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Evaluation of biogas generation from turkey waste

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EVALUATION OF BIOGAS GENERATION FROM TURKEY WASTE

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

Anup Pudasaini

has been approved by the Examining Committee
for the thesis requirement for the Master of Science
degree in Chemical and Biochemical Engineering at the July 2010
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To my parents Achyut Prasad Pudasaini, Punyabati Pudasaini, and my sister Usha Pudasaini, who always supported me unconditionally

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ABSTRACT

This project investigates local industrial biomass streams as feedstocks for the generation of low-cost sustainable energy for The University of Iowa. Methane gas produced during anaerobic digestion would fuel an engine to generate electricity at the University of Iowa Research Park (Oakdale Campus). A current local industry identified for this project is West Liberty Foods (WLF), a turkey processing facility located in West Liberty, Iowa, USA. WLF generates about 6,000 gal/day of blood, 40,000 lb/day of offal (guts), 6,000 lb/day of sludge (process waste water) and 2-4 truckloads/day of feathers as waste streams. To investigate biochemical methane potential, mixed streams and individual streams of WLF were processed anaerobically and incubated at 35 °C. Mixed streams contained blood, offal, and sludge, and individual streams contained offal and sludge. Mixed streams and individual streams generated methane gas. The methane production from mixed streams was achieved on the 11th day of processing, and it was achieved on the 9th day from individual streams. Sludge was the only stream that did not require the addition of acetate for the production of methane gas. Methane production was analyzed using gas chromatography. Methane production was achieved without addition of microbial seed cultures. Cumulative methane and energy produced by the 36th day of processing 6 grams of offal with the addition of acetate are 110 ± 50 mmol/lb and 0.09 ± 0.04 kJ/lb respectively, and without the addition of acetate are 62 ± 2 mmol/lb and 0.054 ± 0.002 kJ/lb respectively. Cumulative methane and energy produced by the 36th day of processing 6 grams of sludge with the addition of acetate are 200 ± 20 mmol/lb and 0.18 ± 0.02 kJ/lb respectively, and without the addition of acetate are 220 ± 60 mmol/lb and 0.19 ± 0.04 kJ/lb respectively. Each average was calculated from three data points with their errors. Reported values are

calculated at 95% confidence intervals. The Oakdale Campus is estimating to produce 5.5 MW energy from renewable sources of energy. The methane production capacity from processing turkey waste based on COD analysis was approximately 1% of the renewable energy target. However, the system is still producing methane gas and the process is not complete yet nor has it been optimized. Benchmarking methane productivity through improved quantitative measures should continue to establish the utility of the process.

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CHAPTER 1: INTRODUCTION

Purpose of the Study

The main goal of this project was to use local industrial biomass waste streams as feed stocks for the generation of low-cost sustainable energy for The University of Iowa. Methane gas produced during anaerobic digestion would fuel an engine to generate electricity at the Oakdale Campus in the University of Iowa. A current local industry identified for this project is West Liberty Foods (WLF) located in West Liberty, Iowa, USA. WLF generates about 6,000 gal/day of blood, 40,000 lb/day of offal (guts), 6,000 lb/day of sludge and 2-4 trucks of feathers per day of turkey as its waste streams.[1] The idea was to be able to identify the potential of WLF turkey waste in generating methane gas during anaerobic digestion in a laboratory set up, and if this process does produce methane gas, then scale it up at the Oakdale Campus.

Hypothesis

1) Microorganisms will convert turkey waste to methane gas
2) The methane gas production from the waste can be scaled up to provide energy for the Oakdale Campus

Individual streams of blood, offal, sludge, and feathers are considered turkey processing waste of WLF. Each of the streams contains organic carbon, and it is hypothesized that mixed cultures (microorganisms) in the streams contain methanogenic strains to convert the streams to methane gas. The conversion of organic waste (turkey waste) to methane gas can be achieved through anaerobic digestion. Anaerobic digestion is the biological decomposition of renewable organic matter by several types of microorganisms in the absence of oxygen to produce biogases.

Biogas produced from anaerobic digestion mainly consists of methane and carbon dioxide as its major products. The interference of air (oxygen) in the digestion of organic waste will produce carbon dioxide and water vapor as its major products. Therefore, maintaining anaerobic environment while processing the turkey waste is very important to achieve the desired product (i.e. methane gas).

Anaerobic digestion of organic matter can be generalized in two phases.[3] The first phase is liquefaction, and the second phase is gasification, shown in Figure 1.

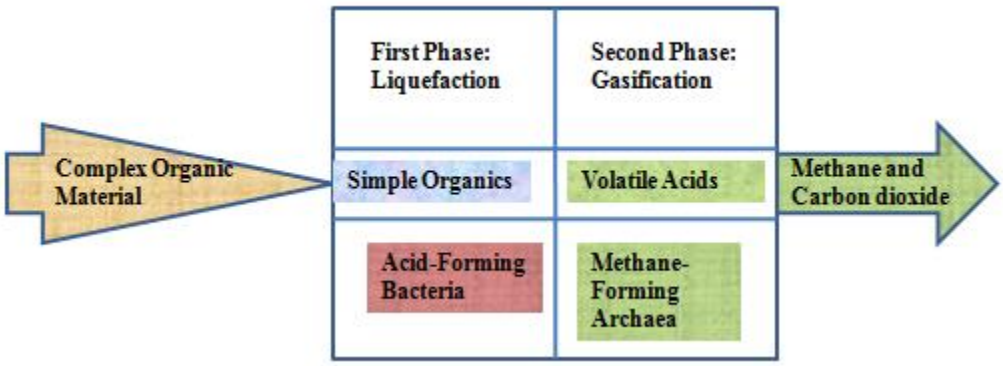


Figure 1: Anaerobic digestion of organic material

The liquefaction phase converts complex organic material (like manure or turkey waste) to simple organics and acid-forming bacteria will convert the simple organics to volatile acids. The gasification phase converts volatile acids to methane, carbon dioxide, and others with the aid of methane-forming archaea. Anaerobic digesters can be operated in mesophilic or thermophilic temperatures with optima at 35 °C and 55 °C respectively.[7] Mesophilic operation may save heat energy over thermophilic operation when this project is finally scaled up at the Oakdale Campus. A few methane-forming microorganisms digesting organic waste anaerobically at mesophilic

temperatures include species from the genera of *Methanobacterium, Methanobrevibacter, Methanosphaera, Methanolobus,* and *Methanococcus*.[8]

Methane gas produced during anaerobic digestion of the turkey waste can fuel an engine to generate electricity at the Oakdale campus. Figure 2 provides a general idea upon processing waste to produce electricity through anaerobic digestion.[3] Waste is fed into an anaerobic digester, and the methane produced fuels a combined heat and power (CHP) unit. This unit consists of an engine or turbine with a heat recovery unit, or steam boiler with a steam turbine. Energy released power a generator to produce electricity. Figure 2 shows CHP unit with turbine. The waste heat is recovered and can be used for maintaining the temperature of the digester, and for auxiliary use. The temperature of the anaerobic digesters at the Oakdale Campus is proposed to be 35 °C. Therefore, the waste heat from the digestion process can be used in maintaining the 35 °C temperature. The remaining heat could be used for auxiliary use such as floor heating, cooking or heating water for shower.

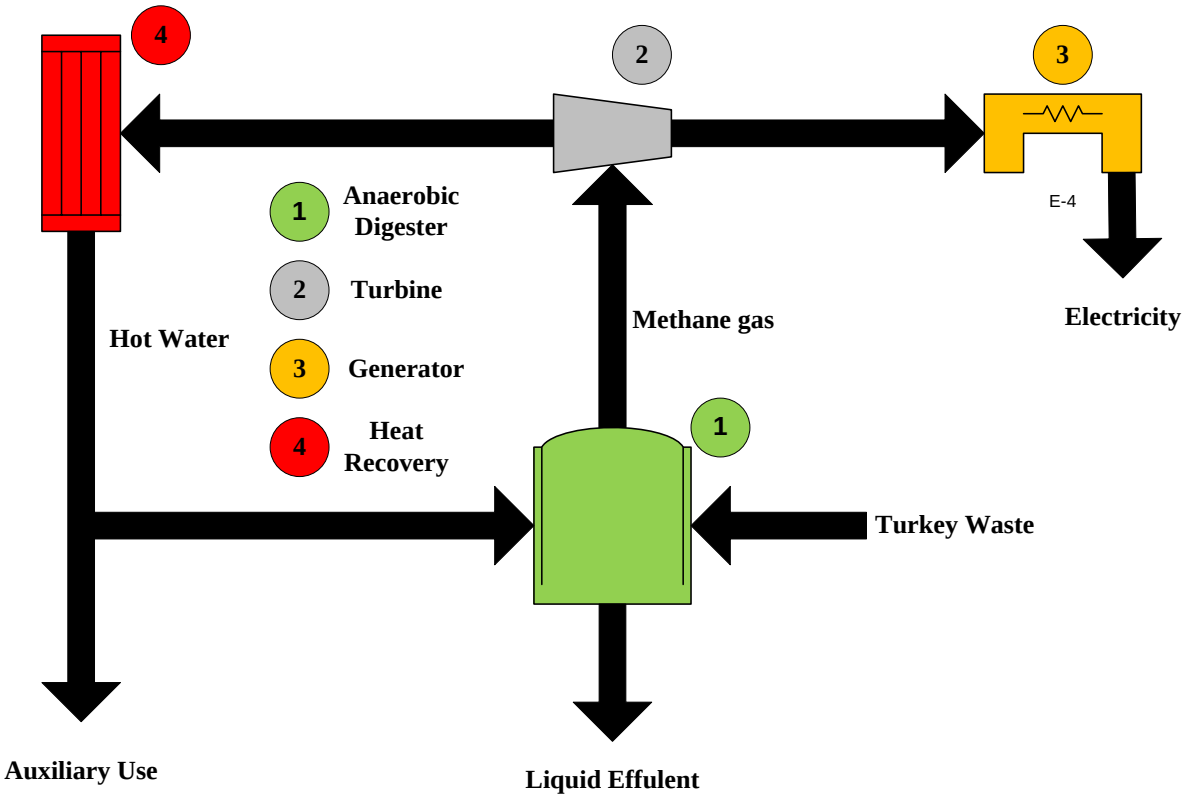


Figure 2: A scale up set for generation of electricity from turkey waste

Specific Aims of this Project

1) To produce and quantify methane gas from turkey waste in small anaerobic digesters (172 mL serum bottles)
2) To provide an energy of methane per pound of turkey waste bench mark for Oakdale Campus

The detection of methane gas can be confirmed by Gas Chromatography (GC). The calibration of pure methane gas using GC quantifies the methane production from turkey waste. A detailed calibration process for methane quantification is presented in Chapter 3 of this thesis.

Providing a benchmark for energy of methane from turkey waste is the eventual goal of this project. Pounds of methane per pound of turkey waste can be multiplied by higher heating value of methane to calculate energy of methane per pound of turkey waste. The higher heating value (HHV) of a fuel is the amount of heat released by a specified quantity once it is combusted and the products have returned to a temperature of 25 °C, and the HHV of methane is 55.5 MJ per kg.[4] Efforts to characterize methane generation on various waste streams are described in this thesis.

Following this brief introduction, this thesis contains background (Chapter 2) on energy and biomass and anaerobic digestion of different waste than turkey waste, methods (Chapter 3) for different tests to evaluate chemical oxygen demand, biochemical methane potential, and methane quantification, results and discussion (Chapter 4), conclusions and future work (Chapter 5). Several appendices (A-E) containing sample calculations, media recipes and sample chromatograms will help the reader with this thesis.

CHAPTER 2: BACKGROUND

Energy and Biomass

Energy is one of the essential needs of today's society. The world population is growing, and so is the demand for energy. The most available and affordable sources of energy in today's world are fossil fuels- about 85 % of all commercial energy is derived from them.[5] Fossil fuels are non renewable. The United States, one of the world's largest economies consumes about 25% of the world's energy, while having less than 5% of the world's population. The average US citizen consumes 100 times more energy than the average person in Bangladesh. This intensity of energy usage for rapid growth of population in the United States is worrisome, and it needs to be controlled, or the efficient development of sustainable energy is required. Biomass is an example of sustainable (renewable) energy.

Biomass is defined as all living plant matter as well as organic wastes derived from humans, animals, and plants. Animal waste, garbage, sewage, and tress are a few examples of biomass. Biomass is widely dispersed as opposed to other non renewable sources (e.g. coal) confined to a few limited sectors of region or country. Energy production from biomass is a great way to manage municipal and agricultural waste. Biomass can undergo a) anaerobic digestion, b) hydrolysis and fermentation, c) oxygen-blown gasification, and d) direct combustion for fuels, chemicals and heat generation.[5] Anaerobic digestion can result in electricity, process heat, and steam. Hydrolysis and fermentation can result in ethanol. Oxygen blown gasification can produce fuel gas. Direct combustion can yield process heat and steam, and they can be converted to electricity through turbines.

Biomass has disadvantages; however. The energy derived from biomass is lower compared to that coal or petroleum derived fuels. Biomass has a higher physically adsorbed

mixture, up to 50% of the total raw material. Overall, electricity and fuel generation using biomass is more expensive than energy generation from fossil fuels. Biomass has environmental impacts. Energy production from biomass causes deforestation, loss of biodiversity, soil erosion, desertification, siltation of rivers, and loss of agricultural productivity. Further, fertilizer contamination, changes in land use patterns, and biodiversity modification are other environmental impacts of processing biomass.

Anaerobic digestion of biomass produces methane gas and carbon dioxide as its major products.[2] Anaerobic digestion could produce up to 60% methane, 35% carbon dioxide, and 5% other gases regardless of the type of waste processed. Equation 1 shows the reduction of biomass into different gases.[2]

$$\text{Biomass} \rightarrow CH_4 + CO_2 + N_2 + H_2S \qquad (1)$$

Turkey waste, cattle waste, swine, poultry are all examples of biomass. Processing each of these wastes through anaerobic digestion would produce methane gas (biogas), which can be converted to electricity. Table 1 shows the potential biogas energy per year in the US from different animal manure.[6]

Table 1: Potential biogas energy per year in the US from different animal manure

Animal Type	Animal units (millions)	Biogas energy per animal unit/day (thousand BTU)	Biogas energy/year (trillion BTU)
Fattened cattle	9.6	25.7	89.9
Milk cows	12.3	20.6	92.4
Other beef and dairy cattle	58.8	23.3	497
Swine	8.5	39.8	124
Poultry	6.1	39.8	125
Total			928

Biogas potential is calculated by using the biogas energy that can be produced per animal unit and the number of the animal units in the US. Each animal unit is defined as 1000 pounds of animal. There are about 95 million animal units in the country that could produce about 928 trillion BTU (about 1 quad) of renewable energy per year, which is approximately equal to 1% of the total US energy consumption. The US consumed about 1000 quads of energy in 2005. Biogas when converted to electrical energy could produce up to 1.8 to 3% of annual electricity consumption in the US.

Table 2 shows possible electrical energy from biogas for each animal type. Larger and smaller generators with efficiencies 34-40% and 25% respectively are considered for the electricity generation. Equation 1 provides a conversion between biogas and electrical energy production (e_{biogas}) from each animal with an efficiency η.[6]

$$e_{biogas} = E_{biogas}[BTU](0.00293)\left(\frac{kWh}{BTU}\right)\eta \qquad (2)$$

where, E_{biogas} is biogas energy production of each animal type.

Table 2: Potential electrical energy production from manure of each animal type

	Possible electrical energy from biogas (billion kWh)	
Animal type	Low Ƞ = 25%	High Ƞ = 40%
Fattened cattle	6.6	10.5
Milk cows	6.8	10.8
Other beef and dairy cattle	36.4	58.2
Swine	9.1	14.5
Poultry	9.2	14.7
Total	68.0	108.8

Greenhouse Gas Reduction

Common greenhouse gases in the atmosphere include water vapor, carbon dioxide, and methane. Excessive presence of these gases can raise the Earth's temperature, and affect living organisms on Earth. Every country is emitting greenhouse gases from automobile to industrial sectors. The most developed countries in the world are more responsible for greenhouse gas emissions due to their many industrial sectors. The United States is alone responsible in releasing 25% of carbon dioxide in atmosphere every year from various industry sectors. Possible industrial sectors include but are not limited to the automobile industry, food processing industry, and power plants. The United States uses fossil fuels such as coal to generate most of the electricity for the country, and this process emits greenhouse gases like carbon dioxide. It is therefore imperative to seek renewable sources of energy to minimize the emission of greenhouse gases to the atmosphere. Table 3 shows the annual greenhouse gas emission (in CO_2 equivalent) by different sectors in the US from 1995-2008.[9]

Table 3: Greenhouse gas emissions from different sectors in the US (in MMT CO_2 eq)[9]

Sector	1990	1995	2000	2005	2006	2007	2008
Energy	5224	5545	6087	6187	6089	6182	5999
Industrial	318	339	351	334	339	350	334
Solvent and other product use	4.4	4.6	4.9	4.4	4.4	4.4	4.4
Agriculture	387	407	410	419	417	423	427
Land use and forestry emissions	15	17	36	28	49	47	32
Waste	177	174	153	158	159	159	159
Total	6126	6489	7044	7133	7059	7168	6956

Processing organic waste through anaerobic digestion reduces greenhouse gas emissions. Figure 3 indicates that the emission of carbon dioxide equivalents due to biogas fired power plant is much lower than the coal fired plant.[6]

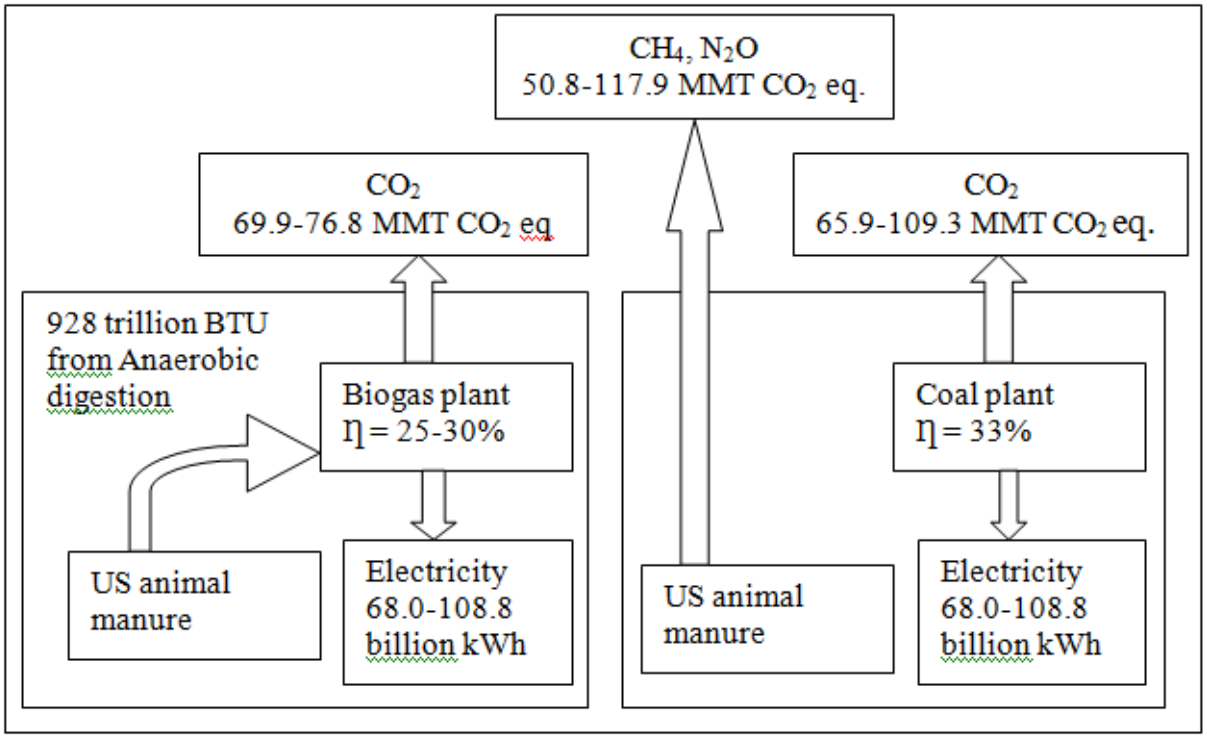


Figure 3: Emissions of carbon dioxide from biogas and coal fired plant[6]

Figure 3 shows the number of emissions of carbon dioxide equivalent (in MMT CO_2 eq.) from biogas and coal fired plant with efficiencies of 25-30% and 33% respectively. The number of emissions of carbon dioxide equivalent from biogas fired plant is 47.1-150.4 million metric tons (MMT) CO_2 eq. lower than from the coal fired plant, which indicates a sharp decrease in the emission of greenhouse gases. Further, there would be reduction of greenhouse gases by 3.9 ± 2.3 % if biogas plants are utilized in the US.

Anaerobic digestion of turkey waste should produce methane gas and help in reducing greenhouse gases. The quantification of methane production and reduction of greenhouse gases in large scale is beyond the scope of this project. The claim could be made however that there would be reduction of greenhouse gases if this turkey waste project is scaled up to the Oakdale Campus since turkey waste can be considered renewable source of energy.

Anaerobic Digestion of *Jatropha curcus* Plants

Figure 4 shows the cumulative methane production yield over time upon processing seed parts and cellulose of *J. curcus.*[10] They were processed in a 5L semi-batch reactor at a mesophilic temperature of 35 °C.

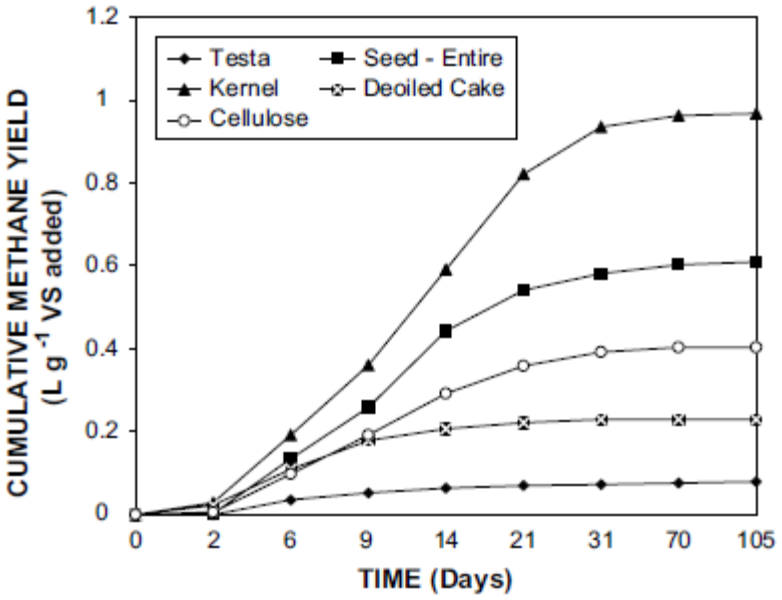


Figure 4: Cumulative methane yield upon processing seed parts and cellulose of *J. curcus*[10]

A similar Figure compared to Figure 4 is expected upon anaerobic processing turkey waste of WLF. Components of turkey waste including blood, offal, sludge and feathers are expected to follow the trends of Figure 4.

Methanogenic Activity

The anaerobic digestion process consists of the hydrolysis of complex organic material, the acid-producing stage, and the methane-producing stage.[11] The methane-producing stage is considered to be the rate limiting stage of the anaerobic digestion process. Methanogenic archaea are very sensitive to environmental conditions such as pH, hydrogen sulfide concentration, and metal sulfides concentrations. The optimum pH range for methanogensis is 6.8-7.2, and either side of this range could reduce the methanogenic activity.[11] Methanogenic archaea which are common in waste processing, take much longer than 24 h to grow. Therefore, no methane production is expected for at least a day after processing anaerobic waste.

Steps of Anaerobic Digestion

The steps of anaerobic digestion of complex organic matter (e.g. turkey waste) to methane gas include hydrolysis, fermentation, acetogenesis and methanogenesis. These conversion processes can be better explained in Figure 5.[18]

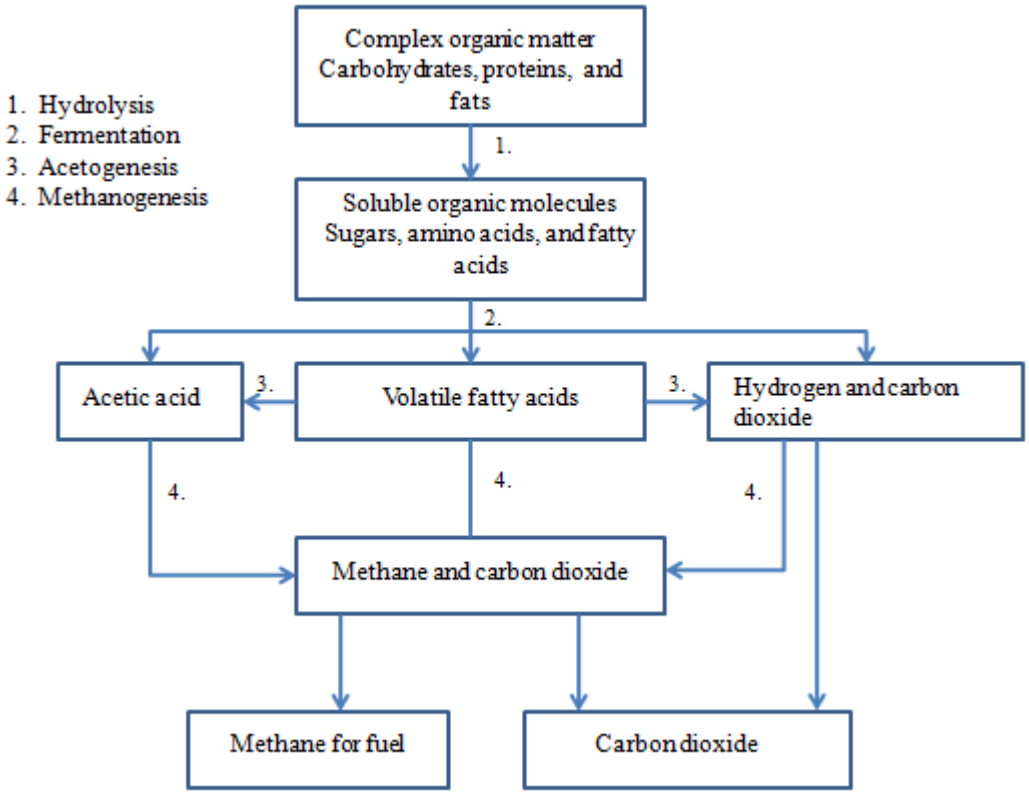


Figure 5: Steps of anaerobic digestion[18]

1) Hydrolysis: Complex organic matter includes carbohydrates, proteins, and fats. Products of enzymatic hydrolysis include soluble organic molecules such as sugars, amino acids, and fatty acids. Hydrolysis of the organic matter can be accomplished by several ways such as chemical or thermal pretreatment or by the addition of enzymes. Further, during the fermentation process, microbes secrete enzymes which hydrolyze complex molecules into smaller compounds which are then taken into the cell for further metabolism.
2) Fermentation: Acid forming bacteria convert soluble organics to carboxylic groups. The other products of this step are carbon dioxide, and water. The nitrogen source required

for this step is provided from ammonia generated in the hydrolysis step. Carboxylic acids, such as propionate and butyrate, are generated in this process.

3) Acetogenesis: Acid forming bacteria convert carboxylic acids such as propionate and butyrate to acetate and carbon dioxide in this step.

   Note both fermentation and acetogenesis steps can be collectively called the acid forming stage.

4) Methanogensis: Methane forming microorganisms convert acetate, volatile fatty acids, hydrogen and carbon dioxide produced in acetogenesis to methane, and carbon dioxide. Methanogens growing at a thermophilic temperature can convert carbon dioxide to methane gas. The methanogenic activity would increase when methane forming archaea digest acetate produced from the acetogenesis step. Optimizing acetate production in the acetogenesis step is very important for methanogensis to produce methane gas.

Biochemical Methane Potential (BMP)

The biochemical methane potential (BMP) assay is a procedure in determining methane yield of organic material during anaerobic digestion by mixed microorganisms in a defined medium. It is performed based on Owen et. al BMP assay with modifications.[12] Waste is mixed with Owen's et.al medium, and processed anaerobically at a mesophilic temperature of 35 °C. Gas produced in serum bottles can be injected in a gas chromatograph (GC) that can detect the presence of methane gas. In some evaluations, the biochemical methane potential is optimized by adding methane producing bacteria to determine whether the organic compounds can support biogas production.[10]

Chemical Oxygen Demand

The Chemical Oxygen Demand (COD) test can be used to indirectly measure the biodegradability of waste.[17] This test shows what would be possible if all of the organic compounds are directed to form methane gas. COD can be expressed in milligrams per liter (mg/L) that indicates the mass of oxygen consumed per liter of solution. The basis for the COD test is that all organic compounds are oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions. The moles of oxygen needed to oxidize an organic compound to carbon dioxide, water and ammonia is shown in Equation 3.

$$C_nH_aO_bN_c + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4}c\right)O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3}{2}c\right)H_2O + cNH_3 \qquad (3)$$

However, this reaction does not include the oxygen demand caused by the oxidation of ammonia into nitrate. Equation 4 shows the oxidation of ammonia into nitrate.

$$NH_3 + 2O_2 \rightarrow NO_3^- + H_3O^+ \qquad (4)$$

Equation 5 provides moles of oxygen needed to convert methane gas to carbon dioxide and water.

$$CH_4 + 2O_2 \rightarrow CO_2 + H_2O \qquad (5)$$

Therefore, the moles of methane per liter of waste can be calculated as:

(COD = mg of oxygen/L of waste) * (1 mole of oxygen/32 g of oxygen)* (1 mole of methane/2 moles of oxygen) = moles of methane/L of waste.

In summary, Chapter 2 presented background on energy and biomass and its advantages in reducing greenhouse gas emissions. This review further presented an anaerobic digestion of organic waste (other than turkey waste) that showed cumulative methane yield over time in Figure 4. In the end, this overview provided basic background of methanogenic activity, detailed steps of anaerobic digestion, the biochemical methane potential test, and the analysis of chemical oxygen demand of organic waste. To evaluate methane potential the aforementioned analyses were applied to the WLF project. Details of methods with modifications are presented in Chapter 3.

CHAPTER 3: METHODS

To evaluate the potential of WLF turkey waste streams to generate methane gas during anaerobic digestion, several analytical procedures were developed. This section describes methods for a) evaluating specific methanogenic activity, b) testing biochemical methane potential, c) determining chemical oxygen demand (COD), d) quantification of methane generation with gas chromatography (GC), and e) determining the methane production in a processed anaerobic bottle.

Specific Methanogenic Activity Test

Specific methanogenic activity test was performed similar to Monteggia with modifications.[13] Waste streams (200 mL of blood, 200 mL of sludge, and 200 mL of offal) from WLF were blended in a Waring blender, mixed together in a 2000 mL serum bottle, and frozen at a 4 °C. Several 6 mL aliquots of the turkey mixture from the 2000 mL serum bottle were mixed with 30 mL biological medium containing nutrient and buffer in 172 mL serum bottles. The mixtures containing medium and waste were evacuated using vacuum pump for 15 min and sparged with helium gas for 20 minutes using a gassing manifold as shown in Figure 6.

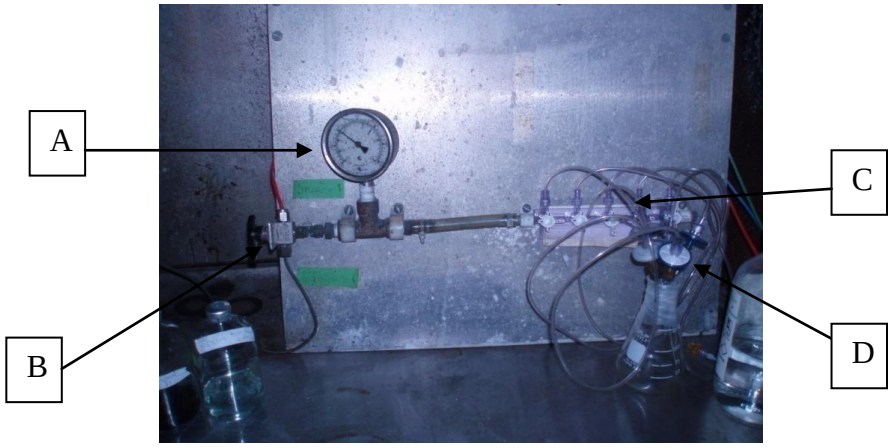


Figure 6: A gassing manifold (A: a pressure gauge, B: 3-way valve, C: multiple gassing ports, D: multiple needles)

Each needle of the gassing manifold in Figure 6 was inserted inside the sealed serum bottles and each head space was evacuated using a vacuum pump for 15 min. The needles were taken off from the bottles, and the gas valve was set at 5 psig. The needles that had gas coming out were injected inside and toward the bottom of the serum bottles at 5 psig. Vent needles were injected to release the build up pressure inside the bottles. Then, the pressure of the sparging gas (helium gas) was raised to 15 psig for 20 min. After 20 min, the pressure of the sparging gas was lowered to 5-6 psig and the vent needles were removed. The sparging needles were taken off of the serum bottle at 5-6 psig to avoid any air interference.

Sodium sulfide (Na_2S) (0.05 mL) was added to each evacuated and sparged bottle to confirm that the system was anaerobic.[12] Sodium sulfide can quench dissolved oxygen in the solutions and turn them black, when anaerobic. Each serum bottle was incubated at a mesophilic temperature of 35 °C. The pH was measured every day by taking a sample carefully with a syringe to avoid any interference of oxygen inside serum bottles. The pH of each solution was found to be near 7 every day.

Individual streams of offal and sludge were also processed and using the same procedure to make the bottles anaerobic. However, offal and sludge were processed by weight instead of volume due to their higher viscosity. Six grams of each (offal and sludge) were processed as opposed to 6 mL of the turkey waste mixture.

Biochemical Methane Potential Test

The biochemical methane potential test was carried out using the method of Owen et. al (1978) with a few modifications. Gas samples were taken from closed serum bottles every two days by syringe and analyzed using a model 8610 C gas chromatograph with a helium ionization detector (SRI Inc., Torrance, CA). Gas volumes in syringe were recorded at atmospheric pressure of 0 psig. The gas chromatograph system contained a 6' X 1/8" stainless steel silica gel packed column (SRI Inc.). Helium was used as the carrier gas (20 mL/min) and make-up gas (40 mL/min). The gas chromatograph displays peak areas for specific gases at specific retention times. Methane calibration was needed to detect and quantify the presence of methane gas from degrading turkey waste. Peak area analysis was performed by Peak Simple (version 3.41, SRI Inc.).

Chemical Oxygen Demand (COD) Test

The COD of turkey waste was calculated using the HACH COD low range (3-150 mg/L) kit. First, the calibration of COD was performed by processing five standard COD solutions of 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 150 mg/L. COD standard solutions (2.5 mL of each) were mixed with a reagent (concentrated sulfuric acid) in a closed glass tube about 1 cm in

diameter, and 10 cm tall. Each of the mixtures in the closed glass tube was heated in a COD reactor at 150 °C for 2 h.

The reacted mixture in the tube was cooled to a room temperature, and the absorbances of all five standard COD solutions were measured using a spectrophotometer at a wavelength of 440 nm. Figure 7 has y-axis for oxygen concentration measured in mg/L, and x-axis for corresponding absorbance.

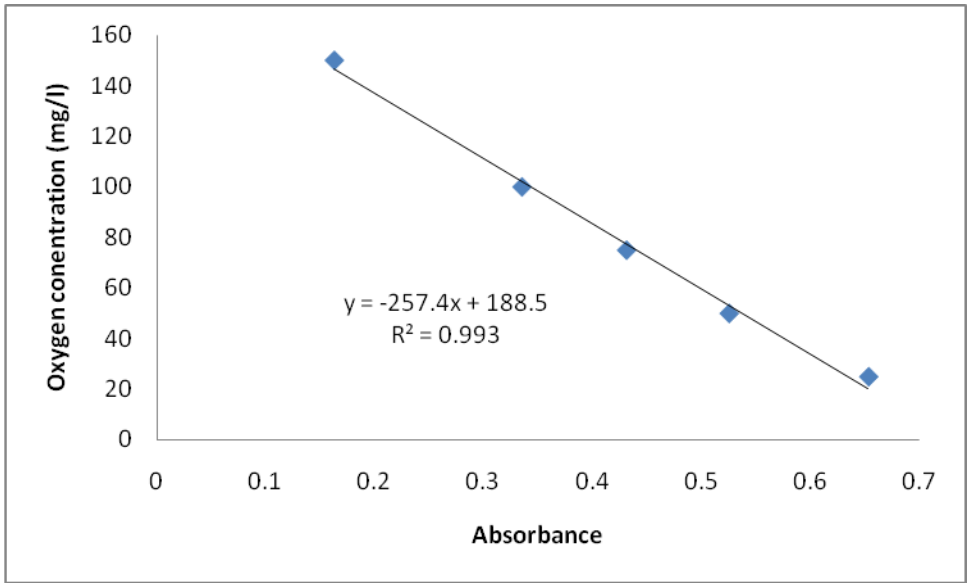


Figure 7: COD calibration. COD low range (3-150 mg/l) kit used processing five standard solutions (25 mg/l, 50 mg/l, 75 mg/l, 100 mg/l, and 150 mg/l). Absorbance measured at 440nm

Figure 7 can be used to calculate the required oxygen concentration (also COD) in mg/L of the turkey waste. The turkey waste was similarly processed as the COD standard solutions in the COD reactor, and absorbance was recorded in the spectrophotometer. The absorbance of the turkey waste can be used to calculate COD (in mg/L) from Figure 7 or the equation of Figure 7.

Methane Calibration for Turkey Waste

Figure 8 shows an initial methane calibration curve. The peak area and the volume of pure methane gas injected are plotted as y-axis and x-axis respectively. The calibration of pure methane gas should provide a linear relationship between volume of gas injected and GC area; however, Figure 8 does not. Components of the gas are separated in the GC column based on the column temperature, carrier gas flow rate, and column packing material. For each component that is ionized in the HID, a peak is detected. The signals for the ionized compounds are processed such that a corresponding peak is plotted on the computer connected to the GC. The corresponding peak is manually integrated by manually integrated by available options in the computer, and the GC area is read. Every injection of gas was done repeating the same steps. The same steps were a) pulling the gas smoothly in the gas syringe from the methane bottle, b) closing the valve of the syringe, c) pushing the plunger in to compress the trapped gas, d) letting the gas equilibrate at atmospheric pressure, e) recording the equilibrated volume while the needle is still inside the methane bottle, f) pulling the needle out from the bottle, g) inserting the needle into the GC port, h) compressing the gas by pushing the plunger in, i) opening the valve, and j) injecting the gas in and starting the GC. The steps f) through j) were done quickly to minimize the air in the needle of the syringe. One of the reasons of discrepancy in Figure 8 could be the injection of air from the needle while injecting methane gas from the syringe. Also, after one injection of methane gas into the GC, the needle was inserted inside the bottle. This needle was exposed to air (for a less than a second) and might have contaminated the pure methane gas in the bottle. The air contamination in the methane bottle increased after every trial, and this might have affected the calibration curve of Figure 8. The other reason could be in the equipment. The equipment might have some internal leaks inside that we were not able to resolve.

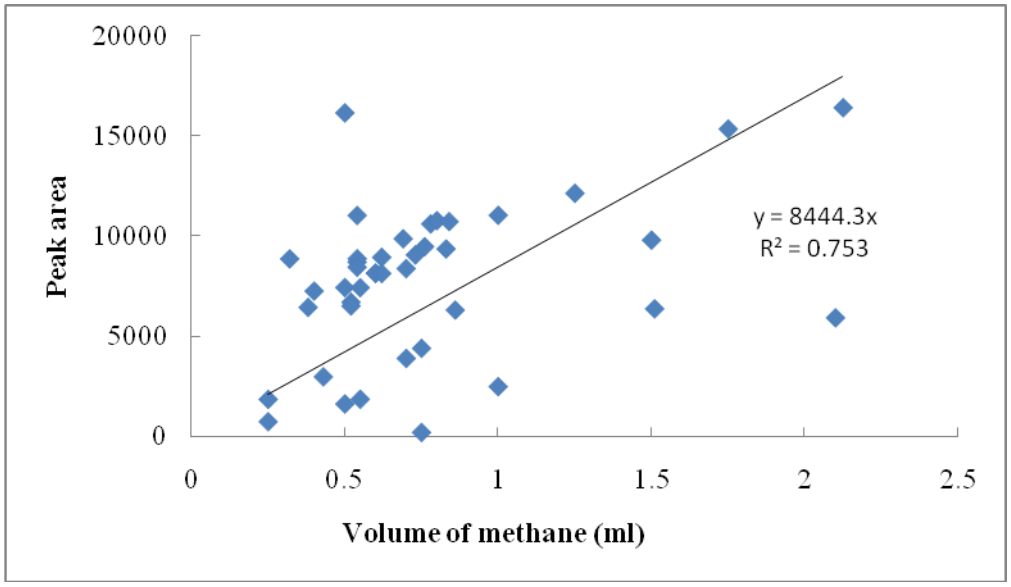


Figure 8: An initial methane calibration curve

Figure 9 shows a poor GC chromatogram achieved from injecting (0.43 mL) pure methane gas. Figure 9 is poor because the methane area is integrated by combining the area of two preceding peaks. The two preceding peaks are due to the presence of air in the needle while injecting pure methane gas in GC.

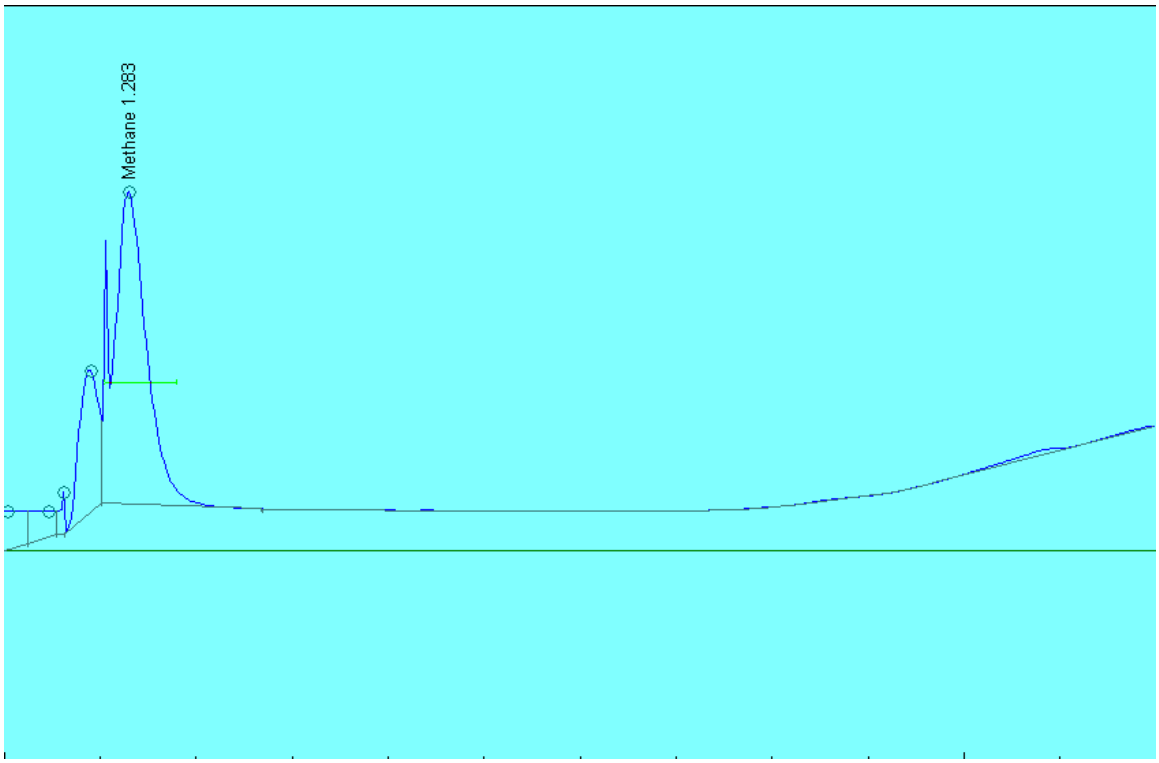


Figure 9: A poor GC chromatogram from injecting 0.43 mL of pure methane

Figure 10 shows a better GC chromatogram from injecting 0.25 mL of pure methane gas. Figure 10 is better in a sense that the methane peak area is calculated by combining a preceding noise peak. Further, the peaks in Figure 10 are more resolved than Figure 9.

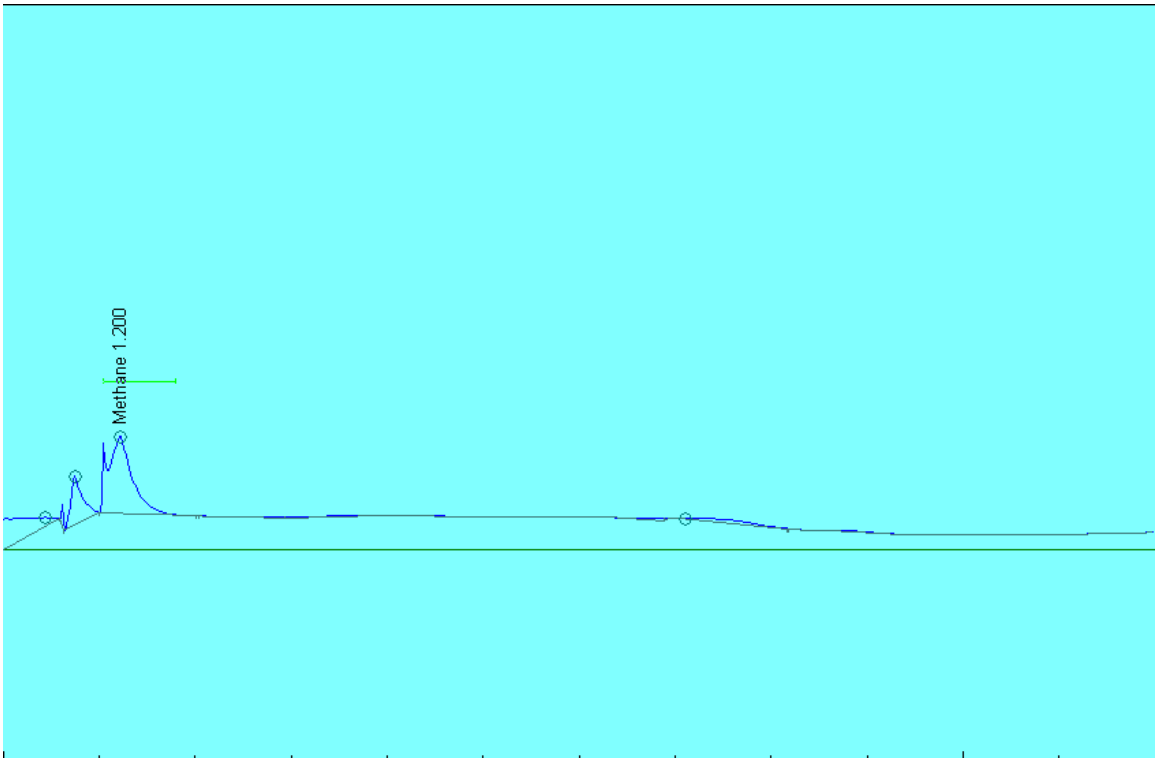


Figure 10: A better GC chromatogram from injecting 0.25 mL of methane

All chromatograms that were similar to Figure 9 were thrown out, while chromatograms similar to Figure 10 were utilized to achieve Figure 11. Figure 11 provided a linear relationship between volume of methane gas injected and peak area. Figure 11 can be best used for our actual samples. The gas generated from our turkey waste bottles can be injected into the GC, and the peak area corresponding to pure methane can be recorded.

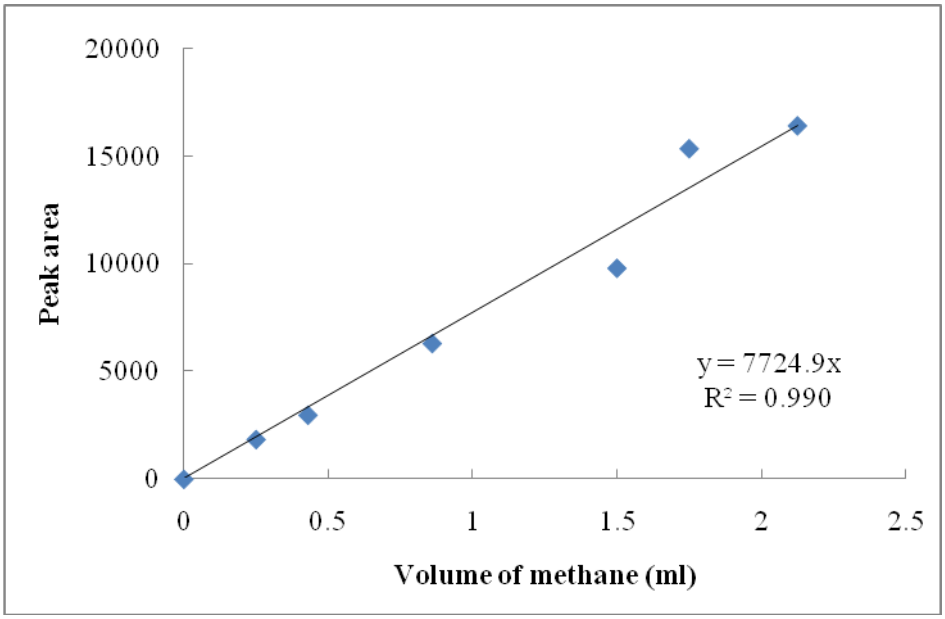


Figure 11: Revised methane calibration

Figure 11 was used to estimate the corresponding moles of methane gas injected into the GC. Equation 2 can then provide the number of moles of methane injected into the GC. The number of moles of gas (methane plus others) produced in the bottle can be calculated using Equation 2. The fraction of moles of methane gas injected into the GC is equivalent to the methane fraction into the bottle. Therefore, the fraction of moles of methane gas can be multiplied by the total moles of gas in the bottle to calculate the number of moles of methane in the bottle. The cumulative moles of methane produced can be calculated by adding the moles of methane in the bottle and the moles of methane in the syringe.

Improved Method with New GC Column

Even though Figure 11 provided a better relationship between peak area and volume injected, Figure 11 still added a noise peak area while calculating the total methane peak area. This noise peak area might have been due to the interference of air while injecting pure methane gas into the GC. The GC contained a 6' X 1/8" stainless steel silica gel packed column. However, this column was replaced with a 15' X 1/8" stainless Carboxen-1000 packed column. The advantage of the new column (Carboxen) was a good separation of methane peak and air peak. Figure 12 shows a good separation between methane and air peaks; however there still exists a noise in the methane peak area. Therefore, this issue is being resolved at this point.

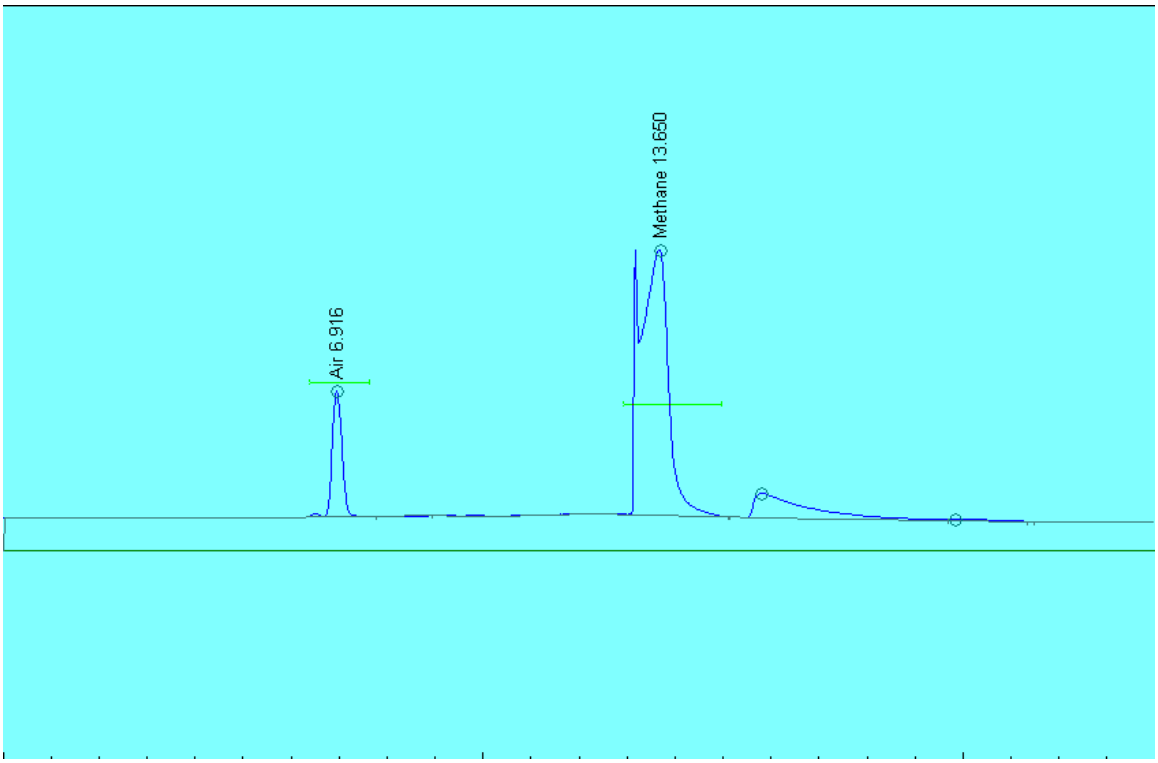


Figure 12: A GC chromatogram from injecting 0.25 mL of pure methane gas. Column used: Carboxen-1000 (15' X 1/8" SS)

Determining Methane Production in a Processed Bottle

A syringe is used to sample out the gas produced from a processed anaerobic bottle. The syringe has a valve that can be closed to trap the gas within. The volume of gas will be equilibrated at an atmospheric pressure, and the number of moles of gas (n) injected into GC at a room temperature can be calculated using ideal gas law in Equation 2.

$$PV = nRT \qquad (2)$$

where, P is pressure, V is the equilibrated volume at pressure P, R is universal gas constant and T is temperature.

The methane gas can be identified as a peak in the computer software that is connected to the GC. The peak corresponds to a certain number of moles of methane. The moles of methane in the syringe can be divided by the number of moles of gas (n) injected, calculated from Equation 2 to determine the mole fraction of methane in the syringe. The mole fraction of methane in the syringe is equivalent to the mole fraction of methane in the headspace at the time when the gas was sampled out of the processed serum bottle. A pressure gauge with a needle can be used to determine the pressure of the gas in the headspace of the bottle. The volume of headspace is known, and thus Equation 2 can be used again to calculate the number of moles of gas produced. However, this number of moles of gas in the headspace can be multiplied with the mole fraction of methane in the syringe to calculate the actual moles of methane in the bottle (headspace).

Wet Weight Determination

Each waste was measured with a balance, and the corresponding volume was recorded. The experiment was repeated three times. The wet weight of turkey waste mixture, sludge, offal, and blood are 2.2 ± 0.3, 2.1 ± 0.5, 2.0 ± 0.1, 2.0 ± 0.1 lbs/L respectively. The errors are calculated based on three data points at a 95% confidence interval.

CHAPTER 4: RESULTS AND DISCUSSION

COD Analysis of Turkey Waste

Turkey mixture contained 200 ml of each blood, sludge, and offal streams of WLF. The pre-COD of each sample in Table 4 was calculated following the COD test in the Methods section of this thesis. Table 4 provides the theoretical moles and energy of methane production possible upon processing turkey waste. The errors in Table 4 are calculated based on 95% confidence interval. Following this COD test, different samples of turkey waste were processed anaerobically. The processed turkey waste is still producing methane gas, so no post COD test is conducted yet.

Table 4: Possible theoretical moles and energy of methane from turkey waste

Waste	COD (mg/lbs)	Methane yield moles/lbs	Production rate (lb/day)	Energy (MJ/day)
Turkey waste mixture	890 ± 80	0.014 ± 0.001	46100	1130 ± 30
Sludge	1110 ± 60	0.017 ± 0.001	6000	93 ± 10
Offal	6500 ± 300	0.101 ± 0.005	40000	3624 ± 80
Blood*	3150 ± 300	0.022 ± 0.004	100	2540 ± 70

*COD of blood is theoretically calculated

The energy (kW) of waste in Table 4 was calculated by incorporating the higher heating value of methane (55.5 MJ/kg). The Oakdale Campus is estimating to achieve 475,200 MJ/day of energy from renewable sources.[19] Therefore, the turkey waste mixture, sludge, blood and offal have potential of producing about 1% of total estimated energy at the Oakdale Campus respectively. The methane production from turkey waste could be optimized by mixing it with

other regional renewable waste. The methane generation can be then evaluated again and compare it the estimated energy from the Oakdale Campus.

Methane Generation from Turkey Waste Mixture

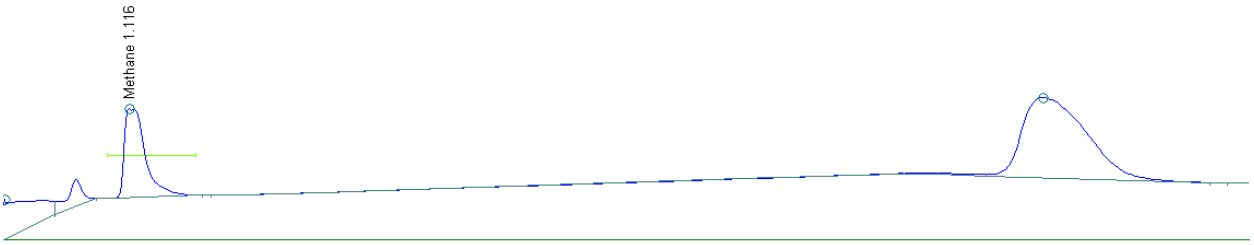
Blood (200 ml), offal (200 ml), and sludge (200 ml) of WLF were mixed together in a 2000 ml bottle. The mixture of total 600 ml was very viscous. Deionized (DI) water was added to make the total solution 1000 ml. Turkey waste mixture (6 ml) from 1000ml total solution was mixed with 30 ml media (similar to Owen et. al). The serum bottle purged out of O_2 and incubated at 35 °C for number of days and gas samples were injected into GC to evaluate any methane generation. However, no methane was detected. Then, the turkey waste mixture was inoculated with a seed that contained methane producing bacteria. The seed was brought as a solution from Iowa City Waste Water Treatment Plant (ICWWTP) in Iowa City, Iowa.

ICWWTP has anaerobic digesters that process sewage and generate methane gas. However, methane gas was not generated even though the turkey mixture had the ICWWTP methane producing seed in it. The seed was processed separately with 30 mL medium and after two days the methane gas was generated. This confirmed that the seed has methane producing microorganisms. This also suggested that the microorganisms were not able to degrade the turkey waste. Methane producing microorganisms convert short chains of volatile fatty acids like acetate, butyrate, and propionate into methane gas. Therefore, adapting microorganisms in turkey waste mixture for the generation of methane gas required the addition of short chain of volatile fatty acids. Thus, acetate, a volatile fatty acid, was added to the turkey waste mixture and six different samples were run. The results of this study are presented in Table 5.

Table 5: Methane generation evaluation from turkey waste mixture

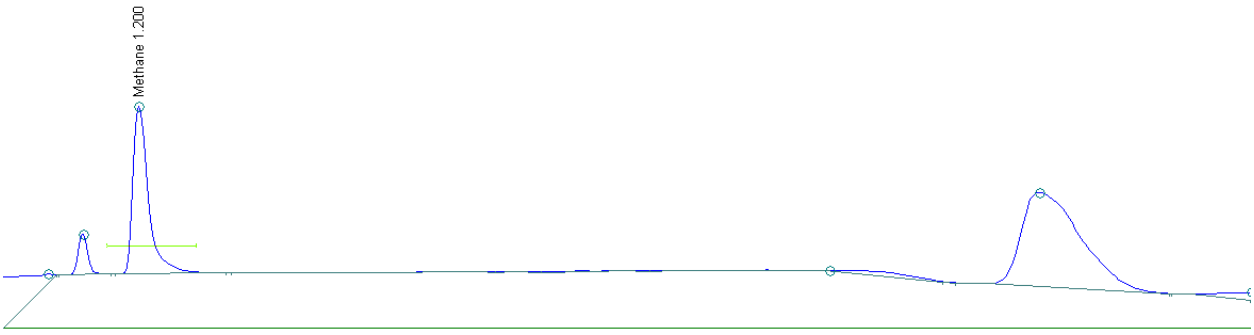
Bottle	Turkey waste mixture (mL)	ICWWTP Seed (mL)	Media (mL)	Acetate (mg)	Methane gas
A	6	0	30	0	NO
B	6	1.5	30	0	NO
C	6	1.5	30	150	YES
D	6	1.5	30	150	YES
E	0	1.5	30	0	YES
F	0	1.5	30	0	YES
G	6	0	30	150	YES
H	6	0	30	150	YES

Methane gas generation was evaluated following the methanogenic activity test described in methods section of this thesis (Chapter 3). Of all the processed bottles, the area of methane peak for bottle H was the largest. This confirms that there was no need of inoculating turkey waste mixture with the seed from ICWWTP. The peak of Bottle C was smaller than the peak of Bottle H; this further suggests that the seed from ICWWTP might be inhibiting the degradation of turkey waste mixture. Two GC chromatograms of Bottle C and Bottle H are presented as Figures 13 and 14. Figure 13 has a GC chromatogram that shows a methane peak with a GC area of 275.36, while Figure 14 has a GC area of 1637.41. Both Figures 10 and 11 were generated upon injecting 1.25ml volume of gas into GC on the 13th day of incubation of serum bottles.



Component	Retention (mins)	Area
Methane	1.116	275.362

Figure 13: A GC output for a 1.25 ml gas injection from Bottle C on the 13th day of incubation



Component	Retention (mins)	Area
Methane	1.2	1642.826

Figure 14: A GC output a 1.25ml gas injection from Bottle H on the 13th day of incubation

The higher GC area of Figure 14 implies that Bottle H is favorable over Bottle C. Bottle C contained seed inoculum while Bottle H lacks it. It implies the production of methane gas was inhibited by the addition of seed inoculum.

Methane Production from Individual Streams

Seed inoculum was inhibiting the methane production from turkey waste mixture, thus it was proposed to process individual streams. The individual streams were not supplemented with any seed inoculum; however, they were supplemented with acetate. The idea was to evaluate the methane production from individual streams, and decide the best mixing ratio between turkey waste streams (i.e., how much blood v/s sludge v/s offal v/s feathers) for optimization. These streams were chosen because the likelihood of having methanogens is higher than in blood or feathers. Feathers were also difficult to process.

Two scenarios were considered while processing offal and sludge. In one scenario, offal and sludge were individually processed without adding any acetate, and in the other they were processed individually with the addition of acetate. Each sample had three replicates to evaluate the reproducibility of data. The total processed samples is therefore 12. Table 6 shows the conditions 12 processed samples. Each sample contained 6 grams of either sludge or offal.

Table 6: Conditions of processed sludge and offal

Samples	Media (ml)	Acetate (mg)
O1, O2, O3	30	150
O4, O5, O6	30	NO
S1, S2, S3	30	150
S4, S5, S6	30	NO

In Table 6, O stands for offal, and S stands for sludge. O1, O2, and O3 are identical, and so are O4, O5, and O6. Similarly, S1, S2, and S3 are identical, and so are S4, S5, and S6.

Methane Production from Offal

Offal processing replicates O1, O2, and O3 produced methane gas. The GC chromatograms showing methane peak areas upon processing the triplicates from 9th day to 26th are shown in Appendix C-H. Identical methane production was not achieved upon processing offal replicates due to the GC issue. Further, the methane productions from replicates were expected to rise and level off over time; however O2 and O3 provided inconsistent results. Again, this error can be attributed to the GC issue. Figure 15 shows methane fraction upon processing O1. O1 is the only sample among the triplicates that showed linear increase in methane production. There were other outliers between 18th and 26th day. Methane fraction by moles over time is plotted for O1 because the pressure in each anaerobic bottle was not measured until 28th day of operation.

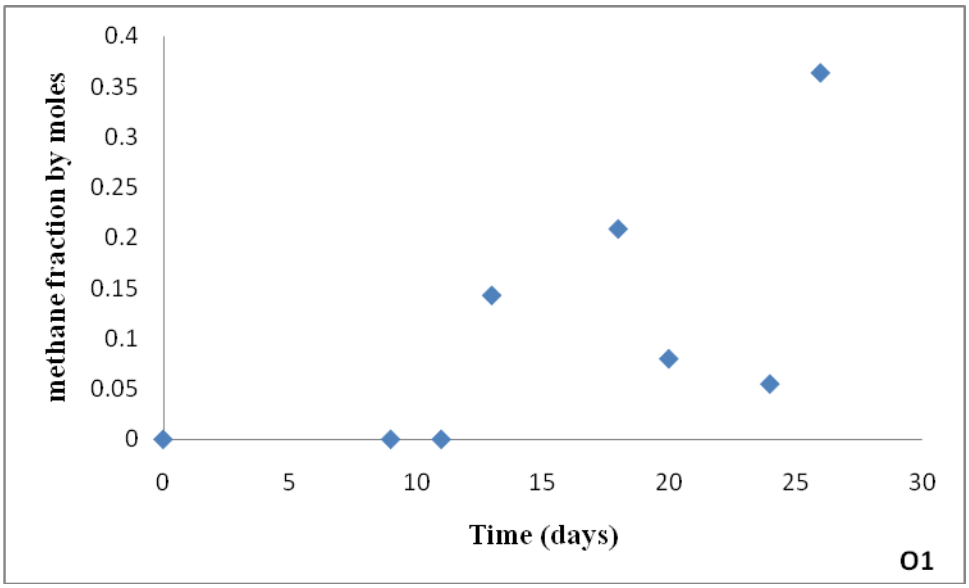


Figure 15: Methane fraction by moles upon processing O1. O1incubated at 35 °C, contained 30 mL media, 6 g of offal and 150 mg acetate

No methane was generated from samples O4 through O6 until 28th day of processing.

Methane fraction over time for sludge

Figure 16 shows methane fraction generated upon processing triplicates sludge S1 through S3.

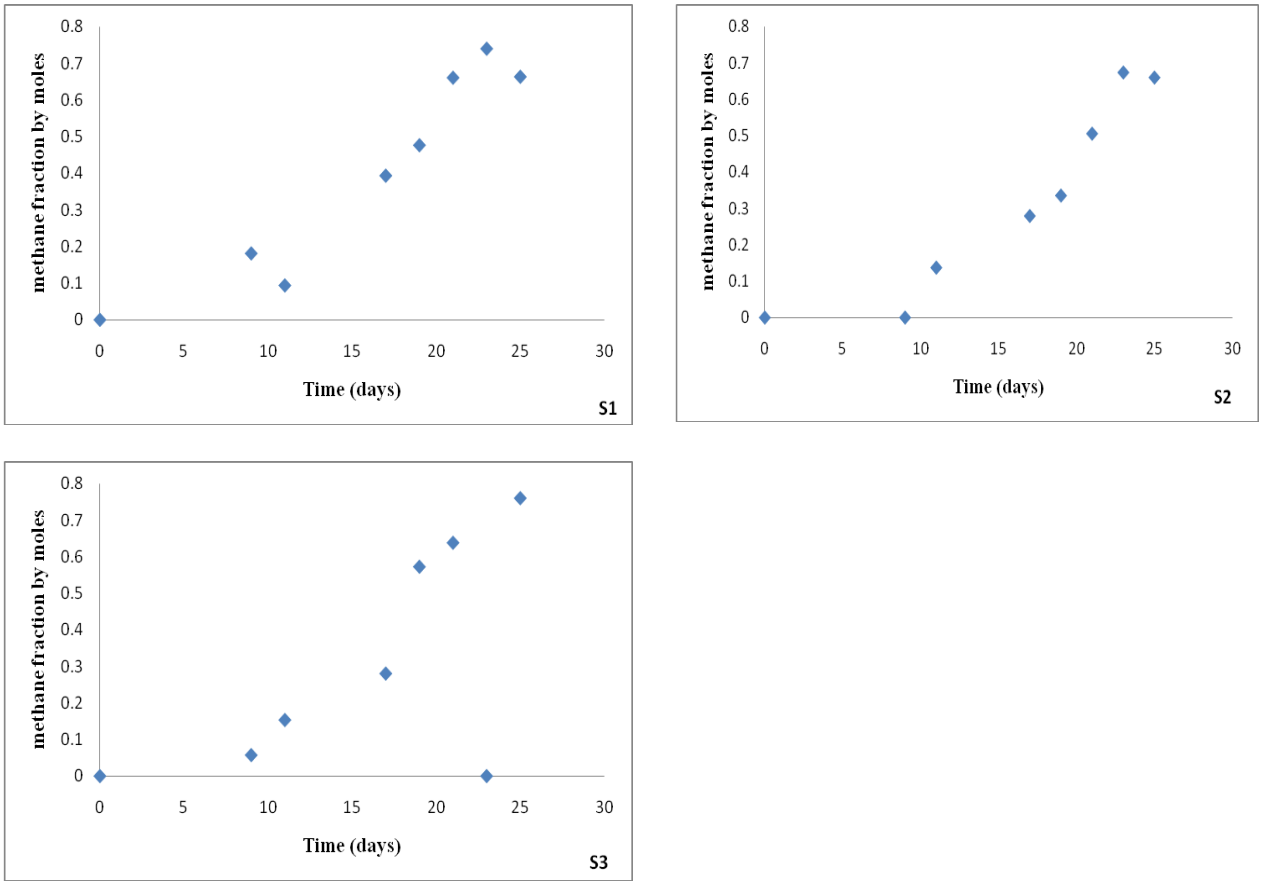


Figure 16: Methane fraction by moles from S1, S2, and S3. Conditions: each contained 30 mL media, 6 g of sludge, 150 mg of acetate, and incubated at 35 °C

Methane fractions upon processing S1, S2 and S3 are higher than O1. The plots of S1, S2 and S3 are somewhat similar to each other in Figure 16. Based on Figure 15 and Figure 16 it appears that sludge might be a better choice for producing methane gas. Figure 17 shows the methane fraction generated upon processing S4 through S6. Note that S4 through S6 did not have any added acetate in them.

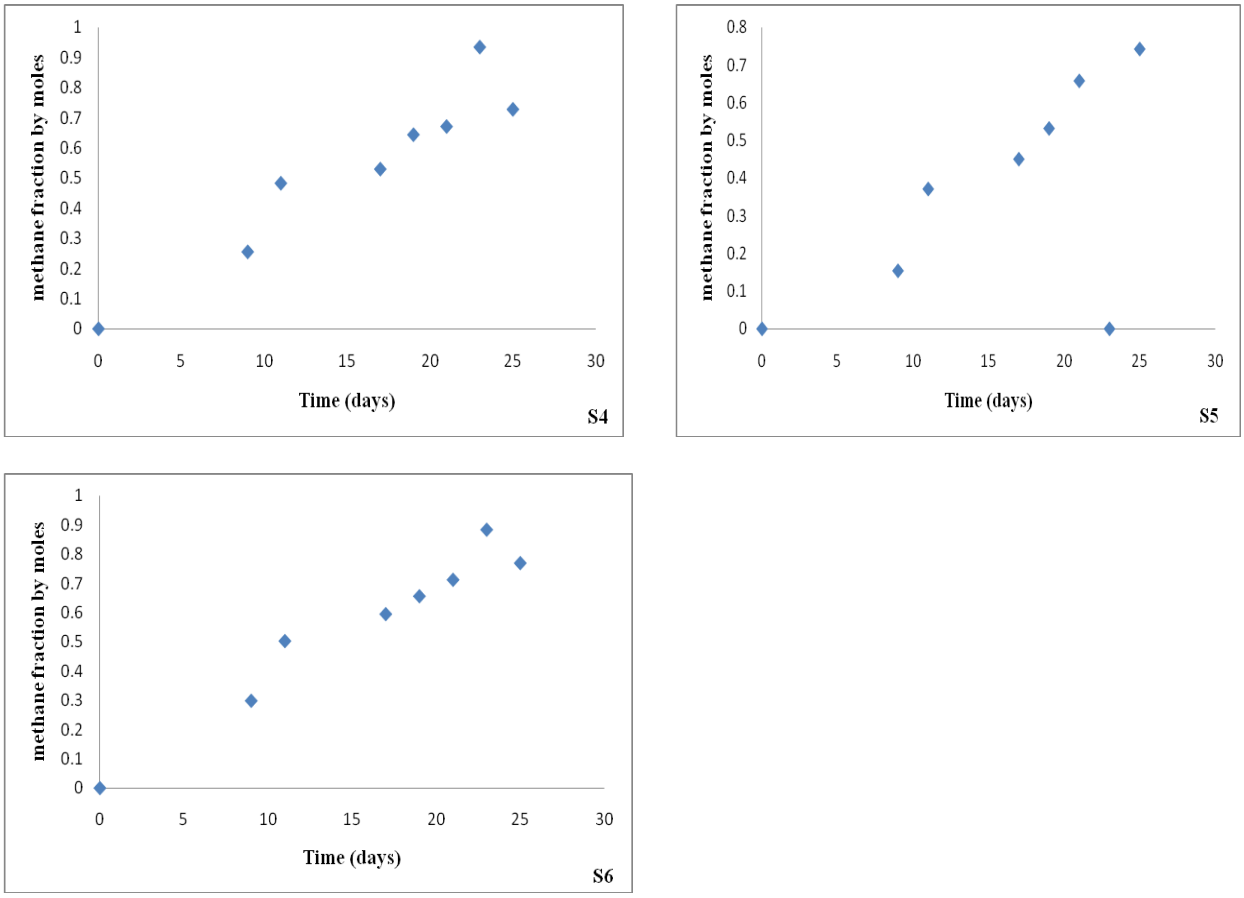


Figure 17: Methane fraction upon processing sludge S4, S5, and S6. Conditions: each contained 30 mL media, 6 g of sludge, and incubated at 35 °C

Again, S4 through S6 are somewhat identical, but there exists discrepancy in data, which is most likely attributed to errors in GC measurements. Figure 17 allows us to conclude that there is no requirement of the addition of acetate to generate methane gas from sludge. This means when this experiment is repeated, sludge can be mixed with offal to provide a fatty acid source for the generation of methane gas. Further, volatile fatty acids (VFA) content will be analyzed in the sludge samples to see if acetate is present initially or is produced by another member of the anaerobic culture during the incubations. This will save the cost of buying acetate.

The pressure of each sample (guts and sludge) was measured from 28th day until 36th day. The pressure of each sample was back calculated using the ideal gas law (Equation 3). The volume of gas was hold in the syringe, and the gas valve in the syringe was closed, then the trapped gas was compressed and was allowed to equilibrate at atmospheric pressure. Equation 3 was established to measure the pressure in each processed bottle. The pressure is expected to vary if there is the production of methane gas in the bottle.

$$P_1 = \frac{P_2 V_2}{V_1} \qquad (3)$$

where, P_1 is the pressure in a anaerobic bottle, V_1 is the hold volume in the syringe (while gas valve in the syringe was still open), V_2 is the equilibrated volume, and P_2 is the atmospheric pressure.

Using the calculated P_1 for each bottle, and Equation 2, the moles of gas generated can be easily calculated by rearranging Equation 2 for n. The headspace in each bottle is 140 ml, which is the volume, V, in Equation 2.

Pressure inside the bottle every day needed to be calculated to calculate the cumulative methane production upon processing guts and sludge.

Cumulative Methane Production from Offal and Sludge

The pressures in the bottles were measured from 28th day until 36th day of processing offal and sludge. The GC chromatograms are presented in Appendix H-M. Table 7 shows cumulative methane, energy production and yield from offal and sludge on 36th day of processing. Errors in Table 7 are calculated from three data points at 95% confidence interval. Table 7 suggests that sludge produced more methane gas than offal and is clearly a better choice. The percentage energy yield was calculated based on COD test in Table 4. The bottles are still running and producing methane gas, and the yield of the waste will increase over time.

Table 7: Cumulative moles and energy of methane from offal and sludge on the 36th day

Sample	Amount of methane (mmol/lb)	Energy of methane (kJ/lb)	% Energy yield based on COD test
Offal with acetate	110 ± 50	0.09 ± 0.04	0.01
Offal without acetate	62 ± 2	0.054 ± 0.002	0.005
Sludge with acetate	200 ± 20	0.18 ± 0.02	0.11
Sludge without acetate	220 ± 60	0.19 ± 0.04	0.12

Based on Table 7, the potential energy from the waste was less than 1% of the potential energy. However, the system is still producing methane gas, and the process is not complete yet nor has it been optimized. Efforts to enhance the liquefaction step in conversion will likely improve overall methane generation results and energy yield estimations.

CHAPTER 5: CONCLUSIONS AND FUTURE WORK

Turkey waste of WLF does produce methane. This implies the first hypothesis of the project is valid. Turkey waste of WLF contains blood, offal, sludge and feathers. However, the processed turkey waste did not contain feathers. It was very hard to achieve fine particles of feathers with a regular grinder in a bench scale. It is thus recommended to utilize better grinder to obtain fine particles of feathers. The turkey waste mixture did not require any addition of seed microorganisms for the production of methane gas. In fact, the production of methane gas from the turkey waste mixture was inhibited by the addition of seed microorganisms from ICWWTP. The processed turkey waste mixture did require the addition of acetate for the methane production.

Individual streams were processed to evaluate if any individual stream is slowing down the methane production in the turkey waste mixture. Offal and sludge were processed because these streams were believed to degrade faster and produce methane gas. Total of 12 samples of offal and sludge were processed. Of 12 samples, 6 samples contained offal and 6 contained sludge. Of each 6, 3 contained the individual stream with acetate, and other 3 contained the individual stream without acetate. Methane production was obtained from every bottle; however the offal bottle that did not have any supplemented acetate showed methane production only after the 28^{th} day of processing. Offal samples supplemented with acetate started producing methane gas from the 11^{th} day of processing. Sludge samples started producing methane gas from the 9^{th} day of processing. The Oakdale Campus is estimating to produce 5.5 MW of energy from renewable sources. At this time, the total energy produced from processing turkey waste was less than 1% of the estimation. However, the system is still producing methane gas, and the process is not complete yet nor has it been optimized. The second hypothesis of the project can

not be validated at this time, since the quantification of methane production from turkey waste is still being continued. Issues with the GC are not yet resolved, however an improved method has been proposed and is being tested. The GC had an old silica column that did not separate methane and air peaks well. The new column (Carboxen-1000) did separate methane and nitrogen peaks widely; however, there was always a noise in methane peak.

Once the GC issue is resolved, it is first recommended to obtain a good calibration curve for pure methane gas. Then, each individual stream (blood, offal, sludge and feathers) can be processed to evaluate the maximum methane gas production from them. The pressures in the processed bottles were measured from the 28th day of processing, however in the next repeat of the experiment, it is highly recommended to keep track of pressure from the very beginning. The pressure was back calculated starting on the 28th day in sludge and offal bottles; however, it may be better to obtain a pressure gauge (0-5psig) that can read the pressure directly in the bottle. The volatile fatty acids tests have not yet been performed. Methane producing archaea convert short chain of volatile fatty acids (VFA) to methane gas; therefore, it is recommended to perform the VFA test that would allow predicting the theoretical methane production.[16] The post-COD after the waste stops to produce methane is also recommended. This difference between post and pre CODs will provide the possible net theoretical moles of methane production from processing the waste.

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APPENDIX A: OWENS ANAEROBIC MEDIA

Table A1: Stock solution for preparation of defined media

Solution	Compound	Concentration (g/l)
S2	Resazurin	1
S3	$(NH_4)_2HPO_4$	26.7
S4	$CaCl_2$.2H2O	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2.4H_2O$	370
S6	$Na_2S.9H_2O$	500
S7	Biotin	0.002
	Folic acid	0.002
	Pyridoxine hydrochloride	0.001
	Riboflavin	0.01
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic acid	0.005
	B_{12}	0.0001
	p-aminobenzoic acid	0.005
	Thioctic acid	0.005

Table A2: Preparation of defined media

Step	Instruction
1	Add one liter of deionized water (18.2 Ω-m) to 2-liter serum bottle
2	Add:
	1.8 ml S2
	5.4 ml S3
	27 ml S4
3	Add deionized water up to 1,800 ml mark
4	Add:
	18 ml S7
	1.8 ml S5
	1.8 ml S6

APPENDIX B: COD CALCULATION OF TURKEY WASTE

$[O_2]$=-257.4* absorbance+188.5

Guts (Offal)	Absorbance	O_2(mg/l)	COD(mg/l)	Ave.COD(mg/l)	moles of CH4/L of waste
100X dilution	0.194	138.5644	13856.44		
	0.23	129.298	12929.8	**13212.94**	**0.206452188**
	0.233	128.5258	12852.58		
50X dilution	0.146	150.9196			
	0.142	151.9492	Out of Range		
	0.141	152.2066			
Sludge	Absorbance	O_2(mg/l)	COD(mg/l)		
100X dilution	0.822	-23.0828			
	0.817	-21.7958	Out of Range		
	0.815	-21.281			
50X dilution	0.648	21.7048	2170.48		
	0.642	23.2492	2324.92	**3435.9**	**0.053685938**
	0.64	23.764	2376.4		
Turkey Waste	Absorbance	O2(mg/l)	COD(mg/l)		
100X dilution	0.661	18.3586	1835.86		
	0.651	20.9326	2093.26	**1921.66**	**0.030025938**
	0.661	18.3586	1835.86		

Conversion: (1mole of methane/2moles of oxygen)*COD(mg/l)*mole oxygen/32g of oxygen

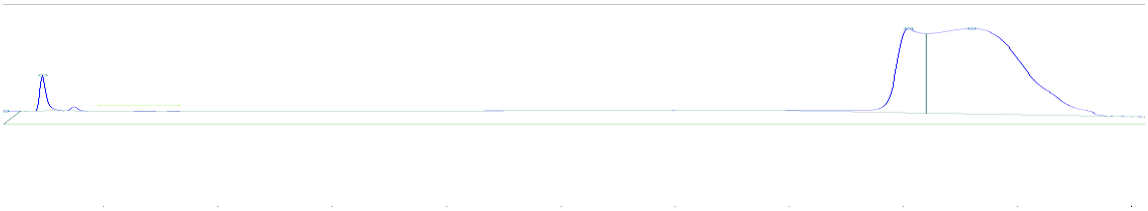
APPENDIX C: A PROCEDURE TO CALCULATE CUMULATIVE METHANE

1) A gas syringe was poked into the serum bottle, and the plunger of the syringe was hold to record the volume (hold volume) of the gas trapped. The pressure of the hold volume is equivalent to the pressure inside the bottle.
2) The gas valve of the syringe was closed and let the trapped gas equilibrate at an atmospheric pressure. The equilibrated volume was recorded.
3) Ideal gas law can be used for hold volume and equilibrated volume at room temperature to calculate the pressure inside the bottle
4) The moles of gas produced in the bottle can be calculated using ideal gas.
5) The moles of the gas trapped inside the syringe was injected into the GC.
6) The peak area corresponding to methane gas was recorded into the computer.
7) The mole of methane gas injected was calculated from the revised calibration curve in Figure 11.
8) The methane fraction was calculated by dividing moles of methane gas with moles of gas injected from the syringe (this can be calculated using ideal gas at atmospheric pressure and temperature)
9) The methane fraction can be multiplied by the moles of gas produced in the headspace of the bottle to determine the actual methane present (in headspace)
10) The moles of methane in the syringe and bottle are added together to calculate the cumulative methane production

APPENDIX D: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 9TH DAY

O1: No Methane

O2: No methane



O3: Methane (No injected volume recorded)

Figure D1: GC chromatograms of offal and sludge on the 9th day of processing

Figure D1 continued

O4: Methane (No injected volume recorded)

O5: Methane (No injected volume recorded)

O6: Methane (No injected volume recorded)

Figure D1 continued

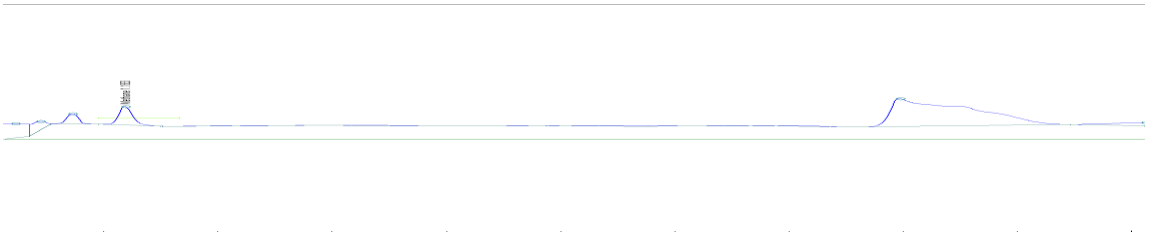
S2: Methane (No injected volume recorded)

S3: Methane (No injected volume recorded)

S4: Methane (No injected volume recorded)

Figure D1 continued

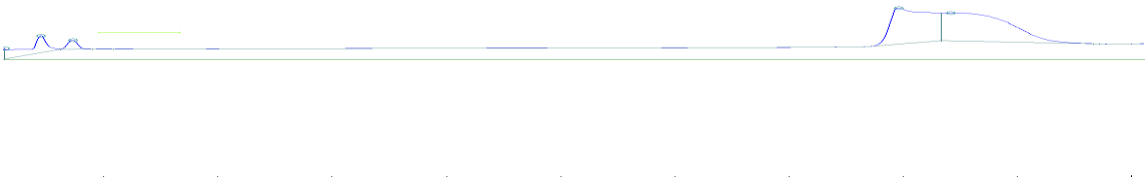
S5: Methane (No injected volume recorded)



S6: Methane (No injected volume recorded)

APPENDIX E: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 11TH DAY

O1 (1.35ml): No methane detected



O3 (2.35ml): No methane detected

O4 (2.30ml): No methane detected

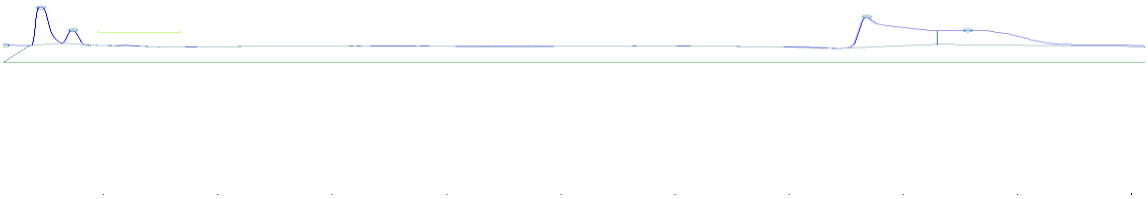


Figure E1: GC chromatograms of offal and sludge on the 11th day of processing

Figure E1 continued

O5 (2.30ml): No methane detected

S1 (2.3ml): Methane GC area: 3230.634

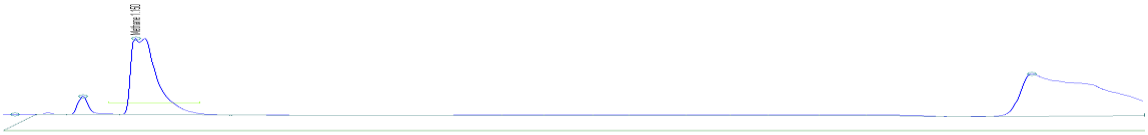
S3 (2.35ml): Methane GC area: 1041.030

S4 (2.4ml): Methane GC area: 4738.172

Figure E1 continued

S5 (2.25ml): Methane GC area: 2675.8660

S6 (1.5ml): Methane GC area: 3462.662



APPENDIX F: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 13TH DAY

O1 (2.3ml): No methane detected

O2 (1.5ml): Methane GC area: 2618.944

.

O3 (1.2ml): Methane GC area: 121.07

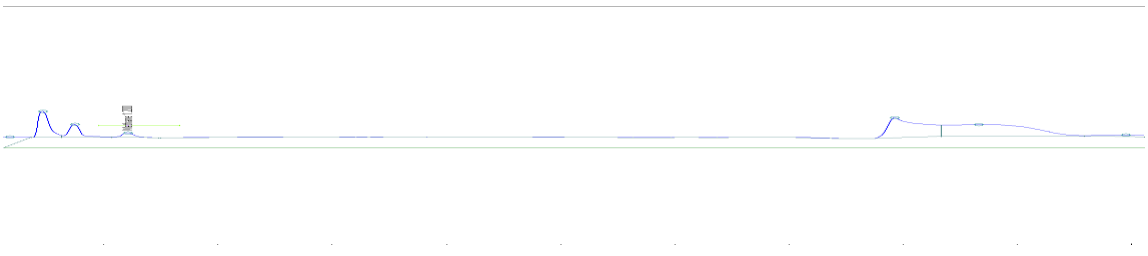


Figure F1: GC chromatograms of offal and sludge on the 13th day of processing

Figure F1 continued

O4 (1.535ml) (NO Methane)

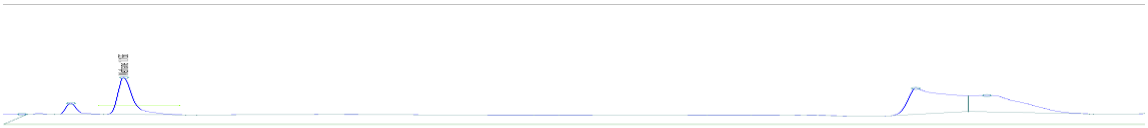
O5 (1.53ml) NO Methane

O6 (2.4ml) NO Methane

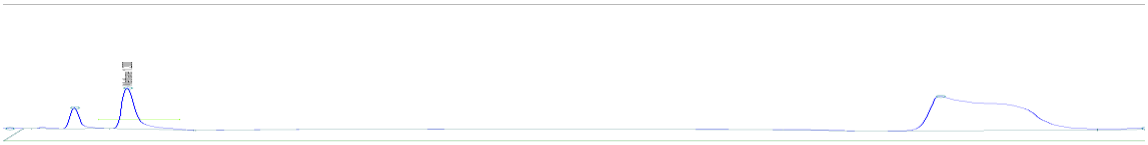
S1 (2.4ml): Methane GC area: 1738.7810.

Figure F1 continued

S2 (1.51 ml): Methane GC area: 1608.67



S3 (1.25 ml): Methane GC area: 1480.658



S4 (1.51ml): Methane GC area: 5623.474

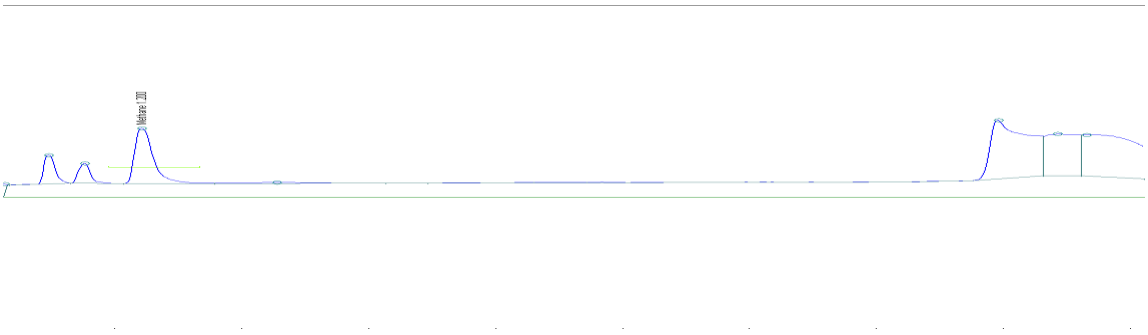
S5 (1.35ml): Methane GC area: 3874.228

Figure F1 continued

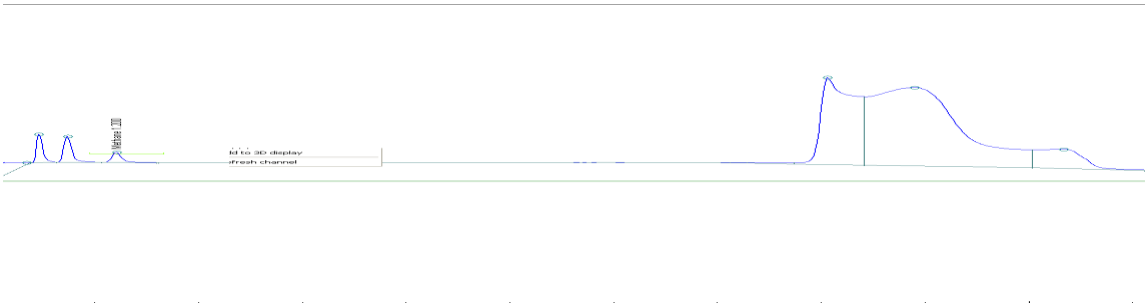
S6 (1.35ml): Methane GC area: 5241.955

APPENDIX G: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 18TH DAY

O1 (1.52ml): Methane GC area: 1679.134



O2 (1.40ml): Methane GC area: 206.122



O4 (1.54ml): No methane detected

O5 (1.505ml): No methane detected

Figure G1: GC chromatograms of offal and sludge on the 18th day of processing

Figure G1 continued

O6 (1.45ml): No methane detected

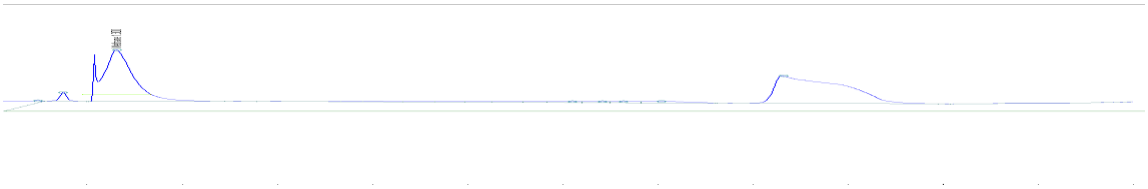
S1 (1.5105ml): Methane GC area: 4622.22

S2 (1.5105ml): Methane GC area: 3253.949

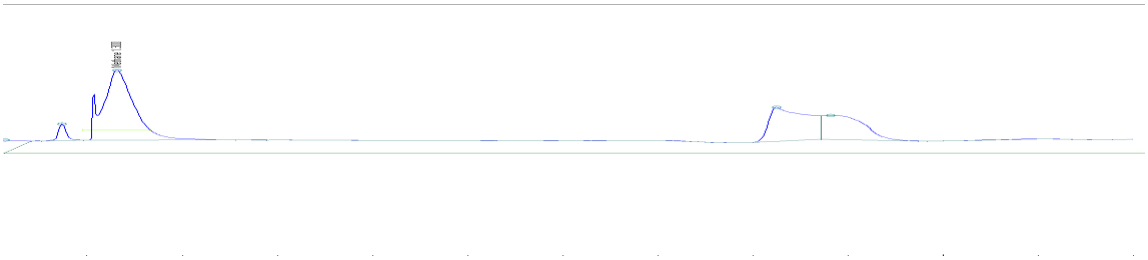
S3 (1.50ml): Methane GC area: 3246.854

Figure G1 continued

S4 (1.505ml): Methane GC area: 6159.446



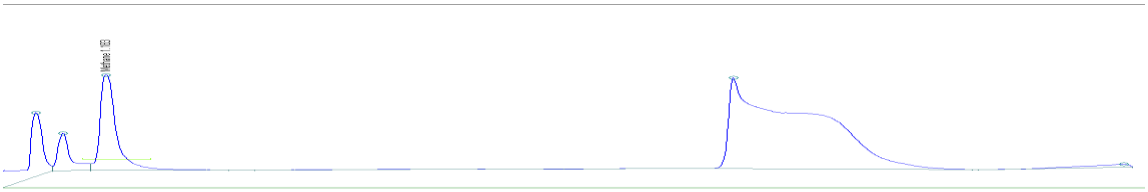
S5 (1.75ml): Methane GC area: 6085.496



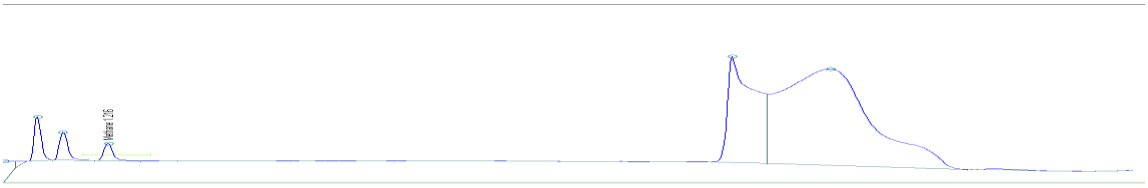
S6 (1.4ml): Methane GC area: 6426.798

APPENDIX H: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 20TH DAY

O1 (2.2ml): Methane GC area: 3557.8



O2 (2.3ml): Methane GC area: 369.255



O3 (2.3ml): No methane detected

O4 (2.25ml): No methane detected

Figure H1: GC chromatograms of offal and sludge on the 22th day of processing

Figure H1 continued

O5 (2.25ml): No methane detected

O6 (2.25ml): No methane detected

S1 (1.53ml): Methane GC area: 5609.467

Figure H1 continued

S2 (1.50ml): Methane GC area: 3882.094

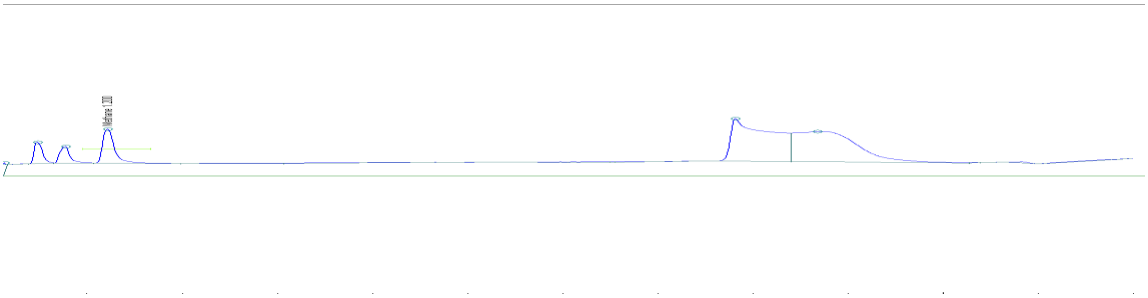
S3 (1.40ml): Methane GC area:6191.314

S4 (1.25ml): Methane GC area: 6215.25

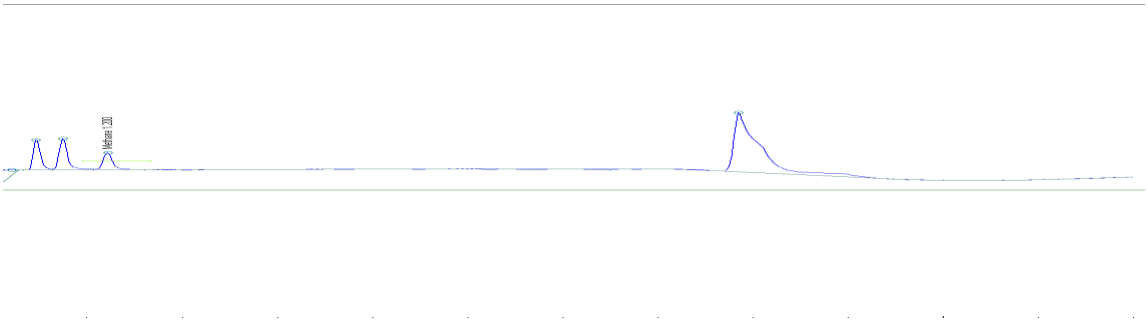
S5 (1.1.515ml): Methane GC area: 6223.258

APPENDIX I: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 24TH DAY

O1 (1.54ml): Methane GC area: 958.48



O2 (2.2ml): Methane GC area: 352.9840



S1 (1.51ml): Methane GC area: 7702.5920

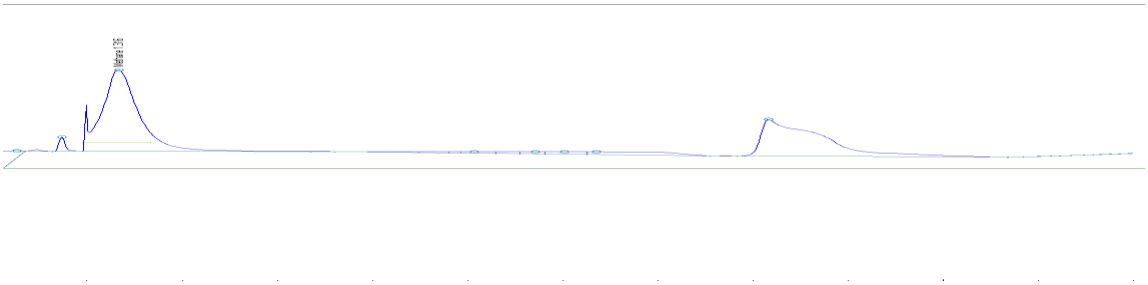


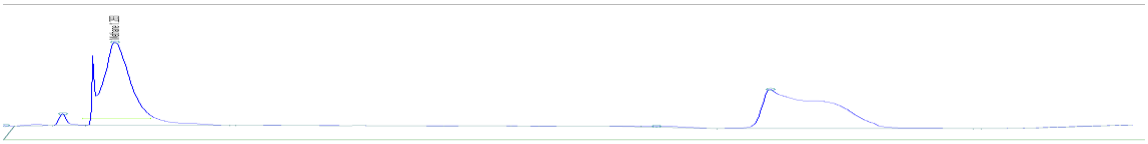
Figure I1: GC chromatograms of offal and sludge on the 24th day of processing

Figure I1 continued

S2 (1.51ml): Methane GC area: 5896.7840

S3 (1.505ml): Methane GC area: 7428.3110

S4 (1.505ml): Methane GC area: 7789.8920



S5 (1.51ml): Methane GC area: 7677.1340

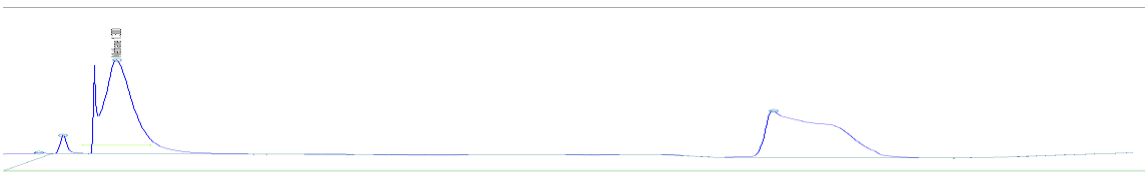
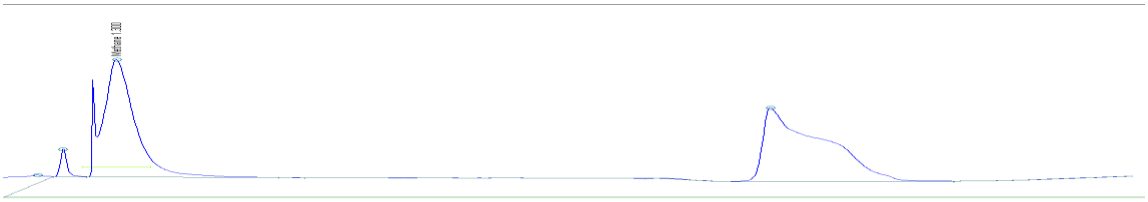


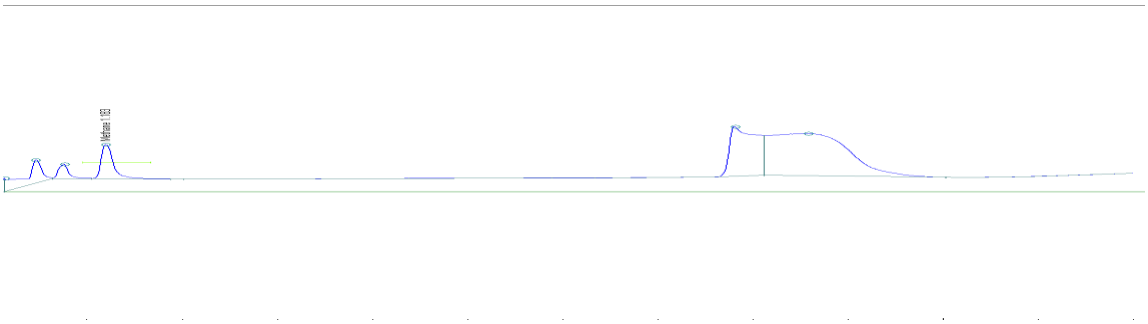
Figure I1 continued

S6 (1.50ml): Methane GC area: 8242.877



APPENDIX J: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON THE 26TH DAY

O1 (2.15ml): Methane GC area: 919.242



O2 (1.1ml): Methane GC area: 143.034

S1 (1.25ml): Methane GC area: 7137.707

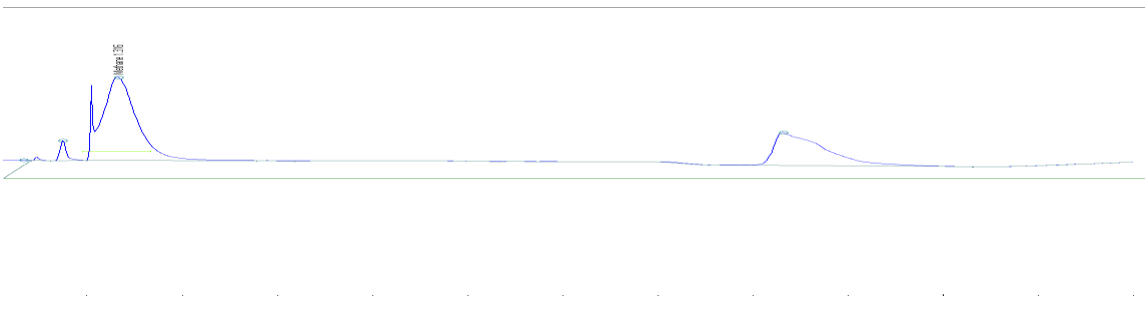
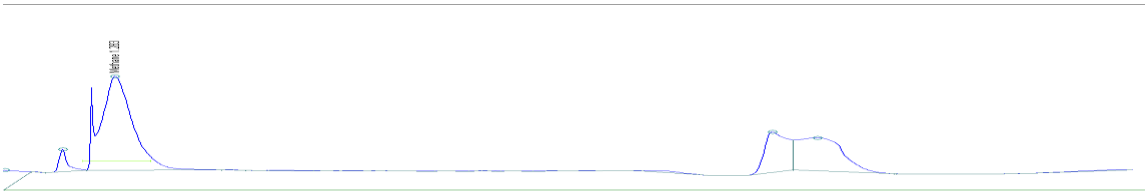


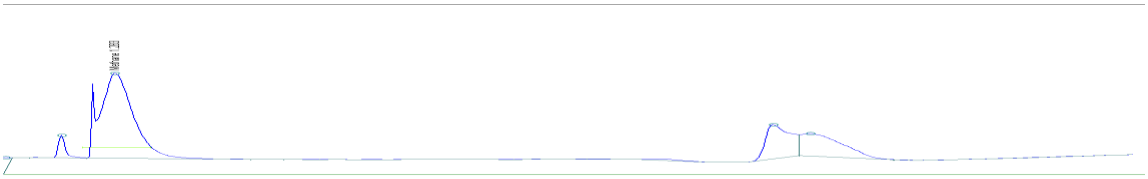
Figure J1: GC chromatograms of offal and sludge on 26th day of processing

Figure J1 continued

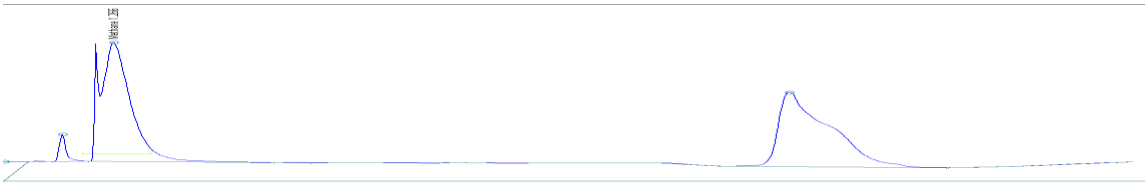
S2 (1.3ml): Methane GC area: 6758.36



S3 (1.3ml): Methane GC area: 6949.3040



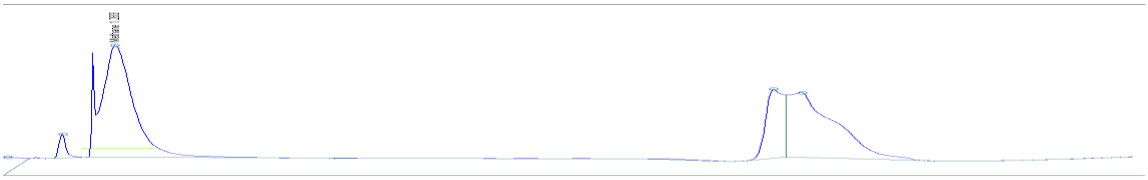
S4 (1.2ml): Methane GC area: 8653.1965



S5 (1.3ml): Methane GC area: 8250.510

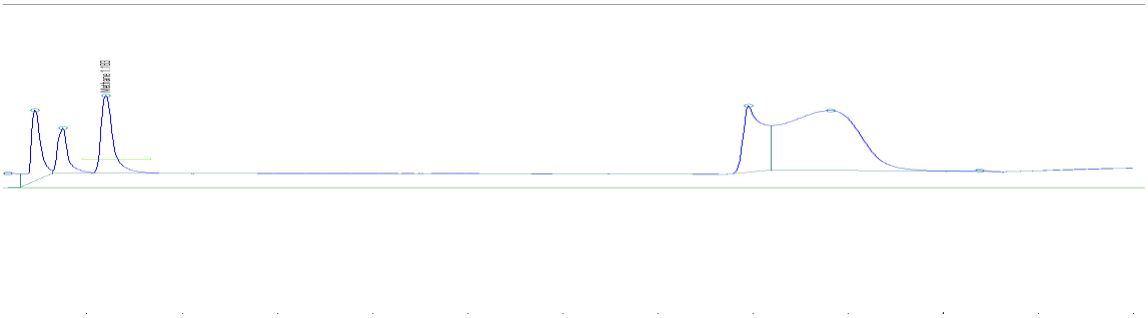
Figure J1 continued

S6 (1.3ml): Methane GC area: 8864.317

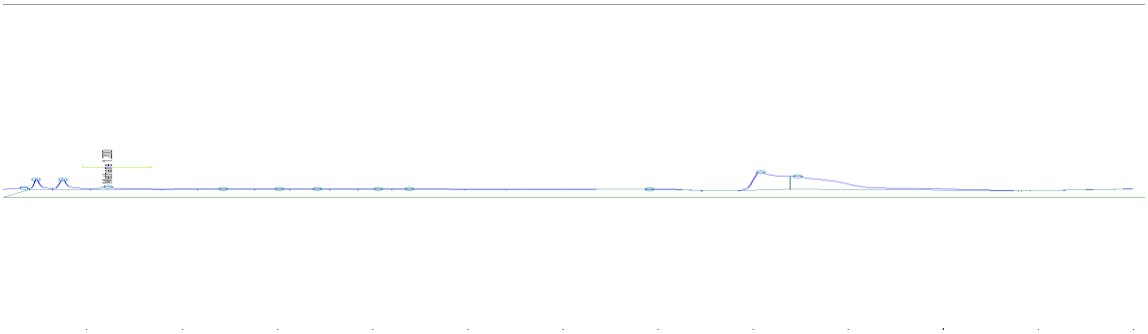


APPENDIX K: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON 28TH DAY

O1 (1.5ml): Methane GC area: 4228.2875



O2 (1.3ml): Methane GC area: 323.0635



S1 (1.40ml): Methane GC area: 6764.9750

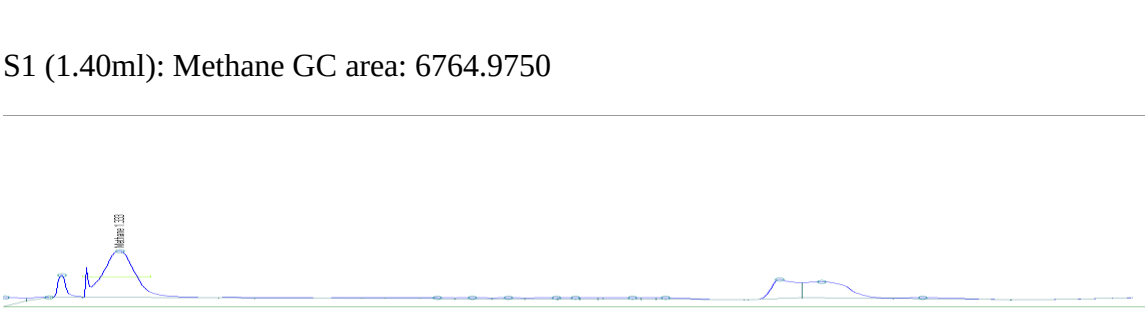
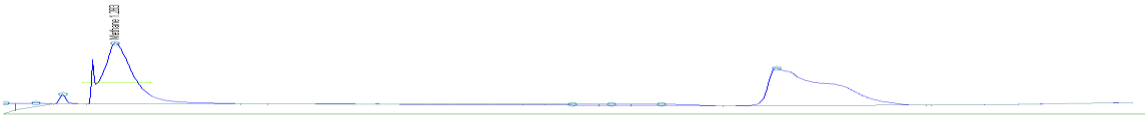


Figure K1: GC chromatograms of offal and sludge on 28th day of processing

Figure K1 continued

S2 (1.30ml): Methane GC area: 6617.9440

S3 (1.35ml): Methane GC area: 7926.1740



S4 (1.35ml): Methane GC area: 7588.3380

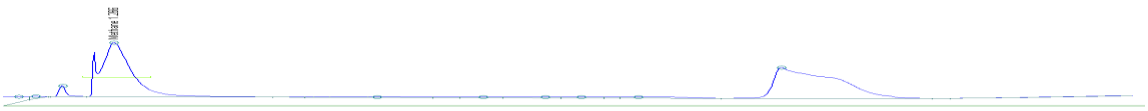


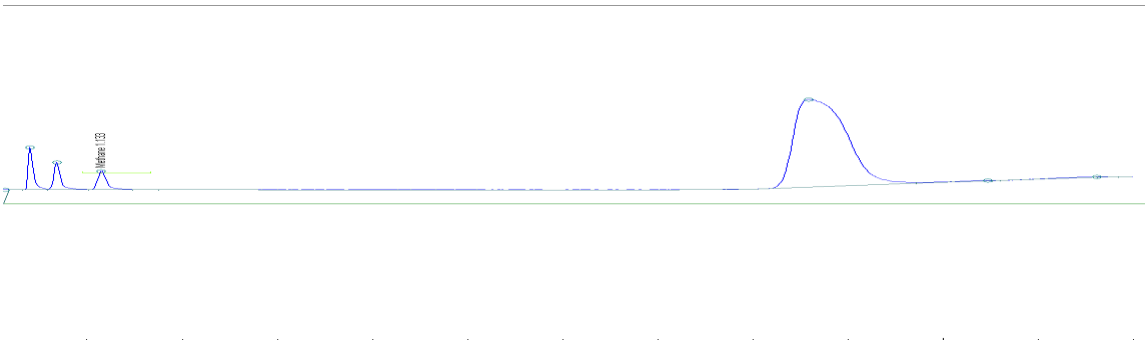
Figure K1 continued

S5 (1.35ml): Methane GC area: 7743.0660

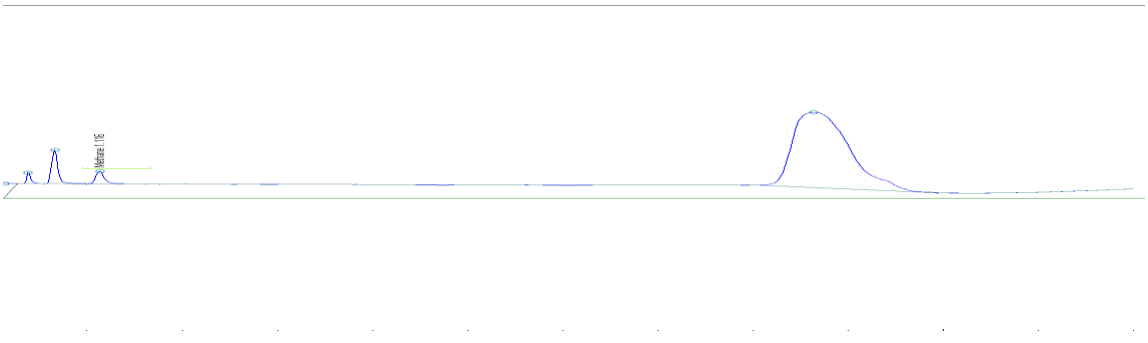
S6 (1.20ml): Methane GC area: 7120.9010

APPENDIX L: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON 30TH DAY

O1 (0.3ml): Methane GC area: 707.8020



O2 (0.3ml): Methane GC area: 461.4100



O3 (0.5ml): Methane GC area: 196.9610

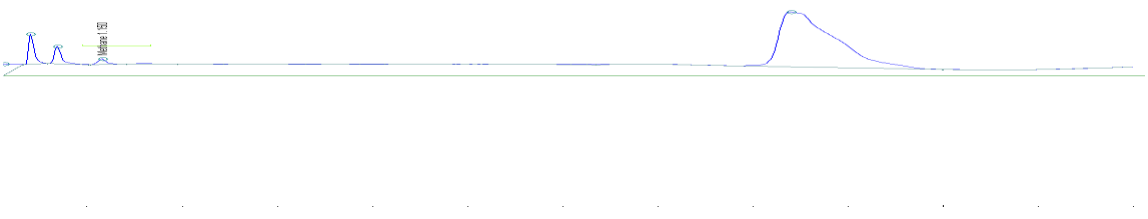


Figure L1: GC chromatograms of offal and sludge on 30th day of processing

Figure L1 continued

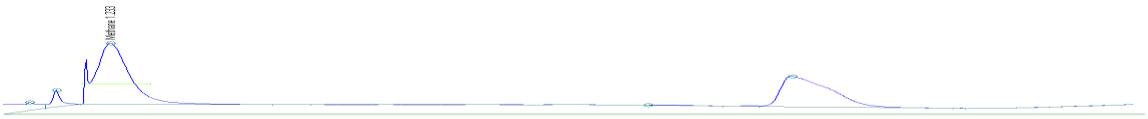
O4 (0.5ml): Methane GC area: 152.5230

O5 (0.5ml): Methane GC area: 139.9040

O6 (0.5ml): Methane GC area: 104.1680

Figure L1 continued

S1 (0.52ml): Methane GC area: 9112.2430



S2 (0.51ml): Methane GC area: 9428.7670

S3 (0.515ml): Methane GC area: 12577.2960

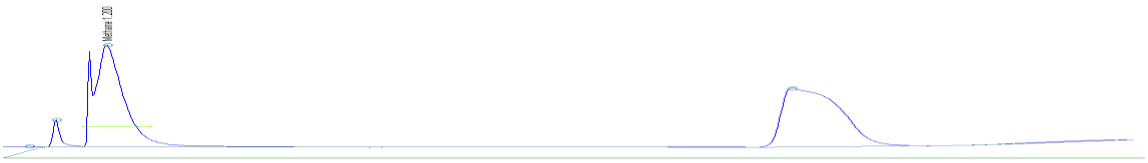


Figure L1 continued

S4 (0.51ml): Methane GC area: 12353.5760

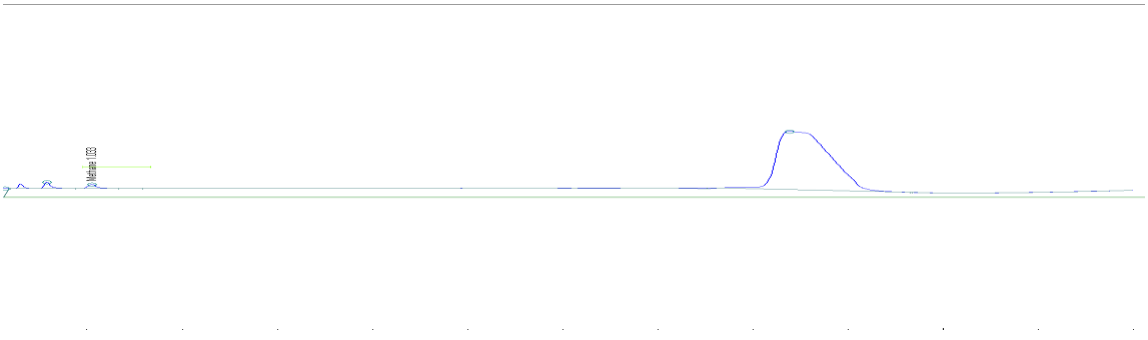
S5 (0.51ml): Methane GC area: 9305.8250

S6 (0.3ml): Methane GC area: 8715.5675

APPENDIX M: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON 32TH DAY

O1 (0.5ml): Methane GC area: 1053.5900

O2 (0.5ml): Methane GC area: 146.4510



O3 (0.5ml): Methane GC area: 113.2940

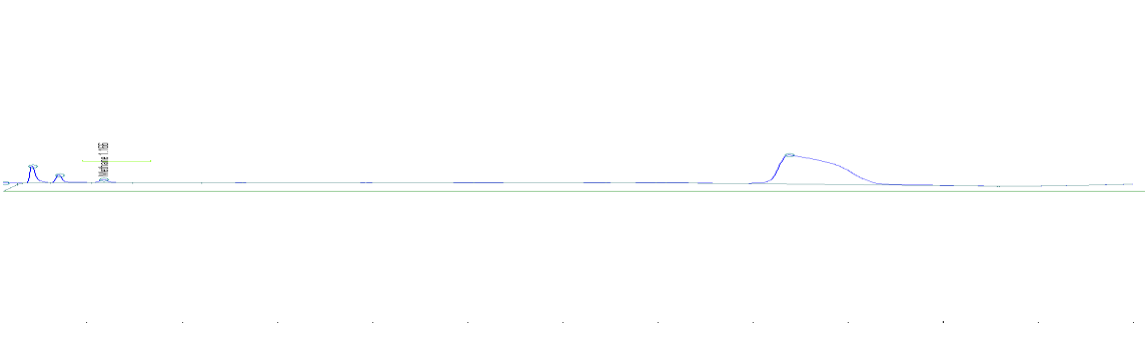
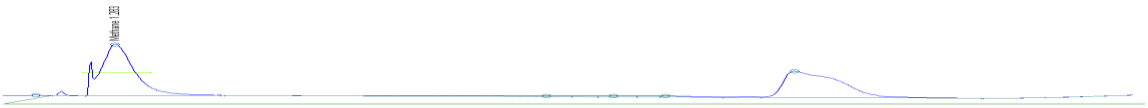


Figure M1: GC chromatograms of offal and sludge on 32th day of processing

Figure M1 continued

S1 (0.75ml): Methane GC area: 7247.0030



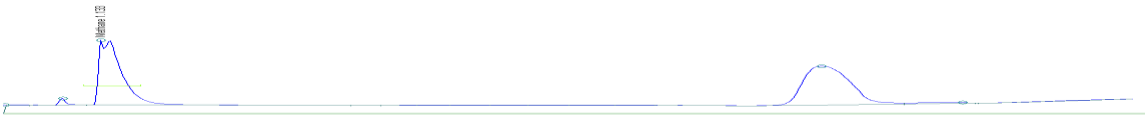
S2 (0.515ml): Methane GC area: 6685.5740

S3 (0.2515ml): Methane GC area: 6414.9895

Figure M1 continued

S4 (0.2515ml): Methane GC area: 6816.2510

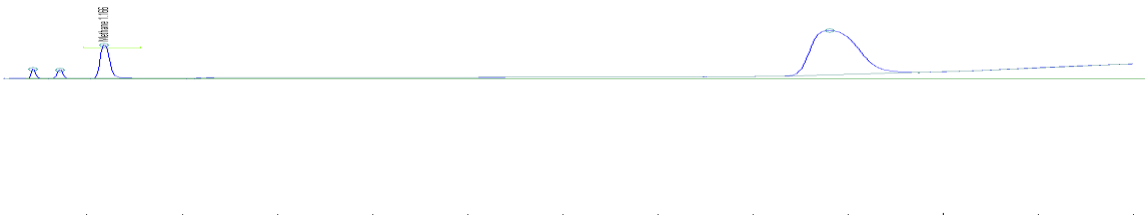
S5 (0.2515ml): Methane GC area: 7009.0520



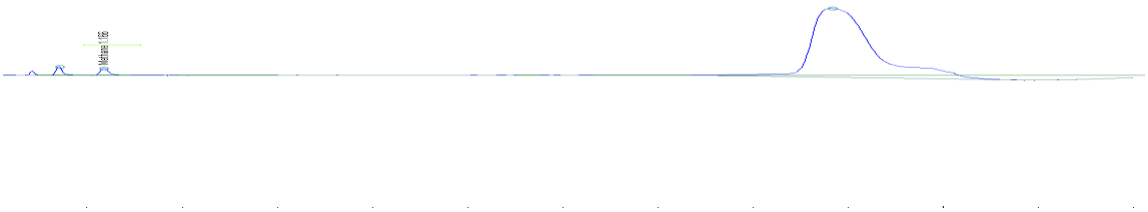
S6 (0.2515ml): Methane GC area: 7442.7960

APPENDIX N: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON 34TH DAY

O1 (0.275ml): Methane GC area: 1346.5160



O2 (0.25ml): Methane GC area: 250.8680



O3 (0.275ml): Methane GC area: 101.1570

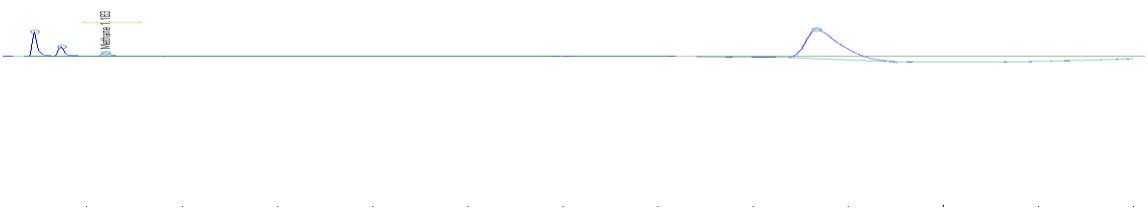
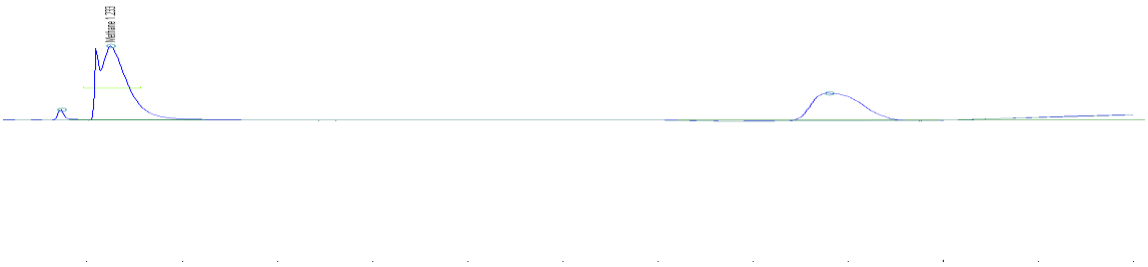


Figure N1: GC chromatograms of offal and sludge on 34th day of processing

Figure N1 continued

S1 (0.4375ml): Methane GC area: 7852.0985

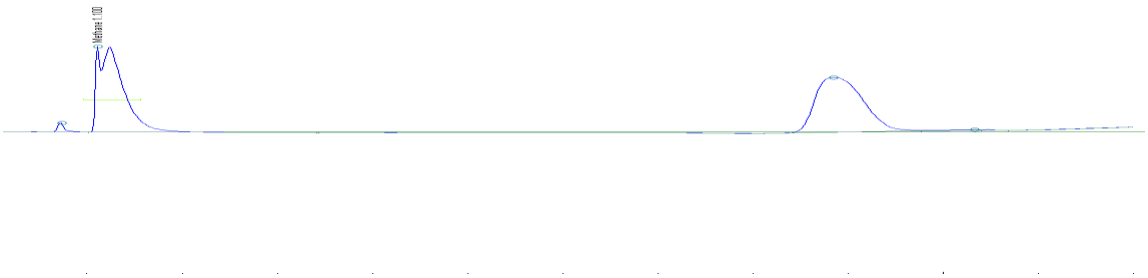
S2 (0.35ml): Methane GC area: 8688.2660



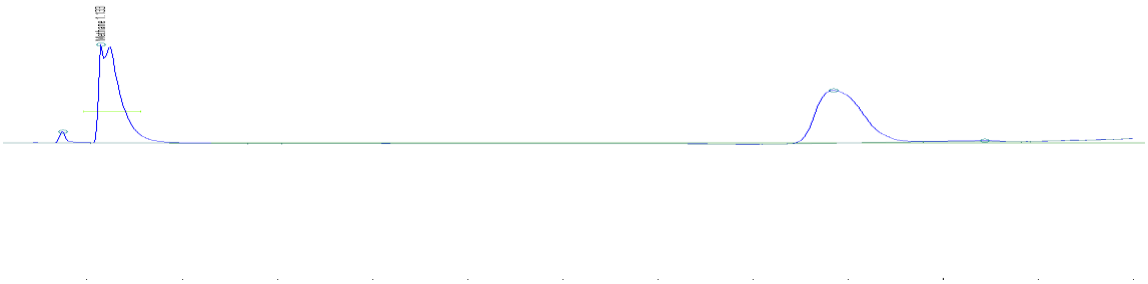
S3 (0.35ml): Methane GC area: 7626.4340

Figure N1 continued

S4 (0.35ml): Methane GC area: 8990.5860



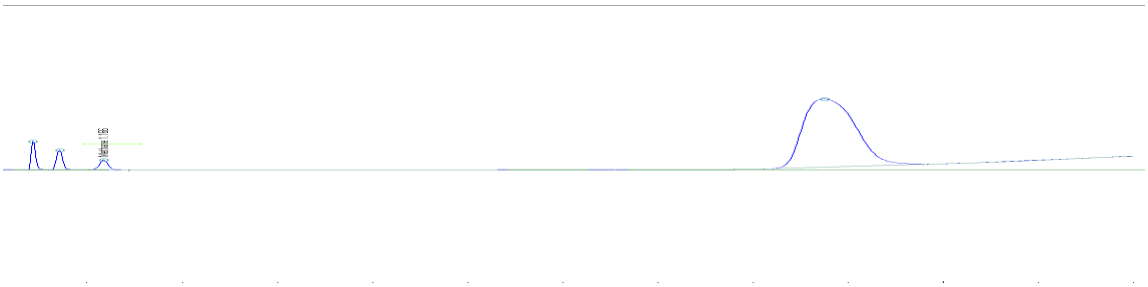
S5 (0.35ml): Methane GC area: 8595.0200



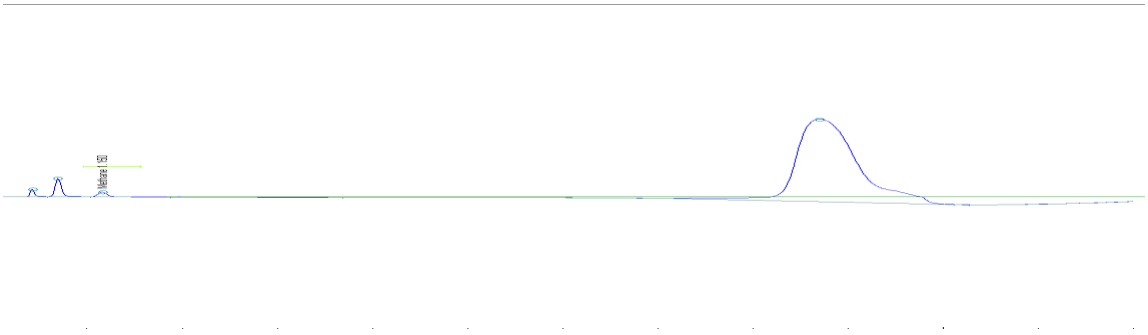
S6 (0.35ml): Methane GC area: 8979.2040

APPENDIX O: GC CHROMATOGRAMS OF OFFAL AND SLUDGE ON 36TH DAY

O1 (0.20ml): Methane GC area: 407.1360



O2 (0.20ml): Methane GC area: 181.2920



S1 (0.8ml): Methane GC area: 16139.1680

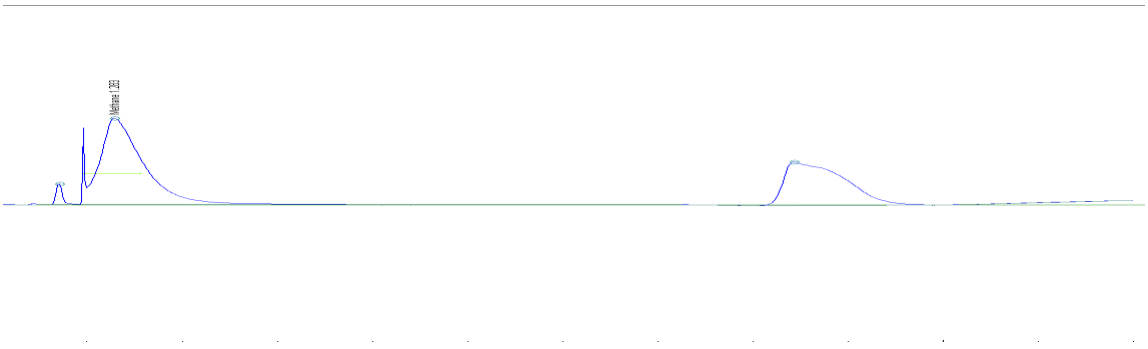
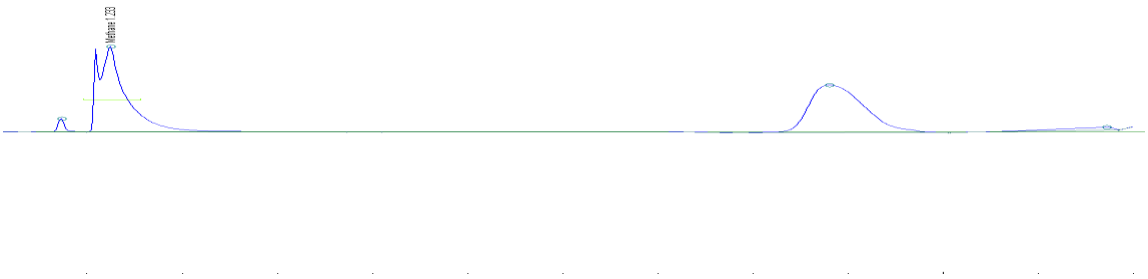


Figure O1: GC chromatograms of offal and sludge on 36th day of processing

Figure O1 continued

S2 (0.35ml): Methane GC area: 9525.8320



S3 (0.35ml): Methane GC area: 9752.90

S4 (0.35ml): Methane GC area: 9735.5630

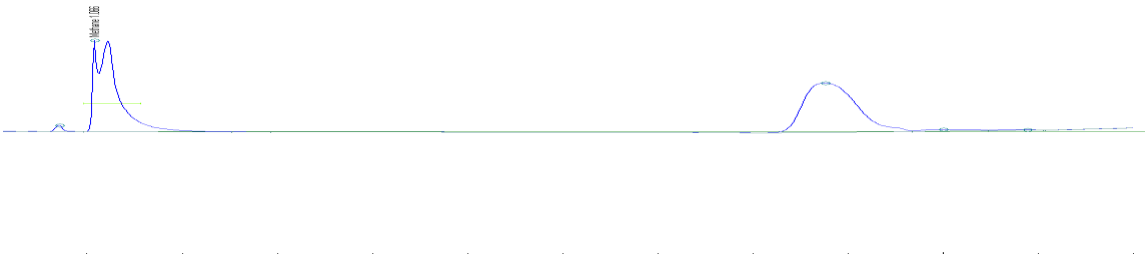


Figure O1 continued

S5 (0.3ml): Methane GC area: 7792.2680

S6 (0.3ml): Methane GC area: 11016.1200