Biological Methane Potential Test Methods Development for The Evergreen State College

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Abstract

The biological methane potentials (BMPs) of three different waste samples were determined in order to determine the amount of methane that could be produced using these waste streams in an anaerobic digester. The samples tested included cow manure from James Road Dairy in Rochester, WA, chicken manure from The Armstrong-Zita ranch in Tumwater, WA, and a co-digestion of 70% cow manure from James Road Dairy and 30% food waste from The Evergreen State College (TESC) in Olympia, WA. The average BMP of cow manure was 21.7 mL CH_4/g VS, chicken manure was 43.6 mL CH4/g VS, and co-digestion was 2.0 mL CH4/g VS and the control was 23.3 mL CH_4/g VS. Average cumulative gas production over the course of 24 days for cow manure was 641.3ml, chicken manure was 605.7mL, co-digestion was 471ml, and the control was 193.3mL. Due to technological and experimental design errors, the data is not reliable and the paper advises on better experimental design for future experiments at TESC.

Introduction

Local organic waste streams have the potential to be a renewable energy resource. Anaerobic digesters turn waste into methane, bedding, and fertilizer while diverting waste from landfills. Methane is a greenhouse gas more than 25 times more potent than CO_2 [10]. Capturing it from decomposing waste streams and combusting it reduces the negative impacts on the atmosphere by transforming methane into carbon dioxide in the the reaction $CH_4 + O_2 = CO_2 + H_2O$. Olympia, WA already uses anaerobic digesters, like those at the wastewater treatment plant LOTT[18], and more could be used to mitigate waste and create renewable energy. In Rochester, WA in southern Thurston County there is a large agricultural presence that could benefit from anaerobic digestion. Currently a consortium of public entities including LOTT Clean Water Alliance, Puget Sound Energy, Washington State University, The Evergreen State College, and others, is assessing the feasibility of anaerobic digestion in Rochester, WA. This experiment was initially designed to assess the BMP of organic waste streams, but due to experimental design and technological errors, the data is inconclusive and the paper will advise on experimental design and materials selection for the next BMP test at TESC.

Cow manure yields a large biogas production and is favorable for biogas plants. Biogas production in manure can be increased through several mechanisms. If the fibers in manure are decreased the biogas yield in manure increases by 30% [1]. According to “Methane Production from Anaerobic Codigestion of Cow Dung, Chicken Manure, Pig Manure and Sewage Waste”, a paper highlighting the effects of co-digestion, combining manure with various food wastes can increase the BMP due to co-substrates and the minimization of high ammonia levels common with mono manure digestion.[19] Food waste like compost collected from The Evergreen State

College's Greenery could increase biogas production at an anaerobic digester in Rochester, WA when combined with cow manure. Chicken manure has a high potential for methane production but due to high levels of ammonia it can be challenging to digest by itself. Dilution or removal of ammonia is suggested for mono manure digestion of chicken wastes.[20]

Anaerobic digestion is a process by which a consortium of microbes turn organic material into methane and carbon dioxide.[15] First, complex organic material is broken down into simpler compounds that microbes can metabolize through hydrolysis.[15] Then, those compounds are metabolized in the acidogenesis stage and hydrogen, carbon dioxide, and acetic acid are produced.[15] The acidogenic microorganisms can tolerate a wide range of environmental conditions.[15] Finally, methanogens convert acetic acid, hydrogen, and carbon dioxide into methane during methanogenesis.[15] Methanogens in anaerobic digesters thrive in narrow environmental conditions.[15] Changes in pH and temperature can inhibit methane production by hindering methanogens' metabolism.[13]

Materials and Methods

Materials

The following equipment and supplies were used:

200mL bottles	Butyl rubber stoppers (20mm)	Aluminum crimp caps	Crimper	De-crimper	Vacuum jelly
Hot water bath	500µL glass syringe with pressure lock	30 mL glass syringe	Methane and carbon dioxide standards	GCMS	Tedlar bags
Blender	Balance	Various beakers	Funnel	Nitrogen rig	pH papers
Ziploc bags	Crucibles	Muffle furnace	Desiccator	Oven	DI water

Samples

Fresh manure samples were obtained January 29th, 2016. The cow manure came from James Road Dairy Farm in Rochester, WA. Five gallons of fresh cow manure was collected by a farm worker. The chicken manure was collected from Armstrong-Zita ranch in Tumwater, WA. One gallon was collected fresh and had small bits of egg breakage, feathers, and bugs in it. The samples were stored at room temperature. A large sample size of food waste was taken and then smaller blended samples were used to more accurately represent the diverse food wastes. A total of nine gallons of food waste was taken over three days and three times a day. The food waste was stored in a freezer until it was blended up and used in the experiment. Sewage sludge from an anaerobic digester was used as the inoculant and control.

Inoculum

The inoculum used in this experiment was sewage sludge from the primary anaerobic digester at the LOTT sewage treatment plant in Olympia, WA. The anaerobic digester operates at mesophilic temperature and its primary feedstock is human sewage.

Experiment Set-Up

The test was carried out in triplicate batch experiments. A total of 25g of sample was added to each reactor and mixed with 25g of inoculum. The headspace of each vial was flushed with nitrogen for three and a half minutes. Permanent line installations were placed in each sample.

The samples were stored in a shaking hot water bath which was originally intended to stay at 35°C. However, due to technical errors the temperature ranged from 19°C to 50°C and was usually around 45°C. The entire experiment lasted 25 days.

Analytical Methods

The biological methane potential was determined based on the total volume of methane produced during the digestion per amount of substrate added (mL CH_4/g VS).

The volume of gas produced by the samples was measured every day except President's Day when the lab was locked. A needle with an airtight plastic line was attached to each sample and volume was measured using a 30cc syringe.

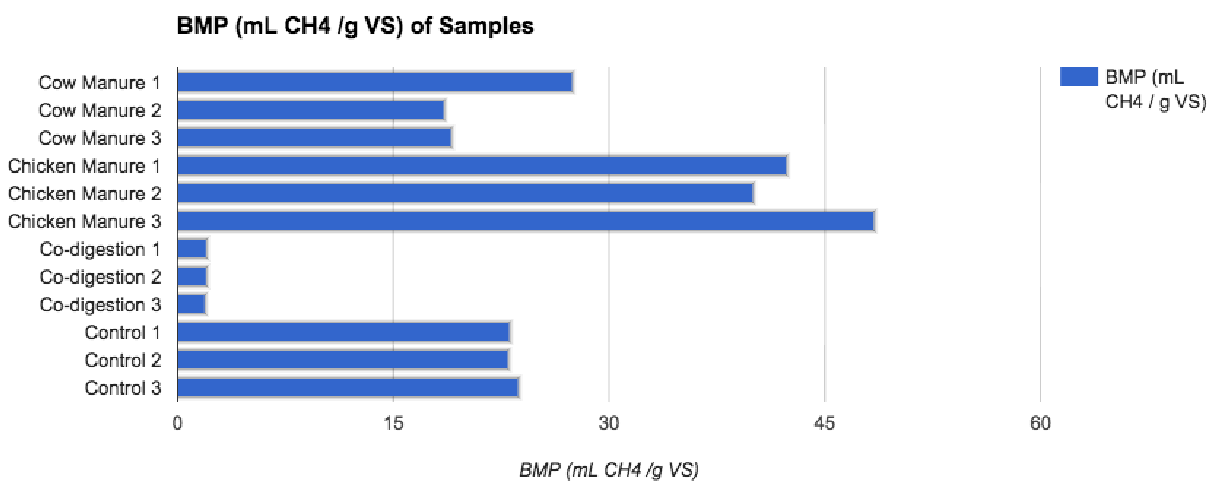
Gas composition was analyzed by taking 20μL of gas from the headspace of the reactors by injecting the needle through the stopper using a 500μl pressure-lock syringe. The gas was then analyzed by a GCMS.

Total Solids (TS) and Volatile Solids (VS) was determined using standard methods. The crucibles were conditioned in a muffle furnace at 550°C for four hours. The weight of the conditioned crucibles was recorded and 10g of sample was added to each crucible. The samples were dried overnight at 105°C. The dried samples were measured to determine the TS and then ashed at 550°C overnight. That night, the lab building flooded and all power went out in the building. The ashed samples were unable to be accessed for several days. The ashed samples were re-dried in an oven at 105°C for four hours once they were able to be accessed. A new balance had to be used for the VS measurements.

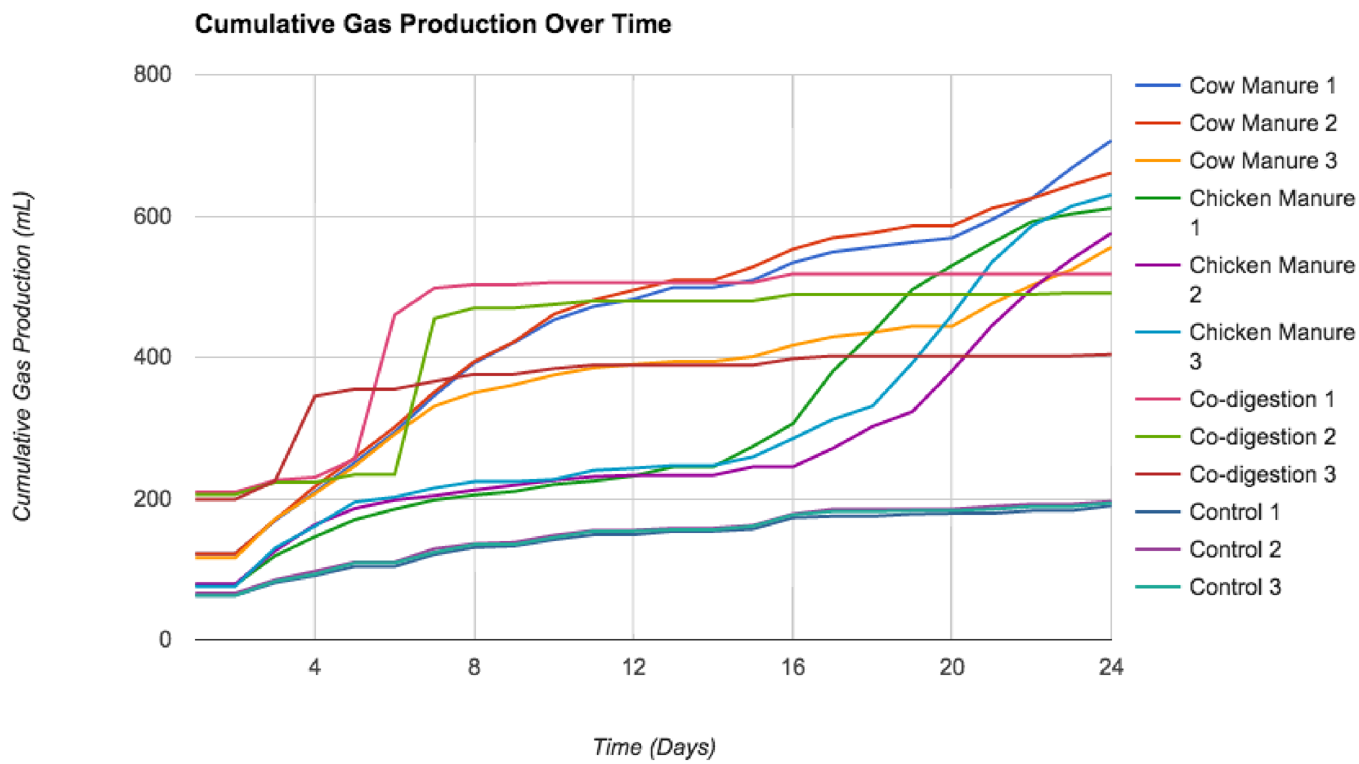
The pH was tested by releasing and measuring the gas samples.Then, the permanent line installation was removed from each sample. Next, approximately 1mL of sample was extracted by inserting a needle through the stopper. The sample was then placed on a pH strip and pH was recorded.

Results

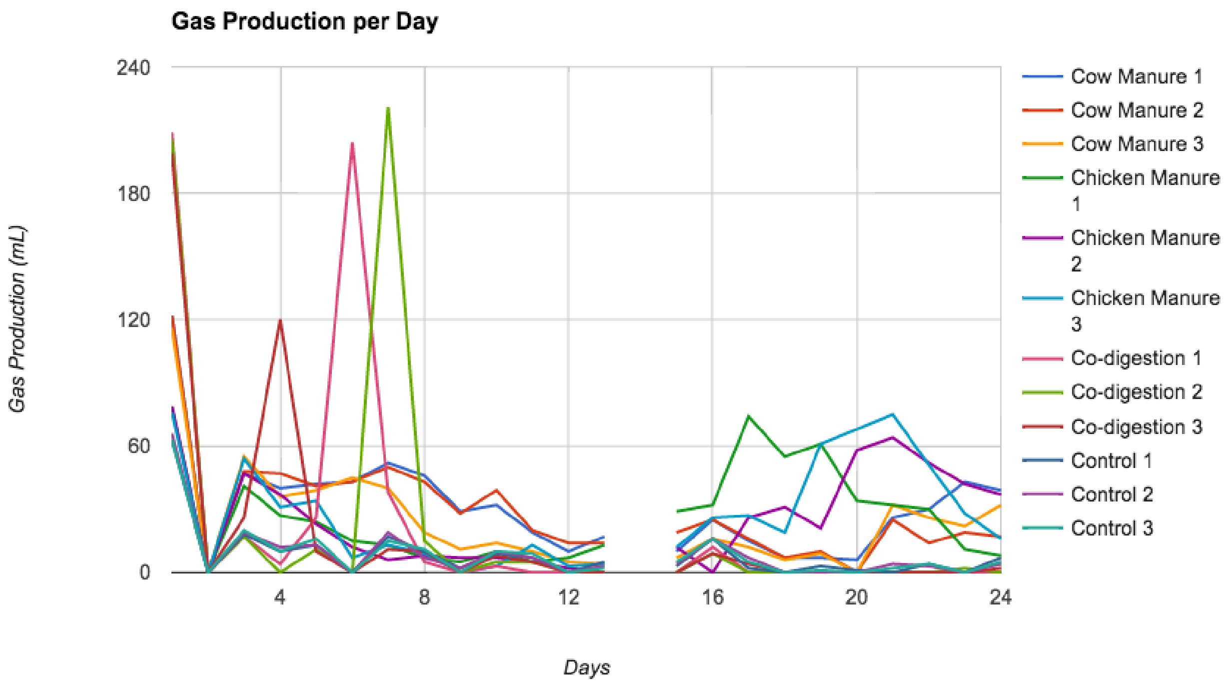
The average BMPs of the samples ranging from greatest to least was chicken manure with 43.6 mL CH_4/g VS, the control with 23.3 mL CH_4/g VS, cow manure with 21.7 mL CH_4/g VS, and co-digestion with 2.0 mL CH_4/g VS. The BMP of cow manure was the most variable with a standard deviation of 4.1mL CH_4 /g VS with values of 27.5 mL CH_4 /g VS for cow manure 1, 18.6mL CH_4 /g VS for cow manure 2, and 19.1 mL CH_4 /g VS for cow manure 3. The standard deviation of the chicken manure samples was 3.5 mL CH_4 /g VS with values of 42.4 mL CH_4 /g VS for chicken manure 1, 40.0 mL CH_4 /g VS for chicken manure 2, and 48.4 mL CH_4 /g VS for chicken manure 3. Co-digestion had the least BMP variability with a standard deviation of 0.1 and BMPs of 2.1 mL CH_4 /g VS for co-digestion 1, 2.1 mL CH_4 /g VS for co-digestion 2, and 1.9 mL CH_4/ g VS for co-digestion 3. The controls' BMPs had a standard deviation of 0.3 and values of 23.1 mL CH_4 /g VS for control 1, 23.1 mL CH_4 /g VS for control 2, and 23.7 mL CH_4 /g VS for control 3. These results are not consistent with published results. According to Labatut et. al., dairy manure had a BMP of 242.7 mL CH_4 /g.[6] A BMP of 351 mL CH_4 /g VS was observed in chicken manure by Li, et al.[7] Co-digestion increased BMP by 67% to 2940% compared to cow manure in the Lisboa et al. study.[8] Sewage Sludge has a BMP of 164.5 in the study, "Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge".[9]



Average cumulative gas production over the course of 24 days for cow manure was 641.3mL, chicken manure was 605.7ml, co-digestion was 471mL, and the control was 193.3mL. However there was variability within the triplicate samples besides the control. The cumulative gas production for the three cow manure samples was 707mL, 661mL, and 556mL with a standard deviation of 63.2mL. The three chicken manure samples' cumulative gas production was 611mL, 576mL, and 630ml with a standard deviation of 22.4mL. Cumulative gas production for the three co-digestion samples was 518mL, 491mL, and 404mL with a standard deviation of 48.6mL. The control cumulative gas production was less variable with values of 190mL, 196mL, and 194mL with a standard deviation of 2.5mL.



Daily gas production was variable and not consistent with other published results.[11] The variability may be due to the temperature being outside of the stable mesophilic range. Co-digestion started out as the most abundant producer but its production plummeted, probably due to its low pH. Gas production data could not be collected on February 15 because it was President's day and the lab building was locked.



The pH of the samples was taken on two occasions, one week and three weeks into the experiment. Average pH was 7.1 for cow manure, 7.5 for chicken manure, 4.8 for co-digestion, and 7.5 for the control. The acidity of the co-digestion samples was likely due to high sugar and carbohydrate concentrations in the Evergreen food waste.

Samples	BMP (mL CH4 /g VS)	Cumulative Gas Production (mL)	% Gas CH4	Total Solids %	Volatile Solids %	pH
Cow Manure 1	27.5	707	11.2	11.4	9.5	7
Cow Manure 2	18.6	661	8.1	11.1	9.2	7.3
Cow Manure 3	19.1	556	9.9	11.3	9.6	7
Chicken Manure 1	42.4	611	15.9	12	7.7	7.5
Chicken Manure 2	40	576	16.4	11.1	6.8	7.5
Chicken Manure 3	48.4	630	17.6	11.3	6.9	7.5
Co-digestion 1	2.1	518	1.6	23.9	N/A	4.5
Co-digestion 2	2.1	491	1.7	23.1	22.3	4.5
Co-digestion 3	1.9	404	1.8	23.9	22.9	5.3
Control 1	23.1	190	12.7	2.8	2.1	7.5
Control 2	23.1	196	12.3	2.7	2.1	7.5
Control 3	23.7	194	12.8	2.7	2	7.5

Discussion

Several experimental design and technological issues caused this experiment to have unreliable results. The initial setup of this experiment resulted in an overproduction of gas. When measuring the samples, the scale was set to DWT (pennyweight) rather than grams. This resulted in adding 50% more of the sample than originally planned. The temperature of the water bath rose to 50°C due to a technological error and the needles used were too short and unable to collect gas through the 20mm stopper. This quickly became an explosion hazard and the experiment was terminated.

The experiment was restarted with a few corrections to experimental design. The samples were measured in grams and only 50g were added instead of 150g. Also, the correct length needles were used so the stopper could be penetrated and gas could be collected. However, the technological error with the hot water bath temperature could not be resolved. It was unable to maintain a steady temperature of 35°C and throughout the experiment temperatures ranged anywhere from 19°C to 50°C. When the temperature dropped to 19°C overnight, the gas production from all of the samples was zero for that day. Reliable results cannot be gathered without controlled temperature at a steady mesophilic or thermophilic range.

Another technological issue arose from inaccurate thermometers used to measure the temperature of the hot water bath. The initial thermometer, which had been showing a temperature of around 45°C on a daily basis, was broken in the middle of the experiment. The next thermometer used showed a temperature of 27°C. Another thermometer was added and showed a temperature of around 45°C. A third thermometer was added and also showed a temperature of 45°C. To avoid issues with inaccurate temperature data, thermometers should be calibrated regularly. Also, at least two thermometers should be in the water bath at all times to help ensure accurate results.

When working with the GCMS there was a problem with gas transfer to and from the syringes. This created atmospheric contamination and skewed early GCMS results. Pressure locked needles and syringes are required to avoid any contamination.

Gas production was ranged from 63mL to 209mL on the first day when the temperature was 50°C. On the second day, when the water bath temperature dropped to 19°C, no samples produced gas. On the third day when the temperature was 40°C, gas production ranged from 17mL to 55mL. Throughout the experiment, temperature and gas production continued to be variable. Towards the latter part of the experiment some unusual gas volumes occurred with the co-digestion samples.The samples would produce no gas one day and then over 100mL the next. A pH test was performed on all of the samples on days eight and nineteen of the experiment. The co-digestion samples showed a more acidic pH then all of the other samples. This was likely caused by high concentration of sugars and carbohydrates in the food waste. Methanogens from anaerobic digesters tend to operate in narrow pH conditions [13, 14] The low pH hindered the methanogens' ability to produce methane which caused the gas production to plummet. Future experiments of this nature should buffer all of the samples or add less food waste to maintain a more neutral pH.

On two occasions the lab was unable to be accessed to take gas measurements. Presidents Day (February 15th) the lab was locked and gas volumes were unable to be collected. Also, due to a water pipe bursting under the lab building gas volumes were unable to be collected on the last day of the experiment and it was terminated early.

Conclusion

Due to the experimental design and technological errors, the results from this experiment should not be considered an accurate representation of the BMP of the tested waste streams. Initially, co-digestion produced the most gas volume, however gas production drastically decreased from low pH. Buffered samples of co-digested TESC food waste could prove to be prolific gas producers. Cow manure produced the most cumulative gas in this experiment.

The mistakes made in this experiment should serve as lessons for the next BMP research project. Either the current hot water baths need to be fixed or different hot water baths or dry incubators should be used. When doing GCMS work pressure-lock needles need to be used to prevent atmospheric contamination. Initially needles and syringes were used that were not gas-tight which caused contamination and leakage problems. We hope that our experiment will add insight to the next group of students who perform BMP tests at TESC.

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