

ASSESSING THE CO-PRODUCTION OF AMMONIA AND METHANE FOR
RESOURCE RECOVERY OPTIMIZATION

by
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ABSTRACT

The wastewater treatment is crucial to safeguard people and ecosystems from health and environmental risks. The treatment requires a significant amount of energy for their processes, and it could represent 30-40% of the total energy spent by municipalities. Moreover, the energy used in wastewater treatment plants is the most significant contributor to carbon emissions in the sector. However, wastewater has immense potential for recovering valuable resources such as methane and ammonia. The recovery of these resources could assist these facilities in offsetting energy expenses and carbon emissions, moving them towards net zero.

This research used microcosms to assess the co-production of methane and ammonia using different solids, including solids from a new technology for primary filtration called cloth media filters (CMF). Primary solids, secondary solids, and anaerobic digester sludge were sourced from 4 major water recovery facilities near Denver, and CMF solids from Mines Park Water Reclamation Facility.

The results showed that the co-production assessment is achievable in a single batch assay using solids concentrations equivalent to the ones used in the operation of real digesters, with similar methane fractions and ammonia in the same order of magnitude. The low C/N ratio was beneficial for accumulating ammonia in the centrate making it an interesting subject for ammonia recovery. For biogas, the pH of the microcosms was the most determinant factor in optimizing the methane fraction in the biogas. The Pearson test showed a positive correlation between pH and methane fraction, and the combinations with a pH higher than 7 had more methane content, following the optimum pH range for methanogenic *Archaea*. The addition of phosphate buffer to maintain the pH values close to neutral was also studied. Results suggest that higher concentrations caused a more significant reduction on the biogas yield, and the phosphate buffer also reduced the VS reduction of a few of the solids, especially the CMF. On the other hand, the final ammonia concentration increased with the addition of phosphate.

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LIST OF ABBREVIATIONS

Anaerobic Digester	AD
Boulder’s Anaerobic Digester Sludge.	AD _B
Boulder’s Primary Solids.	PS _B
Boulder’s Secondary Solids	SS _B
Chemical Oxygen Demand	COD
Cloth Media Filters	CMF
Colorado School of Mines	Mines
Dissolved Air Flotation	DAF
Greenhouse Gases	GHG
Metro’s Anaerobic Digester Sludge.	AD _M
Metro’s Primary Solids	PS _M
Metro’s Secondary Solids	SS _M
Mines Park’s Cloth Media Filter Solids...	CMF _{MP}
South Platte Renew’s Anaerobic Digester Solids	AD _{SPR}
South Platte Renew’s Dissolved Air Flotation Solids (Primary +SS)	DAF _{P+S}
South Platte Renew’s Primary Solids.	PS _{SPR}
South Platte Renew’s Secondary Solids.	SS _{SPR}
Total Nitrogen	TN
Water Resource Recovery Facility	WRRF
Water Wastewater Treatment Plant.	WWTP

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CHAPTER 1 INTRODUCTION

The treatment of wastewater is essential to remove nutrients and pathogens that, once discharged into the environment, can represent great health and environmental risks to humans and ecosystems. Despite its indispensable nature, wastewater treatment is energy-intensive, consuming considerable amounts of energy for various treatment processes.

The energy used in water and wastewater treatment can represent 40% of the total energy spent for some municipalities (1). Countrywide, the electricity consumption represents 3-5% of the total energy expenditure(2). The energy demand was estimated at 30 terawatt hours per year (3), totaling about \$4 billion in annual electric cost (considering a 14 cents kWh energy cost). Energy use is the most significant contributor of carbon emissions in WWTPs (4). It is estimated that wastewater treatment plants (WWTPs) are responsible for nearly 5% of the global non-CO₂ greenhouse gas (GHG) emissions and this is projected to increase by 22% by 2030(5). The energy requirements of these facilities are predicted to increase even more in the following years due to a higher water quality demand, growing population, economic growth, and an increase in the number of people with access to sanitation (6).

In 2020, 83% of the country's energy production—electricity generation and energy consumed directly—relied on petroleum, natural gas, or coal (Figure 1.1). The primary consumer sectors are industrial, transportation, commercial, and residential. The total greenhouse gases (GHG) emitted in 2022 was ~5.5 billion metric tons of carbon dioxide equivalents, about 1% more than the previous year due to an increase in the combustion of fossil fuels (7).

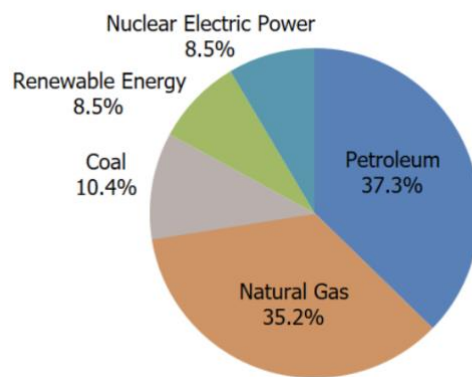


Figure 1.1 U.S Energy Consumption by Energy Source (Percentage) in 2022 (7).

The emissions of GHG by human activities have been pointed out as the major cause of climate change since the Industrial Revolution(8). The consequences of global warming (e.g., heatwaves, droughts, floods, and wildfires) have been reported worldwide, with last year being the warmest on

record, 1.45 ± 0.12 °C above the pre-industrial average (8). Even though there was a unanimous adoption of the Paris Agreement in 2015, this record temperature shows that ambitious actions are required toward decarbonization if nations still want to hold to the promise of keeping the temperature increase at less than 2 °C because, for now, we seem far off to limit the temperature increase to 1.5 °C above pre-industrial levels.

1.1 Recovery of Valuable Resources from Wastewater

Despite being energy-intensive, the wastewater treatment sector also holds immense potential for recovering valuable nutrients and resources, allowing the transformation of treatment facilities into key players in the water-energy-food nexus (9). Addressing challenges in sustainable water management, decarbonization, less dependency on fossil fuels, and recovery and reuse of valuables that otherwise would be discarded. It is estimated that around 9.72 kWh/m^3 of total energy is recoverable from the wastewater, offsetting the total amount of energy required for the operation of a conventional wastewater treatment plant (WWTP), which generally requires $0.3\text{-}0.6 \text{ kWh/m}^3$ (10). The total capacity is not yet understood because there is still an enormous potential to expand coverage and collection. Data from 2019 showed that about 4.5 million people still did not have access to a toilet or one with safe waste management (11).

The transition of wastewater treatment plants into water and resource recovery facilities includes economic and environmental benefits. WWTPs have the potential to become self-sufficient by implementing a cradle-to-cradle concept, in which waste becomes a raw material that can be transformed into resources (12) like biopolymers, cellulose, and bio-crude oil. They could also recover valuables to reuse in their processes such as recovery of volatile fatty acids (VFAs) to use as a carbon source to enhance biological nutrient removal (13), biogas for the generation of electricity and heat in a system called co-generation—offsetting the energy expenditure in the operation of the plants and selling the excess to the energy grid, and biohydrogen [14,15].

The International Fertilizer Association estimated that the nutrients present in wastewater could fulfill 13.4% of the global nutrient demand (16). New studies have been exploring the use of wastewater resources in novel and promising technologies. Such as the use of green ammonia as a hydrogen carrier for fuel cells (17), VFAs to produce sustainable aviation fuel (18), and green ammonia for production of fertilizers. Finally, as regulations tighten regarding the nutrient's discharge limits, investing in and developing technologies to recover these nutrients could be a strategy to stay within the limits and achieve net-zero costs.

1.2 Resource Recovery Technologies

Several new processes and technologies have been suggested to improve the conventional technologies and processes of WWTPs to minimize the overall impacts and increase process efficiency, offering more sustainable options (19).

Among the proposed technologies for advanced primary clarification is primary filtration, where filters replace the conventional primary clarifier. Diverting more organic carbon and nitrogen to the primary sludge sent to anaerobic digesters (AD) instead of aeration for energy recovery (20). A performance evaluation on the first full-scale primary filtration system stated that the total suspended solids removed achieved was 83-85%, compared to an average of 55-60% for conventional primary sedimentation (21).

Because advanced primary treatment technologies divert more solids to the digester instead of secondary treatment, they have advantages related to a reduction in energy requirement for aeration in the secondary treatment and an increase in biogas production and energy recovery in the ADs (22).

1.2.1 Cloth Media Filters

Cloth Media Filters (CMF) (Aqua-Aerobics Systems, Loves Park, IL) were designed to optimize solids removal in water and wastewater facilities with various particle sizes. The cloth media is made of woven fibers, and currently, there are four media technology options and five system configurations to tailor the system to the utilities' needs (23).

The system operates in three modes: filtration, backwash, and solids wasting. As water enters the filtration tank, the solids come in contact with the media and attach to the surface, forming a mat. The filtrate is collected inside the filter structure and discharged. As solids build on the surface of the filter and the water level rises, the backwash starts after reaching the height limit or time predetermined by the operator. The solids on the media are sucked out with the use of manifolds and a pressure pump. The heavier solids that settle in the bottom of the tank are discharged during solids wasting, and the operator also predetermines the frequency of this mode.

This new technology promises to reduce the constructed footprint area, increase the removal of turbidity and contaminants, reduce the electricity demand on secondary treatment and the volume of water used in backwashes, and improve the recovery of ammonia and biogas in the biogas (24). CMF configurations do not always require new constructions; they can be used to retrofit processes and enhance solids collection. If the technology delivers what it promises, it can be a valuable tool in process enhancement and energy savings.

1.2.2 Anaerobic Digestion

Biotechnological processes provide a cost-effective and flexible method for concentrating and converting waste and wastewater resources into valuable products (12). If biosolids had traditionally been seen as an unavoidable byproduct of wastewater treatment, now they are seen as valuable sources of nutrients such as nitrogen and phosphorus (25).

ADs are an old technology developed and utilized with the primary objective of stabilizing and reducing the volume of sludge to be disposed of. They have additional benefits such as lowering odor, pathogens, and vector attraction, which allow using sludge effluent as fertilizer in agriculture. Moreover, biogas is one of the by-products of AD and has always been a source of interest to researchers due to its potential energetic value. However, the early cases' reports of process instability and lack of research data may have caused it to seem unreliable.

As the urgency for more sustainable living practices becomes apparent and more information about the anaerobic digester process becomes available, the system is being given a second look at how it could help society offset the rising energy demand, reduce dependency on fossil fuels, and recover resources present inside the reactor such as ammonia, VFAs, and phosphate.

AD develops in four successive phases that depend on the microorganisms' interaction (26). Phase one is known as Hydrolysis; this phase is carried out by hydrolytic bacteria that break down complex organic matter into simpler molecules that cells can take up; carbohydrates turn into sugars, proteins into amino acids, and lipids into long-chain fatty acids. In phase two, called Acidogenesis or Fermentation, the products from hydrolysis are further converted by oxidation or fermentation into intermediate fatty acids, hydrogen, ammonia, and carbon dioxide by fermentative bacteria (27). The rate of this reaction is known for being faster than the other ones, which can cause the accumulation of VFAs and the failure of the reactor. Known as Acetogenesis, in the third phase, bacteria called acetogens convert the intermediate VFAs and alcohols into acetic acid, hydrogen (H_2), and carbon dioxide. Finally, the Methanogenesis phase is performed by two strictly anaerobe Archaea: acetoclastic methanogens convert the acetate into methane (CH_4) and carbon dioxide (CO_2), and hydrogenotrophic methanogens use H_2 as an electron donor and CO_2 as an energy source to produce CH_4 and water (Figure 1.2).

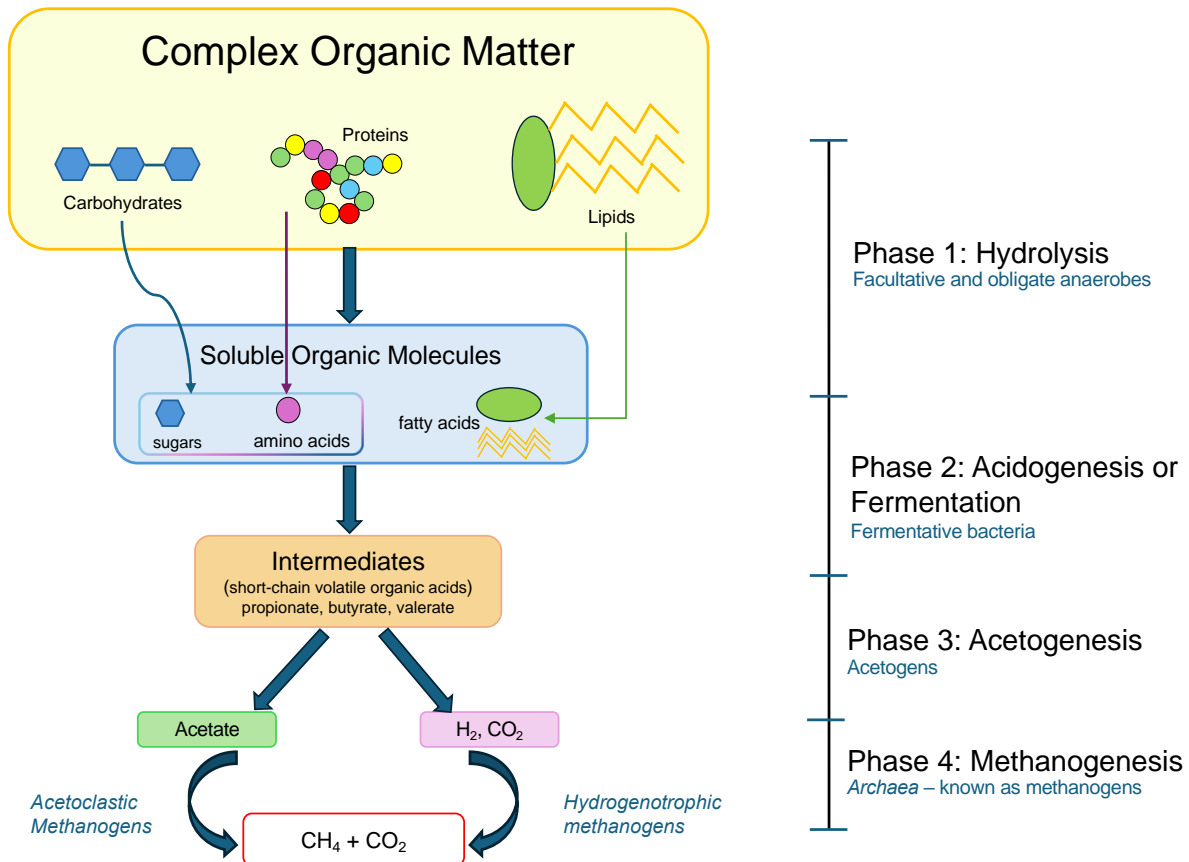


Figure 1.2 The anaerobic digestion is responsible for the oxidation and degradation of complex organic matter in monoatomic carbon, methane, carbon dioxide, and inert material. The digestion is performed by a microbial consortium of bacteria and archaea, and the process is divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

1.2.2.1 Biogas

By harnessing the energy content of biogas, treatment plants can implement combined heat and power systems to generate both heat for the digester and electricity for plant operations. This integrated approach maximizes the utilization of biogas, offsetting the external energy demands of the treatment process and reducing CO₂ emissions. The biogas is composed mainly of methane (50-75%), carbon dioxide (25-50%), and smaller traces of ammonia, hydrogen sulfide, and hydrogen. The methane fraction in the biogas is a result of a relationship between substrate and microorganisms (28).

1.2.2.2 Ammonia

The Haber Bosch is the primary process responsible for the production of synthetic ammonia; it combines nitrogen from the air with hydrogen from natural gas reforming (steam-methane reforming)(29). This process requires high pressure and temperature with the addition of a mostly iron-

based catalytic. This makes it a high-energy demand process with significant emissions of greenhouse gases (GHG).

The Haber-Bosch process currently produces more than 96% of the total ammonia produced (30). However, continuing this process as the primary source of ammonia and its derivatives is environmentally unsustainable. A renewable alternative to the Haber-Bosch process is ammonia recovery from municipal wastewater. Ammoniacal nitrogen ($\text{NH}_4\text{-N}$) naturally occurs in wastewater due to the degradation of nitrogenous organic matter such as urea, protein, and amino acids.

In the hydrolysis phase of the anaerobic digestion, 33% to 80% of nitrogenous material is degraded to ammonium nitrogen ($\text{NH}_4\text{-N}$) (31), making the digester supernatant, also known as centrate an ideal feedstock for nitrogen (N) recovery. It is estimated that the N content in the centrate of sewage sludge and livestock manure could account for 15 % of the global nitrogen fertilizer demand (32).

1.3 Aim of this study

The main objective of this study was to assess the potential of ammonia and biogas co-production using different solid sources, including solids, from a novel technology called CMF, which was designed as an alternative for primary clarification. Understanding how to optimize the production of ammonia and biogas can help water recovery facilities move toward a self-sufficient operation and offset costs. The solids were tested using bench-scale microcosms to mimic the anaerobic digestion conditions observed in real wastewater treatment facilities, and parameters considered to cause impact in ammonia or biogas, such as total nitrogen, carbon to nitrogen ratio, and pH, were used to evaluate the performance.

CHAPTER 2 MATERIALS AND METHODS

Bench-scale anaerobic digesters, comprising 160-milliliter glass bottles, were employed to replicate conditions observed in full-scale digesters. The solids ratio inserted into each bottle mirrored the proportions observed in some full-scale digesters. The microcosms were sealed with self-healing injection ports and crimp caps for recurrent gas pressure measurements (Figure 2.1).



Figure 2.1 The microcosms were assembled using 160 ml bottles, a self-healing injection port, and crimp caps to allow recurrent gas pressure measurements.

2.1 Substrate and Inoculum

2.1.1 Water and Resource Recovery Facilities

Samples were collected from plants of various sizes, operations, and solids characteristics to understand how and which solids characteristics impact ammonia and biogas co-production in AD. All four locations are the vicinity of Denver, Colorado, with two water and resource recovery facilities in the metro Denver area, one in Boulder, and Mines Park Water Reclamation Station (Mines Park) at Colorado School of Mines (Mines) in Golden, CO.

A CMF system (Aqua-Aerobics Systems, Loves Park, IL) designed to treat 5 GPM of raw wastewater was installed at Mines Park to study its performance as a primary treatment. This station is the wastewater laboratory from Mines and handles the wastewater mainly from the student's housing. From this location, only the CMF solids from the backwash were used in the experiments and it will be referred to as CMF Mines Park (CMF_{MP}).



Figure 2.2 Cloth media filter system installed at Mines Park water reclamation station.

Metro Water Recovery's (Metro) Robert W. Hite facility is the largest of the four plants, serving about 2.2 million people. It was designed to treat up to 220 million gallons of wastewater daily and currently treats and recovers 130 million gallons per day (MGD). Metro's anaerobic digestion is operated as two-phase reactors: acidic and methanogen. The hydraulic/solids retention time is about two days for the acidic reactor and 25 days for the methanogen and the digestion temperature is 38.5 °C. The anaerobic digester sludge used in the assembly of the microcosms used only sludge from the second digester, and it will be called (AD_M); other streams collected were Metro's primary solids (PS_M) and secondary solids (SS_M).

The Boulder Water Resource Recovery Facility (Boulder) treats 13 MGD and has a maximum capacity of 25 MGD. Boulder's two digesters are operated in series or parallel, each with a retention time of around 20 days and a temperature of 37 °C. The AD sludge was collected only from the second digester (AD_B), primary solids (PS_B), and secondary solids (SS_B).

South Platte Renew (SPR, Littleton, CO) currently treats 20 MGD, serving nearly 300,000 people. Unlike the previous plants, which concentrate the primary and secondary solids separately, the SPR plant mixes both streams before performing dissolved air flotation (DAF). Its digester operates in only one stage, at around 37 °C. The streams collected at SPR were primary solids (PS_{SPR}), secondary solids (SS_{SPR}), dissolved air flotation after mixing streams (DAF_{P+S}), and anaerobic digester sludge (AD_{SPR}).

Table 2.1 Digester's operation and characteristics by location.

Plant	Digester Type	Temperature (°C)	Solids Retention Time
Boulder	Two digesters used in series or parallel	37	22 days digester one 17 days digester two
Metro	Two-stage digestion	38.5	2 days hydrolysis/acidogenesis 25 days methanogenesis
South Platte Renew	Single digester	37	22 days

2.1.2 Solids Preparation

On the same day, samples were collected at each plant, and the streams were characterized by total solids (TS). It was crucial to match the solids concentrations according to each stream (primary, secondary, and AD sludge) in all the batches to approximate the amount of total solids inserted in each bottle and to achieve a similar headspace since the biogas was measured in pressure.

The total solids samples were prepared in duplicate. Instead of measuring the samples according to their volume, they were weighted using an analytical scale, and total solids were measured in a dry/wet content by weight. The duplicates were placed in the drying oven at 105 °C, remaining overnight to ensure a constant weight was achieved. The samples were placed in the furnace at 550 °C the following day for one hour; therefore, the volatile solids were also measured.

The final concentration of primary and secondary solids inserted in the bottles was based on the concentration used in feeding full-scale digesters: primary solids 5%, secondary solids 4%, and AD sludge was concentrated to 6-7% because of the limited working volume. To achieve the same concentration for all the batch experiments, some samples had to be concentrated using a centrifuge.

After all the solids' concentrations were matched to the desired values, wet chemistry was performed in each of the samples to document values of total nitrogen, soluble total nitrogen, chemical oxygen demand (COD), ammonia, alkalinity, and volatile acids (VFAs) using HACH test-in-tube kits (HACH, Loveland, CO). The solids were stored in a refrigerator until the day they were inserted in the bottles to reduce the microorganisms' activities and the rate of biodegradation and transformation of the solids. All the microcosms were assembled within five days of collecting samples to minimize biological degradation.

2.2 Microcosms Assembly

The microcosms were assembled in triplicates using a dry/wet mass ratio of two parts substrate and one of inoculum (2:1). For this paper, DAF_{P+S}, primary solids, and secondary solids are the substrates, and

AD sludge is the inoculum. A primary solid (PS or CMF) was always paired with a secondary solid, but if DAF was used as substrate, it was used only DAF + inoculum since DAF is already a mix of primary and secondary solids.

It was pre-determined that all the bottles would use 2.4 g of solids, 0.8 g each stream if using PS+SS+AD or 1.6 g DAF_{P+S} + 0.8 g AD. A variation of ±10% on the substrate was also tested to evaluate whether this change would impact the results (Figure 2.3).

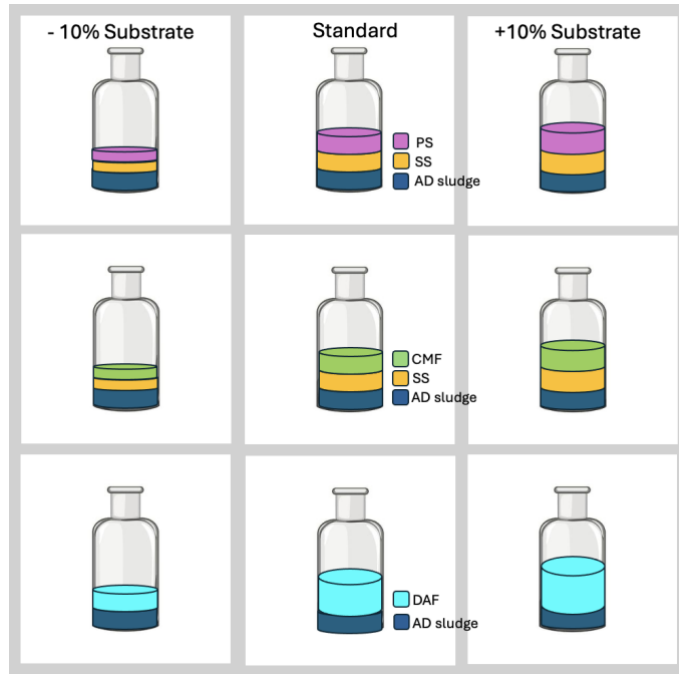


Figure 2.3 Visual representation of the different combinations of microcosm assembled. The substrate is composed by a mixture of primary sludge (PS or CMF) and secondary sludge, or DAF alone for the SPR location since the DAF itself is already a mixture of primary and secondary solids, and inoculum is the AD sludge. The ±10% variation was performed only in the substrate, maintaining the same mass of inoculum.

Different from other studies, the glass bottles were not flushed with nitrogen gas prior to sealing them, since the oxygen existing in the headspace was insignificant when compared to the chemical oxygen demand. This conclusion was a result of the calculation using the constant of the gases' equation, atmospheric pressure in Golden – Colorado, temperature of 20 °C, and 21% O₂ in the air:

$$p V = n R T \quad (2.1)$$

2.3 Operational Conditions

The bottles were placed inside the Isotemp Incubator 6845 (650D) (Fisher Scientific, Hampton, NH) to maintain a constant temperature of 38.5 °C for 20 days. All the combinations were also placed on top of a standard shaker table, model 3500 (VWR, Radnor, PA), at around 400 rpm. Halfway through the incubation period (10 days), one sacrifice bottle was opened, and measurements of ammonia and pH were taken.

2.4 Sampling and Analytical Procedures

2.4.1 Rate of Biogas Production

Due to the limited space inside the incubator, the biogas produced by each bottle was measured using a Digi-Sense digital pressure gauge (Cole-Parmer, Court Vernon Hills, IL). The pressure was measured in psi and converted to the ratio of biogas produced by dividing the biogas pressure by the time the bottle had been in the incubator since the beginning of the experiment's batch.

2.4.2 Carbon and Nitrogen

Carbon and nitrogen are important nutrients required in biological reactions. Carbon is essential for cellular growth and reproduction, and nitrogen for cell synthesis. Total and soluble nitrogen and chemical oxygen demand (COD) were analyzed in each of the solids' streams as they were inserted in the bottles using Hach test-in-tube kits (HACH, Loveland, CO). Samples from each solid's stream were freeze-dried using Free Zone – Freeze Dry systems (LABCONCO, Kansas City, MO). The carbon and nitrogen in the solids were sent to Colorado State University – CSU SPUR TERRA laboratory to be analyzed in terms of carbon/nitrogen ratio, organic carbon, nitrate, and ammonia by dry combustion (1,100 °C) in an elemental analyzer, Vario EL cube (Elementar, Germany).

2.4.3 Ammonia

Ammonia concentrations were measured at four different times throughout the experiment. All tests were performed using ammonia test-in-tube kits (HACH, Loveland, CO) with varying detection ranges. Due to the thickness of the samples, dilutions were required for ammonia analysis. Using an analytical scale, ten (10) and twenty (20) times dilutions were done by the mass ratio of solids and deionized water.

The first ammonia test was performed after the samples had been centrifuged and their concentrations matched the desired values. The following test happened when the three feed seeds were combined and

homogenized before inserting them into the bottles; this allowed us to document the initial concentration in each combination bottle and use it as a base value for comparison on how much ammonia was produced during incubation. Ten to eleven days after the microcosm had been assembled, one sacrifice bottle was opened, and ammonia concentration was measured. Finally, the final concentration was taken at the end of the 20 days.

An ammonia stock solution (100 mg/L) was prepared using potassium nitrate; this known concentration solution was used to ensure that the benchtop visible spectrophotometry DR 6000 (Hach, Loveland, CO) was calibrated and read the correct concentration. The vials with the 100-ppm stock solution were run every six samples, and all the measurements were below a 5% error.

2.4.4 Volatile Solids Reduction

Total solids were obtained using aluminum dishes, a dissector, and an analytical scale; the samples were done in duplicates and dried at 103/105 °C overnight so a constant weight could be achieved. After the values of total solids were measured, the samples were placed in the furnace at 550 °C for one hour for the volatile solids.

The volatile Solids Reduction was calculated using the Van Kleeck Method:

$$\% VSR = \frac{VS_f - VS_b}{VS_f - (VS_f * VS_b)} * 100 \quad (2.2)$$

VS_f = Fractional volatile solids of raw biosolids fed to the digester, kg/kg

VS_b = Fractional volatile solids of digested biosolids, kg/kg

2.4.5 Gas Chromatography

At the end of each experiment, the gas composition was analyzed using a Gas chromatogram—thermal conductivity detector (GC-TCD) TRACE 1310 Gas Chromatogram (Thermo Fisher Scientific, Waltham, MA) and capillary column Carboxen 1010 PLOT (Sigma-Aldrich, San Luis, MO) with helium as the carrier gas.

The methane content in the biogas was quantified in percentage by volume. A calibration curve was created every time (day) the samples were analyzed to avoid possible variations in the machine. The calibration curve was made by injecting gas mixtures of different concentrations by volume; for example, 70% methane and 30% carbon dioxide, 50% methane and 50% carbon dioxide, and 40% methane and

60% carbon dioxide. Gas standard mixtures were created using high-purity methane (99.5% volume) and carbon dioxide (99.99% volume) (GASCO, Oldsmar, FL).

CHAPTER 3 RESULTS AND DISCUSSION

Batch testing has mainly been employed in AD studies to understand biodegradability, inoculum activity, and inhibition (33). The advantages of batch tests lie in their ability to simultaneously compare different solids sources over a short time frame, eliminating the acclimation period required in large-scale digesters. Moreover, it allowed the utilization of the same batch of collected samples for multiple microcosm combinations, avoiding changes in the characteristics of solids within each collection.

While most studies have focused on ammonia inhibition, this paper focused on achieving higher ammonia production for subsequent recovery. The values for ammonia and biogas achieved with the microcosms were compared with those corresponding to their respective water and resource recovery facilities (Table 3.1).

Table 3.1 Comparison of the data shared from the wastewater treatment plants AD to the ammonia and methane content values in the biogas achieved with the microcosms.

	Water and Resource Recovery Facility		Microcosms	
	NH ₄ -N (mg/L)	Methane fraction (%)	NH ₄ -N (mg/L)	Methane fraction (%)
Boulder	Low 1200 Highs 1600	59.5±2.5	Low 1800 Highs 1900	31.8±0.96
Metro	Low 1300 Average 1600 Highs 2100	62.5±0.5	Low 1600 Highs 1900	67.5±2.5
South Platte Renew	Low 1100 Average 1300 Highs 1500	61±1	Low 1400 Highs 1800	49±18

The values were not expected to be identical because an assay is different from real operation. While the microcosms are batch tests, with the substrate added only at the start of the experiment, full-scale digesters at the WRRFPs operate in semicontinuous feeding and discharge, with a different organic loading rate and retention time. The ammonia concentration for Metro bottles achieved values within the observed at the plant and higher values for the other two plants, but the values were in the same order of magnitude and as long as the pH was maintained above 7, the methane fraction was similar to the observed in WRRFs facilities.

3.1 Ammonia

The $\pm 10\%$ variation in the amount of substrate inserted in the microcosms proved insufficient to provoke an increase or decrease in the ammonia concentration achieved in the bottles (Figure 3.1). For each one of the locations and the solids source compared, the concentration in (mg/L) did not follow a trend, suggesting that, at least in these small setups and working mass, higher variability in the substrate is necessary before an impact can be observed.

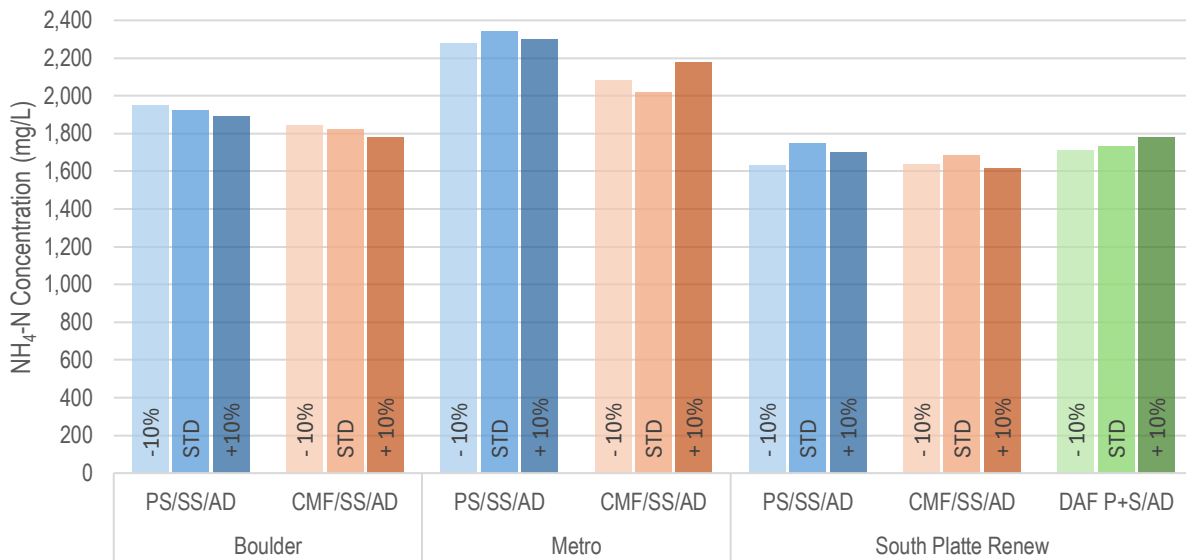


Figure 3.1 Final ammonia-nitrogen (mg/L) concentrations achieved in the microcosms (mg/L) organized by solid source and location. Blue bars are the combinations using primary solids (PS + SS + AD), orange bars are the CMF solids (CMF + SS + AD), and green bars are the DAF combinations (DAF + AD). The numbers near the bottom of the x axis indicate the $\pm 10\%$ variation on the substrate added. For Metro, the plotted values had phosphate buffer added to them while the other locations didn't have buffer.

The significance of the variabilities of concentration values within the same group was tested using the concepts of mean, standard deviation, and coefficient of variation (CV). If the CV value is below 10%, the standard deviation is considered not significant compared to the mean, and the values are considered relatively consistent. For all the groups of solids in all the locations, the CV values were below 2% indicating that the values for ammonia concentration for the substrate variations presented consistent values.

$$\text{Coefficient of variation (CV)} = \left(\frac{\text{Standard Deviation}}{\text{Mean}} \right) \times 100 \quad (3.1)$$

Metro and Boulder’s batch experiments achieved a higher final ammonia-nitrogen concentration and more ammonia was released when compared to how much ammonia the bottles started and end up with. Boulder microcosms released 1,400-1,500s mg/L of ammonia, Metro released 1,300-1,400s mg/L and South Platte Renew around 1100 mg/L (Figure 3.2).

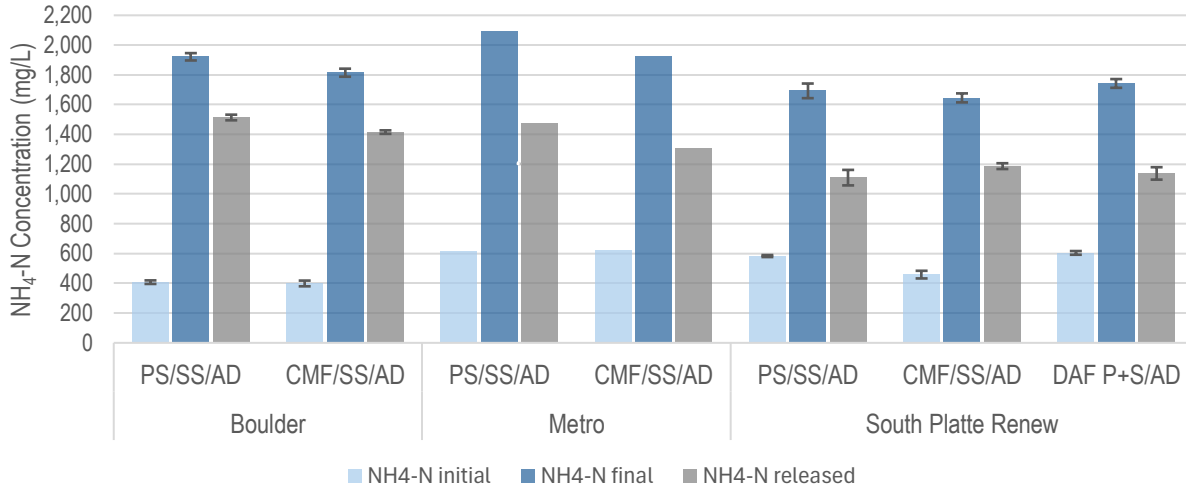


Figure 3.2 Ammonia concentration variability (mg/L) during the incubation period of the microcosms, organized by location and solids source. The values used in this graph were the average of three values with the standard deviation represented by the error bars on top of each series with exception of the Metro’s location data which did not have triplicates.

All the batches were incubated for 20 days and maintained at the same experimental conditions: mesophilic digestion at 38.5 °C, and the pH ranged between 5.8 and 7.3, depending on the batch of solids. While the methanogenesis stage of the anaerobic digestion process requires an optimum pH range for the activity of the methanogenic *Archaea* and production of methane, the pH range didn’t affect fermentation and ammonia production.

The Carbon and nitrogen ratio is an important parameter for the operation of anaerobic digester. A balance of these two nutrients supports the reactor’s performance, increases biogas generation, and controls the digester’s ammonia production. The C/N ratio for each solid combination was calculated using the following equation:

$$C/N = \frac{W1 * C1 + W2 * C2 + W3 * C3}{W1 * N1 + W2 * N2 + W3 * N3} \quad (3.2)$$

where,

W1, W2, W3 = VS weight in a single substrate in the mixture.

C1, C2 and C3 = Carbon content (g Kg⁻¹ VS).

N1, N2 and N3 = Nitrogen content (g Kg⁻¹ VS).

The values obtained for C/N ratio for each solid source, for the substrate, and the bottles' mixtures (primary sludge, secondary solids, and AD sludge) are presented in the following table in addition to total nitrogen (TN) values used to understand why South Platte Renew released less ammonia than the other two plants (Table 3.2).

Table 3.2 Carbon to nitrogen ratio and total nitrogen values to each microcosm's combination.

Microcosm	Boulder		Metro		South Platte Renew		
	PS/SS/ AD	CMF/SS/ AD	PS/SS/ AD	CMF/SS/ AD	PS/SS/ AD	CMF/SS/ AD	DAF _{p+s} / AD
C:N Substrate	8.08	7.98	5.17	15.36	6.77	7.72	7.97
C:N Mixture	7.54	7.44	5.35	8.65	6.62	7.36	7.54
TN	148	142	124	29	133	113	131
Substrate (mg)							
TN Primary substrate (mg)	40	34	120	25	42	21	
Total Nitrogen (mg)	192	186	189	95	159	139	157

The low C:N ratio for all the microcosm combinations reveals that the mixtures are nitrogen-rich, which causes ammonia to accumulate in the centrate, making it an interesting subject for ammonia recovery. In any of the microcosms, the accumulated ammonia caused inhibition of the process. The total nitrogen and the C:N ratio support the idea that the combinations using primary solids release more ammonia than those using CMF in the same batch because they have more nitrogen in the solids.

An intriguing result here, is that the CMF combination at Metro had half of the total nitrogen of the combination using primary sludge and still released a similar ammonia concentration. Additional data added to Appendix A (A.1) shows that 97% of total nitrogen in the primary solids is organic nitrogen, but a further study into the bio-accessibility of organic nitrogen is necessary.

Regarding the ammonia release from the CMF solids compared to the conventional primary, the conventional primary values are statistically different from CMF, meaning they released more ammonia than the CMF solids at Boulder and Metro. An independent student t-test was conducted to compare both groups, with a t-value of $t(4) = 4.14$, $p = 0.014$ (two-tailed). and Metro's location t-value of $t(6) = 2.45$, $p = 0.050$ (two-tailed), and both solids release are statistically the same for South Platte Renew using t-test with $t(4) = 1.16$, $p = 0.31$ (two-tailed).

Even though the results demonstrate that CMF and conventional primary are statistically different, it is important to remember that the final ammonia concentrations were measured using ammonia test kits. Because these kits have a limited detection range, a 20-times dilution was performed on the samples. A 1% dilution error can be translated to a 5% difference in the final result. The CMF and primary ammonia concentrations were different in 10% or less, thus more replicates would have to be performed, or a different detection method be used to affirm that there is a difference between these two solids.

3.2 Biogas

Biogas is typically composed of a mixture of methane (50-75%), carbon dioxide (25-50%), and nitrogen (2-8%) (34), with trace quantities of hydrogen sulfide, hydrogen, water vapor, carbon monoxide, ammonia, volatile organic compounds, and others. The methane fraction for all the microcosms are available in the next figure (Figure 3.3).

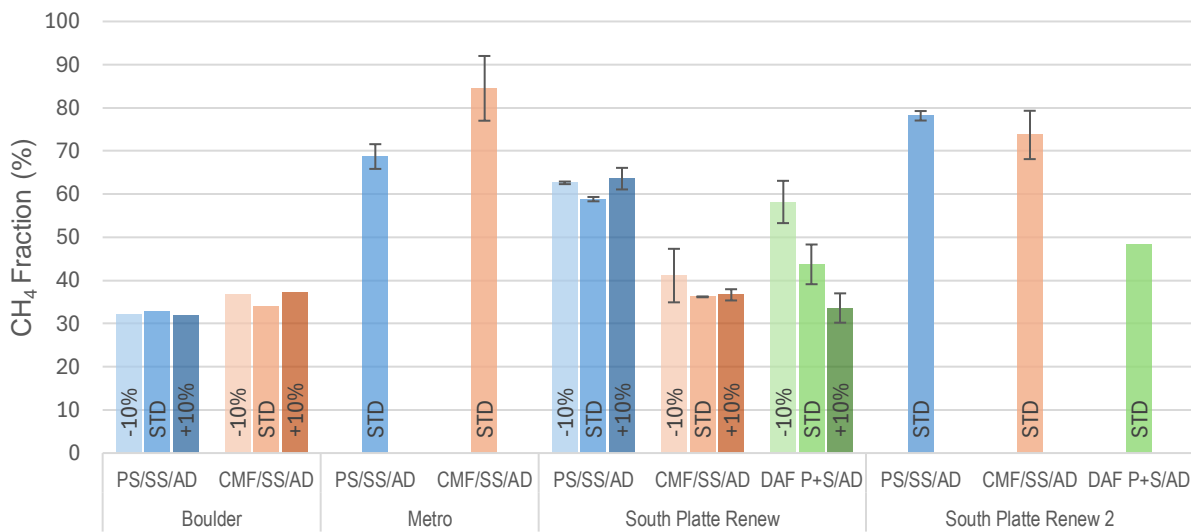


Figure 3.3 Methane fraction in the biogas comparing the combinations using the different substrates. All the combinations with a methane fraction below 50% were due to a drop in pH below neutral during the incubation period.

Preliminary experiments using South Platte Renew and Metro diluted solids didn't present a significant drop in pH values, which remained around 7. Therefore, the experiments were initially set without pH control or adding buffer/alkalinity. The results of the percentage of methane in the biogas showed that there is a correlation between methane fraction in the biogas and pH inside the reactor. In the microcosms combinations with pH 7 or above, over 50% of the volume of the biogas was methane, while

bottles with pH below 6.8 had around 30-40% of methane in their biogas composition, and between 6.8 and 7 there were different fractions (Figure 3.4).

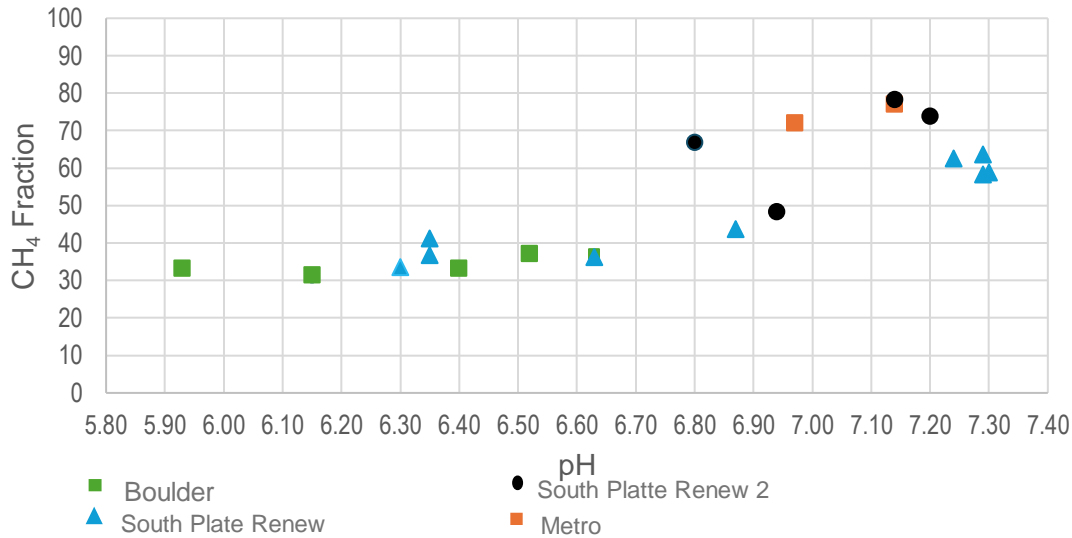


Figure 3.4 Methane percentage in the biogas in each bottle’s combination and the correlation with the pH of the sample. Bottles with pH-neutral or higher presented higher methane content (above 50%).

Through statistical correlation using Pearson’s t test, it was shown that there is a significant positive relationship between the methane content in the reactor and the pH value: $\alpha = 0.05$, $r(21) = 0.85$, and $\rho < 0.001$. Several other publications on methanogens and anaerobic digestion also stated the importance of pH values in maintaining methanogenic activity and methane production [27, 28, 29].

According to the authors (38), methanogens are known for having a narrow pH range of optimal operation 6.3-7.8, while in Angelidaki and Ahring (39) the majority of the methanogens have an optimum pH between 7-8. The inhibition of the methanogenic activity can occur through the accumulation of volatile fatty acids due to large inconsistencies in the feeding that may disrupt the balance of acidogenesis and methanogenesis (40). The rate of acidogenesis is generally fast, and if the uptake of acids, H₂, or CO₂ products during fermentation is slower or inhibited due to disruptions in the organic loading rate, temperature changes, or the presence of toxins, the pH drop may hinder or stop the methanogenic activity (41).

Even though the Boulder batch of microcosms had lower methane in the biogas due to a high organic loading rate and imbalance between acidogenesis and methanogenesis, the pH measurements during the experiment indicated that the pH dropped below six in the initial phase of the incubation, but it was slowly increasing, suggesting that the VFAs were being consumed and if incubation were longer than 20 days, a higher methane fraction could be achieved.

A theoretical C/N ratio value of 20 to 30 is pointed as a desirable value for biogas yield, as low ratios cause accumulation of ammonia and inhibition of methanogenesis, and high ratios mean quick depletion of nitrogen, also suggesting incomplete methanogenesis(42). As mentioned before, because part of this research is interested in maximizing ammonia production, a lower C/N ratio is desired for ammonia accumulation. At least in terms of methane fraction in the biogas, the low C/N ratios from the microcosms did not impact the methane yield in the biogas; as long as the pH was maintained above 7, all the microcosms had a fraction above 50%. A similar outcome was observed in three-year research using C/N ratios varying from 9 to 50 in the digestion of buffalo manure, all samples produced over 50% methane by volume (43) , suggesting that the C/N ratio of 20 to 30 might be more like a recommendation than a determinant factor on the results.

3.2.1 Biogas Increase

The biogas increase was accounted for by considering the pressure increases in the bottles and their methane fraction (Figure 3.5). Biogas leaks caused the challenge of accounting for the gas pressure in the bottles through the rubber septa. As the gas pressure increased and recurrent gas pressure measurements were taken by puncturing holes in the septa, some of the bottles lost biogas through these punctures.

To easily show which bottles had leaked, the following graph was plotted using the gas pressure measurement in two different days, day 7 and day 20 of incubation. The blue bars are the measurements on day 7, and the orange ones are on day 20. If the measurement on the last day was lower than on day 7, it means that there was a biogas leak.

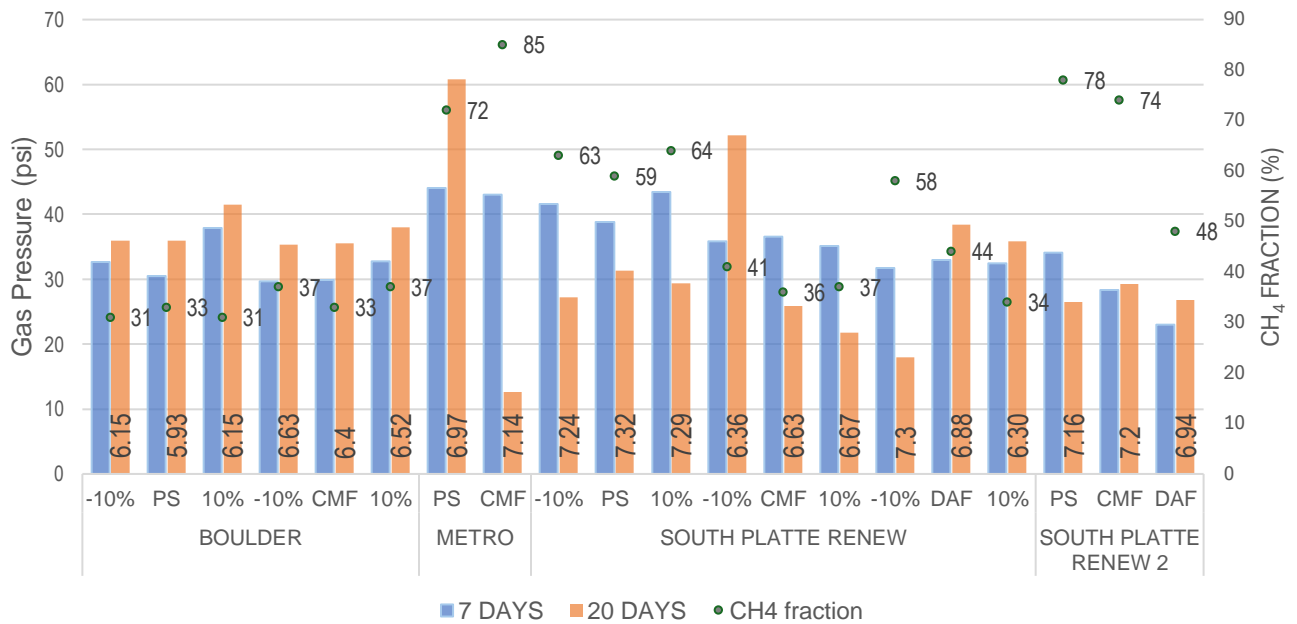


Figure 3.5 The biogas potential used the data from seven days and twenty days of incubation. The methane fraction below 50% happened in the bottles with pH below 7. The pH values are displayed on the bottom of the graph.

Gas pressure and the methane fraction data used together indicate that for the experiments performed, Metro produced a higher volume of biogas with a higher fraction of methane. Still, another batch for Boulder with a pH control would be recommended since its methane fraction was affected by the low pH value. The SPR and SPR2 experiments were conducted with different solids, collected on different days, and showed how the biogas yield can still vary within the same plant.

3.3 Addition of Phosphate pH Buffer

All the initial experiments were set up without adding pH buffer, and most of the bottles presented a pH drop that impacted the biogas production. Therefore, new solids were collected to perform a new batch with phosphate buffer. Otherwise, a wrongful conclusion could be drawn from the results stating that solids like Boulder were outperformed by the solids from the other locations.

The challenge in repeating the experiments to increase the alkalinity and buffer changes in the pH relied on the fact that new batches required the collection of fresh samples. Because the experiments used real wastewater instead of a synthetic mixture of nutrients, the characteristics changed every time. If batch one required a buffer, batch two did not always need it.

A new batch of bottles with solids from Metro with 30 mL⁻¹ phosphate buffer and a batch using 40 mL⁻¹ for SPR was placed in the incubator to fix the pH drop observed in the first batches. The

experimental plan was to buffer the solutions at pH 7.2, adding equal molars of phosphate mono and dibasic.

During incubation, the second batch for these two locations showed enough alkalinity, and the non-buffered bottles didn't have a pH drop. Meanwhile, the bottles with phosphate buffer presented a drop in pH value. The 30 mL⁻¹ phosphate buffer concentration added to the Metro's samples was shown to reduce the biogas yield. Still, the impact is lower than the addition of a higher concentration, 40 mM, to SPR samples when compared to bottles that did not have a buffer (Figure 3.5).

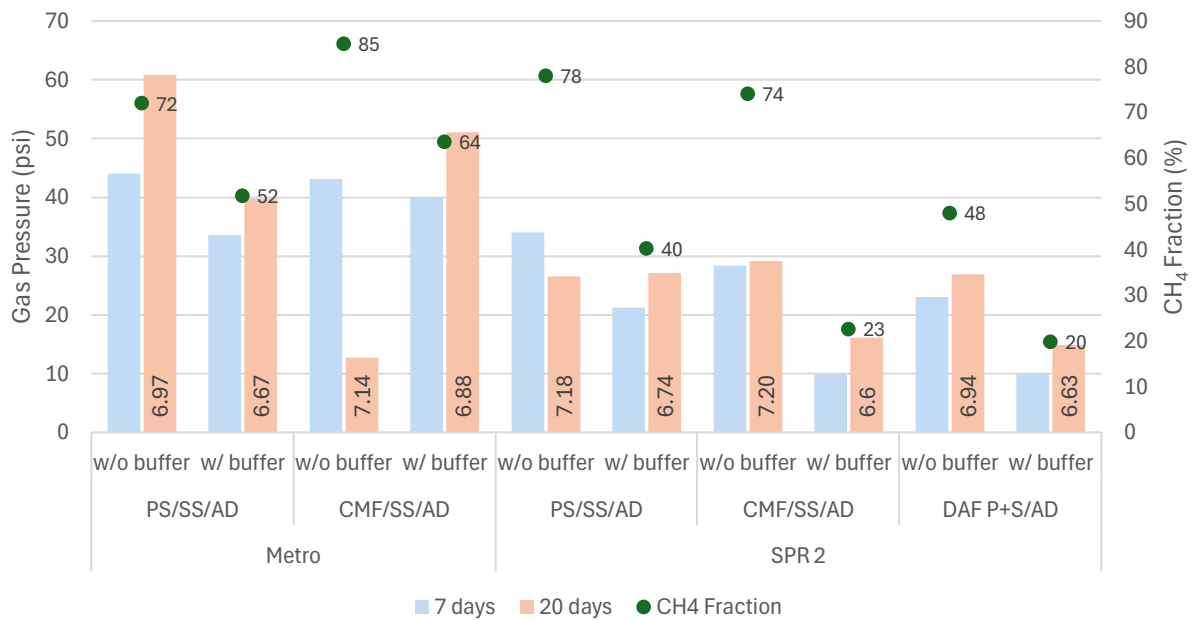


Figure 3.6 Differences in methane concentration in the bottles assembled with and without phosphate buffer. Metro microcosms utilized 30mM of phosphate buffer and SPR 40mM.

Because the buffered combinations' pH dropped below the optimum for the methanogenic, the question became whether the low methane fraction was only related to the low pH value, as with the unbuffered microcosms. Or if it could be associated with the addition of the phosphate buffer. To test these scenarios, a new pH and methane fraction graph was plotted using data only for the buffered bottles (Figure 3.7).

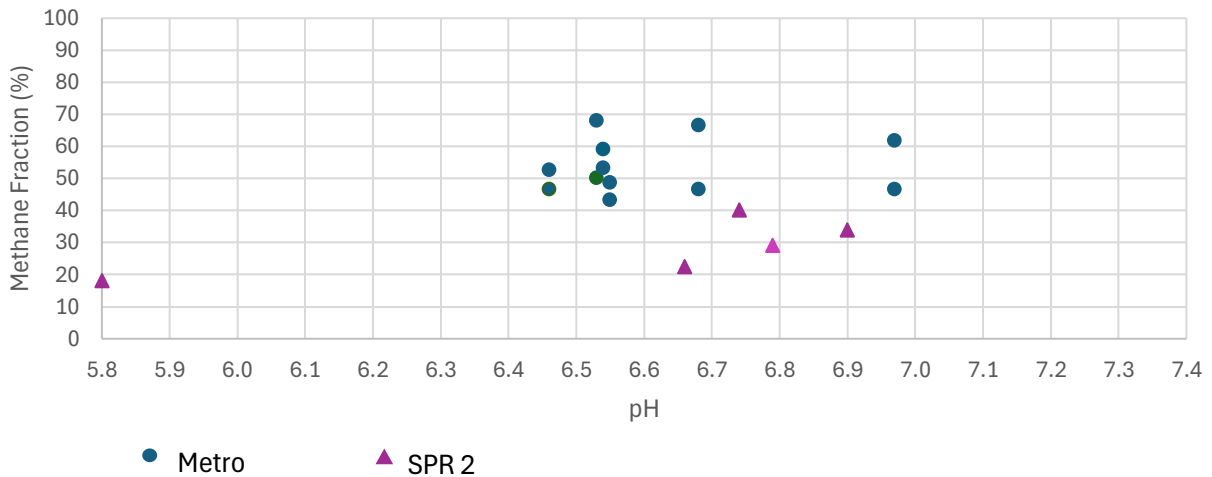


Figure 3.7 pH value versus methane fraction in the biogas for the buffered microcosms. The triangle markers represent the SPR2 batch in which 40mM were added to the microcosms, and the circle markers are the Metro's 30 mM buffered microcosms.

The buffered microcosms revealed that different from the unbuffered ones the correlation of pH and methane fraction in the biogas does not hold. The same Pearson's test was performed for the buffered samples, $\alpha = 0.05$, $r(17) = 0.28$, and $\rho > 0.05$.

The triangle markers represent the SPR3 batch in which the microcosms had a 40 mL⁻¹ of phosphate buffered added to them, while the circular ones are the Metro's microcosms with an addition of 30 mL⁻¹ of buffer. The fact the microcosm with a higher dosage of phosphate buffer had a lower methane content in the biogas could suggest that higher concentrations cause a further reduction in the biogas yield.

A study on the effect of phosphate on rice roots showed that a concentration higher than 20 mM was inhibitory to acetotrophic methanogens, impeding the conversion of acetate to methane. Small amounts of CH₄ were still observed, but due to the reduction of CO₂ (44). Another research studied the effect of different orthophosphate concentration in the digestion of anaerobic sludge. It concluded that smaller concentration help the digestion process by accelerating acidogenesis, acetogenesis and acetotrophic methanogens but further increase in orthophosphate (above 13.4mM) slow down these processes (45).

The effect of phosphate buffer in inhibiting CH₄ in anaerobic digestion was also observed in other studies using different seeds or conditions; thermophilic digesters using carboxymethyl cellulose(46), rice roots (44). Most of the inhibition was caused by a change in the acetoclastic methanogens. For example, it was observed a shift in the methanogens species with more hydrogenotrophic than acetoclastic; the first ones are more resilient to environmental conditions (high temperature, low pH, high ammonia), reduction in the abundance of or impact in the enzymes that participate in the acetoclastic

pathways (47). Depending on the type of substrate, about 70% of the methane in anaerobic digesters is produced from the oxidation of acetate (48).

Phosphate buffers are also responsible for precipitating trace elements such as selenium, cobalt, nickel, iron, molybdenum, and magnesium (49), which are required for biological growth or functions. Otherwise, other studies suggest that phosphate accelerated the biogas production by blocking the inhibition from high ammonia concentrations.

Adding phosphate buffer also decreased the reduction of volatile solids in a few of the microcosms. The solids used to assemble the bottles with and without buffer were the same, and the experiments were conducted in the same controlling environment. Therefore, the results show that adding phosphate buffer decreases the biodegradability of the solids, it appears to cause a greater impact on the CMF solids (Figure 3.8).

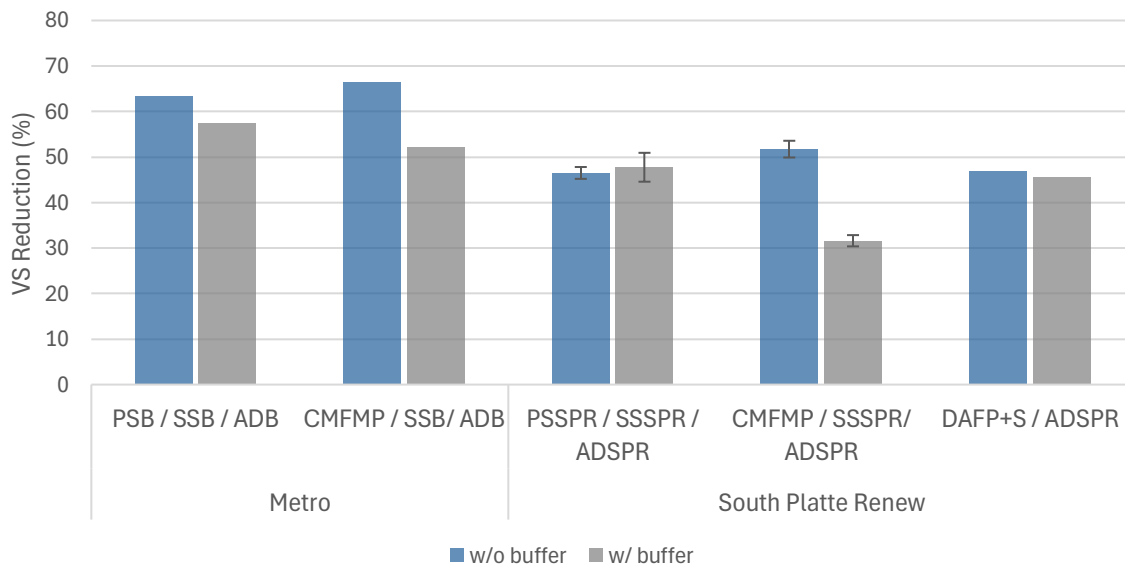


Figure 3.8 Volatile solids reduction comparison between microcosms with and without the addition of phosphate buffer.

If the addition of phosphate buffer reduced the methane fraction of the microcosm and the volatile solids reduction for some of the combinations. Regarding the ammonia concentration, the final $\text{NH}_4\text{-N}$ in mg/L was higher for the buffered bottles (Figure 3.9).

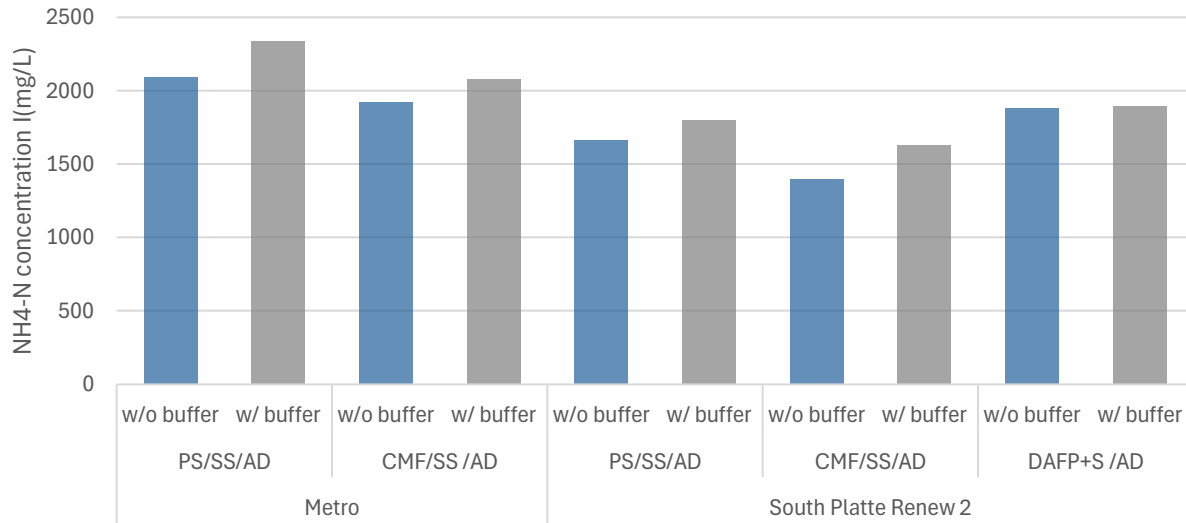


Figure 3.9 The addition of phosphate buffer increases the ammonia concentration in the microcosms (mg/L). The blue bars represent the unbuffered bottles, while the gray bars are the buffered bottles.

The mechanisms involved in the change of ammonia concentration during the anaerobic digestion process is not explored in this paper and it would require further research.

CHAPTER 4 CONCLUSION

This research focused on using a single-batch assay to assess the co-production of ammonia-nitrogen and methane using different solids sources. Optimizing the production of valuable resources embedded in the wastewater means higher recovery, which supports utilities in offsetting the demand for outside resources and becoming net-zero facilities. Microcosms have been extensively used in batch testing to understand different solids' degradation and methane potential. However, there is still a lack of research targeting the ammonia potential and how to assess methane and ammonia potential co-production.

This paper indicates that the co-production of ammonia and methane can be assessed in a single-batch assay using solids concentrations analogous to concentrations used in real anaerobic digesters at WRRFs. This research initiates a discussion and a method development with a matrix relevant to these facilities, with the batch results similar to the actual values.

Future directions of the research presented in this thesis should fix the procedure and analytical barriers encountered in this research. For example, increasing the head space in the bottles solves the gas leak issue. Finding another method to measure the ammonia concentration that doesn't require a significant dilution; dilutions may introduce errors and cause the reliability of the results to be questioned.

Since most of the combinations presented a pH drop using a substrate-to-inoculum ratio of 2:1 (mass basis), a ratio using less substrate is advised, so the control of pH could be done by changing the organic loading rate and not be dependent on buffer solutions.

Moreover, a study using different buffers and understanding how they impact the anaerobic digester process would have an outstanding contribution. As well as understanding how the phosphate buffer impacts the ammonia concentration and through what mechanisms.

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APPENDIX A ADDITIONAL MATERIALS

A.1 Laboratory Results for Carbon and Nitrogen Present in the Solids

Table A1. Laboratory results for carbon and nitrogen in the solids – divided by batch of experiment.

Location	Stream	Total C	Total N	Organic N	NH ₄ -N	NO ₃ -N	C:N
		----- % -----					
Boulder	Primary Solids	46.85	3.01	2.87	0.1372	0.0016	16
	CMF	47.13	2.86	2.65	0.2108	0.0017	16
	Secondary Solids	43.14	8.08	7.88	0.2036	0.0008	5
	Anaerobic Digester	36.69	6.4	5.47	0.932	0.0009	6
Metro	Primary Solids	37.61	8.24	7.99	0.25	ND	5
	CMF	38.11	2.71	2.64	0.07	ND	14
	Secondary Solids	9.93	0.53	0.42	0.11	ND	19
	Anaerobic Digester	38.14	6.71	6.5	0.21	ND	6
SPR	Primary Solids	44.02	4.08	3.73	0.3484	0.0007	11
	CMF	40.1	2.03	1.9	0.1313	0.0003	20
	Secondary Solids	39.27	7.93	7.49	0.4383	0.0006	5
	Anaerobic Digester	31.74	5.43	4.75	0.6801	0.0003	6
	DAF	42.09	5.28	4.81	0.4668	0.0006	8