier to effectively process these high-strength wastewaters at high OLRs. High VFA and TAN concentrations in the substrates inhibited the AD process at the OLR

of 4.7 g COD L^{-1} d^{-1} and feedstock dilution reduced the VFA and TAN concentrations but did not improve methane yield.

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Table 3.1 Operational conditions during testing of digesters

Feedstock Blood: PPWW (%, volume)	OLR (g COD L^{-1} d^{-1})	HRT (day)	Run Duration (day)	Configuration
25:75 (Raw feedstock)	1.1	27.7	25	First phase
	1.8	13.7	14	
	2.9	9.2	28	
	4.7	6.9	38	
25:75 (Two-fold diluted raw feedstock)	3.9	3.7	32	Second phase
	4.6	3.0	25	
	2.5	6.0	38	

Table 3.2 Characteristics of feedstock and inoculum

	Feedstock	Diluted feedstock	Inoculum
pH	6.4±0.3	6.7±0.2	8.0±0.1
COD (mg L^{-1})	29,241±3,299	14,179±1,071	8,700-9,200
TS (mg L^{-1})	21,385±1,900	6,802±2,375	3,610±111
VS (mg L^{-1})	19,164±1,805	5,982±2,413	2,333±102
TSS (mg L^{-1})	4,679±1,299	1,270±596	850-1,050
TN (mg L^{-1})	2,667±828	NA[a]	NA[a]
TAN (mg L^{-1})	267±267	232±119	NA[a]
C [b]	48.5±1.8	NA[a]	NA[a]
H [b]	7.0±0.2	NA[a]	NA[a]
N [b]	13.8±0.3	NA[a]	NA[a]
S [b]	1.0±0.1	NA[a]	NA[a]
C/N	3.5±0.2	NA[a]	NA[a]

[a] NA, not available
[b] w/w, % dry base

Table 3.3 Summary of effluents characteristics and biogas production at different OLRs at steady-state condition for BC digester

	Characteristics of effluents					Biogas production			
OLR (HRT)	COD[a] (mg L^{-1})	pH[b]	TAN[b] (mg L^{-1})	VFA[b] (mg L^{-1})	TSS [b] (mg L^{-1})	L L^{-1} d^{-1}[a]	CH_4% [b]	mL CH_4 g^{-1} COD_{added} [b]	mL CH_4 g^{-1} $COD_{removed}$ [b]
First phase									
2.9(9.2)	17,450±1,213	7.3±0.1	2,520±126	12,253± 428	355±9	0.55±0.01	63.7-63.9	96.2±6.7	220.6±25.4
4.7(6.9)	24,867±375	7.4±0.1	3,277±343	15,035±2,576	808± 59	0.64±0.00	55.9-64.2	81.6±4.9	330.5±37.0
Second phase									
3.9(3.7)	8,350±826	7.1±0.1	1,435±101	7,130±970	955-1,255	0.64±0.04	58.3	81.5±15.1	203.2±51.4
4.6(3.0)	10,300±436	6.8±0.0	1,532±232	6,999±1,074	590-680	0.46±0.00	51.0	46.5±7.1	163.0±51.9

[a] Steady state condition was assumed when Coefficient of Variation of COD of effluent at three consecutive sampling events and biogas production of three continuous days were ≤10.0%.

[b] Parameters here were within results of three consecutive sampling events.

Table 3.4 Summary of effluents characteristics and biogas production at different OLRs at steady-state condition for BB digester

	Characteristics of effluents					Biogas production			
OLR (HRT)	COD[a] (mg L^{-1})	pH[b]	TAN[b] (mg L^{-1})	VFA[b] (mg L^{-1})	TSS[b] (mg L^{-1})	L L^{-1} d^{-1}[a]	CH_4% [b]	mL CH_4 g^{-1} COD_{added}[b]	mL CH_4 g^{-1} $COD_{removed}$[b]
First phase									
1.1(27.7)	14,567±569	6.8±0.1	1,953±26	5,617-8,260	300± 57	0.06±0.00	46.9	24.9±1.1	53.6±4.7
1.8(13.7)	17,183±952	6.7±0.1	2,145±332	6,271±1,337	380±33	0.10±0.01	37.2-38.6	17.5± 2.6	53.9±12.9
2.9(9.2)	26,617±1,862	6.7±0.0	2,548±107	12,042±3,371	1,383±213	0.17±0.00	32.9-34.4	13.6± 1.8	100.9±28.2
4.7(6.9)	31,233±1,741	7.0±0.1	2,923±212	12,012±2,034	2,388±142	0.29±0.02	35.8-39.2	23.9± 4.1	472.7±227.8
Second phase									
3.9(3.7)	11,333±208	6.5±0.1	1,398±123	7,290±359	725-845	0.13±0.01	37.4	10.9±3.4	66.8±42.0
4.6(3.0)	12,567±896	6.5±0.0	1,678±161	6,835±2,557	565-635	0.14±0.00	35.9	10.3±1.3	90.1±62.3

[a] Steady state condition was assumed when CV of COD of effluent at three consecutive sampling events and biogas production of three continuous days were ≤10.0%.

[b] Parameters here were within results of three consecutive sampling events.

Table 3.5 Full-scale digester proposed for a typical poultry processing plant in the US

Birds processed (bird d^{-1})	200,000
PPWW (L $bird^{-1}$)	26
Blood waste (L $bird^{-1}$)	0.068
Feedstock flow rate (L d^{-1})	54,400
HRT (day)	7
Effective volume (m^3)	380.8
Total volume (m^3)	523.6
Biogas production rate (L L^{-1} d^{-1})	0.64
Biogas production (m^3 d^{-1})	243.7
CH_4 content in biogas (%)	60
CH_4 production (m^3 d^{-1})	146.2
Operation temperature (^oC)	35
Feedstock storage temperature (^oC)	20

Table 3.6 Energy and nutrients recovery from full-scale anaerobic digester treating blood wastes in a typical poultry processing plant

Energy recovery	
Energy production	
Methane energy (GJ d^{-1})	5.2
Heat requirement for digester maintenance	
Energy for Preheating feedstock (GJ d^{-1})	3.4
Energy loss (GJ d^{-1})	0.3
Net energy (GJ d^{-1})	1.5
Nutrient recovery[a]	
N (kg d^{-1})	251.5
P (kg d^{-1})	3.0
K (kg d^{-1})	3.7

[a] blood and wastewater nutrients information were obtained from results of Yoon et al. (2014) and Kiepper (2009), respectively.

Figure 3.1 Biogas productions from digesters in the first (a) and second (b) phase. Biogas production is the mean of three days. BC is biochar digester, BB is bamboo digester, and unit of OLR is g L^{-1} d^{-1}.

Figure 3.2 COD removal in the first (a) and second (b) phase. BC is biochar digester, BB is bamboo digester, and unit of OLR is g L^{-1} d^{-1}.

Figure 3.3 Main intermediates of digester effluents in two phases. (1-a) and (1-b) indicate BC and BB digester in the first phase, respectively; (2-a) and (2-b) indicate BC and BB digester in the second phase, respectively. BC is biochar digester, and BB is bamboo digester.

Figure 3.4 Modified Stover-Kincannon model (a) and Grau second-order kinetic (b) for COD degradation in BC digester at two phases. BC is biochar digester.

Figure 3.5 SEM image of surface texture of biochar (a, scale bar=100μm and b, scale bar=10 μm) and bamboo (c, scale bar=100μm and d, scale bar=10 μm).

Figure 3.1

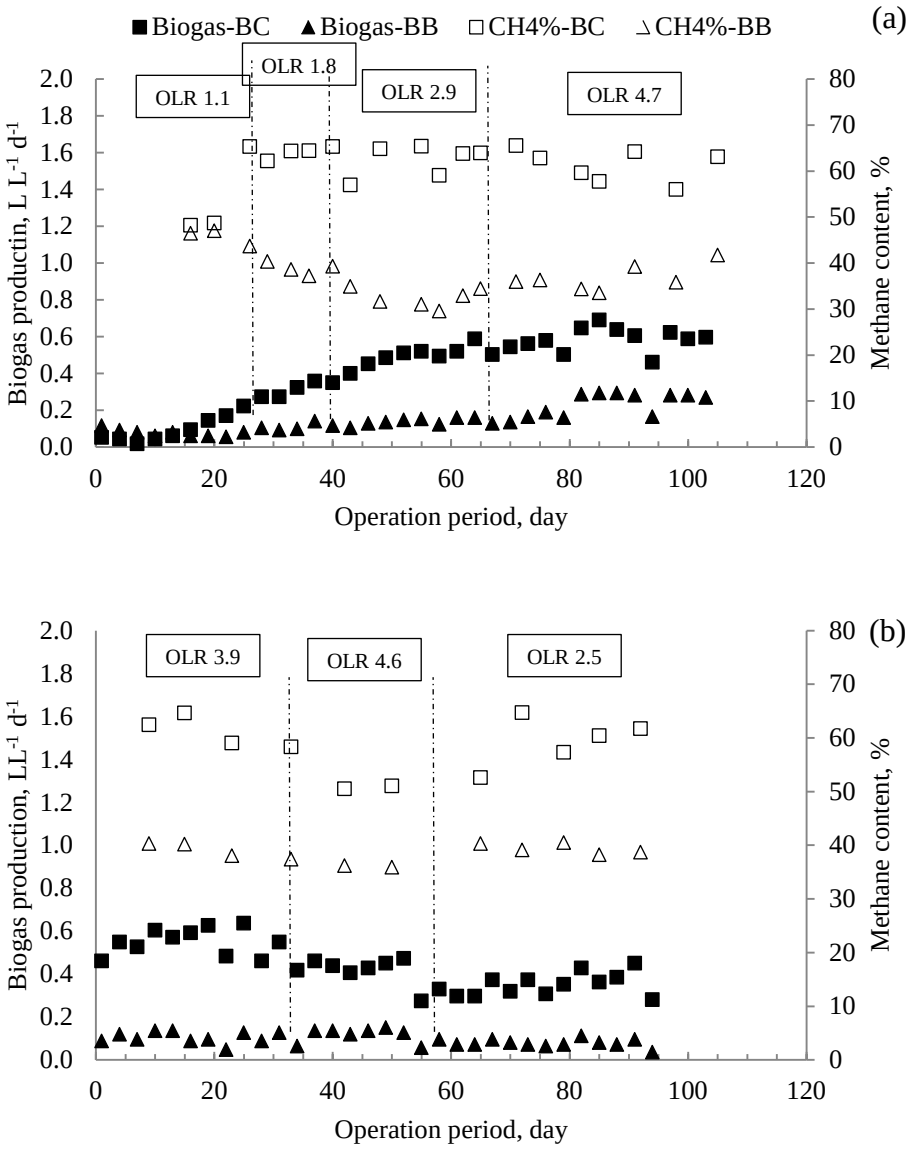

(a)
Biogas-BC
Biogas-BB
CH4%-BC
CH4%-BB
OLR 1.1
OLR 1.8
OLR 2.9
OLR 4.7
Biogas productin, L L-1 d-1
Methane content, %
Operation period, day
(b)
OLR 3.9
OLR 4.6
OLR 2.5
Biogas production, LL-1 d-1
Methane content, %
Operation period, day

Figure 3.2

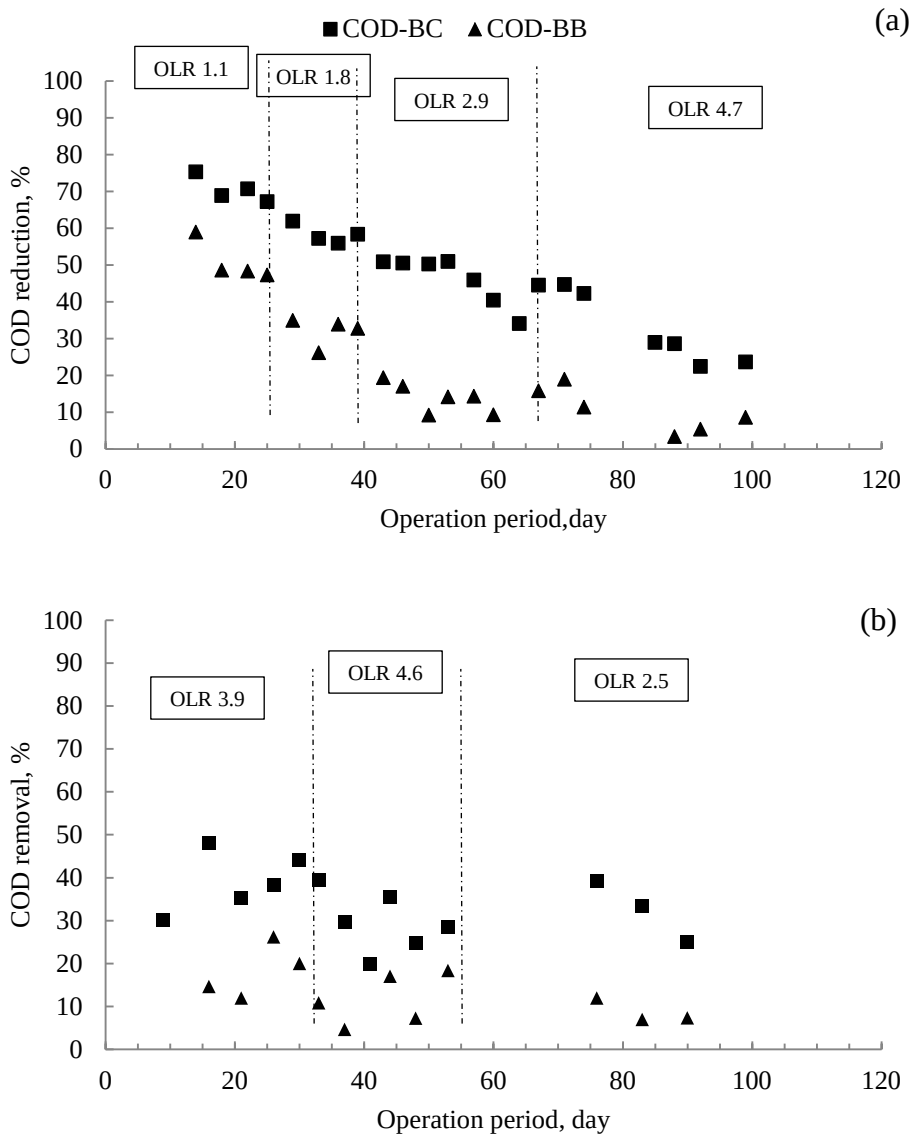

(a)
COD-BC
COD-BB
OLR 1.1
OLR 1.8
OLR 2.9
OLR 4.7
COD reduction, %
Operation period,day
(b)
OLR 3.9
OLR 4.6
OLR 2.5
COD removal, %
Operation period, day

Figure 3.3

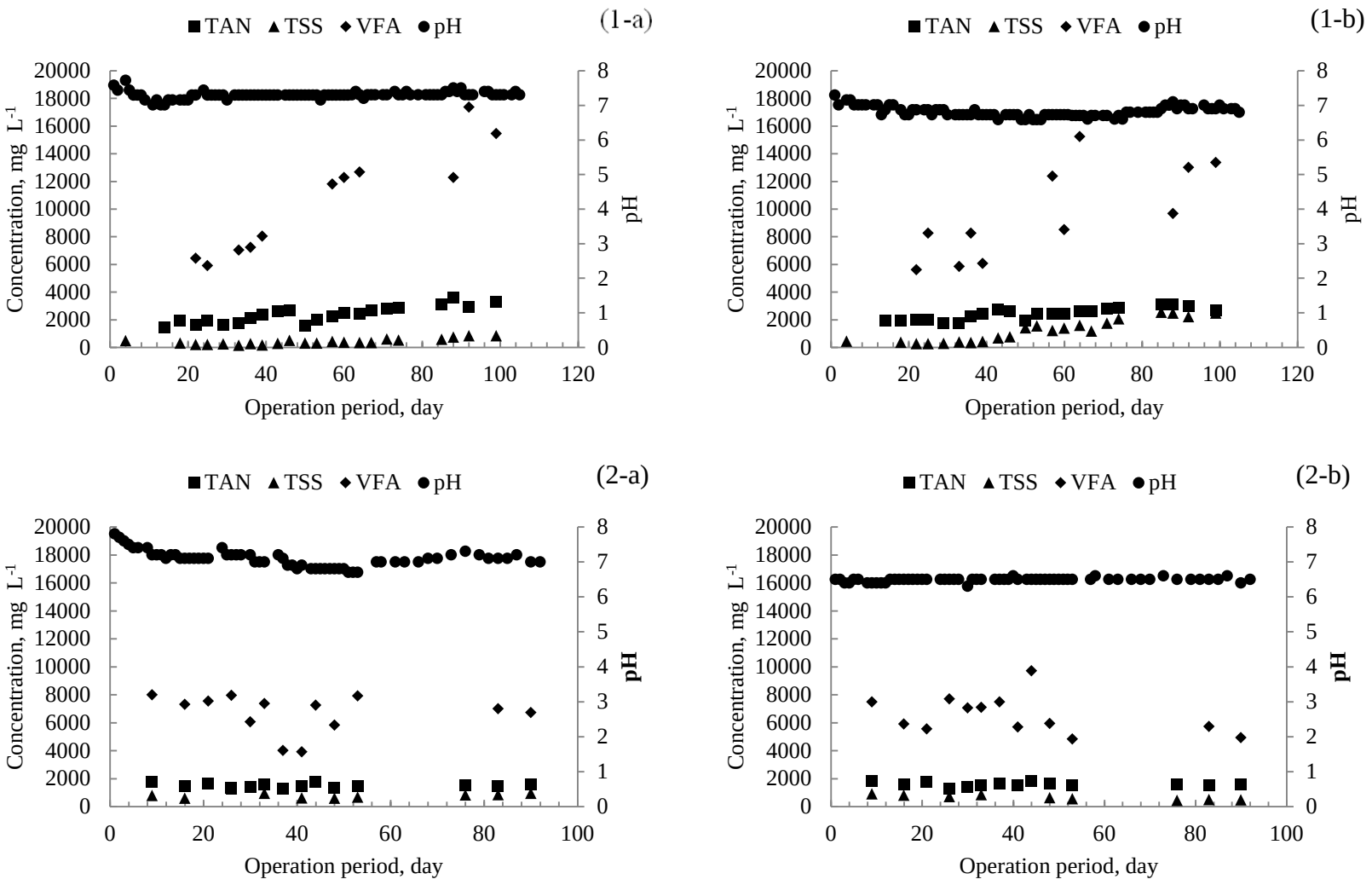

(1-a)
TAN TSS VFA pH
Concentration, mg L-1
pH
Operation period, day
(1-b)
TAN TSS VFA pH
Concentration, mg L-1
pH
Operation period, day
(2-a)
TAN TSS VFA pH
Concentration, mg L-1
pH
Operation period, day
(2-b)
TAN TSS VFA pH
Concentration, mg L-1
pH
Operation period, day

Figure 3.4

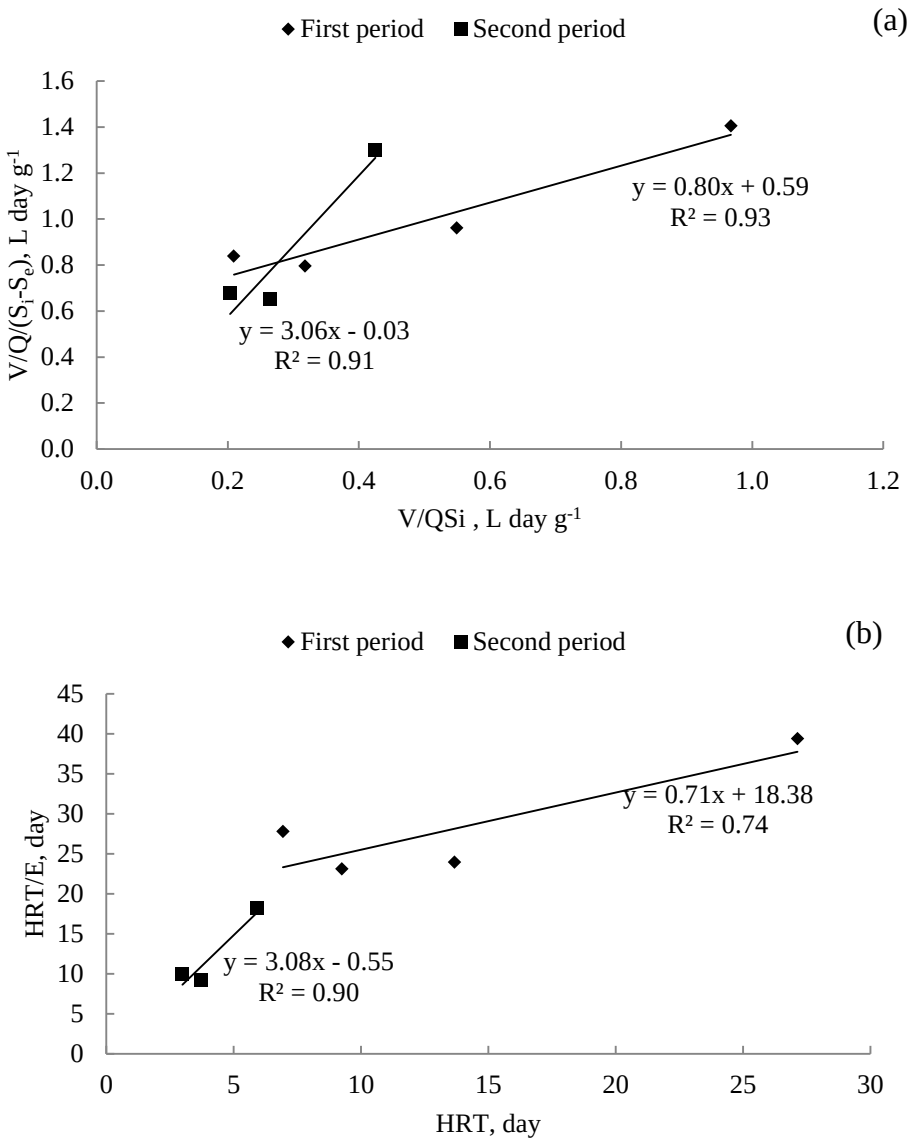
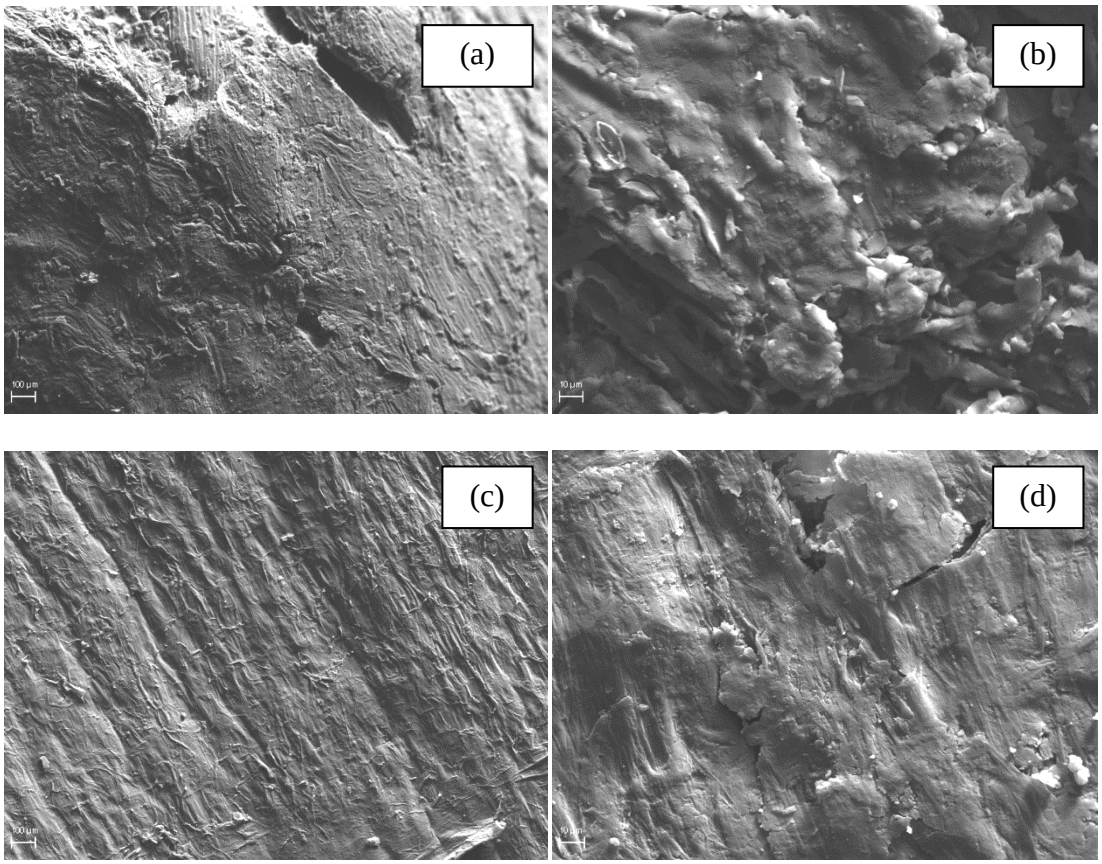

(a)
First period
Second period
y = 0.80x + 0.59
R² = 0.93
y = 3.06x - 0.03
R² = 0.91
V/Q/(Si-Se), L day g-1
V/QSi , L day g-1
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
(b)
First period
Second period
y = 0.71x + 18.38
R² = 0.74
y = 3.08x - 0.55
R² = 0.90
HRT/E, day
HRT, day
0
5
10
15
20
25
30
35
40
45

Figure 3.5



CHAPTER 4

CO-DIGESTION OF SLAUGHTERHOUSE BLOOD WASTES AND WASTEWATERS IN A PILOT SCALE TWO-STAGE ANAEROBIC DIGESTER[1]

[1]Wang, S., B.H. Kiepper, G.L. Hawkins, and K.C. Das. To be summited to *Bioresource Technology.*

Abstract

Recovery of energy from slaughterhouse blood wastes and wastewaters in a pilot two-stage anaerobic digester was investigated at different organic loading rates (OLR). Potential energy recovery efficiency from blood wastes of different animals was estimated based on results of experimental digester operation. Results showed methane yield of 18.7 mL g^{-1} COD_{added} and negligible COD removal in the acidogenic digester (stage 1) under digester conditions of pH 6.4 and OLR 2.3 g COD L^{-1} d^{-1}; and methane yield of 164 mL g^{-1} COD_{added} and 53.7% COD removal in the methanogenic digester (stage 2) at digester conditions of pH 7.3 and OLR 0.6 g COD L^{-1} d^{-1}. The overall two-stage digester yielded higher methane than similar feedstocks reported in the literature treated in single-stage digesters. Energy recovery of 18.4-19.1 kJ kg^{-1} animal weight was estimated available from the two-stage anaerobic digestion of blood wastes at the region of southeast US.

Introduction

The United States (US) meat industry produced 39.3 million metric tons of product including pork, beef and broiler meat in 2013 (USDA, 2014) and discharged approximately the same amount of slaughterhouse wastes (Ockerman and Hansen, 2000). Blood wastes comprised 7-11% of slaughterhouse wastes (Edstrom et al., 2003; Yoon et al., 2014). Blood wastes are typically collected in a storage tanker and shipped to a rendering plant for production of blood meal used in animal food or compounded fertilizer for plant growth. Both energy and chemicals inputs are required for transportation, storage and processing of blood wastes in the rendering plant (Ockerman and Hansen, 2000).

Anaerobic digestion (AD) of slaughterhouse blood wastes is an attractive alternative for energy and nutrients recovery. Methane yield potential reported in the literature is as high as 500 mL g^{-1} VS_{added} and the high protein content (as high as 11%, wet basis) in blood makes it an attractive substrate for nitrogen recovery (Salminen and Rintala, 2002). Co-digestion of slaughterhouse blood wastes with carbon-rich substrates is considered an effective way to provide the required nutrient balance for AD (Lopez et al., 2006). Cuetos et al. (2013) co-digested poultry blood with energy crops in a semi-continuously fed anaerobic digester at 34^{o}C and an organic loading rate (OLR) of 3.1 g VS L^{-1} d^{-1}. They obtained a maximum methane yield of 165 mL $g^{-1}VS_{added}$ and concluded that energy crops comprising 60% VS reduced ammonia inhibition from poultry blood by reducing nitrogen concentrations in the digesters. Zhang and Banks (2012) studied anaerobic co-digestion of sheep blood with the organic fraction from municipal solid waste (OFMSW) which contained 80% VS. They report that ammonia inhibition was alleviated and obtained methane yields of 180 mL g^{-1} VS_{added} at digester temperature of 36^{o}C and OLR of 3 g VS L^{-1} d^{-1}. These studies indicated that when higher fractions of blood are used in the mixes, extremely high total ammonia nitrogen (TAN) concentrations can result, severely inhibiting anaerobic digestion. Therefore, the amount of blood added to the digester feedstock should be carefully thought out and managed to prevent inhibition of performance and potential digester failure.

Co-digestion of slaughterhouse wastewaters with blood wastes can be cost-effective because of wastewater's high lipid content and because both substrates are available at a single location (Kiepper et al., 2008). The volumes of wastewaters discharged from

slaughterhouses are in the range of 400 to 3,100 L per bovine, 58 to 620 L per pig and 19 to 38 L per broiler bird (Kiepper et al., 2008; Tritt and Schuchardt, 1992). There are few studies on anaerobic co-digestion of animal blood waste and poultry processing wastewater (PPWW). Hansen and West (1992) conducted co-digestion of a mixture of blood and condensate discharge (1:49, v/v) at a rendering plant using a 7.8-L UASB operated at 35^{o}C. The system produced 0.06 to 0.11 L_{biogas} $L^{-1}{}_{digester}d^{-1}$ at an organic loading rate (OLR) of 0.34 to 1.01 g COD L^{-1} d^{-1}. Marcos et al. (2010) anaerobically treated a similar ratio of blood to wastewater (1:49, w/w) in a 2-L discontinuous digester (38^{o}C) at different loadings (0.17-0.56 g COD L^{-1} d^{-1}) and obtained the highest chemical oxygen demand (COD) removal of 56.9%. High VFA and TAN concentrations were the primary inhibitors resulting in lower methane production and COD removal in these studies. In addition, high protein and lipid contents in these substrates also possibly caused foaming and clogging within the digesters and pipes, resulting in a reduction of 20-50% in biogas production and higher maintenance costs (Kougias et al., 2014).

In a two-stage digester system, proteins and lipids in the feedstock are hydrolyzed in the first stage (acidogenic digester) and converted to low molecular weight intermediates of VFA and TAN; the VFA are transformed to biogas including methane and carbon dioxide in the second stage (methanogenic digester) (Stoyanova et al., 2014; Wang and Banks, 2003). Two-stage anaerobic digesters have been used to treat slaughterhouse wastes in previous studies with superior performance resulting from reduced TAN and VFA inhibition of methanogenesis (Banks and Wang, 1999; Wang and Banks, 2003). Although

there are lab-scale two-stage anaerobic digesters to treat slaughterhouse wastes, the experience and knowledge at the pilot scale are still very limited.

To fill the gap described above, our objective was to evaluate the performance of a pilot two-stage anaerobic digester treating co-substrates of poultry blood wastes and slaughterhouse wastewaters and determine the optimum OLRs for each stage and the overall system to maximize methane yield, COD removal, and energy recovery.

Materials and methods

Equipment

All experiments in this study were conducted in a semi-stirred acidogenic digester and a down-flow methanogenic digester (Figure 1). The acidogenic digester was a 57-L conical HDPE tank with a working volume of 40-L and the methanogenic digester was a 140-L cylindrical polypropylene tank (diameter of 47 cm and height of 89 cm) with a working volume of 87-L. Digesters were operated in a confined room where the temperature was maintained at 26±2^{o}C using an air conditioner. The acidogenic digester was stirred by recirculating substrates from the bottom to the top using a diaphragm pump (at an approximate flow of 5.7 L min^{-1}) that was operated by a programmable timer for 15-min on and 15-min off. New feedstock was introduced into the acidogenic digester from the bottom using the same diaphragm pump (used for recirculating) and effluent overflowing the acidogenic digester was gravitationally discharged to the methanogenic digester directly or after adjustment of pH, when required. Effluent from the acidogenic chamber was collected in an adjacent tank where pH was adjusted to between 7.2 and 7.4 using a NaOH solution (25 M) and then transferred to the methanogenic digester using a transfer pump

when required. Biogas production of each digester was measured independently using a gas meter.

Feedstock and inocula

The feedstock in this study was a volumetric mixture (1:3, v/v) of poultry blood and PPWW collected from a commercial poultry processing plant. Once every two or four weeks, as needed, blood was manually collected from the top of a portable blood tanker and PPWW was collected from the post-secondary screen pit using a submersible pump. Blood and PPWW were carried in 5-gal (19-L) buckets from the processing plant to the laboratory where the digester was operated. The mixture of blood and PPWW was filtered using a 1-mm screen to remove feathers and large debris, mixed in a plastic tank, and then stored in 5-gal buckets in a freezer at -20^{o}C prior to further use. On a scheduled feeding day, the frozen feedstock was thawed for 3 h using a water bath (50^{o}C), and then diluted with dechlorinated water to achieve a COD of 15 g L^{-1}. Inoculum for the acidogenic digester was obtained from a 1.14-m^3 (300-gal) digester operating at ambient temperature and actively treating a blood-PPWW feedstock for six months. The total solids (TS), volatile solids (VS) and total suspended solids (TSS) of the inoculum were 1.7, 1.1 and 0.3 g L^{-1}, respectively. For the methanogenic digester, the inoculum used for the test period of days 1-99 was the same as that of the acidogenic digester. In the digester recovery period (days 100-140), new inoculum from full-scale anaerobic digesters at a local municipal wastewater plant was introduced to our digesters. The TS, VS and TSS of the new inoculum were 31.4, 21.9 and 27.1 g L^{-1}, respectively.

Experimental procedures

Table 4.1 presents the operational mode of the system at different OLRs. In the acidogenic digester, after 100% volume of inoculum was added, a 3-month startup period was initiated where feedstock was continuously introduced at an OLR of around 3.0 g COD L^{-1} d^{-1} (equivalent to a HRT of 4.8 days). After observing a constant pH in the range of 6.4 to 6.6 for three consecutive weeks, the adaption was considered complete. The digester was further sequentially operated at OLRs of 2.8, 4.3, 2.1, 1.2 and 2.3 g COD L^{-1} d^{-1} (equivalent to HRTs of 4.8, 3.2, 6.3, 11.1 and 6.1 days) for a period of 400 days.

In the methanogenic digester, after 100% volume of inoculum was added, a similar 3-month adaptation period was initiated where feedstock with relatively consistent COD concentration of 14 g L^{-1} was introduced at an OLR of around 1.2 g COD L^{-1} d^{-1}. The feedstock here was the effluent from the acidogenic digester. The operation of the digester can be divided into three periods of differing conditions. In the first period (days 1 to 99), the digester was sequentially operated at OLRs of 1.3, 2.0 and 1.0 g COD L^{-1} d^{-1} (equivalent to HRTs of 10.5, 7.0 and 13.8 days, respectively) with no alkalinity addition. In the second period, because of deteriorating performance (as evidenced by lower biogas production and low pH), a recovery period was initiated on the 100^{th} day and continued to the 140^{th} day. Reviving the digester included three steps that were implemented sequentially and were diluting substrates, alkalinity addition, and introduction of new inoculum as described in Table 4.1. At the end of this period, a mixture of 50% new inoculum and 50% effluent from the acidogenic digester was added in the methanogenic digester. In the third period (days 141 to 400), the pH of feedstock was adjusted to between

7.2 and 7.4 by adding a solution of NaOH before introduction to the methanogenic digester. In this period the digester was sequentially operated at three different OLRs 0.6, 0.4 and 1.0 g COD L^{-1} d^{-1} (equivalent to HRTs of 24.2, 37.8 and 13.2 days, respectively). Each OLR was maintained for a period of time equivalent to three HRTs to obtain a stable performance of digester.

In the experiment period, digesters were fed either daily, every two days or twice a week depending on the feeding strategy which is described in Table 4.1. Feedstock pH was measured at the feeding time. Biogas production was measured daily and biogas composition was tested approximately once a week. Samples of feedstock and all effluents were collected in 240-mL plastic bottles once a week and stored in a refrigerator (4^{o}C) for the further analytical testing.

Analytical methods

Detailed description of most analytical methods used in this study was previously described in Chapter 3. Additionally, protein content was determined by multiplying TN by 6.25 (Yoon et al., 2014) and biogas compositions were measured using GEM-2000 gas analyzer (Lantec, Colton, CA).

Statistical Analysis

A one-way ANOVA was conducted and a Tukey HSD test was used to compare means using JMP Pro 10.

Results and discussion

Feedstock characteristics

The feedstock had C/N ratio of 4.70 and an average TN of 1.32 g L^{-1}, primarily contributed by the blood that was added (Table 4.2). Higher variability in TAN and VFA concentrations compared to that of COD was probably caused by large variations in the condition of the blood, as it was stored on site for as long as 8-h at local ambient temperatures.

Acidogenic digester

The aim of this digester was to hydrolyze proteins and lipids in the feedstock to intermediates including VFA and TAN, with minimal conversion to methane and carbon dioxide. The digester performance was evaluated based on effluent characteristics including pH, COD, TSS, VFA and TAN, and by measuring biogas production, concentration of methane in biogas, and methane yield. The digester operation was considered successful when the biogas production, methane concentration, COD removal and pH were low and stable, while VFA and TAN concentrations reached stable and high levels indicating the proteins or amino acids, main compositions of feedstock, have been completely hydrolyzed.

Under the different OLRs, average volumetric biogas productions ranged from 0.03 to 0.12 L L^{-1} d^{-1}, where more biogas was produced at higher OLRs (Figure 2a). Methane concentration in biogas ranged between 38.1 and 59.2%, except the value at the leakage days. Fluctuation of methane production was observed during the testing period and is attributed to variability resulting from seasonal/plant processing changes that impact feedstock characteristics.

During routine operations of a two-stage system, the acidogenic digester served as a pre-treatment digester that took in feedstock that had day-to-day variations but produced an effluent that was relatively consistent, thus reducing feeding shock to the methanogenic digester. At steady-state of two representative OLRs, namely 1.2 and 2.3 g COD L^{-1} d^{-1} (Table 4.3), biogas productions were 0.03 and 0.09 L L^{-1} d^{-1}, corresponding to methane yields of 15.5 and 18.7 mL $g^{-1}COD_{added}$, respectively. These values were significantly lower than the biogas production of 0.3 to 0.7 L L^{-1} d^{-1} (methane yields of 170 to 400 mL g^{-1} TS_{added}) observed in the single-stage digester treating similar substrates under similar organic loadings (Cuetos et al., 2009; Lopez et al., 2006). At the higher OLR, methane content reduced from the 57% to 44%, a phenomenon similar to that reported by Wang and Banks (2003) who used two-stage digesters to treat similar slaughterhouse wastes. In their study, methane content decreased and methane yield increased with increasing OLRs in the acidogenic digester.

TSS and COD changes in the acidogenic digester were consistent with hydrolysis of organics and observed organic matter loss (expressed as COD). In general effluent TSS concentration decreased with treatment, with the exception of days 40 to 60 when the highest OLR of 4.3 g COD L^{-1} d^{-1} was applied (Figure 4.2b). At steady-state (Table 4.3), TSS removal of 17.2 and 7.8% was observed at OLRs of 1.2 and 2.3 g COD L^{-1} d^{-1}, respectively. Hydrolysis of organics is known to be one of the rate-limiting steps in AD. The lower degradation of proteins or lipids possibly contributed to the observed lower TSS removal in this digester (Eastman and Ferguson, 1981). Furthermore, washout of bacterial biomass at high OLRs also possibly contributed to the lower calculated TSS

removal. However, relatively consistent TSS concentrations were found in the effluent under different OLRs that further confirmed that the limited hydrolysis mainly resulted in lower TSS removal.

In the acidogenic digester, COD of effluents were not significantly different with those of feedstocks (Figure 4.2b) because of primary conversion of organics to intermediates such as VFA and negligible conversion to biogas. At steady-state (Table 4.3), measured COD removal was 1.4% and -7.9 % at OLRs of 1.2 and 2.3 g COD L^{-1} d^{-1}, respectively. The above negative value indicates the possible slight active sludge washout from this digester operated at higher OLR. In this stage, a part of the COD was hydrolyzed to intermediates while the rest remained as particulates and continued to degrade in the second stage methanogenic digester.

VFA and TAN were the primary intermediates after protein and lipid degradation, and their rapid accumulation has been known to result in instability of single-stage digesters (Cuetos et al., 2013). After hydrolysis and acidification, VFA significantly increased from approximately 1.7g L^{-1} in feedstock to 10.0 g L^{-1} in the effluent (Figure 4.2c). At steady state conditions of OLRs of 1.2 g and 2.3 g COD L^{-1} d^{-1} (Table 4.3), effluent VFA were 10.8 and 13.7 g L^{-1}, respectively. In addition, TAN also increased to high levels reaching 1.4 g L^{-1}, approximately equal to the TN of the feedstock in this period (Figure 4.2c). At steady state (Table 4.3), effluent TAN was 1.4 and 1.2 g L^{-1} when OLRs were 1.2 and 2.3 g COD L^{-1} d^{-1}, respectively. Unlike VFA, TAN concentrations seemed to reach a value where it plateaued and did not proportionally increase with increasing OLRs. This seems to suggest that protein hydrolysis was predominantly completed within the

HRT of 6.1 d. These results are in agreement with the finding of Lopez et al. (2006) that in AD blood was completely hydrolyzed within a 5 d retention time resulting in high levels of TAN in the substrate. Increasing VFA concentrations translated to reducing effluent pH to levels in the range of 6.4 to 6.6. Lipids are known to be relatively hard to degrade in the acidogenic phase and previously reported values range between 0 and 13%, while most proteins degrade prior to lipid degradation in this phase (Eastman and Ferguson, 1981; Kim et al., 2010). Zhang and Banks (2012) co-digested sheep blood and other slaughterhouse wastes with OFMSW (20:80, VS basis) in a 4-L CSTR at 36^{o}C and found that digesters treating blood and OFMSW exhibited VFA accumulation reaching values in the range of 11 to 15 g L^{-1} (in conjunction with 7-8g L^{-1} TAN). These concentrations were significantly higher than that for other co-substrates that reached VFA values of 3.5g L^{-1}. Cuetos et al. (2013) also reported high VFA (14g L^{-1}) and TAN (7g L^{-1}) concentrations in the effluent of a 3-L CSTR treating blood and maize residues (40:60, VS basis, 34^{o}C).

In our study, blood protein was quickly hydrolyzed resulting in TAN of 1.4 g L^{-1}, which is close to the level considered inhibitory to methanogens (1.5 g L^{-1}) as reported by Rajagopal et al. (2013). We observed inhibition (reduced biogas production) as a result of high TAN and VFA, which affect methanogenesis. Furthermore, because of reduced methanogenic activity (hence reduced consumption of VFA), VFA accumulated in the digester and resulted in a low pH environment (pH 6.4 to 6.6, Figure 4.2c) (Banks and Wang, 1999).

Methanogenic digester

During the initial days 1 to 140, the methanogenic digester was operated at OLRs of 0.6 to 2.0 g COD L^{-1} d^{-1} in the first period, and because of instability in the second period it was operated on a recovery mode using different methods to stabilize the digester (Table 4.1). In the first period, biogas production, methane concentration in biogas, COD removal and pH were low, confirming inhibition of methanogenesis (Figure 4.3a, b and c). In the second period, attempts were made to gradually revive the digester. However, the recovery was too slow and unsatisfactory. In order to speed up recovery, new inoculum was added to the digester.

In order to prevent complete digester failure, a solution of NaOH (25M) was added to the feedstock to maintain pH of the digester substrate in the range of 7.2 to 7.4 during days 141 to 400 (third period). As expected, decreasing OLR from 0.6 to 0.4 g COD L^{-1} d^{-1} resulted in a reduction of biogas production (Figure 4.3a). However, the biogas production rate did not return to higher values when the OLR was gradually increased to 1.0 g COD $L^{-1}d^{-1}$. It was found that the lower biogas production of 0.06 L L^{-1} d^{-1} occurred between day 312 and 340, compared to the value of 0.08 L L^{-1} d^{-1} between day 214 and 305, at the same OLR of 0.4 g COD L^{-1} d^{-1}. The variation in the methanogenic digester's temperature was a result of atmospheric temperature fluctuations and possibly impacted microbial activities in methanogenesis and caused variations in biogas production (Khanal, 2008; Figure 4.3a). At steady state, biogas production at the three OLRs of 0.4, 0.6 and 1.0 g COD L^{-1} d^{-1} was 0.08, 0.12 and 0.07 L L^{-1} d^{-1}, respectively (Table 4.4). These values were lower than results from other studies using slaughterhouse wastes at higher solids contents (Cuetos et al.,

2009; Cuetos et al., 2013), but higher than results reported by Hansen and West (1992) using similar feedstock and OLRs. Methane concentrations in the biogas in our study ranged between 75 and 79%, while methane yields were 47 to 172 mL CH_4 g^{-1} COD_{added}. These results are comparable to the 60 to 180 mL CH_4 g^{-1} $COD_{added,}$ reported by Hansen and West (1992). In this digester, methanogenic inhibition was reduced after pH adjustment as evidenced by higher methane yields at OLRs of 0.6 and 0.4 g COD L^{-1} d^{-1}, compared to results from the first and second periods. However, decreased methane yields suggest that digester deterioration still occurred when OLR was increased to 1.0 g COD L^{-1} d^{-1} even after the pH of feedstock was adjusted.

Effluent COD concentrations were relatively constant as OLR was increased from 0.4 to 0.6 g COD L^{-1} d^{-1}, but increased gradually at the higher OLR of 1.0 g COD L^{-1} d^{-1} (Figure 4.3b). At steady state (Table 4.4), COD removal was 52.6 and 53.7% at OLRs of 0.4 and 0.6 g COD L^{-1} d^{-1}, respectively. Beyond this point, COD removal reduced to 18.1% at OLR of 1.0 g COD L^{-1} d^{-1}, suggesting reducing efficiency of methanogens. COD of this substrate consists primarily of un-decomposed lipids from acidogenic digester and soluble intermediates of decomposition such as VFA, TAN and trace organics. The COD reduction was primarily a result of VFA conversion to methane and carbon dioxide by the aceticlastic methanogens, because of the low possibility of the existence of a large number of hydrogen producers in this digester (Weillinger et al., 2013). Effluent TSS concentration gradually increased with increasing OLRs (Figure 4.3b). At steady state (Table 4.4), TSS removals recorded were in the range of 31.9 to 46.8% at different OLRs. It is expected that this effluent TSS would contain both un-decomposed lipid particles and microbial biomass that

has been washed out at higher OLRs. Although we did not directly measure lipid content in the substrates, the literature confirms this expectation. For example, it is known that blood contains very little lipids, typically equal or less than 0.3% VS (translating to 0.5 g L^{-1}) (Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012); while the lipid content of typical PPWW (similar to that used in this study) from southeastern U.S. slaughterhouses is estimated to be 1.3 g L^{-1} (Kiepper et al., 2008; Kiepper, 2009). Accounting for dilutions used in this study, the estimated lipid content of the feedstock entering the acidogenic digester would be approximately 0.5 g L^{-1} and effluent lipid content would be approximately 0.1 g L^{-1} based on an anticipated 80% lipid reduction in the methanogenic digester that was observed by Kim et al. (2010). Therefore, the lipid fraction in the TSS is expected to be very low and hence the effluent TSS would be predominantly microbial biomass. In addition, since the methanogenic digester was designed and operated in a down flow mode and the digester exit was 30 cm above the digester floor, solids accumulation would have occurred during the operation at the longer HRT and released during higher flows at the shorter HRT. We observed TSS removal decrease with increasing OLRs, further confirming that possibility. This phenomenon is similar to experiences reported by Wang and Banks (2003) who observed lower TSS removal at higher OLRs.

Effluent VFA was also relatively constant at OLRs of 0.4 and 0.6 g COD L^{-1} d^{-1}, but gradually increased at the OLR of 1.0 g COD L^{-1} d^{-1} (Figure 4.3c), a trend similar to that of effluent COD (Figure 4.3b). At steady state, VFA removals were in the range of 7.3 to 22.5% (Table 4.4). Although we observed higher methane yields (suggesting enhanced methanogenic activity), the VFA concentrations in the effluent remained high and were in

the range of 7.5 to 12.6 g L^{-1}. Kim et al. (2010) reported 80% reduction in lipids in the methanogenic digester while operating at OLRs up to 6.5 g COD L^{-1} d^{-1}. This suggests that un-decomposed lipids coming from the acidogenic digester continued to decompose and produce additional VFA in the methanogenic digester. The high concentrations of accumulated VFA did not significantly inhibit methanogenic activity most of the time because of pH adjustment by alkali addition (Nagao et al., 2012). However, there were signs of inhibition (observed as reduced pH and increased VFA) at the highest OLR of 1.0 g COD L^{-1} d^{-1}. Effluent TAN was consistently around 1.3 g L^{-1} and no further TAN accumulation was observed at all OLRs (Figure 4.3c), suggesting relatively complete hydrolysis of proteins in the acidogenic digester.

Overall evaluation of the two-stage system

Results of studies on co-digestion of slaughter blood and wastewaters done at different scales show relatively low methane yield from these substrates (Table 4.5). Both methane yield and COD removal reported in the literature were either lower than or comparable to our results. We note that our experimental work was conducted at ambient temperatures of 26^{o}C and higher VFA and TAN in contrast to studies in the literature that were done at 35 or 38^{o}C. This two-stage anaerobic digester effectively decoupled the two stages, preventing significant inhibition of methanogens. Methane production and COD removal levels were acceptable for a substrate mixture of relatively high levels of blood content being digested under relatively high OLRs. Nevertheless, there is room for improving the digester performance primarily with respect to TAN inhibition and economic

considerations (e.g. cost of biocarriers, ammonia removal, and potential for using higher amounts of blood.

Energy recovery

In addition to reduction of COD, energy recovery is an attractive benefit of anaerobic treatment of slaughterhouse wastes (Yoon et al., 2014). Energy recovery from AD can be calculated based on results of this two-stage digester and the method used in the study of Bouallagui et al.(2004).

Energy production is in the form of methane produced in the digester, which is calculated as follows:

$$E_{in} = Q_m \times LHV_{methane} \quad (1)$$

Where E_{in} is methane energy production in kJ animal unit^{-1}; Q_m is methane production (L CH_4 animal unit^{-1}); and $LHV_{methane}$ is the lower heating value of methane (35.8 kJ $L^{-1}_{methane}$). The value of Q_m is calculated as:

$$Q_m = 8 \times q \times (R_A \times HRT_A \times P_A + R_M \times HRT_M \times P_M) \quad (2)$$

Where q is the blood collected for anaerobic digestion (L animal unit^{-1}); R_A and R_M are biogas production rates from the acidogenic and methanogenic digesters ($LL^{-1}_{digester}$ d^{-1}), respectively; HRT_A and HRT_M are hydraulic retention times in the acidogenic and methanogenic digesters (day), respectively; and P_A and P_M are the methane concentrations in the biogas from the acidogenic and methanogenic digesters, respectively.

The energy requirement for digester operations is calculated as:

$$E_{out} = E_{heat} + E_{loss} + E_{mechnical} \quad (3)$$

Where E_{out} is energy consumption for the digester operation (kJ animal unit^{-1}); E_{heat} is energy to preheat feedstocks to the operational temperature (kJ animal unit^{-1}); E_{loss} is energy loss from the digester to the environment (kJ animal unit^{-1}); and $E_{mechnical}$ is energy used for mechnical operations such as mixing, pumping, etc (kJ animal unit^{-1}).

E_{loss} in digesters is estimated to be 10% of E_{heat} (Molinuevo-Salces et al., 2012) and $E_{mechnical}$ is assumed to be zero because it is a negligible portion relative to other energy components (Bouallagui et al., 2004). Hence, equation 3 simplifies to:

$$E_{out} = 1.1 \times E_{heat} \quad (4)$$

where E_{heat} is calcualted as:

$$E_{heat} = 8 \times q \times C_p \times \delta \times (T_R - T_o) \quad (5)$$

where q is blood collected for the digestion (L animal unit^{-1}); C_p is specific heat of the feedstock (4.2 kJ kg^{-1} ^{o}C^{-1}); δ is specific weight of the feedstock (1,000 kg m^{-3}); and T_R-T_o is the difference between the digester operating temperature and the feedstock storage temperature (^{o}C).

Parameters used to calculate energy recovery were either results of this study or values obtained from the literature. The OLRs of the acidogenic and methanogenic digesters were set at 2.3 and 0.8 g L^{-1} d^{-1} (corresponding to HRTs of 6.1 and 18.7 days), respectively. Biogas production rates in the acidogenic and methanogenic digesters were set at 0.09 and 0.16 L L^{-1} d^{-1} (corresponding to 44% and 79% methane in biogas), respectively. The digester temperature and average feedstock storage temperature in the slaughterhouse were set at 26 and 20^{o}C, respectively.

The estimate of the blood waste amount was obtained from Marcos et al. (2010) and Kiepper (2007). If 50% of total blood was collected in a storage tank, this translates to 19.7 kg, 5.6 kg and 0.086 kg of blood from a 540-kg bovine, a 160-kg swine and a 2.4-kg broiler, respectively, that is available for AD. Blood was diluted eight times with wastewaters or fresh water before introducing it to the digester.

The net energy recovery from the two-stage digester would be the difference between energy production and energy consumed in the digester as shown below:

$$E_{net} = E_{in} - 1.1 \times E_{heat} \quad (6)$$

The net energy recovery for the three types of animals where blood is processed in a two-stage anaerobic digester is presented in Table 4.6. Calculations show that total energy of 10,330 kJ per bovine, 2,937 kJ per swine and 45 kJ per broiler can be recovered from waste blood produced at slaughterhouses. The normalized energy recovery is quite similar across animal types and is in the range of 18.4 to 19.7 kJ per kg weight, a result that is caused by the fact that the amount of blood in different animals is proportional to its body weight.

The pilot scale AD (operated at 26^{o}C) in this study produced 0.0018 m^3 methane per broiler which is comparable to the 0.0016 m^3 methane per bird reported in the literature based on biochemical methane potential tests conducted by Yoon et al (2014).

Several researchers have reported higher methane yield potentials from slaughterhouse blood wastes by conducting batch tests, bench-scale tests and lab-scale tests (Hejnfelt and Angelidaki, 2009; Yoon et al., 2014; Zhang and Banks, 2012). However, few studies have been conducted at the pilot or full-scale using slaughterhouse blood wastes as

the primary substrate. Our study is one of the few pilot-scale tests and allows better understanding of challenges relating to logistics, digester operations and effluent management. These allow for better designs at full-scale. Energy recovery assessment from blood waste in this pilot digester confirmed the energy and economic benefits of anaerobic digestion of liquid slaughterhouse wastes.

Conclusions

The pilot two-stage anaerobic digester effectively treated a mixture of slaughterhouse blood wastes and wastewaters. In the acidogenic digester, there was low methane yield and negligible COD removal. Protein was completely hydrolyzed to produce high levels of TAN and VFA, while lipids were only partially hydrolyzed and resulted in low TSS removal. In the methanogenic digester, at lower OLRs methane yields and COD removals were found to be acceptable. However, inhibition of methanogenic activity was observed at higher TAN levels. Attractive levels of energy recovery were obtainable from this digester treating slaughterhouse blood wastes.

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Table 4.1 Operational mode of the two-stage anaerobic digester treating a mixture of blood and poultry processing wastewater

Day	OLR and feeding flow rate (g COD L^{-1} d^{-1} and L d^{-1})	
	Acidogenic digester (40-L)	Methanogenic digester (87-L)
1-47	2.8[a] , 8.3	1.3[a], 8.3
48-56	4.3[a], 12.5	2.0[a], 12.5
57-91	2.1[b], 6.3	1.0[b], 6.3
92-99	1.2[c], 3.6	0.6[c], 3.6
100-140		Recovery period Day 100-119: feedstock dilution by adding 1.8 gal tap water approximately every two days Day 120-132: alkalinity addition, adding total 7.2 gal $NaHCO_3$ solution (9.4 g L^{-1}) Day 133-140: new inoculums addition
141-210		0.6[c*], 3.6
211-341		0.4[c*], 2.3
342-347		0.6[c*,**], 3.6
348-356	1.6[c**], 4.7	0.7[c*, **], 4.7
357-400	2.3[c], 6.6	1.0[c*], 6.6

[a] feed daily, [b] feed every two days, [c] feed twice a week

* pH of feedstock adjusted to 7.2-7.4 using NaOH solution, ** Adaption period

Table 4.2 Characteristics of the feedstock mixture of blood wastes and poultry processing wastewater

Parameters	Mean ±Standard deviation
pH	6.8±0.2
COD (g L^{-1})	13.64±1.38
Protein (g L^{-1})	8.24±1.66
TS(g L^{-1})	6.91±1.95
TVS(g L^{-1})	6.07± 1.98
TSS(g L^{-1})	1.59± 0.47
TN(g L^{-1})	1.32± 0.27
TAN (g L^{-1})	0.51± 0.34
VFA(g L^{-1} as acetic acid)	1.68± 1.31
C[a]	43.92± 4.02
H[a]	5.68±0.56
N[a]	9.49±1.85
S[a]	1.22± 0.15
C/N	4.70± 0.52

[a] w/w, % TS

Table 4.3 Performance of the acidogenic digester under steady-state conditions

Parameters	OLR (g COD L^{-1} d^{-1})	
	1.2	2.3
pH	6.6^{a}±0.0	6.4^{b}±0.0
Biogas production ($LL^{-1}d^{-1}$)	0.03^{b}±0.01	0.09^{a}±0.02
CH_4%	57.4^{a}±2.3	44.2^{b}±1.5
CO_2%	35.7^{b}±2.0	52.6^{a}±2.4
COD		
Input (g L^{-1})	13.17±0.67	12.97±0.71
Output (g L^{-1})	12.98^{b}±0.55	13.98^{a}±0.49
Removal (%)	1.36^{a}±0.91	-7.99^{b}±5.04
TSS		
Input (g L^{-1})	1.51±0.36	1.42±0.12
Output (g L^{-1})	1.22^{b}±0.05	1.31^{a}±0.04
Removal (%)	17.18±15.66	7.79 ±5.39
VFA (g L^{-1})	10.82^{b}±0.83	13.67^{a}±1.39
TAN (g L^{-1})	1.39^{a}±0.16	1.16^{b}±0.09
CH_4 yield (mL CH_4 g^{-1} COD_{added})	15.5 ±3.2	18.7± 3.6

Steady-state condition was assumed at the end of each OLR operation. The parameter value was expressed as mean ± standard deviation of three consecutively weekly values; Different letters of each parameter of different OLR indicate significant differences ($P \leq 0.10$).

Table 4.4 Performance of the methanogenic digester under steady-state conditions

Parameters	OLR (g COD L^{-1} d^{-1})		
	0.4	0.6	1.0
pH	7.3^{a}±0.0	7.3^{a}±0.1	7.0^{b}±0.0
Biogas (LL^{-1} d^{-1})	0.08^{b}±0.00	0.12^{a}*±0.01	0.07^{b}±0.01
CH_4%	76.3^{ab}±2.8	79.1^{a}±0.7	74.9^{b}±1.2
CO_2%	21.4^{ab}±0.7	20.8^{b}±0.7	23.0^{a}±0.9
COD			
Input (g L^{-1})	13.43±0.38	14.05±0.91	13.98±0.49
Output (g L^{-1})	6.37^{b}±0.06	6.48^{b}±0.62	11.45^{a}±0.30
Removal (%)	52.58^{a}±1.70	53.65^{a}±6.29	18.09^{b}±2.11
TSS			
Input (g L^{-1})	1.24 ±0.16	1.45 ±0.11	1.31 ±0.04
Output g L^{-1})	0.65^{b}±0.07	0.94^{a}±0.16	0.89^{ab}±0.17
Removal (%)	46.77±11.27	34.23 ±16.21	31.89 ±10.63
VFA			
Input (g L^{-1})	10.27^{b}±0.82	10.13^{b}±1.94	13.67^{a}±1.39
Output (g L^{-1})	9.42^{ab}±1.26	7.51^{b}±1.28	12.59^{a}±3.33
Removal (%)	7.33±18.87	22.45±28.45	8.50±19.34
TAN (g L^{-1})	1.30^{b}±0.01	1.40^{a}±0.04	1.32^{b}±0.02
CH_4 yield (mL CH_4 $gCOD_{added}$)	172^{a}±9	164^{a}±15	47^{b}±4

Steady-state condition was assumed when coefficient variance (CV) of COD of effluent and biogas productions at three consecutive weekly test were ≤10.0%. The parameter value was expressed as mean ± standard deviation of three consecutively weekly values; *One week value was estimated from the six neighbor days beyond this week because the gas meter temporarily did not work in this week; Different letters of each parameter of different OLRs indicate significant differences (P≤0.10).

Table 4.5 Comparison of anaerobic digester performance treating animal blood wastes and wastewaters

Study	Feedstock	OLR (g COD L^{-1} d^{-1})	Methane production (L L^{-1} d^{-1})	COD removal (%)	mL CH_4 g^{-1} COD_{added}	Digester configuration
Marcos et al., 2010	2% blood and 98% wastewaters	0.17*	0.04*	56.9	224	2-L batch digester, 38°C
Hansen and West, 1992	2% blood and 98% rendering condensate	1.01	0.06	28.0	60	7.8-L UASB, 35±1°C
		0.54	0.06	50.9	100	
		0.34	0.05	65.9	140	
This study	Approximate two-fold dilution of 25% blood and 75% wastewaters	0.7	0.05	11.7	72	127-L two-stage digester, 26±2°C
		0.4	0.07	50.8	189	

*This value is calculated from reported results.

Table 4.6 Energy recovery from the two-stage anaerobic digestion of slaughterhouse blood wastes

	Cattle	Swine	Broiler
Energy production			
Methane production (m^3 $unit^{-1}$)	0.411	0.117	0.0018
Methane energy (kJ $unit^{-1}$)	14,699	4,178	64
Energy requirement for digester operation			
Feedstock preheating (kJ $unit^{-1}$)	3,972	1129	17
Digester heat loss (kJ $unit^{-1}$)	397.2	112.9	1.7
Net energy recovery (kJ $unit^{-1}$)	10,330	2,937	45
Net energy recovery (kJ kg^{-1} animal weight)[a]	19.1	18.4	18.8

[a] The unit weight was 540-kg per cattle, 160-kg per swine and 2.4-kg per broiler

Figure 4.1 Schematic of the pilot two-stage anaerobic digester treating a mixture of blood and poultry processing wastewater.

Figure 4.2 Weekly averaged methane production (a), COD and TSS changes (b) and changes in intermediates (c) during the operation of the acidogenic digester. The operational duration of each organic loading rate (OLR) is presented in Table 4.1. Biogas production during day 155 to 210 was not available due to the accidental gas leakage from the sealed digester cover. The suffix of “f” and “e” denote feedstock and effluent, respectively. Volatile fatty acid concentrations of feedstock after day 330 were not available because the presence of excess foam interfered with the distillation process.

Figure 4.3 Weekly averaged methane production (a), COD and TSS changes (b) and changes in intermediates (c) during the operation of the methanogenic digester. The operational duration of each organic loading rate (OLR) is presented in Table 4.1. The suffix of “f” and “e” denote feedstock and effluent, respectively.

Figure 4.1

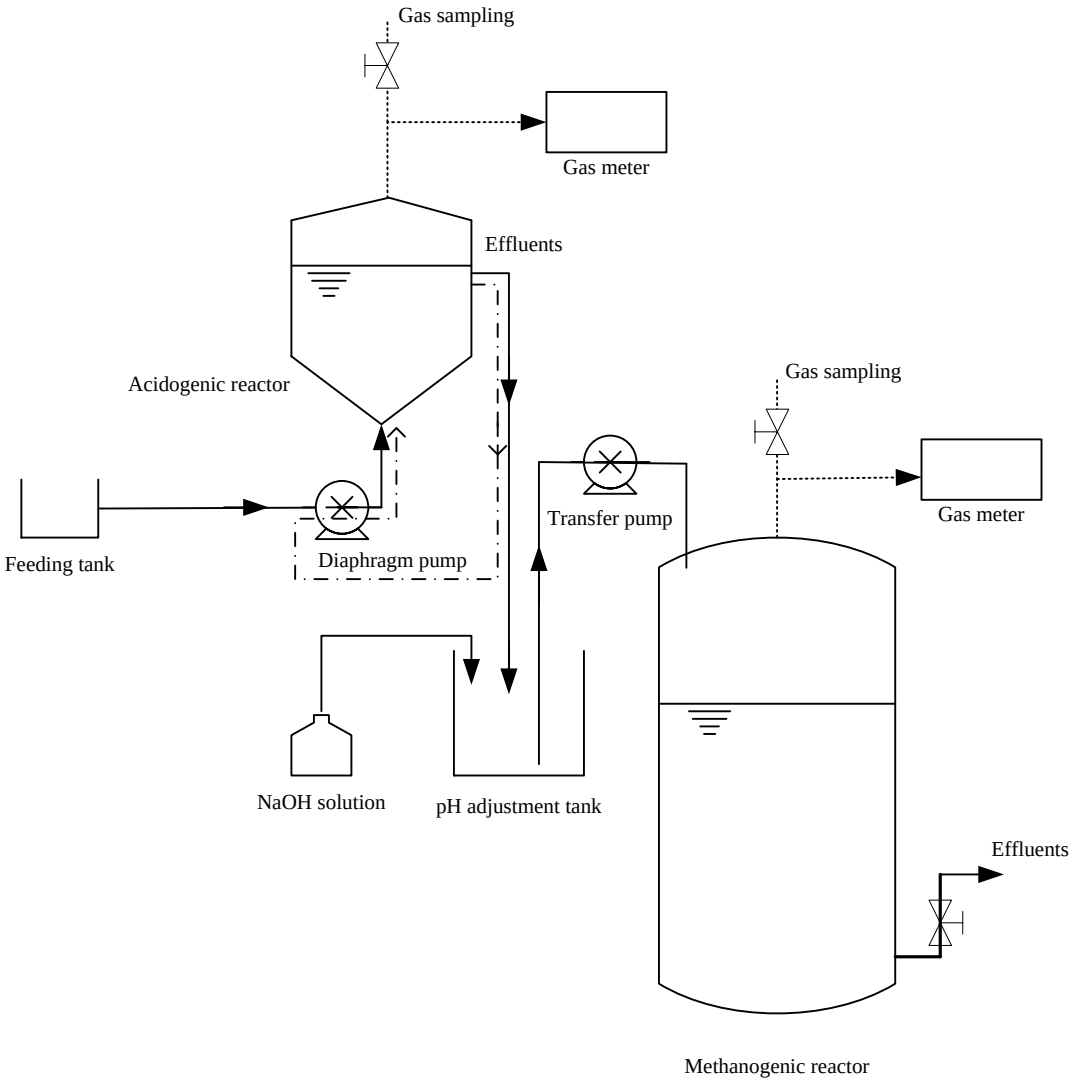

Gas sampling
Gas meter
Effluents
Acidogenic reactor
Gas sampling
Transfer pump
Gas meter
Feeding tank
Diaphragm pump
NaOH solution
pH adjustment tank
Effluents
Methanogenic reactor

Figure 4.2

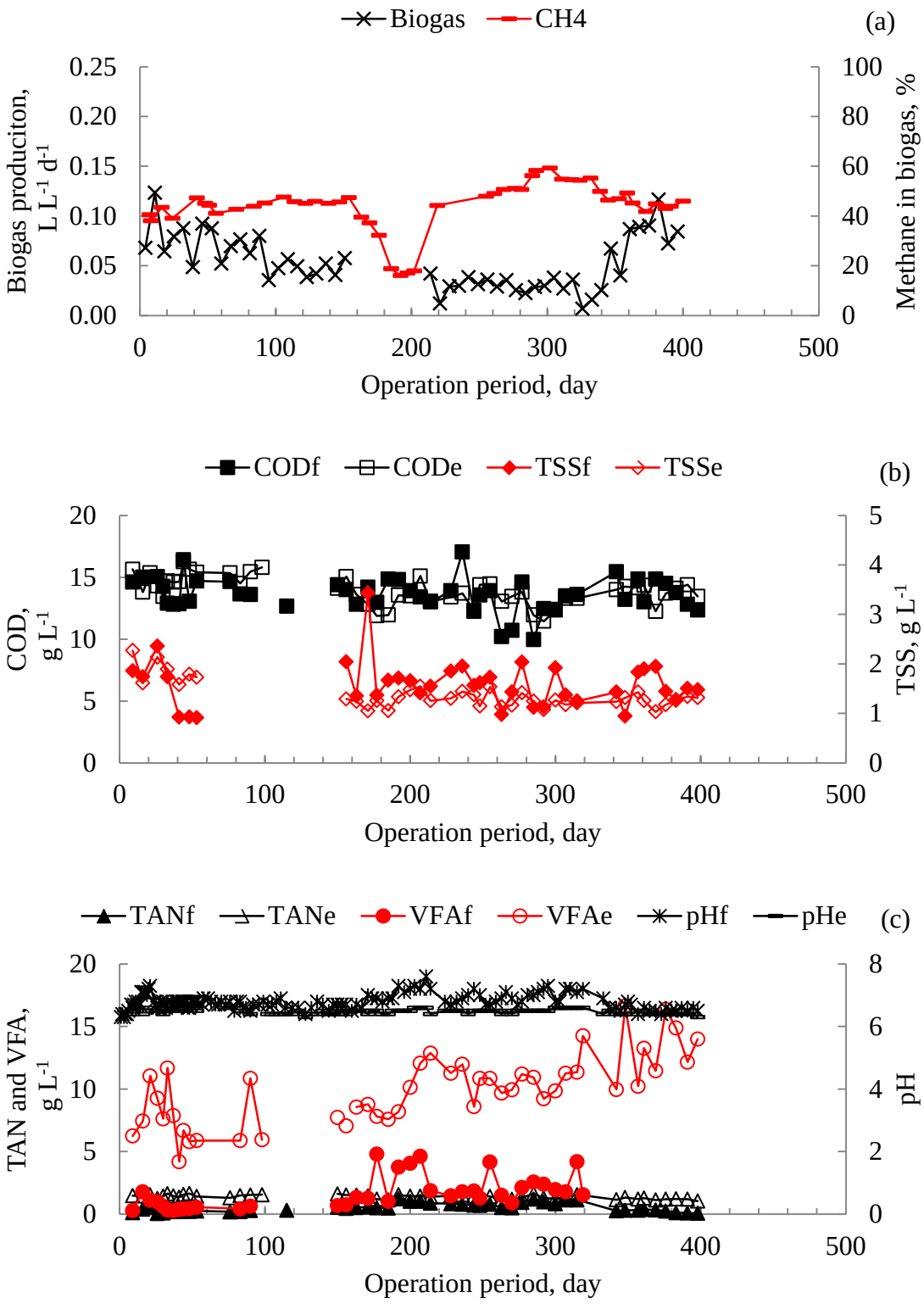
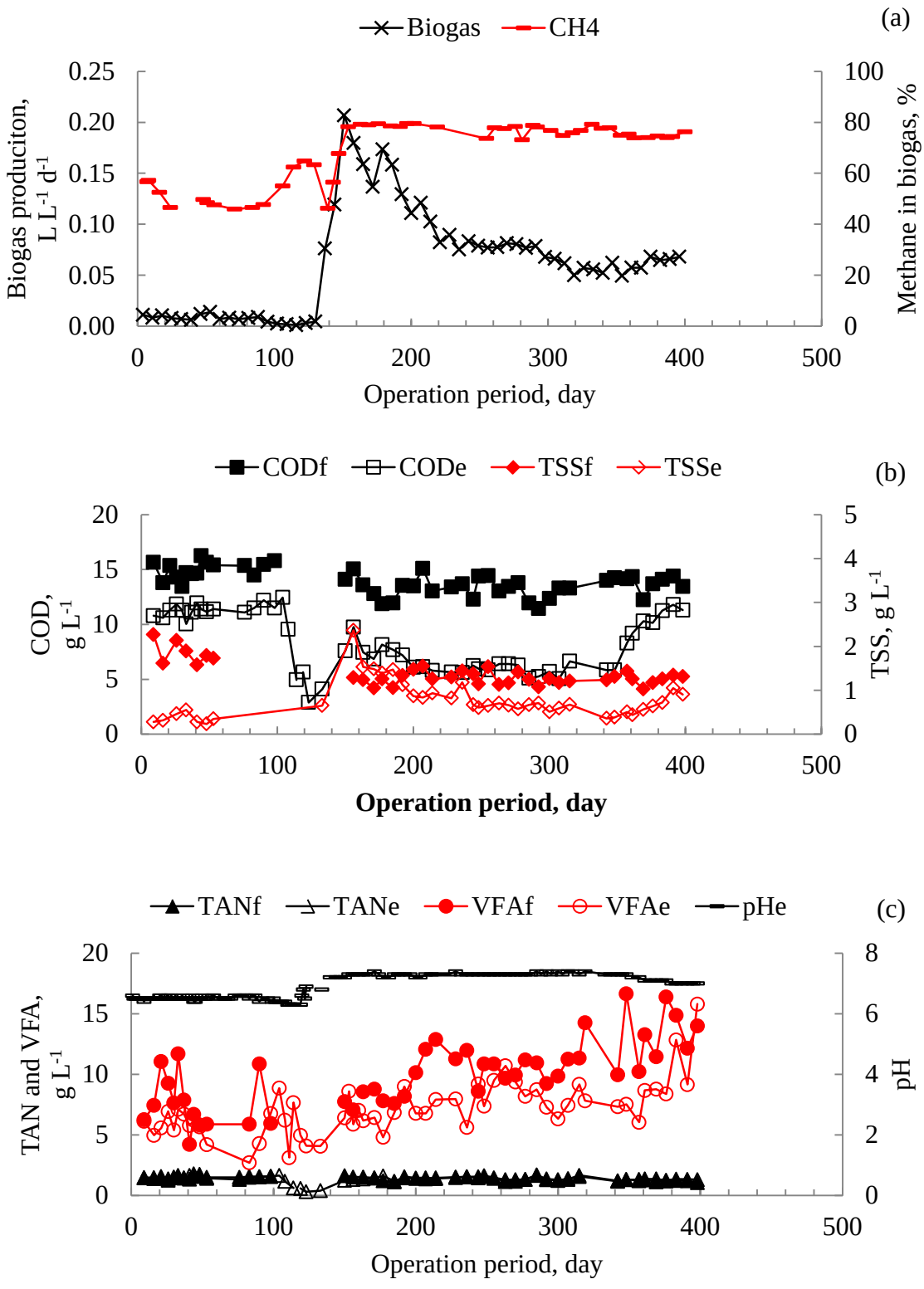

Biogas
CH4
(a)
Biogas produciton, L L-1 d-1
Methane in biogas, %
Operation period, day
CODf
CODe
TSSf
TSSe
(b)
COD, g L-1
TSS, g L-1
Operation period, day
TANf
TANe
VFAf
VFAe
pHf
pHe
(c)
TAN and VFA, g L-1
pH
Operation period, day

Figure 4.3


(a)
Biogas
CH4
Biogas produciton, L L-1 d-1
Methane in biogas, %
Operation period, day
(b)
CODf
CODe
TSSf
TSSe
COD, g L-1
TSS, g L-1
Operation period, day
(c)
TANf
TANe
VFAf
VFAe
pHe
TAN and VFA, g L-1
pH
Operation period, day

CHAPTER 5

USE OF LOW-COST BIOCARRIERS TO IMPROVE THE PERFORMANCE OF TWO-STAGE ANAEROBIC DIGESTERS TREATING POULTRY PROCESSING WASTES[1]

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Abstract

Biocarriers that serve as matrices for microbial attachment used in anaerobic filters and moving bed digesters can improve the performance and economics of anaerobic digestion (AD). Three low-cost biocarriers, bamboo, biochar, and seashell were evaluated as candidates for improving performance in pilot two-stage digesters treating poultry processing wastes. Digesters with and without biocarriers were evaluated at different OLRs in terms of methane production and COD removal. Results show that bamboo was superior to other biocarriers and maintained physical integrity after 400 days of operation. The acidogenic digester filled with bamboo cylinders had higher treatment effect on proteins and lipids and lower COD loss at the highest OLR tested of 2.8 g COD L^{-1} d^{-1}. The methanogenic digester filled with bamboo cylinders had the highest methane yield of 359 mL g^{-1} COD_{added} and COD removal of 68% at an OLR of 0.6 g COD L^{-1} d^{-1}. Observations at the end of the trial showed that 46% of total bamboo cylinders were floating in the substrate within the methanogenic digester, potentially contributing to enhancement of mass transfer between the organic substrate and the microflora attached to the bamboo.

Introduction

Approximately 39.3 million metric tons of high-strength slaughterhouse wastes were generated from meat processing in the U.S. in 2013 (Ockerman and Hansen, 2000; USDA, 2014). These included intestinal contents, hides, bones, blood, etc. which contain proteins and lipids and are typically processed in a rendering plant where they are converted to blood meal, bone meal, compounded fertilizer or as protein and lipid supplement in animal food (Ockerman and Hansen, 2000). This method of management through rendering is energy intensive. In addition to significant energy consumed for transportation of these materials, the processing

requires storage of large volumes of feedstock and product, and energy-intensive operations such as centrifuging, cooking or drying. Rendering also requires the use of expensive chemicals such as anticoagulants, antioxidants, and others that are used for preservation of feedstocks and products (Ockerman and Hansen, 2000). Anaerobic digestion (AD) is an attractive alternative that removes organic pollutants and pathogens while providing the opportunity for energy recovery (via methane production) and nutrient (e.g. nitrogen and phosphorus) recycling (Arvanitoyannis and Ladas, 2008; Salminen and Rintala, 2002).

In AD, significant amounts of ammonia (measured as total ammonia nitrogen, TAN) and volatile fatty acids (VFA) are produced from initial degradation of proteins and lipids in the acidogenic stage of digestion. In contrast to acidogens, methanogens, especially acetotrophic methanogens, are sensitive to the presence of TAN at high concentrations (Banks and Wang, 1999; Demirel and Scherer, 2008). Inhibiting concentrations of TAN range between 1.5 to 6 gL^{-1} depending on digester conditions such as pH, temperature and microflora adaption (Rajagopal et al., 2013). At high TAN levels, because acidogens continue to remain active while methanogens are inhibited, ammonia continues to be generated and TAN accumulation reaches extreme levels resulting in low pH and severe inhibition of methanogens or complete process failure. In addition, the proteins and lipids also contribute to foaming and clogging in digesters and piping systems, which lowers process efficiency and increases operating and maintenance costs (Kougias et al., 2014).

A two-stage anaerobic digester can potentially alleviate above-mentioned problems and enhance the process stability and efficiency when treating slaughterhouse byproducts. This approach decouples acidogenesis and methanogenesis and provides an optimal environment for each stage, specifically by reducing the impact of TAN and VFA on methanogenesis (Ke et al.,

2005). Recognizing this benefit, several researchers have used two-stage AD to treat slaughterhouse wastes. Wang and Banks (2003) developed a laboratory scale two-stage digester to treat mixed abattoir wastes and found it had higher processing efficiency compared to a single stage digester. Similarly, Beux et al. (2007) documented high COD removal of 73.9% in a lab scale two-stage anaerobic digester treating high-strength slaughterhouse wastewaters. In the above studies, plastic rings were placed in the methanogenic digester to serve as matrices for the attachment of bacteria thus preventing its washout at high OLRs.

Immobilizing the microflora by using inert biocarriers was also reported as an effective way to reduce inhibition and process instability in AD (Rajagopal et al., 2013). Biocarriers made from petroleum derivatives such as nylon fiber, polyurethane foam, polyvinyl chloride rings (Chaiprasert et al., 2003; Lima et al., 2005; Rajakumar et al., 2012) are used in AD of high strength wastewaters because of their resistance to biological and physico-chemical degradation. However, the high cost of such biocarriers remains a limiting factor for biocarriers-based digestion approaches (Harper et al., 1990). Therefore, identifying low cost alternatives is a necessity for wider adoption of biocarriers-based digester designs.

Different types of byproducts including wood, straw, bamboo, coconut coir, seashell, charcoal, lava rock etc. have been used as biocarriers in AD to effectively treat high strength wastewaters (Lo and Liao, 1987; Tritt, 1992; Andersson and Björnsson, 2002; Acharya et al., 2008; Najafpour et al., 2008). Specific features of such biocarriers such as high surface area, porosity, and bio-resistance make them attractive for immobilizing bacteria and effective as biocarriers. These byproducts are also typically produced as low-cost byproducts of local industries, making them easy to access and reducing the cost of the AD operation. These natural

materials are also easy to dispose or recycle at the end of their useful life making them more environmental sustainable.

The primary goal of this study was to investigate the performance of low-cost biocarriers (biochar, bamboo and seashell) in pilot two-stage anaerobic digesters treating co-substrates of poultry blood and wastewaters at different OLRs. Performance was quantified in terms of methane yield and COD removal from the substrates.

Materials and methods

Equipment

Three 57-L conical HDPE tanks were used as acidogenic digesters that had working volumes of 40-L each. The acidogenic digesters received either no biocarriers (control) or 10-L each of bamboo rings or biochar. To provide sufficient mixing, substrates were recirculated from the bottom of the digester to the top using a diaphragm pump set to a timer of 15-min on and 15-min off. The feedstock was fed into the acidogenic digester from the bottom using the same diaphragm pump used for recirculation and effluent from this first stage digester was gravitationally discharged to a collection tank (when pH adjustment was required) or directly to the second-stage methanogenic digester. A tipping-bucket gas flow meter was connected to the top of the digester to measure biogas production.

Three 140-L cylindrical polypropylene tanks (47-cm diameter and 89-cm tall) with a working volume of 87-L were used as methanogenic digesters. These digesters contained either no biocarriers (control) or were filled with 43.5-L bulk volume of bamboo rings or seashells. The digester was fed from the top and discharged from the bottom. The feedstock to these digesters were effluents from the acidogenic digesters either directly flowing gravitationally, or captured in an intermediate container then adjusted to pH 7.2-7.4 using NaOH solution and then pumped

into the methanogenic digester from the top. Effluents discharged from the methanogenic digester by which was displaced by the feedstock was collected in a storage tank. A tube from the top of the digester was connected to a gas flow meter to measure biogas production volumes daily. The entire digester setup was placed in an air-conditioned room controlled at temperature of 26±2^{o}C (Figure 5.1).

Feedstock and inocula

Operation schedule and OLRs at different points in time are documented in Table 5.1 and feedstock characteristics are presented in Table 5.2. Characteristics of the inocula were previously described in Chapter 4.

Biocarriers

Biochar, bamboo, and seashell were used as biocarriers in the two-stage digesters (Figure 5.2). Biochar was the byproduct of pyrolysis of pine wood pellets (500^{o}C for 0.5-1.0 hour), bamboo was cut from naturally dried bamboo poles obtained from a local private bamboo garden in Athens, GA, U.S.A. Seashells were obtained from a commercial seashell supplier in Florida, U.S.A (Shellhorizons.com) and consisted of a mix of different types of shells collected from various locations in the Indian ocean. All biocarriers were washed and immersed in tap water for 24 hours and dried at 50^{o}C to remove any impurities and toxins before use in the experiments.

Biocarriers were characterized by measuring particle size, porosity and bulk density. Biochar, and seashell were used as received. Fifteen pieces of biochar were randomly picked from the biochar bulk and particle sizes were measured using a digital caliper (CEN-TECH, Virginia, USA). Bamboo was cut using a table saw into 1.5-cm pieces from 2.4-m bamboo poles with a diameter of 1.9 cm. Porosity and bulk density were measured the procedure described by Akdeniz et al. (2011) with minor modifications. In the porosity measurement, modifications

included using a 2-L plastic beaker fully filled with biocarriers dropped from a 5-cm height to settle the biocarriers. The biocarriers level was marked and tap water was added into the beaker to fill the voids to the mark level. In the density measurement, a 1-L glass beaker was fully filled with biocarriers and weighed.

Experimental procedures

In the acidogenic digesters, after 100% inoculum addition and three months startup (continuous feed at OLR of 3.0 g COD L^{-1} d^{-1}), digesters were run at an OLR of 2.8 gCOD $L^{-1}d^{-1}$ (Table 5.1). As the feedstock composition remained relatively constant throughout the experimental period, changing applied OLRs changed HRTs. The digesters were operated at different OLRs of 2.8, 4.3, 2.1 and 1.2 g COD L^{-1} d^{-1} in sequence, corresponding to HRTs of 4.8, 3.2, 6.3 and 11.1 days, respectively, for a total run duration of 319 days.

In the methanogenic digester, after 100% inoculum addition and three months startup adaptation (continuous feed at OLR of 1.2 g COD L^{-1} d^{-1}), experimental data used in performance evaluation were collected. The operation of methanogenic digesters is divided into three periods, namely, days 0-99 where only feedstock was added; days 100-140 when digester rehabilitation was implemented by diluting its contents, adjusting pH, and adding fresh inoculum; and days 141 to 400 when alkalinity was added to control inhibition caused by accumulating VFA and changes in pH (Table 5.1). In the first period, the digesters were operated at OLRs of 1.3, 2.0 and 1.0 g COD L^{-1} d^{-1} (equivalent to HRTs of 10.5, 7.0 and 13.8 days, respectively). In this period, digester performance began to deteriorate as indicated by reducing biogas production and reduction in pH. At the end of the digester rehabilitation in the second period, 50% new inoculum and 50% effluents from the acidogenic digester was added to the methanogenic digester. In the third period, the pH of feedstock was adjusted to 7.2-7.4 using

NaOH solution (25M) before feeding to the methanogenic digester. The digester was operated at three OLRs, 0.6, 0.4 and 1.0 g COD L^{-1} d^{-1} in sequence (equivalent to HRTs of 24.2, 37.8 and 13.2 days, respectively). Each OLR was maintained for a period equivalent to three HRTs to establish a steady state condition.

Analytical methods

Analytical methods used to test the substrates were identical to those previously described in Chapter 4.

Statistical Analysis

Statistical analyses used were identical to those previously described in Chapter 4.

Results and discussion

Feedstock for acidogenic and methanogenic digesters

The characteristics of feedstocks to the acidogenic and methanogenic digesters are presented in Tables 5.2 and 5.3, respectively. Characteristics of feedstocks to the methanogenic digesters were generally similar. The slightly lower TAN concentration in the effluent from the BB-1 digester which is described in Table 5.3 was potentially caused by ammonia emission from a leak in the acidogenic digester. This TAN difference was very slight (less than 6%) and is unlikely to have negatively that impacted the performance of that methanogenic digester.

Biocarriers characteristics

Biochar granules had diameter of 0.49±0.03 cm and length of 0.85±0.13 cm, sizes of seashell were in the range of 1.3 to 3.8 cm and the size of bamboo rings was around 1.9 cm diameter and 1.5 cm long. The bulk density of biochar, bamboo, and seashell were 387.5±14.1, 182.7±8.3 and 635.9±10.9 kg m^{-3}, respectively. The porosities of these were 66.0±1.4%, 79.3±2.1, and 71.3±2.5%, respectively.

Biocarriers effects in the acidogenic digester

Biogas production and its methane concentration from acidogenic digesters were compared for the test period of 319 days (Figure 5.3). The biochar digester produced the highest volume of biogas ranging between 0.04 and 0.28 L L^{-1} d^{-1} with methane concentrations around 50% (Figure 5.3). The bamboo and control digesters produced comparable levels of biogas ranging between 0.03 and 0.17 L L^{-1} d^{-1} and containing approximately 45% methane. An incident of methane concentration drop occurred when a temporary leak of digesters occurred, however, once leaks were sealed the digesters recovered to normal levels of methane. This confirms others' findings that short-term oxygen exposure to the anaerobic digester does not cause permanent negative impact to the methanogens as most of the oxygen is rapidly consumed by the acidogens and very little to no oxygen reaches the methanogens (Botheju and Bakke, 2011). At the steady-state conditions in two representative OLRs (2.8 and 1.2 g COD L^{-1} d^{-1}) the biochar digester had significantly higher biogas production and methane yields compared to the other two digesters (Table 5.4). This shows active methanogenesis in the biochar digester.

The pHs of effluents from the three digesters were comparable. The COD and TSS, however, were significantly lower in the biochar digester compared to the other two digesters (Figure 5.4). At steady state (Table 5.4), effluent COD and TSS of the biochar digester were lower than other digesters, confirming higher degradation of organics supported by stronger hydrolysis in this digester. At the lower OLR of 1.2 g COD L^{-1} d^{-1}, the bamboo and control digesters had similar effluent CODs and TSS, however, at the higher OLR of 2.8 g COD L^{-1} d^{-1} the control digester had significantly higher effluent CODs and TSS than the bamboo digester, potentially caused by partial microbial biomass washout.

TAN in effluents of the three digesters were relatively similar during the entire test period (Figure 5.5). High TAN accumulation is a result of complete hydrolysis of blood proteins for test periods where HRT was longer than 5 days (Lopez et al., 2006). Similarly, VFA in effluents of three digesters were also comparable during the entire test period (Figure 5.5). The amount of carbon conversion did not seem to have an impact on the VFA concentrations. At steady state (Table 5.4), there were no significant differences between effluent TAN and VFA among the three digesters.

The higher methane yield and COD reduction in the biochar digester at different OLRs suggests that biochar is not suitable as a biocarier in the acidogenic digester where the desired outcome is conversion of complex organics to intermediate VFAs (and not complete conversion to methane). The performance of bamboo and control digesters were similar at the lower OLR of 1.2 g COD L^{-1} d^{-1}, however, at the higher OLR of 2.8 g COD L^{-1} d^{-1} the control digester had effluents of higher TSS and COD, suggesting digester instability. Based on these data we conclude that bamboo is a superior biocarrier for use in the acidogenic digester at high OLRs.

Biocarriers effects in the methanogenic digester

Operation of methanogenic digesters is divided into three periods as described earlier, namely, no alkalinity addition in days 1-99, recovery period in days 100-140, and alkalinity addition in days 141-400.

In the first period, the control and bamboo digesters showed very low biogas production, low methane content, low pH, and low COD reduction. The seashell digester performed better with respect to biogas production, methane concentration, and having higher pH compared to the other digesters (Figure 5.6 and 5.7). Seashells are predominantly $CaCO_3$ that could have dissolved and provided buffering capacity to the substrate and resulted in higher methanogenic

activity (Hamestera et al., 2012). In the second period, the digesters were placed on a rehabilitation mode by first diluting the substrate in the digester and then adding alkali to raise the pH. The COD, TAN and VFA in the digesters were reduced because of dilution, and process inhibition was alleviated to some extent. These digesters showed signs of performance recovery with gradually increasing biogas production and methane concentrations. However, the recovery was very slow; therefore new inoculum was introduced to these digesters to speed up recovery.

In the third period, the bamboo digester showed the highest performance with respect to biogas production and COD removal. However, methane contents in biogas from all three digesters were relatively the same and around 79%. This value observed in our work is close to values in the range of 81 to 84% previously reported by Hansen and West (1992). It is interesting to note that although the control digester had low biogas production, the concentration of methane was still high. One reason for this could be the effect of adding NaOH. It is known that carbon dioxide produced from biological activity remains largely in solution at relatively low temperatures (solubility of 0.15 kg-CO_2 per kg water at 1 atm and 20 ^{o}C) (Appels et al., 2008). This could have reacted with NaOH to form $NaHCO_3$ thus reducing the carbon dioxide concentration in the biogas. This phenomenon and our supporting data show that AD of mixtures of blood and wastewater can produce high quality of biogas, thereby reducing costs for further biogas upgrading.

At steady state (Table 5.5), in the control and seashell digesters biogas production gradually increased initially and then dropped when OLRs increased from 0.4 to 1.0 g COD L^{-1} d^{-1}. However, the bamboo digester continued to produce higher levels of biogas even when operated at an OLR of 1.0 g COD L^{-1} d^{-1}, confirming high methanogenic activity. Higher methane yield was also found in the bamboo digester (178-378 mL $g^{-1}COD_{added}$) relative to the

other two digesters. Effluent pH was initially in the preferred range of 7.1 to 7.5 in all three digesters, however, it gradually decreased with increasing OLR suggesting possible organic overloading of the system. COD removal also tended to decrease when increasing OLR. At the OLR of 0.4 g COD L^{-1} d^{-1}, the minimum effluent COD reached 0.80, 2.45 and 4.75 g L^{-1} for bamboo, seashell and control digesters, corresponding to 93.5%, 79.9% and 61.1% of COD removal, respectively. The TSS of effluents from the bamboo digester was higher than that seen in the other two digesters (Figure 5.7). At steady state (Table 5.5), the bamboo digester had the highest COD reductions of 67.7 to 80.1% at lower OLRs, and 31.1% at the highest OLR of 1.0 g COD L^{-1} d^{-1}.

Effluent TAN and VFA were at similar levels of concentration in the three digesters (Figure 5.8). VFA gradually accumulated in the effluents of all digesters when the OLR increased to 1.0 g COD L^{-1} d^{-1}. Bamboo digester had slightly lower VFA compared to that of other digesters, possibly because of volatilization in this digester that had the highest biogas production. At steady state (Table 5.5), VFA reduction initially increased and then decreased when OLRs were raised from 0.4 to 1.0 g L^{-1} d^{-1}. Higher VFA accumulation at the higher OLRs resulted in inhibition of methanogenesis and declining digester performance.

The seashell digester performed better when no alkali was added to the digester, because of the $CaCO_3$ buffering described earlier. However, when alkali addition was initiated the bamboo digester performed the best. The surface texture and shape of bamboo rings seem to have particularly good for microbial attachment, growth, and prevention of washout, contributing to the higher performance of this digester (Figure 5.2; Habibi and Lu, 2014). Seashells have a relatively flat surface structure potentially making it harder to create a biofilm attachment

(Figure 5.2). Overall, bamboo was identified as the superior biocarrier for use in the methanogenic digesters treating blood and wastewaters.

Performance comparison of digesters with and without biocarriers

Performance of different digester configurations treating co-substrates of poultry blood and wastewater is summarized in Table 5.6. At OLRs of 0.4 to 0.7 g COD L^{-1} d^{-1} and 26^{o}C, the two-stage pilot digester with bamboo biocarriers had higher methane production, methane yield, and COD removal compared to an identical digester without biocarriers. Besides, this digester also had higher performance compared to batch digesters and continuous lab-scale digesters treating similar substrates at 35-38^{o}C. These results confirm that adding biocarriers to the digester can enhance AD performance.

Observations on the biocarrier at the end of the test of the Bamboo Digester

After the 16-month operation, the digester filled with bamboo, which had the best performance, was cut open from the top to visually observe biocarriers distribution and biosludge in the digester (Figure 5.9).

In the acidogenic digester, up to 3 cm foam was found floating on the substrate surface and additional foam on the inner surface of the digester lid. These findings agree with observations from previous studies that protein and lipid wastewater can easily cause foam build up and impact the AD process (Kougias et al., 2014). Approximately 2.5 gal (9.5 L) bulk volume of bamboo was found on the bottom of the digester along with a slurry of microbial biomass. The substrate in the digester had very high TS, VS and TSS compared to the feedstock (Table 5.7). The bamboo rings seemed to have retained a high level of physical integrity as observed when picked up from the digester bottom using tweezers.

In the methanogenic digester, approximately 5.5 gal (20.8 L) bulk volume of bamboo (about 17.8 cm thick layer) was found floating on the substrate surface and 6.5 gal (24.6 L) bulk volume of bamboo (about 21.6 cm thick layer) was found settled at the bottom along with a thick slurry. Only a few pieces of bamboo rings (<10) were suspended between the top and bottom layers. The inner surface of the digester lid was relatively clean and had no evidence of foam formation. The distribution of bamboo rings in the substrate seems to have enhanced mass transfer between the biocarrier biofilm and substrates. The effective volume of digester, containing substrate, bamboo and microbial slurry was divided roughly into three parts, namely, the top, middle and bottom with each layer having an approximate depth of 23 cm. Bamboo rings and substrate from each of these layers were collected for further examination. Visual observations showed no apparent outer structural differences between bamboo rings from the top and bottom layers. However, bamboo from the bottom layers had more slurry/biofilm attachment to it. As seen in the acidogenic digester, the bamboo rings from the methanogenic digester also maintained a high level of physical integrity as observed when picked up from the digester bottom using tweezers. These finding agree with observations reported in previous studies that bamboo rings had higher resistance against biodegradation in long term operation (Camargo and Nour, 2001; Colin et al., 2007).

Conclusions

Addition of low-cost biocarriers improved the performance of two-stage digesters treating blood and wastewater by enhancing hydrolysis and acidogenesis in the first stage and increasing methane production and COD removal in the methanogenic digester. Bamboo rings were found to be superior biocarriers among those tested biocarriers. Bamboo rings randomly distributed in the acidogenic digester bottom (which was periodically mixed), while in the

methanogenic digester approximately half of the bamboo rings floated at the surface. Bamboo rings also maintained a high level of physical integrity over the 400 day test period.

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Table 5.1 Operational mode of 400 days operation

Day	OLR and feeding flow rate (g COD L^{-1} d^{-1} and L d^{-1})	
	Acidogenic digester (40-L)	Methanogenic digester (87-L)
1-47	2.8[a] , 8.3	1.3[a], 8.3
48-56	4.3[a], 12.5	2.0[a], 12.5
57-91	2.1[b], 6.3	1.0[b], 6.3
92-99	1.2[c], 3.6	0.6[c], 3.6
100-140		Recovery period Day 99-119: feedstock dilution by adding 1.8 gal tap water approximately every two days Day120-132: alkalinity addition, 7.2 gal $NaHCO_3$ solution (9.4 g L^{-1}) Day 133-140: new inoculums addition
141-210		0.6[c*], 3.6
211-341		0.4[c*], 2.3
342-347		0.6[c*,**], 3.6
348-356	1.6[c**], 4.7	0.7[c*, **], 4.7
357-400	2.3[c], 6.6	1.0[c*], 6.6

[a] feed daily, [b] feed every two days, [c] feed twice a week, no feeding was on day 319-400 for acidogenic digester filled with biochar.
* pH of feedstock adjusted to 7.2-7.4 using NaOH solution, ** Adaption period.

Table 5.2 Characteristics of feedstock of blood wastes and poultry processing wastewaters

Parameters	Mean ±Standard deviation
pH	6.8±0.2
COD (g L^{-1})	13.64±1.38
Protein (g L^{-1})	8.24±1.66
TS(g L^{-1})	6.91±1.95
VS(g L^{-1})	6.07± 1.98
TSS(g L^{-1})	1.59± 0.47
TN(g L^{-1})	1.32± 0.27
TAN (g L^{-1})	0.51± 0.34
VFA(g L^{-1} as acetic acid)	1.68± 1.31
C[a]	43.92± 4.02
H[a]	5.68±0.56
N[a]	9.49±1.85
S[a]	1.22± 0.15
C/N	4.70± 0.52

[a] w/w, % TS

Table 5.3 Characteristics of acidogenic digester effluents as feedstock for methanogenic digester

	BB-1	BB-2	BB-3
pH	6.4 ±0.1	6.5±0.1	6.4±0.1
COD (g L^{-1})	13.41±1.06	13.32±1.01	13.11±0.91
TSS (g L^{-1})	1.21±0.16	1.19±0.17	1.16±0.17
TAN (g L^{-1})	1.29^{b}±0.14	1.32^{ab}±0.15	1.37^{a}±0.15
VFA (g L^{-1})	10.71±3.14	10.23±2.85	9.84±2.78

BB-1, BB-2 and BB-3 denoted three identical acidogenic digesters filled with bamboo. The effluents from three digesters were used as the feedstock of methanogenic digester filled with control (CT), seashell (SS) and bamboo (BB), respectively.
Different letters of each parameter indicate significant differences ($P \leq 0.10$) .

Table 5.4 Performance of acidogenic digester filling different biocarriers at steady-state condition

OLR ($g\ COD\ L^{-1}\ d^{-1}$)	2.8			1.2		
	BB	CT	BC	BB	CT	BC
Biogas ($L\ L^{-1}\ d^{-1}$)	0.09^{b}±0.02	0.07^{b}±0.02	0.13^{a}±0.01	0.03^{b}±0.01	0.03^{b}±0.01	0.06^{a}±0.01
CH_4%	40.6^{b}±1.6	43.7^{b}±3.4	50.6^{a}±2.3	51.0^{b}±3.8	54.5^{ab}±0.3	56.4^{a}±1.8
pH	6.5^{b}±0.0	6.5^{b}±0.0	6.6^{a}±0.1	6.6±0.1	6.6±0.0	6.6±0.1
COD ($g\ L^{-1}$)	12.08^{b}±0.81	14.26^{a}±0.57	10.96^{b}±0.81	12.68^{ab}±0.15	12.98^{a}±0.55	11.98^{b}±0.48
TAN ($g\ L^{-1}$)	1.34±0.19	1.45±0.16	1.47±0.13	1.28±0.08	1.39±0.16	1.32±0.07
VFA ($g\ L^{-1}$)	8.45±1.30	9.11±1.86	7.41±0.81	12.09±1.72	10.82±0.83	12.06±2.57
TSS ($g\ L^{-1}$)	1.32^{b}±0.10	1.87^{a}±0.28	1.03^{b}±0.07	1.21^{a}±0.05	1.22^{a}±0.05	1.05^{b}±0.05
CH_4 yield ($mL\ g^{-1}$ COD_{added})	11.9^{b}±1.6	10.7^{b}±3.0	22.5^{a}±0.7	13.1^{b}±3.2	15.6^{b}±3.2	27.6^{a}±5.4

BB-Bamboo digester-2, CT-Control digester, BC-Biochar digester; Steady-state condition was assumed at the end of each OLRs operation. The parameter value was expressed as average of three consecutively weekly values; Different letters of each parameter the same OLR indicate significant differences ($P \leq 0.10$) .

Table 5.5 Performance of methanogenic digesters under steady-state condition

Parameters	OLR (g COD L^{-1} d^{-1})								
	0.4			0.6			1.0		
	CT	SS	BB	CT	SS	BB	CT	SS	BB
Biogas (LL^{-1} d^{-1})	0.08^{c}±0.00	0.12^{b}±0.01	0.18^{a}±0.01	0.10^{c}±0.01	0.17^{b}±0.01	0.24^{a}±0.02	0.05^{c}±0.00	0.10^{b}±0.00	0.22^{a}±0.02
CH_4%	78.1^{b}±0.6	80.0^{a}±0.4	77.5^{b}±0.4	78.6^{b}±0.1	80.4^{a}±0.5	78.2^{b}±0.7	76.2±0.8	77.0±1.1	77.6±1.0
pH	7.4^{b}±0.0	7.5^{ab}±0.1	7.5^{a}±0.0	7.3±0.1	7.3±0.1	7.3±0.0	7.1±0.0	7.1±0.0	7.2±0.0
COD									
Input (g L^{-1})	13.27±1.08	13.75±0.26	14.20±0.48	13.58±1.46	13.18±0.51	12.75±0.75	13.03±0.75	12.45±1.04	12.72±0.20
Output (g L^{-1})	6.08^{a}±0.36	4.37^{b}±0.12	2.82^{c}±0.46	5.62^{a}±0.25	4.70^{b}±0.36	4.13^{b}±0.56	11.75^{a}±0.57	11.00^{a}±0.20	8.77^{b}±0.68
Removal (%)	54.1^{c}±1.3	68.2^{b}±1.5	80.1^{a}±3.4	58.3^{b}±5.4	64.2^{ab}±4.1	67.7^{a}±2.7	9.7^{b}± 6.3	11.2^{b}±7.8	31.1^{a}±5.2
VFA									
Input (g L^{-1})	10.91±0.80	10.63±1.31	10.35±0.99	9.98±2.73	9.65±2.09	9.61±2.32	13.36±2.15	12.65±3.10	12.05±1.45
Output (g L^{-1})	7.30±1.09	8.52±0.67	8.33±1.56	5.26±1.27	6.37±1.87	4.85±1.23	11.36±1.79	11.67±2.71	10.54±2.46
Removal (%)	33.2±6.1	19.2± 9.6	19.0±17.3	46.0±11.9	34.6±5.6	47.6±16.0	12.3±25.1	1.3±39.8	10.0±32.8
TAN									
Input (g L^{-1})	1.07^{b}±0.12	1.07^{ab}±0.03	1.22^{a}±0.03	1.33±0.04	1.32±0.01	1.31±0.12	1.19±0.06	1.11±0.10	1.14±0.08
Output (g L^{-1})	1.08^{b}±0.02	1.17^{a}±0.03	1.22^{a}±0.01	1.20±0.15	1.21±0.07	1.36±0.09	1.33±0.09	1.34±0.07	1.35±0.06
Removal (%)	-2.3±10.3	-9.3±5.7	-0.3±3.7	9.5±10.6	8.6±4.9	-4.0±9.6	-11.6±8.3	-20.5±4.6	-18.5±4.4
TSS									
Input (g L^{-1})	1.08±0.24	1.28±0.11	1.13±0.19	1.28±0.08	1.10±0.11	1.06±0.14	1.11±0.06	1.10±0.11	0.99±0.11
Output (g L^{-1})	0.64^{b}±0.16	0.64^{b}±0.05	0.98^{a}±0.05	0.76^{b}±0.06	0.74^{b}±0.04	1.41^{a}±0.10	0.74^{b}±0.06	0.93^{a}±0.06	0.83^{ab}±0.11
Removal (%)	40.2^{a}±13.6	49.8^{a}±5.7	10.9^{b}±17.2	40.5^{a}±8.1	31.7^{a}±10.7	-29.2^{b}±21.5	33.0±7.5	14.6±7.9	12.7±14.2
CH_4 yield (mL $g^{-1}COD_{added}$)	190^{c}±13	255^{b}±17	378^{a}±30	148^{c}±30	250^{b}±18	359^{a}±11	38^{c}±4	85^{b}±9	178^{a}±14

CT-control digester, SS-Seashell digester, BB-Bamboo digester; Steady state condition was assumed when CV of effluent COD or COD removal and biogas production at three consecutive weekly values were ≤10.0%; Different letters of each parameter in the same OLR indicate significant differences (P≤0.10) .

Table 5.6 Comparison of anaerobic digester performance treating animal blood wastes and wastewaters

Study	Feedstock	OLR (g COD L^{-1} d^{-1})	Methane production (L L^{-1} d^{-1})	COD removal (%)	mL CH_4 g^{-1} COD_{added}	Digester
Marcos et al., 2010	2% blood and 98% wastewaters	0.17*	0.04*	56.9	224	2-L batch digester, 38°C
Hansen and West, 1992	2% blood and 98% rendering condensate	1.01	0.06	28.0	60	7.8-L UASB, 35±1°C
		0.54	0.06	50.9	100	
		0.34	0.05	65.9	140	
Chapter 4-no biocarriers	Approximate two-fold dilution of 25% blood and 75% wastewaters	0.7	0.05	11.7	72	127-L two-stage digester, 26±2°C
		0.4	0.07	50.8	189	
This study-bamboo biocarriers		0.7	0.13	32.4	196	
		0.4	0.13	68.6	361	

*This value is calculated from reported results.

Table 5.7 Characteristics of biosludge in two-stage pilot digester filled with bamboo

	Biosludge location	TS ($g\ L^{-1}$)	VS($g\ L^{-1}$)	TSS($g\ L^{-1}$)
Acidogenic digester	Mixture of whole biosludge in whole digester			
		12.0±0.3	10.2±0.3	8.8±0.3
Methanogenic digester	Distance from the digester bottom, cm			
	0-23	18.3±0.1	13.2±0.1	15.6±0.1
	24-46	3.8±0.4	2.2±0.4	0.7±0.0
	47-69	3.0±0.1	1.5±0.1	0.3±0.0

Figure 5.8 Effluents characteristics of methanogenic digesters filled with or without biocarriers by time: TAN (a) and VFA (b). CT, SS and BB denote digesters filled with no biocarriers, seashell and bamboo, respectively.

Figure 5.9 The two-stage pilot digester after 16 months operations. AG is acidogenic digester and MG is methanogenic digester.

Figure 5.1

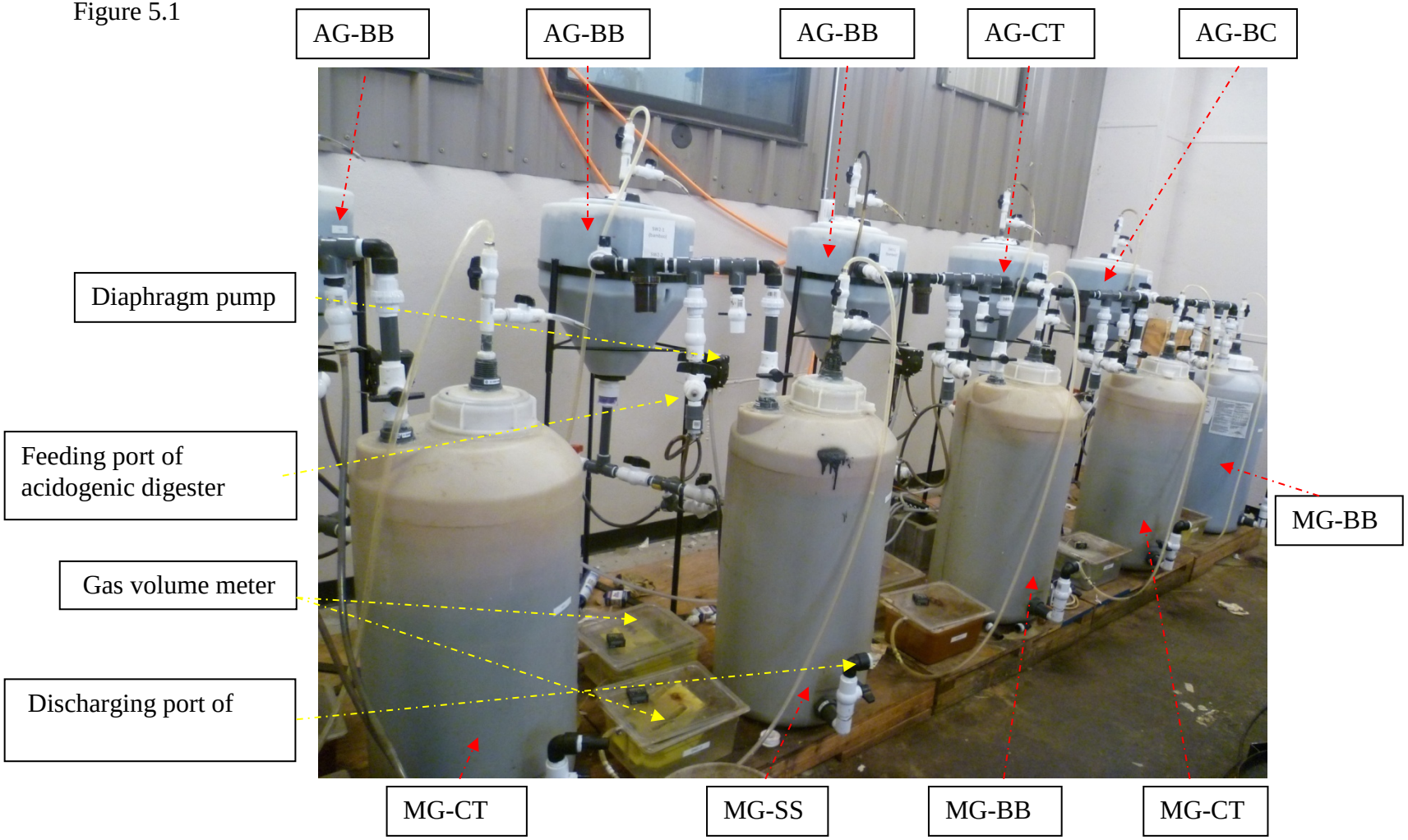

AG-BB
AG-BB
AG-BB
AG-CT
AG-BC
Diaphragm pump
Feeding port of acidogenic digester
Gas volume meter
Discharging port of
MG-BB
MG-CT
MG-SS
MG-BB
MG-CT

Figure 5.2

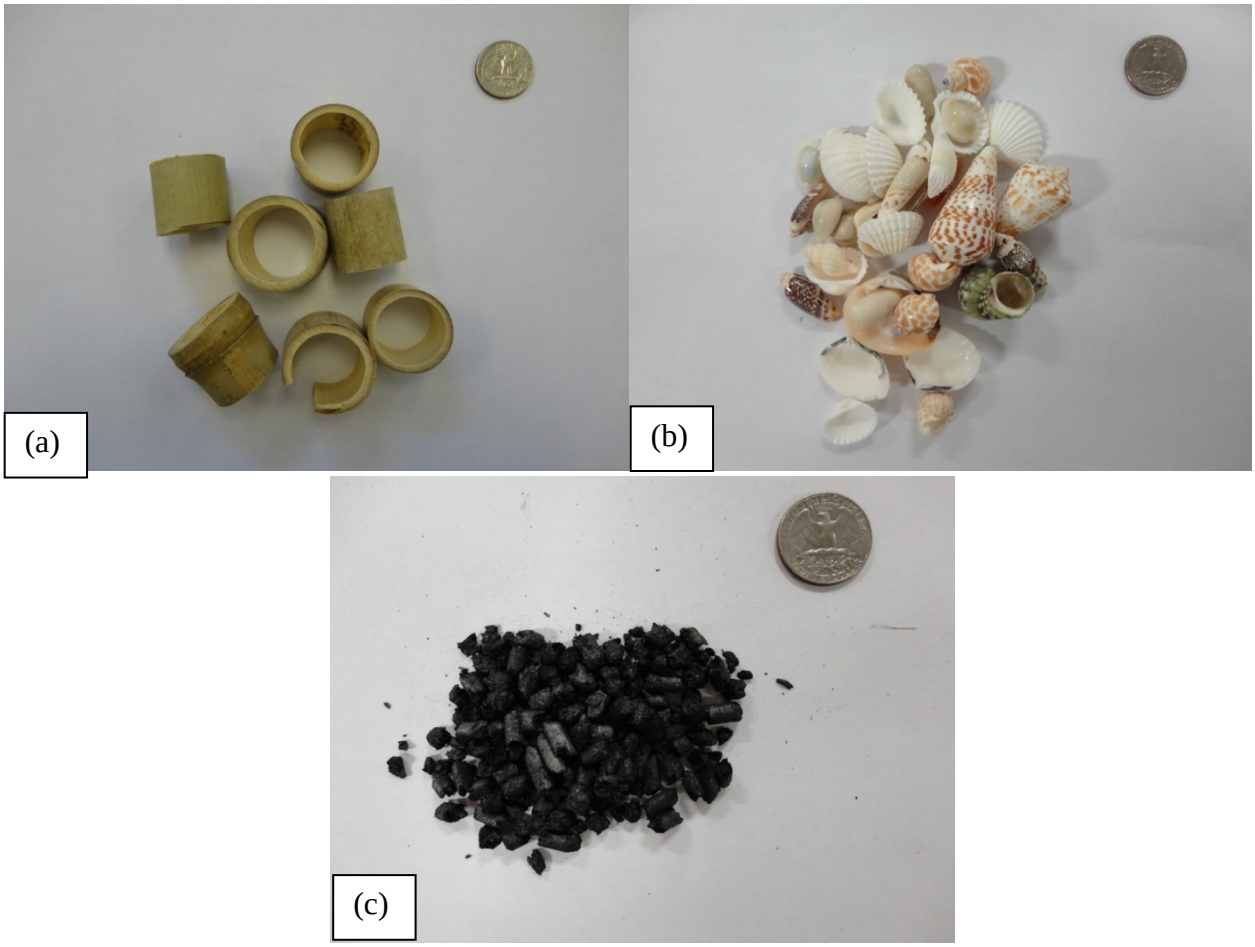
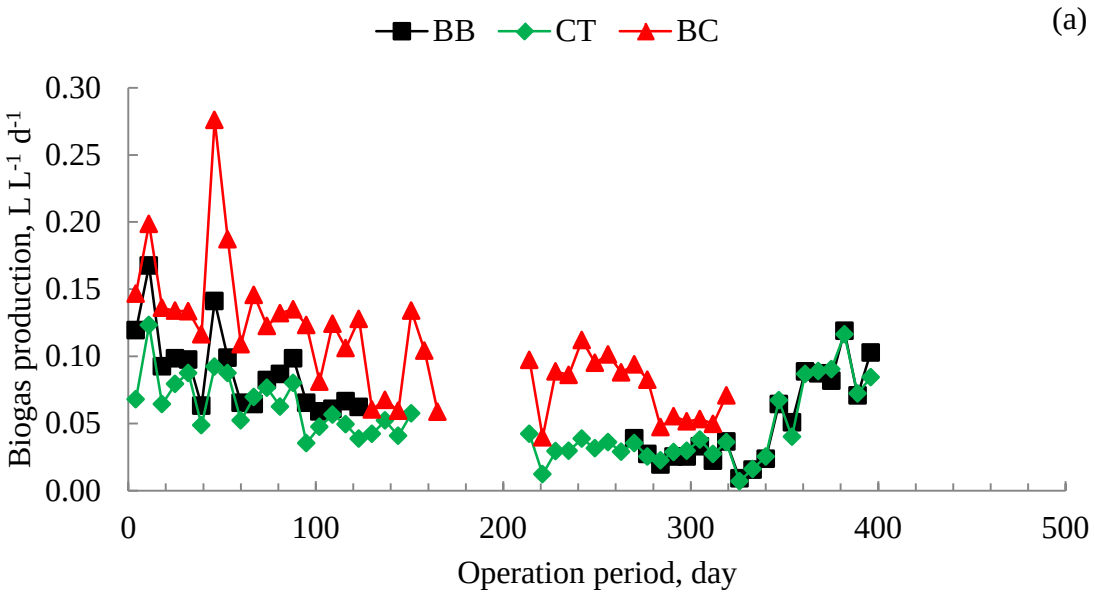


Figure 5.3


(a)
BB
CT
BC
Biogas production, L L-1 d-1
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
Operation period, day

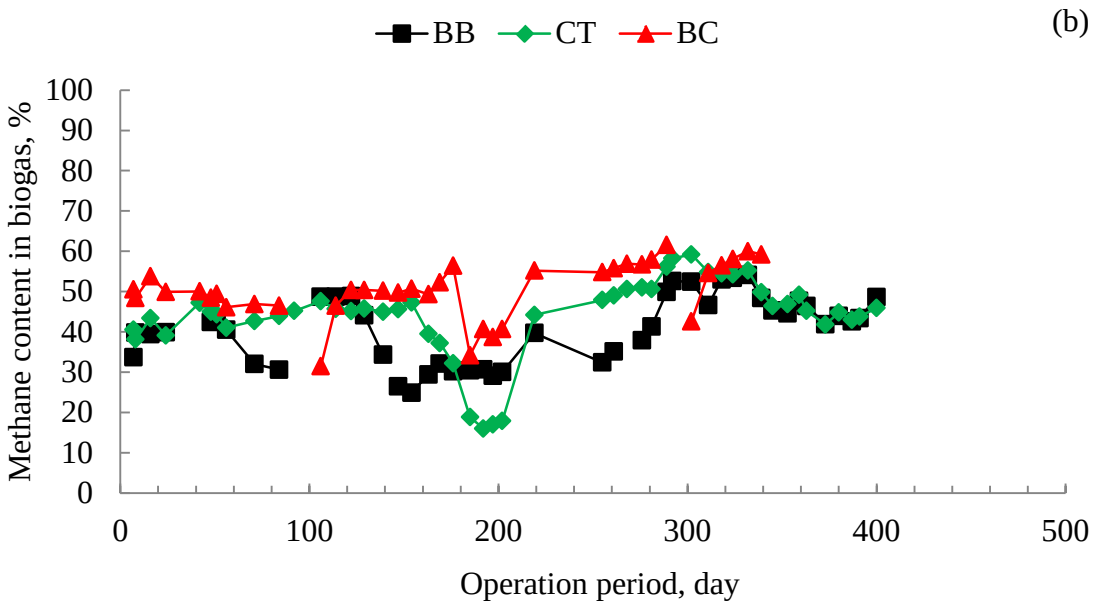

(b)
BB
CT
BC
Methane content in biogas, %
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
Operation period, day

Figure 5.4

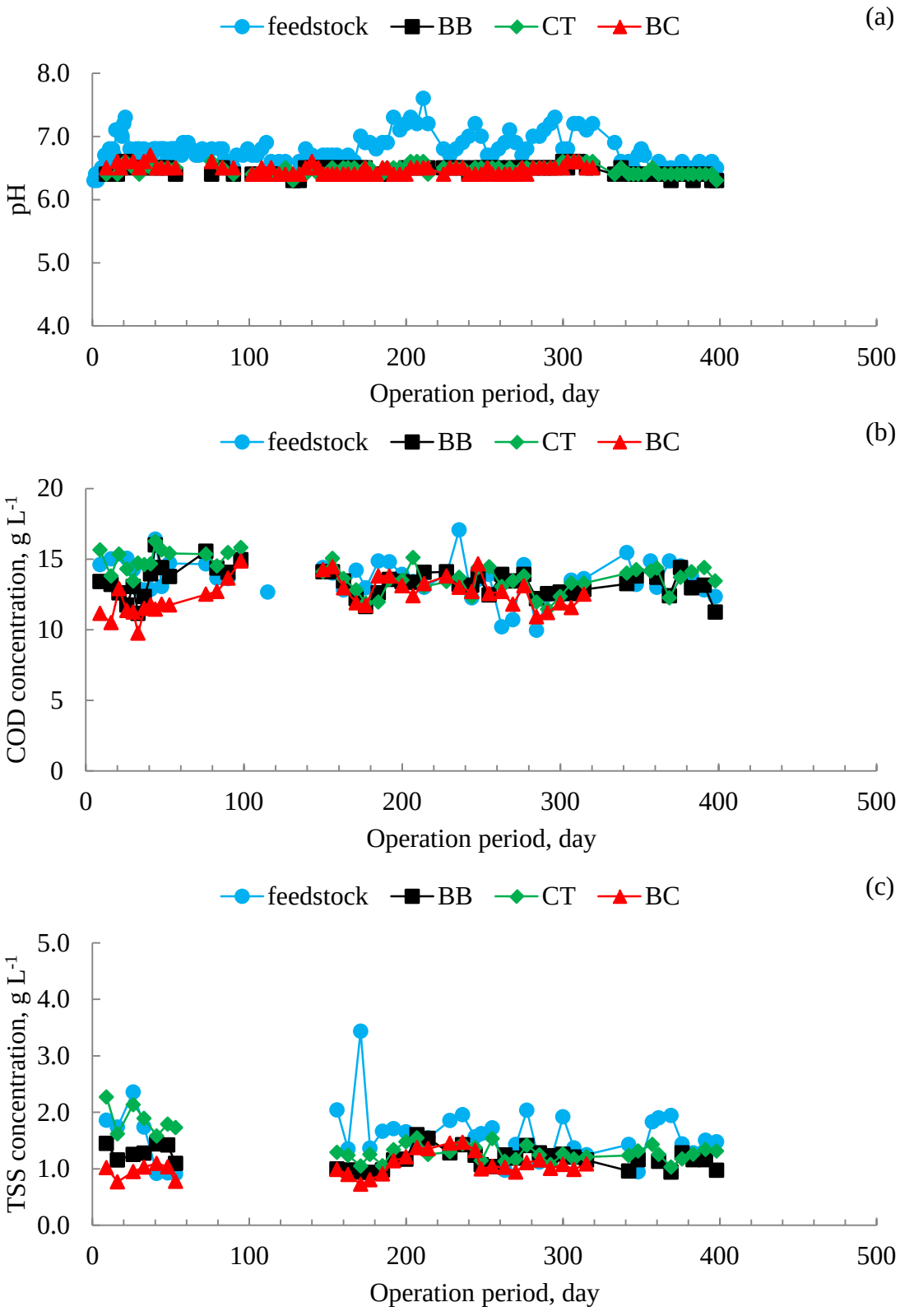
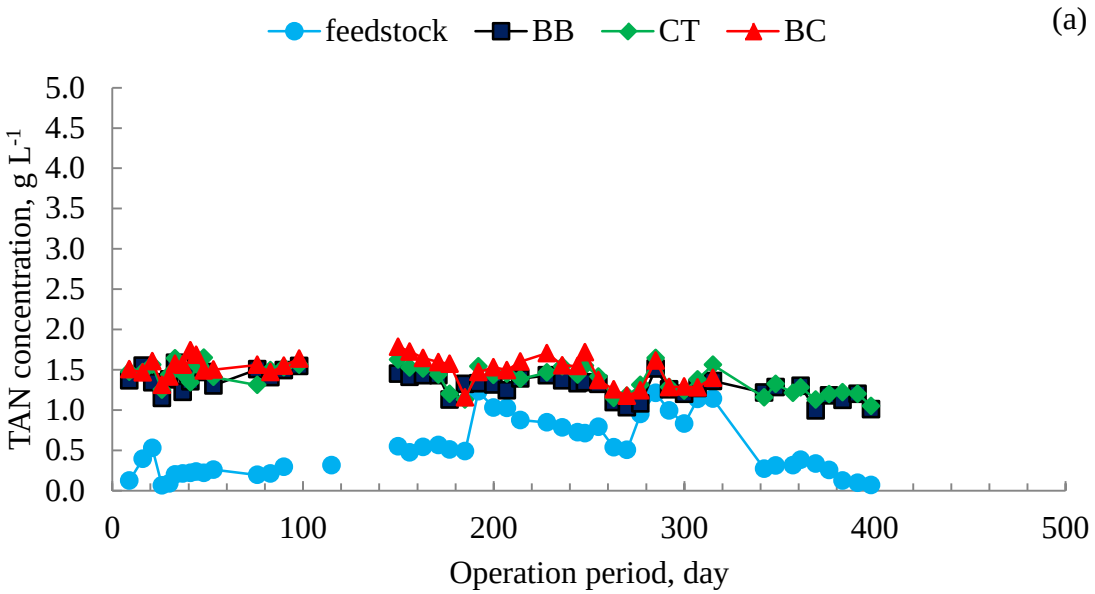

(a)
feedstock
BB
CT
BC
pH
8.0
7.0
6.0
5.0
4.0
0
100
200
300
400
500
Operation period, day
(b)
feedstock
BB
CT
BC
COD concentration, g L-1
20
15
10
5
0
0
100
200
300
400
500
Operation period, day
(c)
feedstock
BB
CT
BC
TSS concentration, g L-1
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Operation period, day

Figure 5.5


(a)
feedstock
BB
CT
BC
TAN concentration, g L-1
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Operation period, day

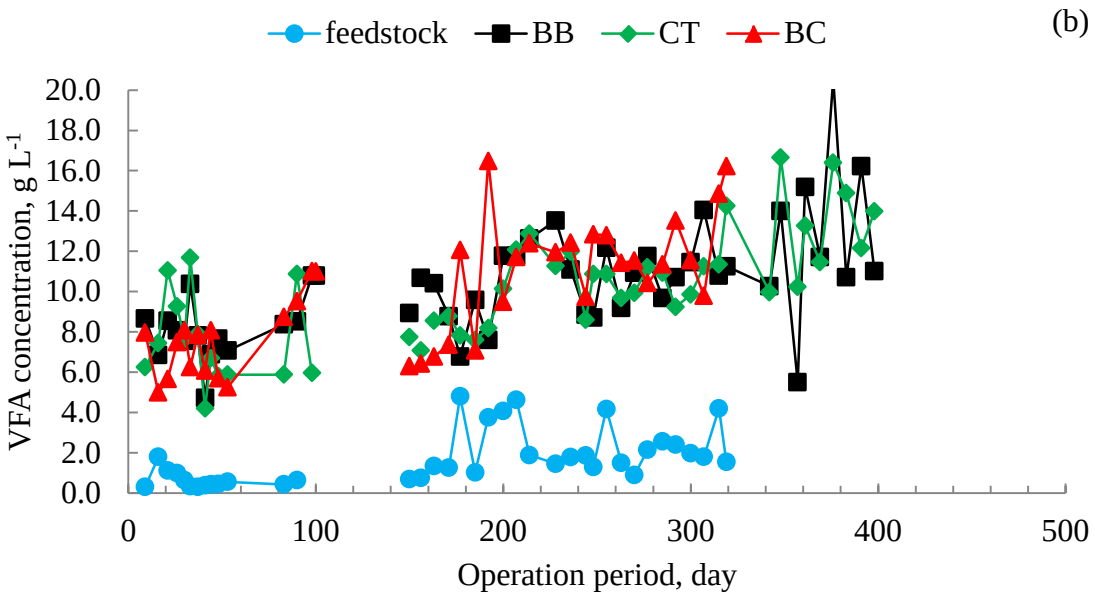
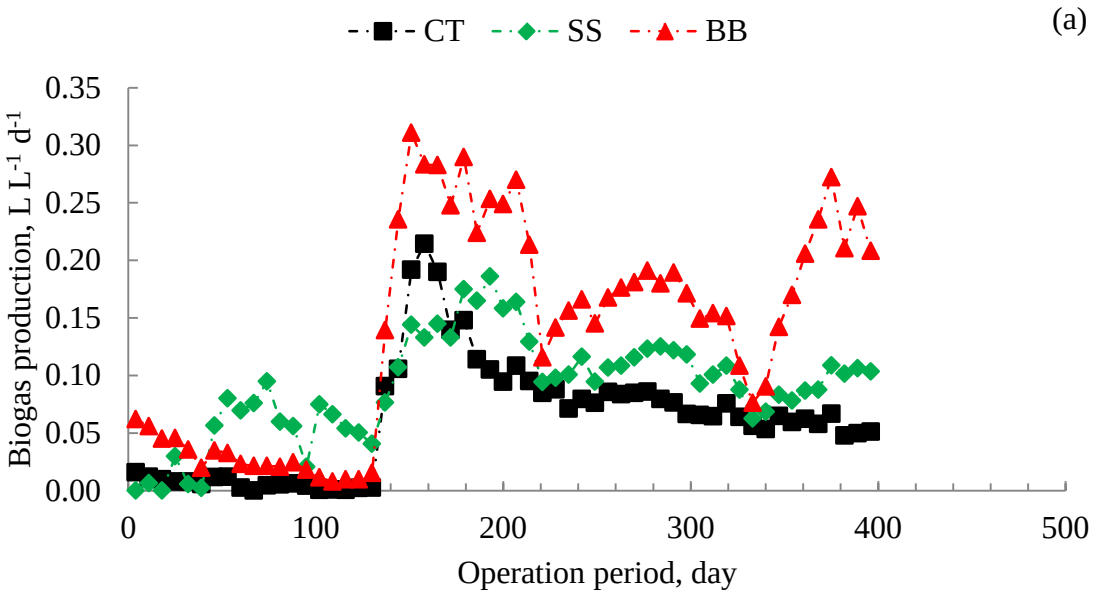

(b)
feedstock
BB
CT
BC
VFA concentration, g L-1
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
100
200
300
400
500
Operation period, day

Figure 5.6


(a)
CT
SS
BB
Biogas production, L L-1 d-1
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
Operation period, day

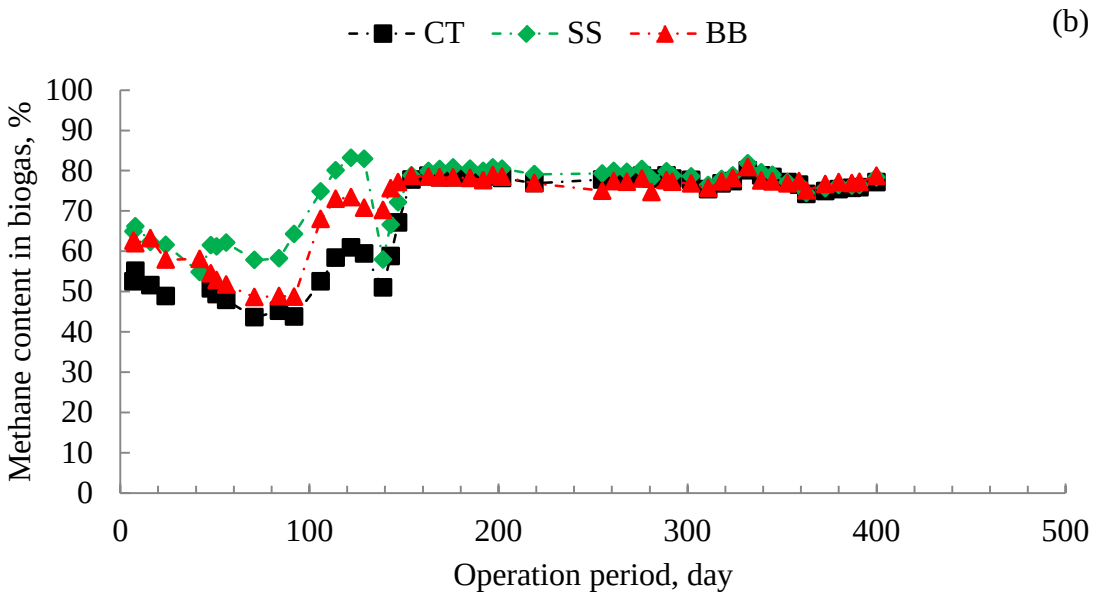

(b)
CT
SS
BB
Methane content in biogas, %
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
Operation period, day

Figure 5.7

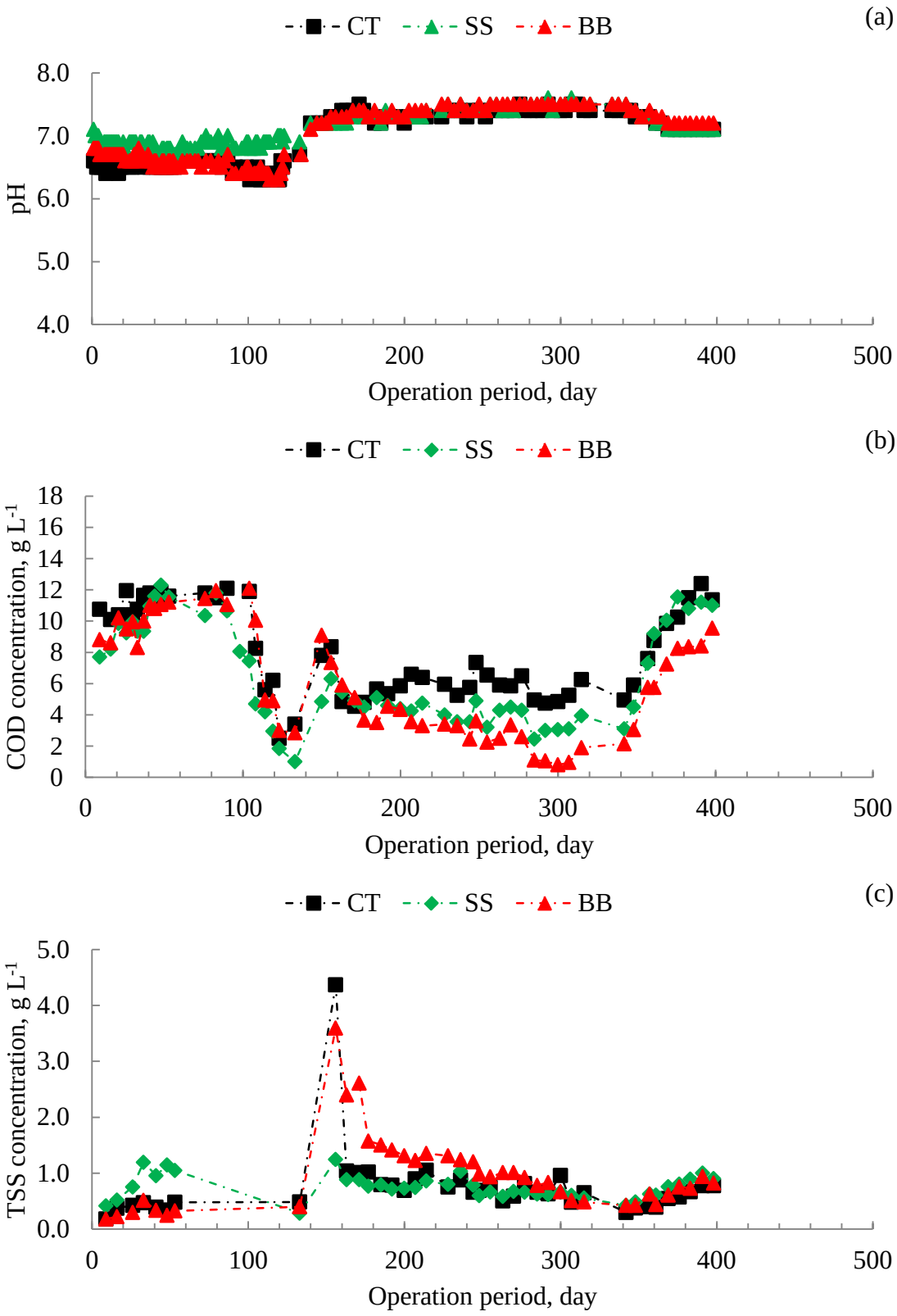
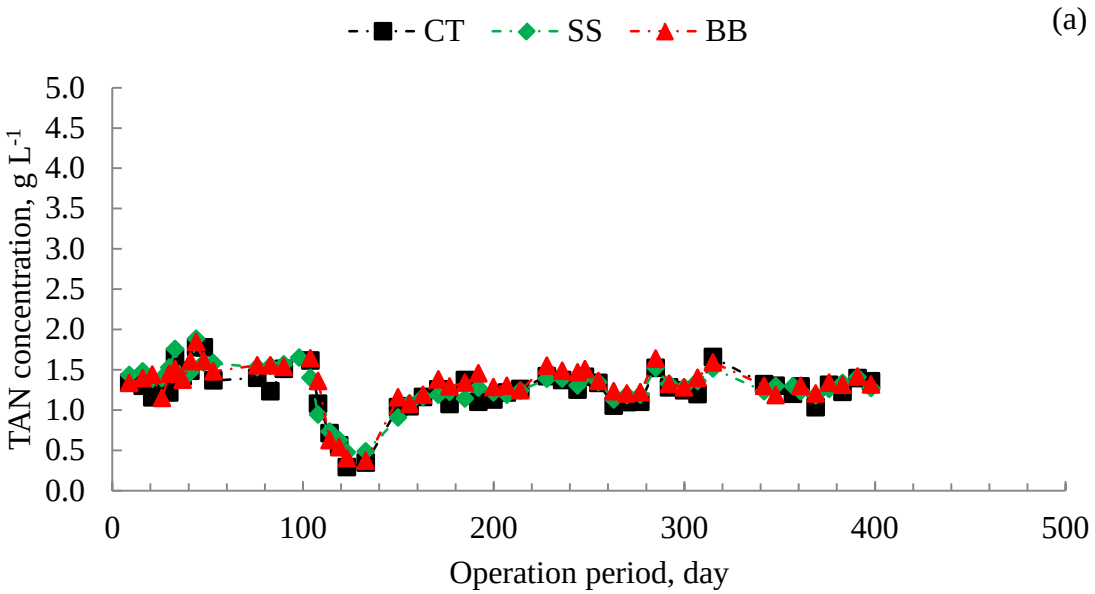

(a)
CT
SS
BB
pH
8.0
7.0
6.0
5.0
4.0
0
100
200
300
400
500
Operation period, day
(b)
CT
SS
BB
COD concentration, g L-1
18
16
14
12
10
8
6
4
2
0
0
100
200
300
400
500
Operation period, day
(c)
CT
SS
BB
TSS concentration, g L-1
5.0
4.0
3.0
2.0
1.0
0.0
0
100
200
300
400
500
Operation period, day

Figure 5.8


(a)
CT
SS
BB
TAN concentration, g L-1
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Operation period, day

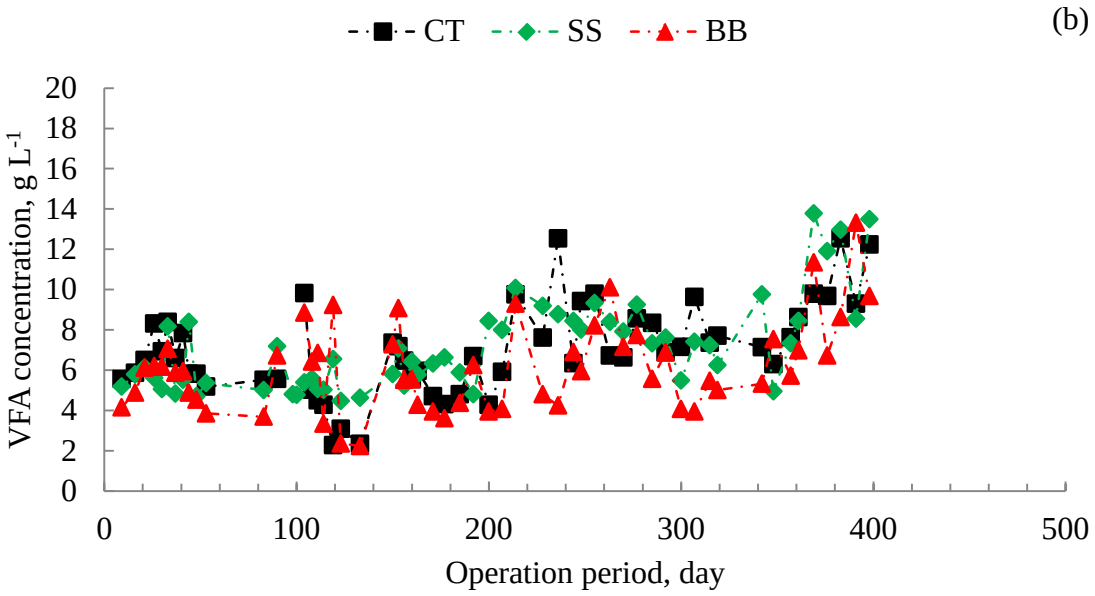

(b)
CT
SS
BB
VFA concentration, g L-1
20
18
16
14
12
10
8
6
4
2
0
0
100
200
300
400
500
Operation period, day

Figure 5.9

Two-stage pilot digester with bamboo biocarriers

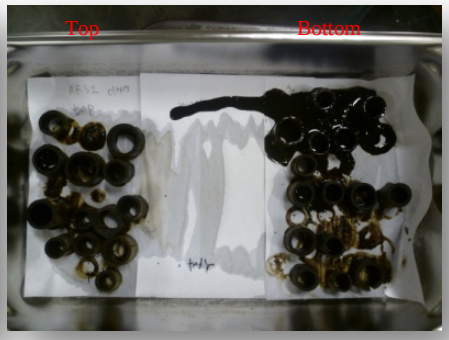
Top of AG

Top of MG

Top of AG substrate

Top of MG substrate

Bamboo in AG



Bamboo in MG

CHAPTER 6

STRUVITE PRECIPITATION AS A MEANS OF RECOVERING NUTRIENTS AND MITIGATING AMMONIA TOXICITY IN A TWO-STAGE ANAEROBIC DIGESTER TREATING PROTEIN-RICH FEEDSTOCKS[1]

[1]Wang, S., B.H. Kiepper, G.L. Hawkins, and K.C. Das. To be summited to *Water Research.*

Abstract

Accumulation of ammonia (measured as total ammonia nitrogen, TAN) a product of protein decomposition in slaughterhouse wastes can inhibit the AD process, reducing digester productivity and leading to failure. Struvite precipitation (SP) is an effective means to remove TAN and enhance the buffering of substrates. Different magnesium (Mg) and phosphorus (P) sources were evaluated as reactants in SP in acidogenic digester effluents to reduce its TAN levels. In order to measure impact of TAN removal, a standard biochemical methane potential (BMP) test was conducted to measure methane yield from treatments that had the highest TAN reductions. SP results showed 6 of 9 reagent combinations resulted in greater than 70% TAN removal. The BMP results indicated that SP treated substrates had similar or higher methane yields, higher nitrogen recovery, and higher buffering capacity than substrates that were not SP treated. The best performance was found where SP was achieved by adding $Mg(OH)_2$ and H_3PO_4 and resulted in 74.1% nitrogen recovery and 29.4% increase in methane yields relative to the substrate without SP.

Introduction

Anaerobic digestion (AD) is an attractive technology to treat high-strength slaughterhouse wastes as it can provide for energy recovery (methane), nutrient recovery (nitrogen and phosphorus), and pathogen destruction in wastewaters (Salminen and Rintala, 2002; Heinfelt and Angelidaki, 2009). Energy content in the form of methane recovered by treating slaughterhouse wastes in AD was reported to be 1,300 MJ per bovine, 140 MJ per pig and 1.3 MJ per broiler (Edström et al., 2003; Bouallagui et al., 2004; Yoon et al., 2014a). From poultry slaughterhouse wastes, as much as 23.0 g of nutrients per bird including nitrogen, phosphorus and potassium are potentially recoverable (Yoon et al., 2014a). With respect to

pathogens destruction, both fecal coliform and salmonella were completely eradicated in a thermophilic digester (50^{o}C). In addition, 99.9% and 90-99% of oocysts of *Eimeria tenella* were inactivated in a thermophilic digester and a mesophilic digester, respectively (Salminen and Rintala, 2002).

In AD, proteins and lipids are hydrolyzed and acidified to intermediates including hydrogen, NH_4 (measured as total ammonia nitrogen or TAN) and volatile fatty acids (VFA) in the acidogenic stage of the process. Hydrogen and VFAs are then converted to methane and carbon dioxide by a different group of bacteria in the methanogenic stage. High concentrations of TAN are known to severely inhibit methanogenesis reducing performance and leading to digester failure. Strategies of TAN management are needed when treating such high protein wastes in AD.

One of the best management strategies is the use of a two-stage AD system where acidogenesis (and the production of TAN and VFA) are physically separated from methanogenesis to reduce TAN and VFA inhibition of methanogens. The two-stage pilot digester studied in Chapter 4 was used to improve the performance of AD treating blood wastes, a high-protein waste. This digester produced 189 mL CH_4 g^{-1} COD_{added} and removed 50.8% of COD at an OLR of 0.4 g COD L^{-1} d^{-1} and 26^{o}C, which is higher than results from a single-stage digester treating similar feedstocks (Hansen and West, 1992). However, because TAN concentration (~1.4 g L^{-1}) in the substrate was close to the inhibitory level of 1.5 g L^{-1} (Rajagopal et al., 2013), the performance of this digester did not reach optimum values generally considered as over 300 mL CH_4 g^{-1} COD_{added} and 80% COD removal. The reduction of TAN in the substrate could possibly enhance the digester performance. Considering TAN is not biologically removed in AD, removing it via stripping or precipitation from the acidogenic

digester effluents could be one way to reduce TAN concentrations and associated inhibition in the methanogenic digester.

Magnesium ammonium phosphate (MAP) commonly known as struvite is a compound with low aqueous solubility under alkaline conditions (Lee et al., 2004). When concentrations of TAN, magnesium and phosphorus reached the certain values, struvite is naturally formed and is known to attach to and clog pipes in wastewater treatment. Struvite is also a slow release fertilizer with commercial value, so its precipitation from many types of high-ammonia wastewaters, including AD effluents has been studied extensively (Çelen and Türker, 2001; Nelson et al., 2003). Çelen and Türker (2001) reported 50 to 90% TAN removal from AD effluents through SP using phosphoric acid and magnesium oxide or magnesium chloride under short reaction times (~ 10 min), high pH (~9.0) and room temperature (25^{o}C). In order to improve AD performance by TAN reduction, in some cases magnesium (Mg) and phosphorus (P) were directly added to the digester (Lee et al., 2004; Romero-Güiza et al., 2014). Lee et al. (2004) reported reduction of 67% TAN and 73% of P in the substrate after adding $MgCl_2$ to a food waste digester thus increasing methane yields from 180 to 290 mL g^{-1} COD_{added}. Romero-Güiza et al. (2014) added a low-grade MgO byproduct to a digester treating pig manure and found methane yields increased from 130 mL g^{-1} VS_{added} (before MgO addition) to 190 mL g^{-1} VS_{added} (after 30 kg MgO m^{-3} addition). SP directly in the digester has been shown to significantly improve methane yields, however, accumulation and deposit of struvite within the digester gradually reduce the effective digester volume. In addition, once settled inside digesters the precipitate is very hard to remove. Establishing a struvite precipitator external to the AD digester, between the 1st and 2nd stages of the two-stage digester, is a practical way to remove TAN from substrates before feeding to the methanogenic digester.

Integrating an external SP with a two-stage digester treating high-protein substrates can result in nitrogen recovery, reduce TAN inhibition, and increase buffering in the methanogenic digester. In particular, conducting SP between the acidogenic and methanogenic phases would provide the desired results. To evaluate this plan, we conducted the following study with the goals of: (1) selecting most suitable reagents that maximize N recovery while minimizing residual methanogenic toxins, (2) testing the Mg and P sources and quantifying TAN removal from the feedstock to the methanogenic digester, and (3) performing a biochemical methane potential (BMP) test to quantify the methane yields from the treated substrates.

Materials and methods

Substrates

Acidogenic digester effluents were collected from three 40-L pilot acidogenic digesters treating co-substrates of poultry processing wastewater (PPWW) and poultry blood (3:1, v/v) that was diluted 50% with de-chlorinated water to reduce its initial organic strength. The digesters had been actively operating at mesophilic conditions ($26\pm2^{o}C$) for several months at the point of sampling. Collected samples were stored in a refrigerator ($\leq4^{o}C$) for further testing prior to use.

Experiment 1 - Optimization of struvite precipitation

Reagents

Five Mg compounds, namely, MgO, $MgCl_2.6H_2O$, Mg $(OH)_2$, $MgCO_3$ and $MgHPO_4.3H_2O$ and three P compounds, namely, 85%H_3PO_4, $NaH_2PO_4.H_2O$ and $MgHPO_4.3H_2O$ were used in this experiment. These compounds were selected based on the market availability and the low concentration of residual toxins that remain after the reactions (Table 6.2). A solution of 300 g NaOH L^{-1} was prepared and used for pH adjustment in the SP protocol. Nine

combinations of reagents, namely, MgO+85%H_3PO_4 (G1), MgO+$NaH_2PO_4.H_2O$ (G2), $MgCl_2.6H_2O$+85%H_3PO_4 (G3), $MgCl_2.6H_2O$+$NaH_2PO_4.H_2O$ (G4), $Mg(OH)_2$+85%H_3PO_4 (G5), $Mg(OH)_2$+$NaH_2PO_4.H_2O$ (G6), $MgCO_3$+85%H_3PO_4 (G7), $MgCO_3$+$NaH_2PO_4.H_2O$ (G8), and $MgHPO_4.3H_2O$ (G9), were evaluated to rank their performance in TAN removal.

Reactions equations

Reactions occurring during SP in reagent combinations G1 to G9 are listed below in equations (1) to (9) as follows:

$$MgO + H_3PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + H_2O + H^+ \quad (1)$$

$$MgO + NaH_2PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + H_2O + Na^+ \quad (2)$$

$$MgCl_2 + H_3PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + 2Cl^- + 3H^+ \quad (3)$$

$$MgCl_2 + NaH_2PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + Na^+ + 2Cl^- + 2H^+ \quad (4)$$

$$Mg(OH)_2 + H_3PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + 2H_2O + H^+ \quad (5)$$

$$Mg(OH)_2 + NaH_2PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + 2H_2O + Na^+ \quad (6)$$

$$MgCO_3 + H_3PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + CO_2 \uparrow + H_2O + H^+ \quad (7)$$

$$MgCO_3 + NaH_2PO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + CO_2 \uparrow + H_2O + Na^+ \quad (8)$$

$$MgHPO_4 + NH_4^+ + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow + H^+ \quad (9)$$

Struvite precipitation protocol

The amount of reagents to add to 500 mL of substrates was calculated so as to result in a molar ratio of 1:1:1 for NH_4: Mg: PO_4, including the Mg, NH_4 and PO_4 present in the substrate. The substrate TAN was measured to be 1.36 g L^{-1}, while Mg nor P in the feedstock were measured for this experiment because their amounts in the substrate are very few (based on

values in the range of 6.0 to 65.5 mg L^{-1} obtained in preliminary studies). Reagents and substrates were reacted in 500-mL Erlenmeyer flasks with aluminum covers and a magnetic stir bar placed in the flask to stir the substrates at 500 rpm on a stir plate. A volume equal to 510 mL substrates were placed in the Erlenmeyer flask and heated to room temperature in 1 to 3 minutes using a water bath at 50^{o}C. A 10-mL aliquot of substrates was sampled at the start of the experiment (T0). Subsequently, Mg and P reagents were added to the substrates in sequence. After adjusting substrate pH to 8.5, 10-mL substrate was sampled at 20 (T20), 40 (T40) and 60 minutes (T60) time points. A 1-mL aliquot from the T0 samples and from the supernatants of the T20 samples (1 hour after sample collection) was analyzed for TAN concentrations. Visualization of static samples of T0, T20, T40 and T60 were used to evaluate the completeness of struvite formation at different reaction time after one hour of each samples collection. Each treatment was replicated twice.

Experiment 2 - Biochemical methane potential of TAN reduced substrate

Treatments used in experiments

Due to different levels of TAN removal observed with different reagent combinations, treatments G1, G3, G5, G7 and G8 were selected for further BMP analyses. A volume equal to 1,700 mL of substrates was placed in a 2,000-mL Erlenmeyer flask and processed exactly as was done in the SP experiment described earlier. After 20 minutes, substrate stirring was stopped and the substrate was allowed to stand in the flask for one hour to allow for gravitational settling of the struvite precipitates that were formed. The supernatant was poured slowly into storage bottles and stored in the refrigerator ($\leq$4^{o}C) for further use in chemical analyses and BMP testing.

Inoculum

Inoculum used in the BMP tests was collected from an 87-L methanogenic digester treating similar effluents from a 40-L acidogenic digester. The inoculum was placed in a pre-incubated anaerobic digester at 38^{o}C for three days to deplete any un-degraded biological residues present in the inoculum before use in the BMP assay. The characteristics of inoculum were 2.9±0.1 g L^{-1} of TS, 1.3±0.1 g L^{-1} of VS, 1.4±0.0g L^{-1} of TAN, and 1.6±0.4 g L^{-1} of COD.

Biochemical methane potential (BMP) Assay

The BMP was tested using batch anaerobic digesters of 500 mL with an effective substrate volume of 300 mL incubated at 38^{o}C. Substrate to inoculum ratio was 70/30 (v/v) which corresponded to 3 g-$VS_{untreated\ substrate}$ to 1 g-$VS_{inoculum}$ (Yoon et al., 2014b). Because different treatments had different final TAN concentrations after SP, the TAN concentration substrates used in the BMP were adjusted to 0.6 g L^{-1} by adding untreated substrates, which had a higher TAN concentration. The pH of the mixture of untreated/treated substrates and inoculum was adjusted to 7.3 using HCl solution before placing the BMP test digesters. The headspace of the digesters was purged using N_2 gas and sealed using butyl rubber stoppers and aluminum crimps. To obtain a complete profile, the BMP digesters were incubated for 278 days for the untreated substrate and the 5 treatments with each replicated three times. Blank digesters of triplicate were run using the inoculum and DI water replacing substrates. Methane yields were calculated at standard temperature and pressure (STP) and expressed as mL CH_4 g^{-1} COD_{added}. Biogas production and methane concentration in the biogas was measured every 4 to 12 days depending on the level of activity. The pH was measured every 2 to 8 weeks by collecting 1-mL sample from serum bottles and measuring pH using a laboratory pH probe.

Calculation of cumulative methane production

The cumulative methane production was calculated using the following equation

$$M_n = \sum_{1}^{n}(m_n * C_n) + H * C_n \quad (10)$$

Where M_n is the cumulative methane production till the n^{th} day (mL); m_n is the biogas production on the n^{th} day (mL); C_n is the methane content in biogas on the n^{th} day; H is the headspace in the bottle and equal to 239 mL.

In this equation, $\sum_{1}^{n}(m_n * C_n)$ is the cumulative methane volume measured by discharging biogas and testing methane percentage in biogas on the n^{th} day and $H * C_n$ is the methane volume in the bottle headspace on the n^{th} day.

Modeling the kinetics of methane production

A modified Gompertz model was used to model cumulative methane production during the incubation period (Yoon et al., 2014a) as shown below:

$$M = P * \exp\{-\exp[\frac{R_m * e}{P}(\lambda - t) + 1]\} \quad (11)$$

Where M is the cumulative methane production (mL); e is 2.718282; R_m is the maximum specific methane production rate (mL d^{-1}); P is methane production potential (mL); and λ is the lag phase time (days).

Analytical methods

Methods for measuring pH, COD, TS, VS, TN and TAN were previously described in Chapter 3. Micronutrients and chloride concentration of samples were analyzed at the University of Georgia's Soil, Plant, and Water Analysis Laboratory. For micronutrients analysis, 0.5 g or 1

mL sample was added to 5 mL of concentrated HNO_3 and digested in a microwave oven following the US EPA method 3051A. The digested solutions were analyzed using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES) (Spectro Arcos FHS16 AMETEK ICP-OES). For Cl^- analysis, the samples were diluted 100 to 2,000 fold using DI water and filtered through a 0.45-µm syringe filter. Further analysis was carried out in an ion chromatograph (Metrohm 861 Advanced Compact IC) running at a flow rate of 0.7 mL min^{-1}. The CHNS concentrations were measured using a FLASH 2000 CHNS-O analyzer (Thermo Fisher Scientific, Waltham, MA, USA) on freeze dried samples. Approximately 1 mg dried samples were weighed in tin capsules and placed in the instrument that quantified elements by combustion and detection of elements in the off gases. Volume of biogas produced was measured by volume-displacement in a Eudiometer water column (Selutec, Germany), while methane concentration was measured using a GC-FID (SRI310C, SRI Instruments, Torrance, CA). The method used a stainless steel column (80/100 HayeSep D 6' ×1/8''); oven and detector temperatures of 40^oC and 380^oC, respectively; Carrier gas, fuel gas and oxidizing gas were helium (10 mL min^{-1}), hydrogen(25 mL min^{-1}) and air (250 mL min^{-1}), respectively. The Total biogas volume generated was measured by puncturing the rubber lid of each digester with the needle and syringe, which was connected to the Eudiometer by airtight tubing. Biogas samples (0.1-mL) were taken from the headspace of each digester using a gastight syringe and tested in the GC.

Statistical and regression method

A one-way ANOVA test and Tukey HSD test were used to compare methane yields and COD and TAN removal from different substrates in the BMP study using JMP Pro 10 software.

Differences between treatments were considered significant at $P \leq 0.10$. The nonlinear regression of the modified Gompertz model was performed using Sigmaplot 12.

Results and discussion

Selection of Mg and P sources for SP test

In previous studies (Table 6.1), different Mg and P compounds were used to precipitate and remove TAN from different AD effluents. Table 6.2 summarizes the Mg and P sources that can be used in SP and residual toxins that could remain, along with their inhibition thresholds in AD. $MgCl_2$ and $MgSO_4$ have high solubility in water and are often used as Mg sources in SP. MgO has low solubility in water, however, it can dissolve in substrates containing acids, and can remove as much as 25.7 to 54.4% TAN (Table 6.1). Both $Mg(OH)_2$ and $MgCO_3$ are rarely used in SP because of their low water solubility and relatively higher cost. However, they can provide buffering in AD effluents and for this reason may be preferred Mg sources to neutralize acidogenic digester effluents. $MgHPO_4$ contains Mg and P together and hence for convenience is also an attractive candidate for SP. Soluble phosphate salts/acid that are summarized in Table 6.2 are often used in SP of AD effluents based on their availability and cost.

Since the substrates after SP are fed to the methanogenic digester, the criteria for selecting Mg and P sources included both highest TAN removal and least amount of residual toxins (e.g. ions and elements such as N, S, Na^+, K^+ or Cl^-) that could potentially inhibit methanogenic activity. Since these elements are highly soluble in the substrates and will remain in the treated substrates, the reagent dosages would have to be calculated to minimize inhibition of methanogenesis. Therefore, $MgCl_2$, MgO, $Mg(OH)_2$ and $MgCO_3$ were selected as Mg sources and H_3PO_4 and NaH_2PO_4 were selected as P sources in this study. $MgHPO_4$ was also tested because of the advantage of adding Mg and P together as one compound (Table 6.3).

Experiment 1

TAN of substrates was 1.3±0.1 g L^{-1} a value that could have led to methanogenesis inhibition and lower process efficiency and TAN removal is expected to reduce these impacts (Table 6.4). Ca, P and Mg in the substrate were at very low levels to contribute to the reactions of SP, which was found in the preliminary test (not reported in this study).

Treatments G1and G5 using H_3PO_4 had TAN removal of more than 70% (Table 6.5), while G2 and G6 using NaH_2PO_4 were less than 60%. H_3PO_4 is a strong acid and the low soluble Mg sources of MgO and $Mg(OH)_2$ could be more readily dissolved in the H_3PO_4 to provide more active Mg^{2+} for the SP relative to NaH_2PO_4 (Uludag-Demirer et al., 2005; Zhang et al., 2014). After the Mg and P sources were added at the start of the experiment, substrate pH dropped to between 5.8 and 5.9 in G1 and G5 and 6.4 to 6.5 in G2 and G6 treatments. The pH was brought back to 8.5 by adding NaOH at the dosage of 4.8 g $L^{-1}_{substrate}$ for G1 and G5 treatments and 2.0 to 2.6 g $L^{-1}_{substrate}$ in G2 and G6 treatments. TAN removals in G1 and G5 treatments were higher than previous experiments conducted under similar conditions (Table 6.1). In particular, in G1 TAN removal was 77.5 %, compared to 54.4 and 25.7% removal reported by Celen and Turker (2001) and Yetilmezsoy and Sapci-Zengin (2009), respectively. Such large differences could be caused by higher availability of Mg^{2+} formed from the dissolution of MgO that is enhanced by the presence of H^+ from H_3PO_4 and the high levels of VFA in the substrates (Uludag-Demirer et al., 2005). Substrates used in the above studies had a pH of 7.9 and after adding equimolar H_3PO_4, MgO dissolved in the substrate was relatively small. Very few studies using $Mg(OH)_2$ in SP of wastewater are reported in the literature. Based on general reaction chemistry, in SP $Mg(OH)_2$ produces only one more molecule of water than MgO, which does not apparently impact the effective $[H^+]$ in the solution. Therefore, the results of TAN removal using these two

Mg sources were assumed to be comparable. This assumption had been proved in this study (Table 6.5). The substrate used in this study was from the acidogenic digester and had high concentrations of VFAs and a pH of 6.4. The higher H^+ concentration in substrate enhanced the solubility of MgO and $Mg(OH)_2$ and provided more Mg^{2+} for the SP.

The treatments of G2 and G6 using NaH_2PO_4 had lower TAN removal of 50.5 to 58.8%. After adding Mg and P sources, substrate pH became 6.4 to 6.5 and relative to G1 and G5 only half the amount of NaOH was required to increase the pH of the solution. The lower availability of Mg^{2+} and PO_4^{3-} due to the lower H^+ concentration contributed to lower TAN removal in these treatments. These results indicate higher removal of TAN compared to Li et al (2012) who treated pharmaceutical wastewater with pH of 12.2 and NH_4-N of 1.12 g L^{-1} by adding MgO and NaH_2PO_4 (Mg^{2+}: NH_4-N: PO_4-P=1:1:1) and maintaining pH of 9 with mixing of 15 minutes. The NH_4-N removal in the above study was reported as less than 40%, which was lower than the 50.5% TAN removal we observed. Celen and Turker (2001) reported that the SP for TAN removal operated at the 5-minute point and pH between 8.0 and 9.0 was completed. Therefore, the higher TAN removal reported in this study is a result of higher availability of Mg^{2+} caused by the higher concentration of H^+ in the acid substrate. Treatment G6 had similar TAN removal to G2, because of the similar chemical reaction of MgO and $Mg(OH)_2$ in SP.

Treatments of G3 andG4 containing $MgCl_2$ had higher TAN removals of 88.3-89.0%. These values were comparable to many studies treating different wastewaters using SP (Celen and Turker, 2001; Altinbas et al., 2002a; Altinbas et al., 2002b). $MgCl_2$ is a highly water soluble Mg compound and provides the highest ion concentrations of Mg^{2+} for SP without the addition of acids, resulting in higher TAN removals in treatments containing $MgCl_2$ regardless of the strength of acids used (e.g. H_3PO_4).

Treatments containing $MgCO_3$ also had similar high TAN removals in the range of 75.9 to 76.5%. In contrast to treatments using less soluble MgO and $Mg(OH)_2$, with $MgCO_3$ large amount of CO_2 bubbles were created after Mg and P sources were added to the substrates. This bubbling of CO_2 resulted in some mixing of the solution and potentially enhanced mass transfer and ionization of $MgCO_3$ to Mg^{2+}. Furthermore, we required 56% more NaOH in these treatments compared to treatments containing MgO and $Mg(OH)_2$. It is likely that a part of the CO_2 created remained dissolved in the substrate and provided buffering to pH increase.

It was surprising that treatment G9 containing $MgHPO_4$ produced a very low TAN removal of 5.2%. This result is not in agreement with previously reported results in the literature. For example, Sugiyama et al. (2005) reported TAN removals of 49% and 77% for 1 and 3 hour operation, respectively, using $MgHPO_4$ at pH of 8 and temperature of 25^{o}C using an equimolar ratio of NH_4-N to $MgHPO_4$. This difference in performance could be explained as follows. In this study, the $MgHPO_4$ powder was dissolved in the HCl solution first and then added to the substrate. The Mg^{2+} and PO_4^{3-} were released in the acid solution and possibly precipitated as other compounds such as $Mg_3(PO_4)_2$ instead of struvite (Turker and Celen, 2010). A dark colored precipitate was also seen with treatment G9, different from other treatments which had a white precipitate characteristic of struvite, showing possibility of different compounds precipitated other than struvite. A longer reaction time is probably necessary to form struvite when using $MgHPO_4$ as the sole Mg and P reagent. These possibilities needed further validation.

After the 20-min reaction, treatments G1 to G8 produced a white precipitate of approximately 0.5 to 1.0 mL per 10 mL substrate, and no additional precipitation was observed

at the 40 and 60 minutes times. This confirms that SP was completed in 20 minutes, agreeing with findings in previous studies (Celen and Turker, 2001; Li et al., 2012).

TAN was also potentially removed by free ammonia volatilization from the substrate which was kept stirred at high pH and temperature (Yetilmezsoy and Sapci-Zengin, 2009; Rajagopal et al., 2013). In our study, which was operated at 25^{o}C, 20 min, and pH 8.5, only 15.2 % of TAN was in the form of free ammonia (Cuetos et al., 2009). Therefore, considering the high solubility of ammonia in water, ammonia volatilization is not expected to have a significant impact on TAN reduction, compared to TAN removal by SP (Yetilmezsoy and Sapci-Zengin, 2009; Li et al., 2012).

Experiment 2

In this experiment, the SP in large volumes of substrates was redone following the procedure of experiment 1 to make sufficient substrate for the BMP test in experiment 2**.** Characteristics of treated substrates for BMP testing are shown in Table 6.6.

Untreated substrates had very low concentrations of Mg, Na and P to impact SP in any appreciable manner. There was also an absence of micronutrients at toxic concentrations for AD (Schattauer et al., 2011; Hamilton, 2013). After the SP, no significant COD change was observed between treated and untreated substrates ($P>0.10$). Previous researchers have reported COD reduction in wastewaters after SP ranging between 10 and 20% (Li et al., 2012) and 22.4 to 53.3% (Yetilmezsoy and Sapci-Zengin, 2009). These COD reductions are typically caused by co-precipitation of organics and struvite (Uludag-Demirer et al., 2008; Yetilmezsoy and Sapci-Zengin, 2009). In our study, because of prior treatment through acidogenesis, organics were mostly converted to soluble VFA and did not participate in the co-precipitation. TAN removal of each treated substrate ranged between 57.4 and 93.8%, agreeing with results of experiment 1 in

this study. It should be mentioned that in treatment G1 using MgO, we observed TAN removal of 57.4%, which was lower than the 77.5% removal observed in experiment 1. This is potentially a result of the limited reaction between reagents. After the SP it was noticed that a small quantity of white MgO powder was stuck at the inner corner of the 2-L flask, a result of incomplete stirring using the stir bar. The SP treatment substantially increased TS and VS of substrate because of salt residues such as NaOH and $Mg(OH)_2$ which decomposes at 177^{o}C and 350 ^{o}C, respectively (chemicalbook.com, 2015), however, did not appreciably change feedstock rheology which makes the treated substrate easily pumped into the liquid-state anaerobic digester. The C/N of substrates increased from 5.0 to 22.7 as a result of TAN removal, providing the additional benefit of nutrient balance in AD. It is not surprising that concentration of certain reagent residual elements such as Na, P and Mg remained in the substrates. After SP, Na concentrations were 3,526 to 7,432 mg L^{-1} depending on the amount of NaOH addition used for pH adjustment. High Na concentrations in substrates can potentially inhibit the AD (Hamilton, 2013). However, inhibition can be alleviated if microorganisms are properly acclimatized in AD (Chen et al., 2008). Residual substrates had equimolar ratios of TAN and P, but less than equimolar contents of Mg. This suggests that TAN and P removal were directly in the form of struvite, however, in addition to contributing to struvite, Mg was also removed through the precipitation in the form of other compounds. Magnesium was reported as the limiting factor in SP in previous studies (e.g. Stratful et al., 2001), confirming our observations. Residual Mg in the treated substrates ranged from 23.96 to 90.44 mg L^{-1}, far less than the moderately inhibitory threshold of 1000-1500 mg L^{-1} (McCarty, 1964). Residual total P concentrations were in the range of 195 to 1,246 mg L^{-1}. P is an important requirement for living microorganisms and plays a vital role in their growth and metabolism (Wang et al., 2015). Based on our knowledge, few studies have looked at the impact

of high P concentrations on AD. Lei et al (2010) reported that 465 mg P L^{-1} in the substrate including rice straw and anaerobic sludge can accelerate the biogas production, compared to the value of 155 and 775 mg P L^{-1} in their study. Similarly, Wang et al (2015) found that 414 mg P L^{-1} in synthetic sludge enhanced AD and resulted in higher methane yields. Methane production gradually decreased when the P concentration increased from 414 to 1,489 mg L^{-1}. Therefore, the residual P in substrates from our experiments (except G3) was relatively high and could potentially inhibit the AD process. Addition of excess Mg could enhance SP and reduce residual P. Due to the different final TAN concentrations in different treatments, we normalized TAN concentration to 0.6 g L^{-1} prior to conducting the BMP test (Table 6.7).

Cumulative methane production and yields are presented in Figure 6.1. As expected, the control with only inoculum had negligible methane production compared to other treatments, confirming the very low biodegradable organic fraction in the inoculum. All treatments showed long lag phases in the range of 30 to 140 days, which is longer than reported in other previous similar work (Yoon et al., 2014a; Hejnfelt and Agelidaki, 2009). This could potentially be a result of retardation of microbial growth caused by the high VFA concentrations in the substrate, combined with minor inhibition caused by TAN, Na and P residuals. The inoculum from the methanogenic digester operated at 26^{o}C also required some time to adapt to the higher test temperature of 38^{o}C. The pH in all treatments increased from 7.3 to around 7.6 and then stabilized for most the rest of duration of the BMP, with a slight pH drop that occurred at 122nd day (Figure 6.2). The buffering effects to maintain an acceptable pH were from TAN and P compounds including solution of Na_3PO_4, Na_2HPO_4 or NaH_2PO_4. As we know, these compounds have a high buffering capacity and are often used to make common buffering reagents.

Table 6.8 shows performance parameters of each treatment. The BMP results of treated substrates did not show a systematic improvement over the untreated control. Treatment BG0 which contained untreated substrate, described in Table 6.7, had a relatively longer lag phase than most other treatments. However, methane yield of BG0 was in the range of most other treatments with the exception of BG5. These results indicated that SP did not negatively impact BMP. Among BMP of treated substrates, COD and TAN of feedstocks had negligible differences (Table 6.9). The major differences impacting BMP test were residual Cl^-, Na (in form of sodium ion) and P (total phosphorus). Chloride toxicity was reported at a concentration of 5,500 mg L^{-1} in the case of 0.6 g L^{-1} tannin input in AD (Vijayaraghavan and Ramanujam, 1999). Since our measured levels (335 to 3,026 mg L^{-1}) were significantly lower, we conclude that chloride toxicity was not a factor in our experiment. The inhibition level of Na was reported as 3,500 mg L^{-1} in AD (McCarty, 1964) and since our measured Na levels in all treatments were lower, we anticipated no negative impacts.

Treatment G5 had the shortest lag phase (8.7 days) and highest methane yield (180.2 mL $g^{-1}COD_{added}$) in our experiment. BG1 with 897 mg L^{-1} of P had the longest lag phase of 125.7 days, suggesting that microorganisms required a long adaption period to adjust to high P substrate. The best performance of BG5 showed that 623 mg L^{-1} of P was an optimum level, compared to the 408 to 422 mg L^{-1} of P in treatments of BG7 and BG8. This result is slightly higher than results in a previous study (Lei et al., 2010) that reported that the optimum P concentration was 465 mg L^{-1}. COD removal of all treatments ranged from 42 to 65%.

Optimization of SP treatment

In the SP treatments, TAN was significantly removed from substrates, which improved methane yield. However, residuals such as Na, Cl^-, Mg (total magnesium) and P were introduced

at different levels to the substrate. If not properly managed, the high levels of such compositions can result in inhibition of AD, economic loss of P as a valuable nutrient, and eutrophication of water bodies if discharged.

Actions should be taken to minimize the residual Na and P in the treated substrates. Adding extra Mg source and reducing the reaction pH can facilitate the P precipitation and reduce the usage of NaOH for pH adjustment, respectively. Because of the low solubility of most Mg sources and high solubility of P sources, the addition of excess Mg source is required to provide more active Mg^{2+} to improve SP. Celen and Turker (2001) reported that when TAN and P equal molar concentrations, SP was improved when Mg concentration was increased. Yetilmezsoy and Sapci-Zengin (2009) also concluded that TAN removal efficiency increased when excess Mg was added using $MgCl_2$ and K_2HPO_4. Few studies have looked at using $MgCO_3$ in SP in wastewaters, probably because of its higher price compared to MgO, $MgCl_2$ or $Mg(OH)_2$. The Mg provided by MgO is cheaper than that of $MgCl_2$ (Celen and Turker, 2001). To keep cost down, low-grade MgO can be used to remove the TAN (e.g. Romero-Guiza et al., 2014). The residual Na can be decreased if the reaction pH is controlled at 7.5 or 8.0 without seriously impact of SP effect (Celen and Turker, 2001).

Treatment BG5 had 29.4% more methane yields than treatment of BG0 and obtained 74.1% nitrogen recovery in the form of struvite. The SP treatment in other treatments recovered more than 70% nitrogen but did not impact the methane yields, though the longest lag phase occurred in treatment BG1. Finally, based on the performance in nitrogen recovery and BMP we conclude that the tested treatment of BG5 was the best candidate and can be used in further AD treatment.

Conclusions

The SP treatment using different groups of Mg and P sources recovered nitrogen and improved methane yields, while increasing buffering capacity of acidogenic digester effluents. The treatment using $Mg(OH)_2$ and H_3PO_4 was the best candidate for higher TAN removal and higher methane yields, compared to controls. Residual components after SP, including P and Na, could lead to inhibition of AD and the loss of valuable nutrients. This could be minimized by process control strategies such as addition of extra Mg and lowering the operational pH.

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Table 6.1 A summary of relevant previous studies on TAN removal by struvite precipitation

Substrates source	Mg+P	Molar ratio (M:N:P)[1]	Initial TAN ($g\ L^{-1}$)	TAN removal (%)	pH	Citation
	$MgCl_2.6H_2O+H_3PO_4$	1:1:1	1.4	83.4	8.5	Celen and Turker, 2001
Molasses based	$MgO+H_3PO_4$	1.2:1:1.2	1.4	54.4	8.5	Celen and Turker, 2001
	$MgCl_2.6H_2O+KH_2PO_4$	1:1:1	1.318	85.4	9.0	Yetilmezsoy and Sapci-Zengin, 2009
	$MgSO_4.7H_2O+Na_2HPO_4.7H_2O$	1:1:1	1.318	83.1	9.0	Yetilmezsoy and Sapci-Zengin, 2009
Poultry manure	$MgO+85\%\ H_3PO_4$	1:1:1	1.318	25.7	9.0	Yetilmezsoy and Sapci-Zengin, 2009
Baker's yeast industry effluent	$MgCl_2.6H_2O+NaH_2PO_4 \cdot 2H_2O$	1:1:1	0.735	84.0	9.2	Altinbas et al., 2002a
Landfill leachate	$MgCl_2.6H_2O+NaH_2PO_4 \cdot 2H_2O$	1:1:1	2.24	85.0	9.2	Altinbas et al., 2002b

[1] M:N:P is the molar ratio of Mg: NH_4-N: PO_4-P

Table 6.2 Magnesium and phosphorus sources potentially used in struvite precipitation

	Molar weight[1] ($g\ mol^{-1}$)	Solubility[1] (g per 100g water, 25°C)	Toxic element[4]	Final concentration[2] ($g\ L^{-1}$)	Inhibition threshold[3] ($g\ L^{-1}$)
Mg source					
$MgCl_2$	95.2	55.5	Cl	7.1	n/a
MgO	40.3	0.0086 (30°C)	no	no	no
$Mg(OH)_2$	58.3	0.00064	no	no	no
$MgCO_3$	84.3	0.0139	no	no	no
$MgSO_4$	120.4	37.4	S	3.2	0.05
$Mg(NO_3)_2$	148.3	72.7	N	2.8	1.5
$MgHPO_4$	120.3	Slightly soluble	no	no	no
P source					
H_3PO_4	98.0	599.3 (24°C)	no	no	no
NaH_2PO_4	120.0	85.2 (20°C)	Na	2.3	
Na_2HPO_4	142.0	12.0	Na	4.6	
Na_3PO_4	163.9	14.5	Na	6.9	3.5 – 5.5
KH_2PO_4	136.1	25.1	K	3.9	
K_2HPO_4	174.2	168.4	K	7.8	
K_3PO_4	212.3	105.9	K	11.7	2.5 – 4.5

[1] Obtained from http://chemister.ru/Database/search-en.php, and http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-261.pdf

[2] Calculated based on 1.4 gL^{-1} TAN in the substrate

[3] Obtained from Gerardi, 2003 and Hamilton, 2013

[4] Cl, S, N, Na and K denote the element of Chloride, Sulfur, Nitrogen, Sodium and Potassium, respectively

Table 6.3 Dosage of magnesium and phosphorus sources used in experiment 1

Group	Mg and P source	Molar weight ($g\ mol^{-1}$)	Amount (g)	Ions left in the solutions ($mg\ L^{-1}$)	
				Na^+	Cl^-
G1	MgO	40.3	1.94		
	H_3PO_4 (85%)	98.0	5.6	0	0
G2	MgO	40.3	1.94		
	$NaH_2PO_4.H_2O$	138.0	6.7	2233	0
G3	$MgCl_2.6H_2O$	203.3	9.86		
	H_3PO_4 (85%)	98.0	5.6	0	6887
G4	$MgCl_2.6H_2O$	203.3	9.86		
	$NaH_2PO_4.H_2O$	138.0	6.7	2233	6887
G5	$Mg(OH)_2$	58.3	2.82		
	H_3PO_4 (85%)	98.0	5.6	0	0
G6	$Mg(OH)_2$	58.3	2.82		
	$NaH_2PO_4.H_2O$	138.0	6.7	2233	0
G7	$MgCO_3$	84.3	4.08		
	H_3PO_4 (85%)	98.0	5.6	0	0
G8	$MgCO_3$	84.3	4.08		
	$NaH_2PO_4.H_2O$	138.0	6.7	2233	0
G9	$MgHPO_4.3H_2O$	174.3	8.45	0	0

Table 6.4 Primary characteristics of substrates used in experiment 1(mean± standard deviation)

Parameters		Sample[1]#
pH	6.4±0.1	62
TS (g L^{-1})	2.7±0.2	23
VS (g L^{-1})	1.7±0.1	23
TSS (g L^{-1})	1.2±0.2	23
COD (g L^{-1})	13.3±0.7	25
NH_4-N (TAN) (g L^{-1})	1.3±0.1	25
VFA (g acetate L^{-1})	10.6±1.8	27

[1] The samples were collected in the period operated at the same OLR

Table 6.5 TAN removal performance of the different magnesium and phosphorus reagent combinations tested

Group	TAN at T0[1] ($g\ L^{-1}$)	TAN at T20[1] ($g\ L^{-1}$)			TAN Removal (%)	NaOH used ($g\ L^{-1}_{substrate}$)	pH_0[2]	pH_{20}[3]	T_{20}[3] (^oC)
		Rep 1	Rep 2	AVE					
G1	1.386	0.345	0.277	0.311	77.5	4.8	5.9	8.5	26.7
G2	1.336	0.714	0.609	0.661	50.5	2.6	6.4	8.5	25.5
G3	1.409	0.159	0.150	0.155	89.0	12.5	3.2	8.4	25.0
G4	1.514	0.177	0.177	0.177	88.3	8.3	5.4	8.7	23.8
G5	1.386	0.377	0.341	0.359	74.1	4.8	5.8	8.5	24.2
G6	1.364	0.632	0.491	0.561	58.8	2.0	6.5	8.9	22.5
G7	1.455	0.364	0.336	0.350	75.9	7.5	5.8	8.5	22.1
G8	1.459	0.318	0.368	0.343	76.5	3.6	6.3	8.4	21.4
G9	1.473	1.386	1.405	1.395	5.2	0.4	6.6	8.2	20.8

[1] T0 andT20 denote the reaction time at 0 and 20 minutes, respectively.
[2] pH was measured right after adding Mg and P sources.
[3] These parameters were measured at T20.

Table 6.6 Characteristics of substrates after struvite precipitation used for BMP testing

Parameters[1]	G0[3]	G1	G3	G5	G7	G8
pH	7.1	9.0	8.7	9.2	8.9	8.9
TS (g L^{-1})	2.9±0.2	14.3±0.1	21.8±0.2	13.9±0.1	16.5±0.2	17.1±0.2
TVS (g L^{-1})	1.8±0.2	5.9±0.2	4.9±0.1	5.9±0.0	5.6±0.1	5.9±0.1
COD (g L^{-1})	14.7±3.3	13.3±1.2	12.5±2.0	12.2±0.7	11.4±0.1	12.6±1.6
TN (g L^{-1})	1.5±0.0	0.7±0.0	0.3±0.0	0.5±0.0	0.4±0.0	0.4±0.0
TAN (g L^{-1})	1.4±0.0	0.6±0.0	0.1±0.0	0.4±0.0	0.3±0.0	0.3±0.0
C (%)[2]	31.5±11.0	18.0±7.8	15.4±0.4	23.6±1.1	22.4±0.5	22.0±1.1
N (%)[2]	6.3±2.2	1.2±0.3	0.7±0.1	1.4±0.2	1.0±0.0	1.0±0.2
C/N	5.0	14.8	22.7	17.0	22.1	21.8
M:A:P[4]	1:310:8	1:11:11	1:6:6	1:18:18	1:19:19	1:24:23
Micronutrients (ppm or mg L^{-1})						
Al	1.79	1.53	8.81	<0.50	4.42	0.86
B	<0.20	<0.20	<0.20	<0.20	0.46	0.43
Ca	27.88	33.10	21.22	22.96	37.22	47.74
Cd	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Cr	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Cu	0.75	1.00	0.41	0.38	0.31	0.27
Fe	15.35	12.39	11.71	10.86	9.83	12.18
K	123.0	121.6	96.2	118.8	124.4	117.5
Mg	7.83	90.44	26.52	42.70	28.60	23.96
Mn	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Mo	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Na	269	3526	7432	3618	4856	4892
Ni	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
P	79	1246	195	1005	706	726
Pb	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
S	54.56	56.94	49.32	52.78	55.84	56.46
Si	7.56	7.60	20.54	10.45	12.64	10.11
Zn	0.40	0.28	<0.10	<0.10	0.19	0.26

[1] Triplicate for each sample except micronutrients
[2] w/w, dry base
[3] G0 denotes the untreated substrate
[4] Molar ratio of Magnesium to TAN to Phosphorus

Table 6.7 BMP assay

Treatments[1]	DI water (mL)	Inoculum (mL)	Untreated substrate (mL)	Treated substrates[2] (mL)				
			G0	G1	G3	G5	G7	G8
BI	210	90	0	0	0	0	0	0
BG0	0	90	210	0	0	0	0	0
BG1	0	90	0	210	0	0	0	0
BG3	0	90	81	0	129	0	0	0
BG5	0	90	34	0	0	176	0	0
BG7	0	90	53	0	0	0	157	0
BG8	0	90	51	0	0	0	0	159

[1] BI is control, and BG0 is the treatment of untreated substrate; BG1, BG3, BG5, BG7 and BG8 denote treatment mainly using the treated substrate after struvite precipitation using the Mg and P sources of Group 1,3,5,7 and 8, respectively.

[2] All the treated substrates were normalized to have 0.6 g L^{-1} TAN by adding untreated substrate.

Table 6.8 Methane yields and modified Gompertz model parameters of methane production from each treatment

Parameters	BG0	BG1	BG3	BG5	BG7	BG8
P[1] (mL)	593.2±213.1	367.9±12.0	516.8±326.5	559.3±22.4	430.7±27.9	400.8±61.3
R_m[1] (mL day^{-1})	2.9±0.4	4.3±0.1	1.6±0.6	2.6±0.4	1.9±0.0	2.8±1.4
λ [1](day)	80.5±9.7	125.7±7.6	20.9±29.6	8.7±4.3	18.3±0.4	68.6±27.5
Methane yield[2] (mL g^{-1} COD_{added})	127.2^{b}±9.3	116.8^{b}±4.6	123.3^{b}±33.7	180.2^{a}±9.2	125.8^{b}±2.1	112.6^{b}±4.8
Methane yield[2] (mL g^{-1} $COD_{removed}$)	263.7±38.1	233.9±7.8	267.5±131.7	277.5±10.2	255.6±7.9	270.6±52.6

[1] No statistical analysis was done for these parameters because there are only two effective values in BG3, BG5, BG7 and BG8 after the non-linear regression.
[2] Different letters indicate significant differences ($P \leq 0.10$).

Table 6.9 Characteristics of the substrate before and after BMP testing

	COD ($g\ L^{-1}$)			TAN ($g\ L^{-1}$)			Na ($mg\ L^{-1}$)	P ($mg\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)
	Before	After	Removal[1] (%)	Before	After	Removal[1] (%)	Before	Before	After
BG0	10.770	5.453±1.250	49±12	1.424	1.257±0.031	12^{a}±2	336	80	336
BG1	9.790	4.900±0.254	50±3	0.856	0.793±0.031	7^{ab}±4	2,616	897	335
BG3	9.824	4.697±1.832	52±19	0.854	0.847±0.035	1^{b}±4	3,416	130	3,026
BG5	9.303	3.263±0.200	65±2	0.854	0.843±0.025	1^{b}±3	2,300	623	352
BG7	9.043	4.587±0.205	49±2	0.853	0.843±0.006	1^{b}±1	2,736	408	364
BG8	9.657	5.563±0.580	42±6	0.854	0.797±0.029	7^{ab}±3	2,786	422	360

[1]Different letters indicate significant differences ($P \leq 0.10$).

Figure 6.1 Cumulative methane production (a) and yields (b) of struvite precipitated substrates from the acidogenic digester treating poultry blood and wastewaters. Each point denotes the average value of three replicates.

Figure 6.2 pH change in the BMP treatments. Each point denotes the average value of three replicates.

Figure 6.1

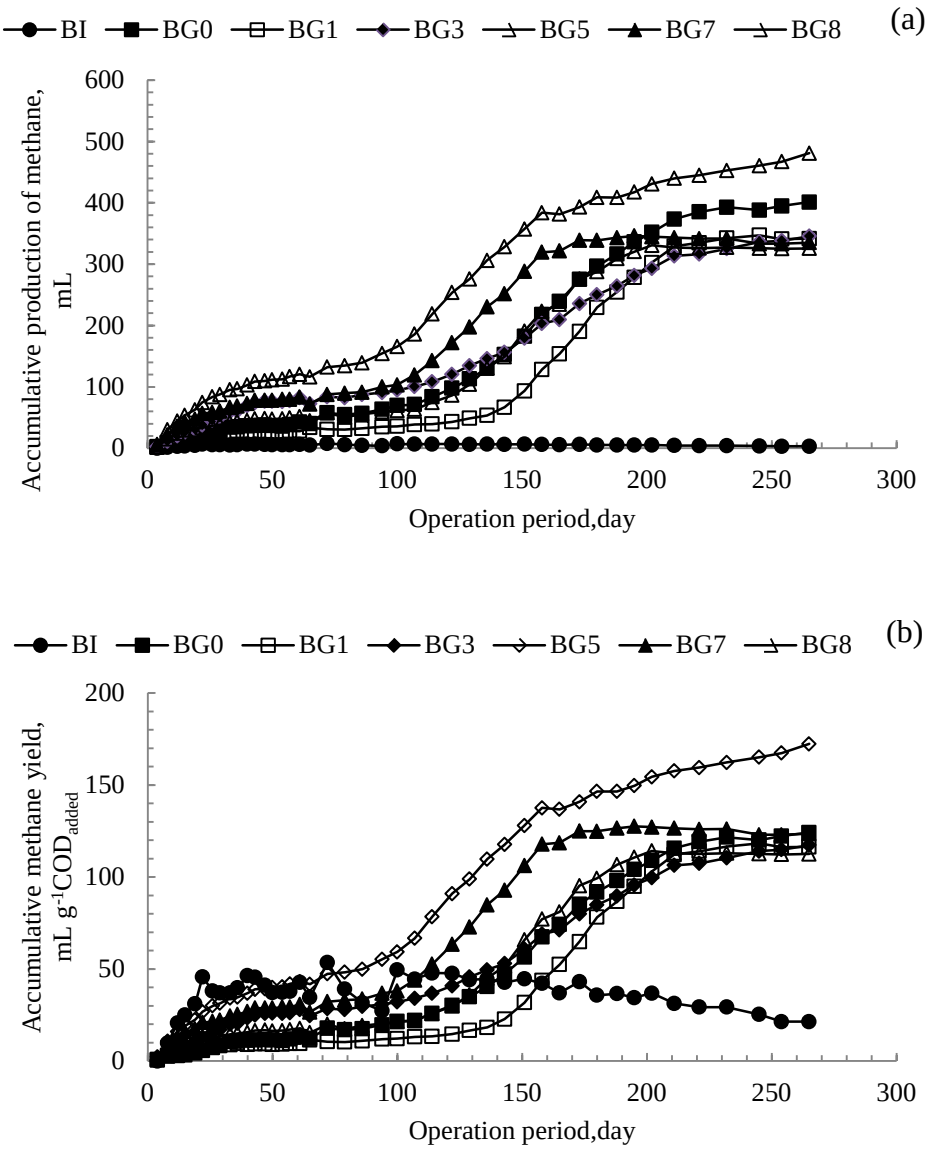
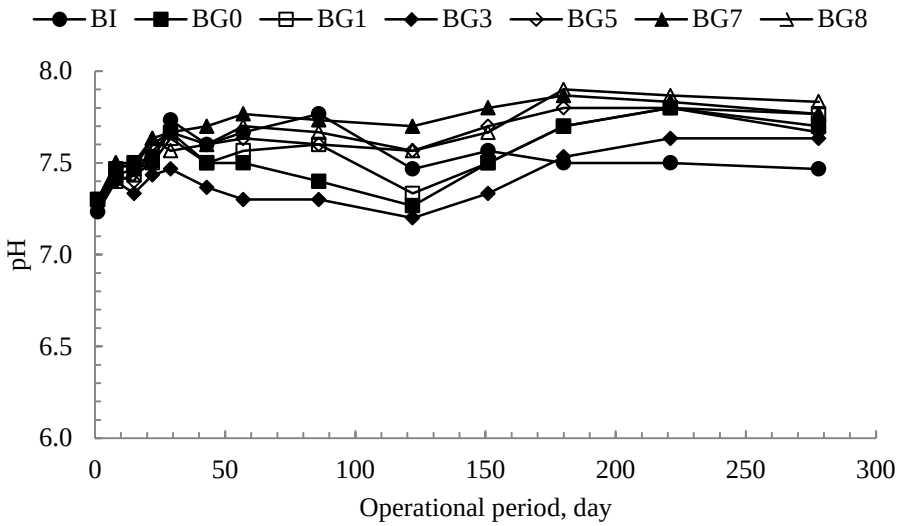

(a)
BI
BG0
BG1
BG3
BG5
BG7
BG8
Accumulative production of methane, mL
600
500
400
300
200
100
0
0
50
100
150
200
250
300
Operation period,day
(b)
BI
BG0
BG1
BG3
BG5
BG7
BG8
Accumulative methane yield, mL g-1CODadded
200
150
100
50
0
0
50
100
150
200
250
300
Operation period,day

Figure 6.2


BI
BG0
BG1
BG3
BG5
BG7
BG8
8.0
7.5
7.0
6.5
6.0
pH
0
50
100
150
200
250
300
Operational period, day

CHAPTER 7

CONCLUSIONS

Poultry broiler processing in the U.S. results in the discharge of large amounts of blood wastes which requires treatment to ensure environmental protection and public health. Anaerobic digestion (AD) of blood has many potential advantages compared to other alternatives, however, evaluations of AD in the past have been very limited and a significant knowledge gap exists. Studies in this dissertation were designed to identify energy and nutrient recovery from blood through different configurations and strategies of AD, namely, the use of biocarriers, two-stage digesters, and struvite precipitation.

Major findings of this work are:

Poultry blood waste and poultry processing wastewaters can be effectively anaerobically co-digested in the semi-continuous mesophilic upflow anaerobic filters containing biochar granules as biocarrier. At the higher OLR of 4.7 g COD L^{-1} d^{-1}, the digester had a methane yield of 331 mL g^{-1} $COD_{removed}$ and volumetric biogas production of 0.64 L $L^{-1}d^{-1}$. Ammonia and volatile fatty acids accumulated to the maximum concentrations of 3,277 mg L^{-1} and 15,035mg L^{-1}, respectively. Estimated recovery of energy and nutrients from the full-scale AD processing of these wastes in a typical processing plant are 1.5 GJ d^{-1} and 252 kg-N d^{-1}, 3.0 kg-P d^{-1} and 3.7 kg-K d^{-1}.

Two-stage pilot anaerobic digesters were designed and tested to investigate energy recovery from slaughterhouse blood wastes and wastewaters at different organic loading rates (OLR). Results showed low methane yield of 18.7 mL g^{-1} COD_{added} and negligible COD removal

in the acidogenic digester operated at OLR 2.3 g COD L^{-1} d^{-1}; and acceptable methane yield of 164 mL g^{-1} COD_{added} and 53.7% COD removal in the methanogenic digester operated at OLR 0.6 g COD L^{-1} d^{-1}. The overall two-stage digester had higher methane yield than a single-stage digester treating similar feedstocks reported in the literature. The estimated energy recovery from a two-stage blood wastes anaerobic digester operating in of at the region of southeast US is 18.4-19.1 kJ kg^{-1} animal weight.

Three low-cost biocarriers, bamboo, biochar, and seashell were evaluated for improving performance in pilot two-stage digesters treating poultry processing wastes. Results showed that bamboo was the superior biocarrier and maintained physical integrity after 400 days of operation. The acidogenic digester filled with bamboo cylinders had higher treatment effect on proteins and lipids, and lower COD loss at the highest OLR tested of 2.8 g COD L^{-1} d^{-1}. The methanogenic digester filled with bamboo cylinders had the highest methane yield of 359 mL g^{-1} COD_{added} and COD removal of 68% at an OLR of 0.6 g COD L^{-1} d^{-1}. Approximately 50% of total bamboo cylinders were floating in the substrate within the methanogenic digester at the end of the trial, potentially contributing to enhancement of mass transfer between the organic substrate and the microflora attached to the bamboo.

Struvite precipitation (SP) is an effective means to remove TAN and enhance the buffering of substrates. SP results showed 6 of 9 reagent combinations resulted in greater than 70% TAN removal. The biochemical methane potential (BMP) results indicated that SP treated substrates had similar or higher methane yields, higher nitrogen recovery, and higher buffering capacity than substrates that were not SP treated. The best performance was found where SP was achieved by adding $Mg(OH)_2$ and H_3PO_4 and resulted in 74.1% nitrogen recovery and 29.4% increase in methane yields relative to the substrate without SP.

Studies in this dissertation explored and tested several strategies to optimize the performance of anaerobic digesters treating poultry blood wastes. The long-term goal of efficiently obtaining bioenergy and recovering nutrients through AD requires:

First, the development of solid-state AD and co-digestions to treat blood wastes having 13 to 20% total solids. Second, an integration of AD with SP, nitrification-denitrification, or land application to optimize effluent management of AD treating blood wastes rich in nitrogen. Third, life cycle assessment (LCA) to evaluate the environmental impacts of AD and other alternatives, such as composting and rendering, when treating blood wastes.