



Article Methane Production from a Rendering Waste Covered Anaerobic Digester: Greenhouse Gas Reduction and Energy Production

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Abstract: Livestock wastes can serve as the feedstock for biogas production (mainly methane) that could be used as an alternative energy source. The green energy derived from animal wastes is considered to be carbon neutral and offsetting the emissions generated from fossil fuels. In this study, an evaluation of methane production from anaerobic digesters utilizing different livestock residues (e.g., poultry rendering wastewater and dairy manure) was carried out. An anaerobic continuous flow system (15 million gallons, polyethylene-covered) subjected to natural conditions (i.e., high flow rate, seasonal temperatures, etc.) containing poultry rendering wastewater was set up to evaluate methane potential and energy production. A parallel pilot-scale plug-flow anaerobic digestion system (9 m³) was also set up to test different feedstocks and operating parameters. Biogas production was sampled and monitored by gas chromatography over several months of operation. The results showed that methane production increased as the temperature increased as well as depending on the type of feedstock utilized. The covered rendering wastewater lagoon achieved an upward of 80% (v/v) methane production. The rates of methane production were 0.0478 g per g of COD for the poultry rendering wastewater and 0.0141 g per g of COD for dairy manure as feedstock. Hence, a poultry processing plant with a rendering wastewater flow rate of about 4.5 million liters per day has the potential to capture about two million kilograms of methane for energy production per year from a waste retention pond, potentially reducing global warming potential by about 50,000 tons of CO₂ equivalent annually.

Keywords: livestock waste; rendering; anaerobic digestion; greenhouse gases; methane; covered pond

1. Introduction

Millions of tons of livestock waste from animal-rearing operations are generated in the U.S. each year to meet the high demand for protein consumption. Animal manure has been traditionally land-applied as natural fertilizer for crop production. However, the land application of livestock wastes is limited due to problems associated with potential groundwater contamination, air quality, and limited immediate availability of agricultural land. The runoff and leachate could cause eutrophication of waterways. In addition, animal waste is a major source of anthropogenic greenhouse gas (methane, carbon dioxide, and nitrous oxide) emissions and offensive odors. Livestock manure emission contributes to roughly 10 to 18% of total greenhouse gas emissions [1–4]. Swine waste, dairy manure, and poultry litter contribute to about 38%, 21%, and 9% of methane emissions, respectively [2–5].

Thus, alternative waste management and treatment are sought. Anaerobic digestion of animal wastes can serve as an alternative waste treatment practice that could reduce air pollution (odors and greenhouse gases) and generate energy for on-farm use. Animal wastes can serve as the feedstock for biogas production (mainly methane) that could be



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). used as an alternative energy source. The green energy derived from animal wastes is considered to be carbon neutral and offsetting the emissions generated from fossil fuels, especially poultry rendering plant wastewater that has very high organic content, a total solid (TS) content of above 10–15%, and consists of mostly animal proteins and fats [6–9]. Poultry manure and feathers contain about 8% fat by weight and about 60% protein. Poultry blood contains about 3% fat and about 90% protein, whereas intestine residues contain about 29% fat and 46% protein. Therefore, poultry rendering wastewater would be a good source or feedstock for anaerobic digestion to obtain methane for a bioenergy source.

Previously, poultry waste from rendering plants such as blood, feathers, and other internal residues have mostly been utilized and recycled as animal feedstock or resources for the production of other agricultural commodities. Furthermore, the rendering wastewater is mostly stored in retaining ponds or lagoons, which creates many environmental problems such as air pollution and odor issues. If the processing plants are near municipal wastewater treatment plants (MWTP), the rendering wastewater is de-gritted, and fats and grease are removed before being transferred to MWTP for further treatment and then released to natural water bodies. Otherwise, the rendering wastewater is sent to retaining ponds or open lagoons.

The literature on covered lagoons of animal wastes for energy production and air pollution reduction is very scarce. Most of the previous studies were conducted to characterize the poultry wastes for mixed substrate as feedstock for digestion, examine the different poultry residues for biomethane potential, and improve digestion by using poultry waste as a co-substrate [6,10–16]. Other studies have dealt with the recycling and reuse of livestock wastewater [17–19]. However, there are not a lot of studies on energy recovery and biomass reduction (which leads to air pollution controls) on full-scale rendering wastewater processing plants.

Traditionally, animal wastes (liquid form) are stored in retaining ponds or lagoons. These receptacles are major sources of greenhouse gas emissions. Many studies have shown that these receptacles are major sources of odors and greenhouse gas emissions [20–26]. Therefore, the objective of this study was to evaluate the efficacy and effectiveness of covered lagoons of poultry rendering wastewater for energy production via anaerobic digestion and reduction in greenhouse gases, mainly methane.

2. Materials and Methods

2.1. Large Covered Lagoon

A 1.2-hectare retaining lagoon was lined with a polyethylene sheet (5 mm thick). Previously, it was an open retaining pond with a depth of about 4 m. It has a working volume of about 57 million liters (15 million gallons) receiving about 4.5 million liters per day (MLD) of poultry rendering wastewater. The poultry slaughterhouse is located in western Kentucky (USA) with a slaughtering capacity of 200,000 birds per day. The covered lagoon has a similar setup as a plug-flow anaerobic digestion system resembling Figure 1 and pictured in Figure 2 with a recirculation stream of about 0.5 million liters per day. Downstream to the anaerobic covered lagoon is an activated sludge treatment along with a disinfectant system for polishing and further treatment of organic materials in the incomplete digestate for reuse and discharge. However, for this paper, only the capture of methane and power generation from the lagoon will be discussed. The electrical output from the combustion of the biogas was generated using a 999 kW generator (Jenbacher Gas Engine, Jenbach, Austria) equipped with a gas flow meter. HOBO temperature probes (6) (Onset Computer Corp., Bourne, MA, USA) were located spatially in the lagoon. The biogas, electrical output, and temperatures are reported in this paper on a weekly average basis for smoothness. Liquid samples were collected weekly and COD, TS, pH, ions, and VFA were analyzed accordingly based on standard methods [27].



Figure 1. Schematic of anaerobic digestion of poultry rendering wastes.



Figure 2. Picture of the lagoon (pond) anaerobic digester (57 million liters).

2.2. Pilot-Scale Plug-Flow Digester

A pilot-scale plug-flow anaerobic digestion system (9 m³) was also set up to test different parameters similar to the large covered digestion system and schematically similar to Figure 1 (pictured in Figure 3). Different parameters such as pH, temperature, flow rates, and substrates were tested to gain insights into obtaining the best operating parameters for suggestion and recommendation for an actual system.

The inoculum used for methane production consisted of anaerobic sludge collected from a 1000 L geomembrane bag biodigester fed with a mixture of cow manure and water (7–10% TS, pH 6.83 \pm 0.14). The biodigester was operated at ambient temperature (20 \pm 8 °C) with a solid retention time of 30 d. Depending on the flow rate used for each experiment, the recirculation was set to be about ten percent of the influent volumetric flow rate. The collected inoculum was degassed at room temperature (19.7 \pm 7.0 °C) for 10 days. The TS and VS contents of the inoculum were 6.86 \pm 0.06% on a wet basis and 57.14 \pm 0.54% on a dry basis, respectively.



Figure 3. Picture of the plug-flow anaerobic digester (9 m³) for testing system parameters.

2.3. Theoretical Calculation

Methane chemical oxidation equation:

$$CH_4 + 2O_2 == \Rightarrow CO_2 + 2H_2O \tag{1}$$

Two moles of oxygen are required for the oxidation of one mole of methane. Therefore, one mole of methane requires 64 g of oxygen or COD. The relationship between a mole (N) of methane and COD can be rewritten as Equation (2).

$$N_{CH4} = COD/(64 \text{ g/mole})$$
(2)

The theoretical volume of methane produced per mass of COD can then be calculated based on natural gas law or ideal gas law (Equation (3)):

P

$$V = NRT$$
(3)

where R is the gas constant (R = 0.082 atm L/mole K), T is the standard temperature in Kelvin, P is the standard pressure, and V is the volume of gas.

The hydraulic retention time (HRT) of a plug-flow system is calculated as follows:

$$HRT = V/Q \tag{4}$$

where V is the volume of a system, and Q is the volumetric flow rate.

2.4. Analytical Methods

Determination of the TS, VS, COD, and other contents was performed according to standard procedures [27]. The pH was measured using the method reported by Kang et al. [28]. The pH measurement of the samples from the biogas tests was performed by shaking the sample manually, after which it was left to stand for 10 min and the supernatant reading was taken.

Biogas quality was sampled and monitored using a gas chromatograph (GC), Varian Model CP-3800 (Varian Associates, Palo Alto, CA, USA). The GC was equipped with a model 1041 on-column injector operated at 75 °C and 263 kPa which was connected to a 10-port gas sampling valve and pressure-actuated solenoid valve. A gas sample (5 mL) was injected using a syringe at a temperature of 35 °C. The syringe was then flushed for 30 s with 250 μ L of the sample transferred onto a 1.8 m by 1.6 cm o.d. column packed with 80/100 mesh Hay Sep Q (Varian Associates) with a He flow rate of 55 mL min⁻¹ for methane analysis. A thermal conductivity detector (TCD) operated at 120 °C and with a filament temperature of 200 °C was used for carbon dioxide analysis. After the sample passed through the detector, the sample went to a flame ionization detector operated under

N₂ makeup gas of 15 mL min⁻¹, H₂ 30 mL min⁻¹, air 300 mL min⁻¹, and temperature of 275 °C. Fatty acids (acetic, propionic, butyric acids, and others) were determined by high-performance liquid chromatography (Ultimate 3000 HPLC, Dionex Corporation, San Francisco, CA, USA). Total nitrogen analysis was carried out using a total nitrogen analyzer (Shimadzu TNM-L, Kyoto, Japan), and total carbon was analyzed using a total organic carbon analyzer (Shimadzu TOC-L, Kyoto, Japan). Table 1 shows the range of important chemical properties of various livestock wastes used in this study. Other livestock wastes such as poultry litter, swine manure, and others were also utilized in our study. However, only the dairy manure and poultry rendering wastewater results will be used for comparison and validation in this paper since they have similar properties (Table 1).

Table 1. Range of important chemical characteristics of the livestock wastes used.

	Poultry Rendering Waste	Dairy Manure
COD (mg/L)	10,000–14,000	7500–12,000
TS (mg/L)	2400-4000	3000-6500
pH	6.3–7.8	6.2–9.2
N (%)	6.5–17.4	2.2-4.2
C (%)	35.3–58.7	25.0-35.0
$SO_4 (mg/L)$	81.2-125.3	112–247
$NH_4 (mg/L)$	15.0-47.4	35.0-62.0

3. Results and Discussions

3.1. The Covered Poultry Rendering Wastewater Lagoon

The covered poultry rendering wastewater lagoon (57 million liters) in this study receives a wastewater flow rate of 4.5 million liters per day (MLD) with an average influent chemical oxygen demand (COD) of about 12,000 mg/L, spanning a range from 10,000 to 14,000 mg/L. The COD range seems high. However, it is similar to the range reported by Bustillo-Lecompte and colleagues [29]. The hydraulic retention time (HRT) is equivalent to about 12.6 days for the system (based on Equation (4)). This is very short for ideal methanogenesis. The process needs at least around 30 days for optimal digestion of organic matter. Total solid (TS) content ranges from 2400 to 4000 mg/L in the influent to about 300 to 800 mg/L in the effluent. In any case, the system was able to produce substantial biogas. Figure 4 shows biogas production during the second year of operation along with the temperature profile of the covered lagoon. As can be seen, the biogas production is correlated with the temperature—as temperature increases, the biogas production increases (Figure 5). A simple linear regression line between weekly gas volume and weekly temperature showed a correlated R square value of 0.57. We only use the second year of data for this regression due to the fact that the digestion system appears to be somewhat stable in the second year of operation, whereas the system is deemed a little bit unstable during the first year of operation. Even though the correlated value is not quite 100%, the simple regression analysis still gives us an idea of how the temperature affects the biogas production (i.e., not optimum conditions for microbial growth and respiration). It should also be noted that these data include winter months when temperatures are quite low and not ideal for biogas production. It would be more robust and accurate if we could include other variables, such as the COD, pH, the amount and type of microorganisms, and other parameters in regression analysis and utilize modeling methods such as neural networks and others. However, for this paper, predictive modeling is not the main focus.

The biogas production ranges from 14,000 cubic meters per week to about 68,000 cubic meters per week (Figure 4). Methane concentrations range from 68% to 80%, and the rest is balanced by carbon dioxide (20–32%) and other minor gases such as ammonia and hydrogen sulfide. Since the gas flow meter from the gas engine can only detect total biogas volume, methane concentrations are determined based on grab samples once a month using GC analyses. The temperature of the covered lagoon ranges from 15 to 28 °C. However, there were some weekly dips in biogas production. This may be due to the overloading of

the system (insufficient HRT) which may cause a reduction in methanogenesis. Seasonality also seems to affect biogas production. Methanogenesis appears to slow down during cold months as well (e.g., January and February). The biogas yields in wintertime are equivalent to about 40% of those in the summer months. Figure 6 shows the corresponding power generation from the biogas production. The power generation ranges from about 40,000 kWh to 170,000 kWh. As can be seen, the power generation mirrors the biogas production (Figures 4 and 6). The amount of power generated was enough to run the daily energy requirement for the poultry processing plant and the surplus amount was sold to the local power grid.



Figure 4. Weekly temperatures and biogas volumes for the second year of operation, starting with the last two weeks of December (D) of the previous year. The letters on the x-axis represent the weekly data for each month of year. The solid line is the temperature, and the dashed line is the biogas.



Figure 5. Linear regression of gas volumes (solid dots) versus temperatures showing a regression line (dashed line) with an R² value of 0.57.



Figure 6. Weekly energy production (second year of operation) from December (D) to December in kilowatt-hours (kWh). The letters on the x-axis represent the weekly data for each month of the year.

Looking at Figure 4, it may not be quite clear that overloading is the cause of reduced methanogenesis (the final step of methane production from short-chain fatty acids). However, by examining Figure 7, it may be observed that the system crashed once (between weeks 13 and 17) before a dissolved air floatation system (DAF) was installed. A DAF is used to remove fats and grease. Table 2 confirms this phenomenon. As can be seen, concentrations of long-chain fatty acids and sterols (e.g., zoosterols) were very high before the installation of the DAF. The analysis for fatty acids before DAF installation was carried out only once and the subsequent analyses after DAF installation were carried out once a month or so. The DAF was able to reduce long-chain fatty acids by 34% to 48% from the system. Another indication of the overloading and rate-limiting step at methanogenesis is the high concentrations of short-chain fatty acids such as formic, acetic, butyric, propionic, and valeric acids. The total concentrations of short-chain fatty acids range from 109 to 125 mg/L after the installation of the DAF. For a well-running anaerobic lagoon, the system will yield a ratio of less than unity between short-chain fatty acids and long-chain fatty acids. For this start-up poultry rendering processing anaerobic lagoon, the ratios range from 1.4 to 1.6. These concentrations appear to be accumulating over time in the system. In addition, the reduction in COD was observed to be only in the 50% to 65% range. Therefore, a long HRT may be helpful in increasing biogas production from the accumulation of these fatty acids. However, we could not modify the current feedstock flow rate since that would alter the processing plant operation. This is our limiting factor in constructing this semi-effective anaerobic digestion lagoon. The processing plant utilizes a consistent amount (4.5 MLD) of washed and cleaned wastewater. For the digestion system to work efficiently, additional downstream polishing treatment systems are necessary to further treat the undigested residues to meet regulatory discharge requirements. However, additional treatment systems are not the subject of this paper.



Figure 7. Weekly biogas volumes and temperatures of the lagoon anaerobic digester for the first two years of operation. A dissolved air floatation system (DAF) was installed in the second quarter of the first year. The solid line is the lagoon temperature, and the dashed line is the biogas volume.

	Systems	
	Before DAF (mg/L)	After DAF (mg/L)
Palmitic acid	23.4	11.2–15.3
Stearic acid	25.1	13.7–16.2
Myristic acid	15.7	8.7–9.5
Óleic acid	29.2	17.4–21.6
Palmitoleic acid	18.5	8.4–12.4
Linoleic acid	13.5	5.2-7.4
Cholesterol	7.2	3.7–5.1
Lanosterol	9.5	4.1-6.4
Desmosterol	6.3	3.4–5.1

Table 2. Long-chain fatty acids and cholesterols in the effluent.

3.2. Pilot-Scale Validation Using Dairy Manure

The main objective of this study was to assess the efficacy and effectiveness of turning an open retaining pond of poultry rendering wastewater into an anaerobic digestion system to produce bioenergy via biogas production and reduce potential greenhouse gas emissions. However, due to operational constraints at the actual poultry rendering wastewater anaerobic lagoon, a plug-flow anaerobic digestion system (pilot scale, 9 m³) was set up to examine the effect of physicochemical factors on biogas production to gauge the important parameters affecting biogas production. Since our lab is quite far away from the poultry processing plant, we were not able to transport the large quantity of the rendering wastewater for our pilot-scale validation study. Instead, dairy manure was used since it has similar chemical characteristics to poultry rendering waste as feedstock.

Using dairy manure as feedstock, it was found that temperature and pH had a major effect on biogas production as well as the flow rates (i.e., the retention time of feedstock in the digestion system). It appeared that the optimum production of biogas was observed in

the temperature range from 25 to 35 °C (mesophilic conditions; Figure 8) and pH in the neutrality zone. However, the pH of the feedstock (influent) appeared to have a major effect on the biogas production. The high pH of the feedstock appeared to reduce gas production substantially (Figure 8). Our results corroborated previous studies where pH and temperature affected the growth of microorganisms, especially methanogenic and acidogenic microorganisms [30–34]. There seems to be a decrease in biogas yield, however, from about the 120th day onward. During this time, the pH is on the rise along with the temperature (effluent temperature). This increase in pH and temperature toward the thermophilic range may have caused the system to be unstable. This time period coincided with the hot summer weeks. Even though the thermophilic range of operation should create a larger yield, this is not the case. The high heating of the polyethylene cover (mostly exposed) may have caused a shock to the system (potential death of methanogens) since the depth of the digester is less than a meter. Once the dying-off has started, it takes a little while for the system to stabilize again, which was evident by the long lag time of about 60 days for the yield to increase again. The microbial concentration decreased from 10^7 cfu/mL to 10^4 cfu/mL during this time. However, this paper will not go into further detail on microbial profiles. Optimum biogas production was observed when the pH of the feedstock was close to neutrality. Even though these facts have long been established, the results of this study still hold true with the feedstock utilized.



Figure 8. The effect of temperatures and pH on biogas yield using dairy manure as feedstock.

Optimum biogas production was also obtained with a long retention time: biogas production increased as HRT increased (Table 3). The average methane concentrations range from 3.7 to 5.5×10^5 ppm for feedstock hydraulic retention time from 12.8 to 90 days. A simple linear regression line shows a correlated R square value of 99% between hydraulic retention time and average methane production (Figure 9). Therefore, HRT plays a major role in obtaining the optimum methane production from plug-flow anaerobic digestion systems.

Table 3. Testing the effect of HRT on methane production using a pilot-scale plug-flow (9 m^3) anaerobic digester.

Hydraulic Retention Time (Day)	Average CH ₄ (×10 ⁵ ppm)
90.0 45.0	5.5 4.5
30.0 18.0	4.2
10.0 12.8	3.7



Figure 9. Linear regression line (dashed line) of hydraulic retention time (HRT) versus the average methane concentration ($\times 10^5$ ppm) as solid dots with an R² value of 0.99.

3.3. Greenhouse Gas and Biomass Reduction

Utilizing Equations (2) and (3), the theoretical methane yield could be determined and has a value of 0.35 L of methane per gram of COD. Covered poultry rendering wastewater lagoons could reduce greenhouse gas (GHG) emissions and produce renewable green energy. For the 4.5 MLD poultry rendering wastewater system, there was a potential reduction of two million kilograms of methane annually (calculated using Equations (2) and (3))—equivalent to about 50,000 tons of CO_2 eq. in global warming potential (GWP) based on the fact that methane has 25 times the GWP of carbon dioxide [2]. By the same token, about 8000 tons of COD could be removed annually based on calculations using Equations (1) and (2). This is only about 40% removal of annual COD (~20k tons) in the feedstock. Thus, further downstream treatment such as an activated sludge treatment system is needed to remove the leftover COD. Based on our experimental results, the rates of methane production from poultry rendering wastewater and dairy manure feedstocks are 0.0478 and 0.0141 g per g of COD, respectively. A larger COD amount was converted to methane using poultry rendering wastewater as feedstock than dairy manure (about 19% vs. 5%). This may be due to the available COD (soluble form) for biodegradation in the poultry wastewater than in the dairy manure. There are also other factors that may contribute to the overall low COD conversion to methane as well, such as high concentrations of sulfate and ammonia in the system along with low concentrations of metals such as iron, copper, aluminum, and others. The high concentrations of sulfate (>200 mg/L) and a reduction rate of 70% may have created a system with a high concentration of sulfur reducers that may compete with methanogens (i.e., lower conversion to methane). Also, the high concentration of free ammonia (>250 mg/L) along with a high concentration of LCFA and SCFA in the system may be inhibitory to methanogenesis, similar to previous studies [35]. In addition, the low concentrations of iron and copper (<0.5 mg/L) and high concentrations of zinc (>6 mg/L) may reduce the rate of methane production as well.

4. Conclusions

Covered poultry rendering wastewater lagoons could be quite beneficial to reducing air pollution such as greenhouse gases and odors, biomass, and, at the same time, obtaining renewable bioenergy for on-farm use. Due to the constraint on physical size (volume) and the high throughput of wastewater influent, the hydraulic retention time (HRT) was reduced below the ideal HRT for an effective anaerobic digestion system. However, biogas production was still very high, but incomplete digestion of organic materials persisted. This is evident in the high concentrations of long-chain fatty acids and short-chain fatty acids in the effluent. Furthermore, due to the nature of the system being subjected to natural environmental conditions, biogas production fluctuated quite a bit due to seasonal environmental conditions, namely temperatures. With the appropriate downstream treatment systems to further reduce organic materials in the incomplete digestate, the anaerobic covered lagoon (with short HRT) could be continuously sustained and meet the regulatory discharge requirements. This was supported by results obtained from a parallel study utilizing a pilot-scale plug-flow anaerobic system to examine the effect of other physic-ochemical parameters on the anaerobic digestion similar to the covered lagoon systems. Nevertheless, future studies in the microbial structure and behavior in a covered rendering wastewater lagoon are needed to truly understand the efficacy and effectiveness of the system in achieving the optimum biogas (mainly methane) production. In addition, HRT could be improved by creating a larger lagoon or adding an additional one downstream to accommodate the high throughput of the current flow rate to obtain complete digestion and maximum biogas production.

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