FINAL REPORT: BIO TOWN AG BMP MONO-DIGESTION EXPERIMENT

Prepared by Jennifer Rackliffe

Technical advisors: Dr. Jiqin Ni and Dr. Nathan Mosier

Purdue University, Agricultural and Biological Engineering

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Contents

Introduction

This report describes the procedure and results from the manure biomethane potential (BMP) test conducted at Purdue University from April 5 to May 14, 2021. The objective of the experiment was to determine the methane potential and kinetic characteristics from eight feedstocks that Bio Town Ag (BTA) currently uses for anaerobic digestion. Physical and chemical characteristics of the feedstocks were also measured. The ultimate goal of this research is to determine how feedstocks behave during digestion when combined, specifically looking for any synergistic or antagonistic effects on methane yield, kinetics, or digester performance. This first experiment provides baseline information for each of the feedstocks digested alone (mono-digestion). Subsequent experiments may examine various combinations of feedstocks to support later optimization of BTA's operations and feedstock selection. For this experiment, the following feedstocks were tested:

1. Feedstock 01: Pad Manure - freshly collected manure from cattle pens as received to anaerobic digester pad (concrete pad close to digester)
2. Feedstock 02: Food Grade Starch – Off-spec starch used for food manufacturing
3. Feedstock 03: Slaughter Waste that runs through wastewater treatment at slaughter plant.
4. Feedstock 04: Dewatered waste activated sludge coming off Membrane Bioreactors – corn processing facility collects all processing waste and runs through onsite treatment plant.
5. Feedstock 05: Soapstock from glycerin refining
6. Feedstock 06: Food Waste - Filter press slurry from food grade water treatment facility; facility generates various cheeses, salad dressings, condiments
7. Feedstock 07: Food Waste – DAF from treatment plant that makes an assortment of snack-type foods high in oil & grease (potato chips/dips/etc.)
8. Feedstock 08: Ammonia recovery effluent

Overview of Experimental Procedure

- The tests were conducted using 30 1000-mL lab-scale anaerobic digesters at Purdue University.
- The anaerobic digestion was performed at mesophilic conditions of 101°F (38.3°C), using temperature-regulated water baths.
- For most digesters, the test lasted for 30 days, until daily biogas production a small fraction of the cumulative biogas production. Eight digesters (2 × Blank, 3 × Slaughter-house waste, and 3 × Soapstock) ran for 37 days to allow additional biogas production as they were still producing substantial amounts of gas.
- Each substrate was tested alone (mono-digestion) with inoculum in triplicate.
- A single co-digestion treatment was tested in triplicate that combined all eight feedstocks, where the ratios of each are proportional to the amounts of each feedstock used in the digesters at Bio Town.
- Controls included negative control (blank) inoculum-only digesters, tested in duplicate, alongside a single positive control digester (cellulose and inoculum).
- Many of the digesters were too thick with the initial ratios and 26 experienced foaming, expansion, or excessive gas production that resulted in overpressure of the digester. Dilutions were made during the experiment to prevent additional explosions. The final dilution is shown in Table 1.
- Table 1 contains details of the experiment, including both initial and final amounts of inoculum and feedstock. The working volume in all cases was 1000 mL, which means that the rest of the mass was made up by deionized water.
- Additional details regarding digester set up, intermediate dilutions and tear down are contained in Appendix A. This includes dates and reasons for the intermediate dilutions.

BMP Tests

Table 1: Experimental digester treatment details. Average weight percent refers to the mass of the inoculum or feedstock out of the total digestate mass (approximately 1000 g). Deionized water was used to make up the digester volume to 1000 mL.

Treatment	**Initial preparation (average wt %)**		**Final dilution (average wt %)**		**Number of digesters**	**Digester ID**
	% Inoculum	**% Feedstock**	**% Inoculum**	**% Feedstock**		
Blank	100	0	86	0	2	1-2
Cellulose control	83	9	56	6	1	3
F1: Pad manure	63	38	31	18	3	4-6
F2: Starch	72	24	51	17	3	7-9
F3: Slaughterhouse waste	59	35	16	9	3	10-12
F4: Waste activated sludge	38	38	25	25	3	13-15
F5: Soapstock	9	91	3	32	3	16-18
F6: Filter press slurry	63	37	7	21	3	19-21
F7: Food waste DAF	42	31	23	17	3	22-24
F8: AR effluent	20	80	20	80	3	25-27
Mixture	48	50 (F1: 20, F2: 2.7, F3: 3.7, F4: 4.7, F5: 4.9, F6: 1.8, F7: 2.9, F8: 10.5)	25	26	3	28-30

Sampling and sample delivery process

Purdue employees collected substrate and inoculum from Bio Town Ag. The inoculum was collected one week prior to the substrate collection to allow it to de-gas prior to the beginning of the experiments. No grinding of the samples was done as it was determined that the feedstock particle size would not be further reduced in the BTA digester.

Biogas collection and analysis

Biogas produced from each lab-scale digester was individually collected into a 3-L gas bag for the experiment. The gas bags were connected to each digester at all times during the test. The gas production was measured daily initially and subsequently as needed, based on the biogas production rate. The biogas volume in the bags was measured with a syringe.

The biogas compositions, including concentrations of methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S) in collected gas bags was measured with a BIOGAS 5000 Gas Analyzer (LANDTEC North America, Inc., Colton, CA). This analyzer has measurement ranges of the four gases as: CH_4 (0–100%); CO_2 (0–100%); O_2 (0–25%), H_2S (0–10,000 ppm) and balance (nitrogen) in biogas.

Results

Bio Town Ag Digestate Methane Potential

<u>Summary</u>

Approximately 7.6 L biogas/L raw digestate (5.6 L methane/L raw digestate, average 73.7% methane concentration) was produced over 45 days. More than 50% of the methane was produced during the first 7 days. All gas volumes are represented at standard temperature and pressure (32°F, 1 atm pressure).

<u>Measurements</u>

Digestate was collected from one of the BTA digesters seven days prior to the beginning of the BMP experiment to be used as inoculum in the BMP experiment. It was allowed to "de-gas" under controlled temperature conditions (38.3°C) for 7 days. Biogas volume and methane content was measured throughout this week-long period. At the end of the de-gas period, two "blank" digesters were set up with the de-gassed digestate (inoculum) only during the BMP experiment and were monitored for gas production volume and composition.

<u>Results</u>

Figure 1 shows the cumulative gas production during the course of the de-gas and BMP experiment. Approximately 7.6 L biogas/L raw digestate (5.6 L methane/L raw digestate) was produced over 45 days (7 days of de-gas followed by 38 days of BMP experiment).

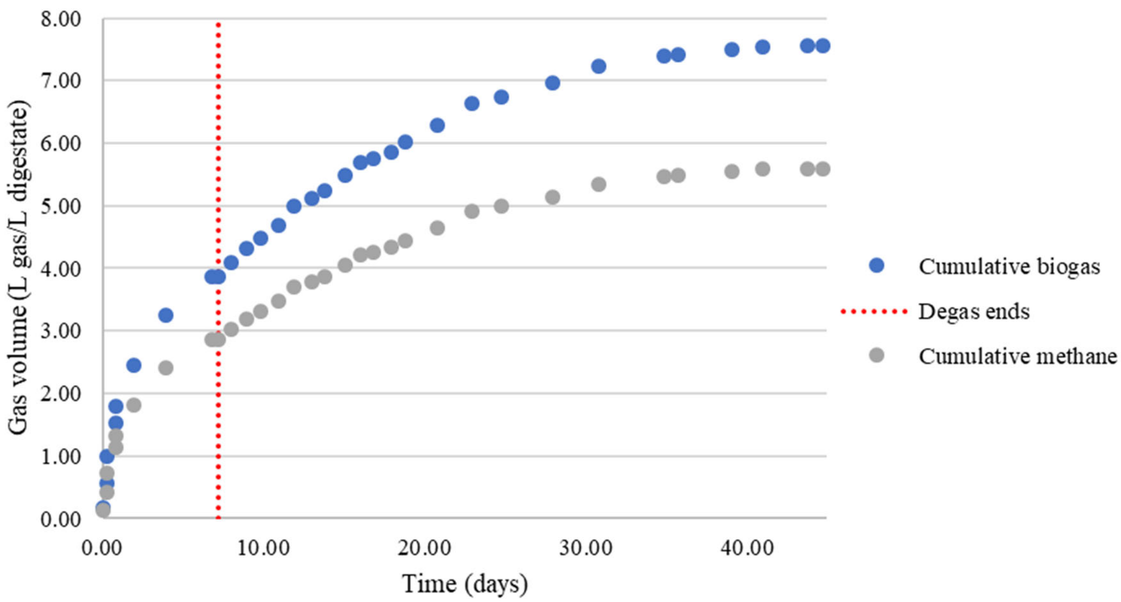


Figure 1: Raw digestate (inoculum) biogas and methane production potential.

Discussion

The raw digestate collected on March 29, 2021 still contained a substantial amount of biogas. Approximately 51% of the biogas and methane was produced during the initial seven-day de-gas period. Bio Town Ag might consider methods of capturing this residual gas, either through operational changes (longer hydraulic retention time) or recycling the digestate (instead of just the ammonia recovery effluent) back into the digester(s). These two approaches would each require reduction of feedstock input to the digesters or additional digesters. Other options may include: 1. Expand working volume of digesters to improve performance by removing the built-up sludge; 2. Cover the digestate storage lagoons to recover methane.

Biomethane Potential (BMP) and Kinetics of Mono-digestion

<u>Summary</u>: Overall, most replicates gave similar biomethane potentials as measured by cumulative methane produced per unit volatile solids added. Most also exhibited fairly typical kinetic patterns, where the majority of the biogas is produced within the first few to several days, with a gradual tapering. Table 2 contains the biogas and methane potentials of each digester, corrected for inoculum. Zero values indicate that the inoculum likely contributed all of the methane measured during the experiment, and perhaps that there was some digestion inhibition. All gas volumes are represented at standard temperature and pressure (32°F, 1 atm pressure).

Table 2: Specific biogas and methane production per gram volatile solids added after inoculum subtraction.

Feedstock	**Digester**	**Biogas (mL biogas/g VS added)**		**Methane (mL CH_4/g VS added)**	
		Individual digester	Feedstock average	Individual digester	Feedstock average
Blank	1	0	0	0	0
	2	0		0	
Cellulose	3	209	209	86	86
F1	4	316	373	202	228
	5	363		228	
	6	440		254	
F2	7	32	47	0	0
	8	28		0	
	9	80		0	
F3	10	75	135	51	100
	11	238		179	
	12	93		71	
F4	13	101	116	73	91
	14	165		124	
	15	81		75	
F5	16	193	212	159	173
	17	168		134	
	18	275		226	
F6	19	39	40	0	2
	20	42		3	
	21	41		2	
F7	22	188	183	134	133
	23	202		143	
	24	158		120	
F8	25	56	78	56	72
	26	78		71	
	27	99		87	
Mix	28	45	47	0	6
	29	16		0	
	30	80	209	17	

Cumulative Biogas and Methane Production by Treatment

The graphs in the following section show the cumulative biogas and methane production over time. *None of these graphs have been corrected for inoculum contribution*, so the final numbers will vary slightly from Table 2. They should be used as a general visual of the approximate trends of the rate of gas production.

For rapid comparison of digestion rate, Table 3 is also provided with cumulative specific biogas and methane production potential to reflect the approximate amount of methane recovery that could be expected at varied hydraulic retention times. These values have been corrected for inoculum contribution, with the exception of the blank, which is shown as the raw, uncorrected value. The blank also includes the one-week de-gassing period, rather than only showing the amount of gas produced since the beginning of the BMP test. There are a few cases where the cumulative gas production decreases over time. This is due to the method of calculating inoculum removal and likely indicates some inhibition of the digestion process. In contrast to Table 2, these values are calculated based on the feedstock mass rather than the volatile solids mass. The gas volumes are still calculated in terms of standard temperature and pressure (32°F, 1 atm pressure).

Table 3: Cumulative biogas and methane production potential per pound raw feedstock at three different hydraulic retention times.

Feedstock	**Biogas (ft^3/lb)**			**Methane (ft^3/lb)**		
	12-day	**21-day**	**30-day**	**12-day**	**21-day**	**30-day**
Blank	0.09	0.11	0.12	0.07	0.09	0.11
Cellulose control	3.30	3.19	3.21	1.40	1.31	1.32
F1: Pad manure	1.36	1.52	1.59	0.80	0.92	0.97
F2: Starch	0.23	0.28	0.33	0.01	0.00	0.00
F3: Slaughterhouse waste	0.18	0.25	0.29	0.00	0.04	0.11
F4: Waste activated sludge	0.17	0.19	0.20	0.13	0.15	0.16
F5: Soapstock	0.13	0.16	0.13	0.09	0.12	0.11
F6: Filter press slurry	0.25	0.22	0.21	0.04	0.02	0.01
F7: Food waste DAF	0.25	0.28	0.30	0.17	0.20	0.22
F8: AR effluent	0.03	0.03	0.04	0.03	0.03	0.03
Mixture	0.13	0.11	0.12	0.02	0.01	0.01

Comparison of Average Biogas Production

As shown in Figure 2, the soapstock (F5) and slaughterhouse waste (F3) treatments were both producing substantial biogas on a daily basis at the end of the originally planned 30 days. As a result, they were both maintained for another week to recover more biogas. The blank digesters were also maintained during that time so that the inoculum contribution could be accurately subtracted.

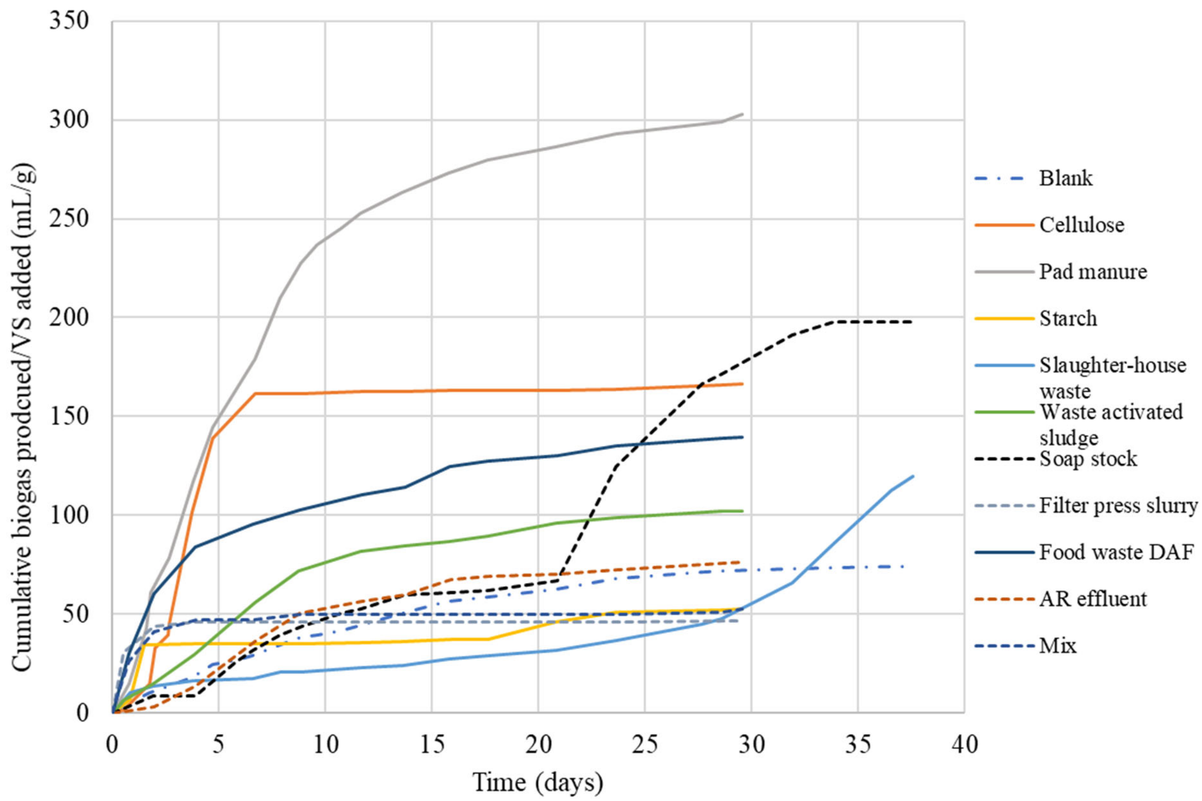


Figure 2: Average biogas production of all treatments during experiment.

For each of the following treatments, a solid line is used to indicate the average for the treatment cumulative biogas production, and a dotted line is used to indicate the average for the treatment cumulative methane production.

Blank

The blank (inoculum-only) replicates (1-2) performed very similarly to each other, with a final coefficient of variation of 7% for average biogas production and less than 1% for average methane production. These results give us confidence in our calculations to remove the inoculum biogas and methane productions from the mono-substrate and mixture digesters.

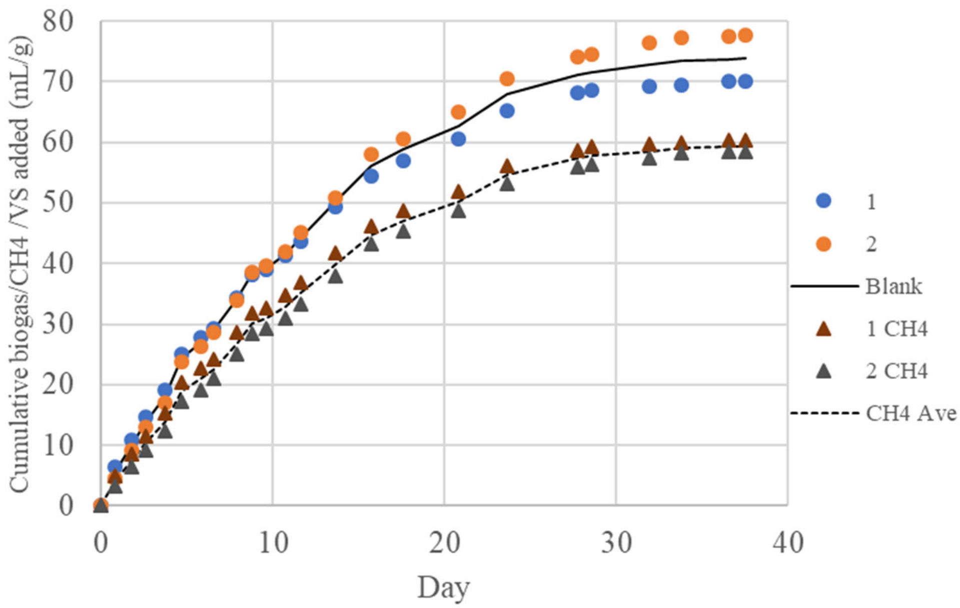


Figure 3: Blank (inoculum only) cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

Cellulose control

The positive (cellulose) control did not perform as well as expected (~350 mL methane/g VS cellulose). However, this may be the result of high starting total solids, which required substantial dilution during the experiment. Although these results control for that, it is possible that more VS were lost than calculated initially. Despite the low BMP, the curve for the cellulose control is reasonably shaped. A future experiment may explore other possibile causes for the discrepancy between what we observed and what was expected.

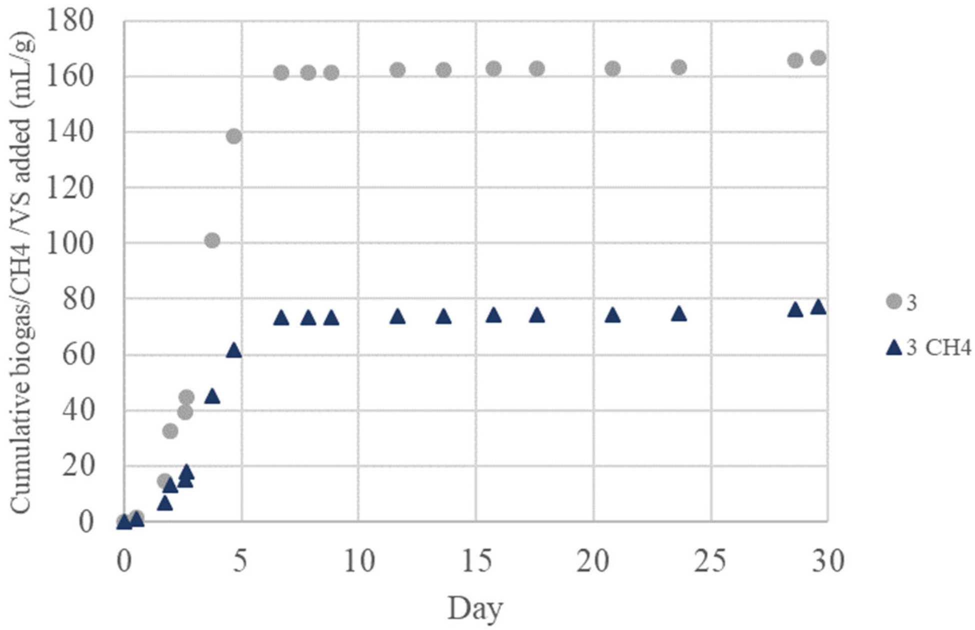


Figure 4: Positive control (cellulose) cumulative biogas and methane production.

F1: Pad manure

The pad manure digesters performed similarly and as anticipated based on previous manure results. The replicates are even closer in performance when using cumulative methane as a metric rather than biogas. As shown in Table 2, the pad manure had the highest BMP of all feedstocks tested.

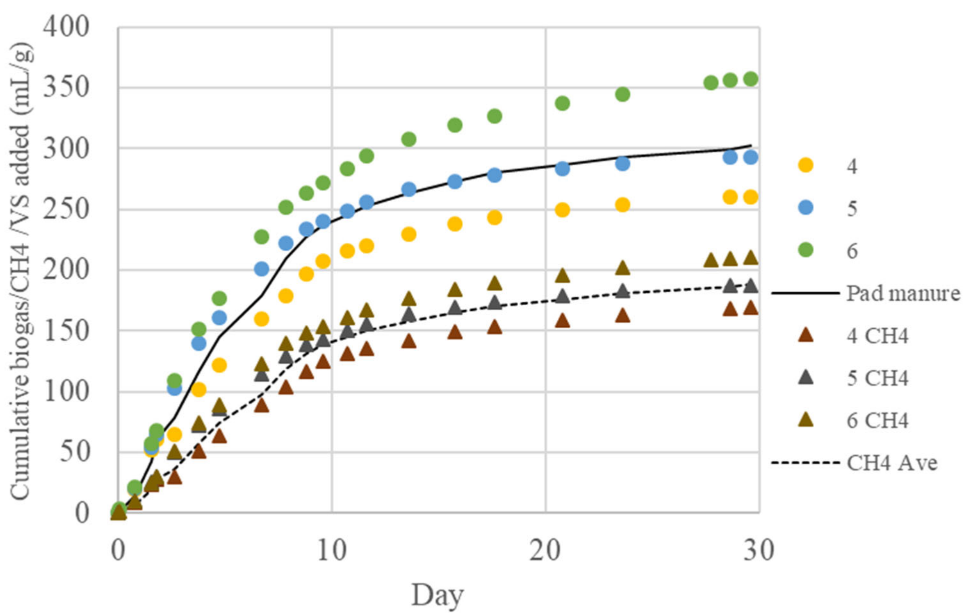


Figure 5: F1: Pad manure cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F2: Starch

The starch digesters all rapidly produced essentially all of their methane at the very beginning of the experiment, within the first 2-4 days, and little to none the rest of the experiment. Although there is an unusual increase in biogas production at the end of the experiment from one of the digesters, very little of it was methane. The BMP was one of the lowest among the feedstocks.

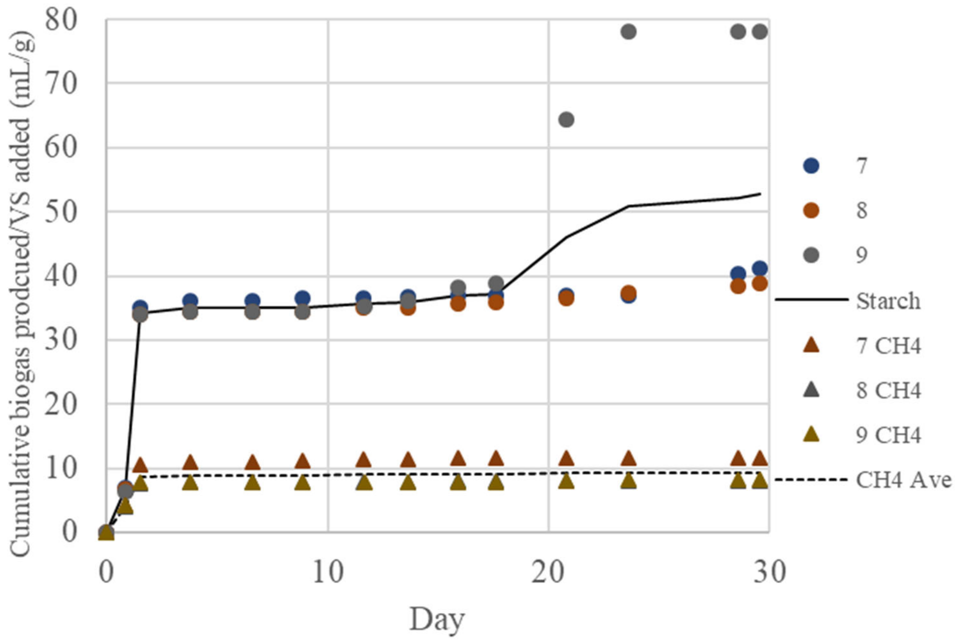


Figure 6: F2: Starch cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F3: Slaughterhouse waste

The slaughterhouse waste digesters generated an unusual cumulative gas production curve. Initially, they started producing a small amount of gas then temporarily appeared to be tapering off. However, towards the end of the experiment, they all began to produce an exceptionally high amount of gas and began foaming to the point of clogging gas bags. These digesters were all run a week longer than the original proposal of 30 days in order to observe this increase. This very unusual behavior may be attributable to the low carbohydrate content and high protein and lipid content of the feedstock. This will be investigated further. This feedstock in particular may benefit from co-digestion with another feedstock that can balance out the unusual kinetic behavior shown here, but more research is needed.

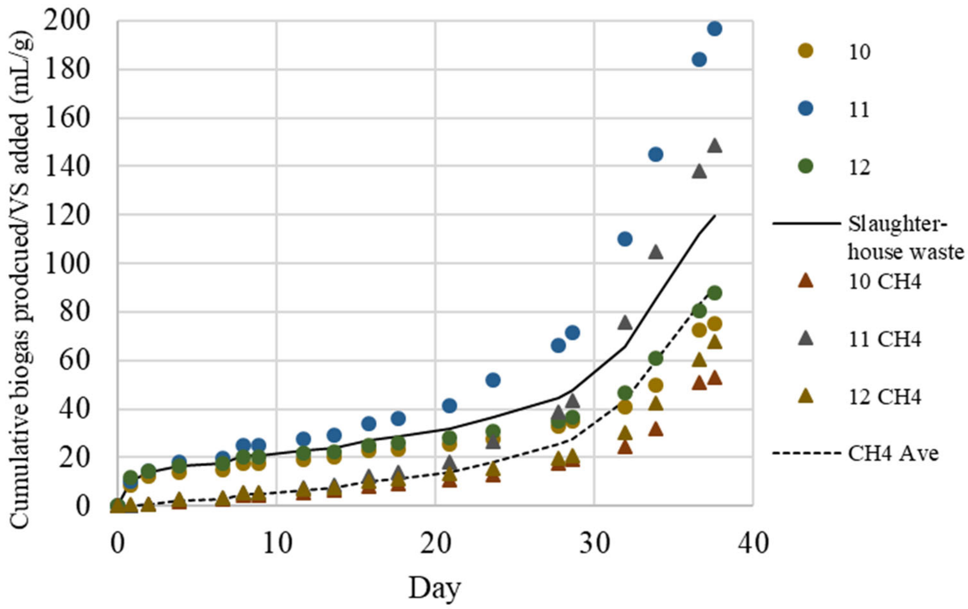


Figure 7: F3: Slaughterhouse waste cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F4: Waste activated sludge

This feedstock appears to be relatively normal in terms of rate of gas production. One of the digesters did generate an unusually high amount of gas compared to the other two, but this difference is expected occasionally in digesters as it is a biological process.

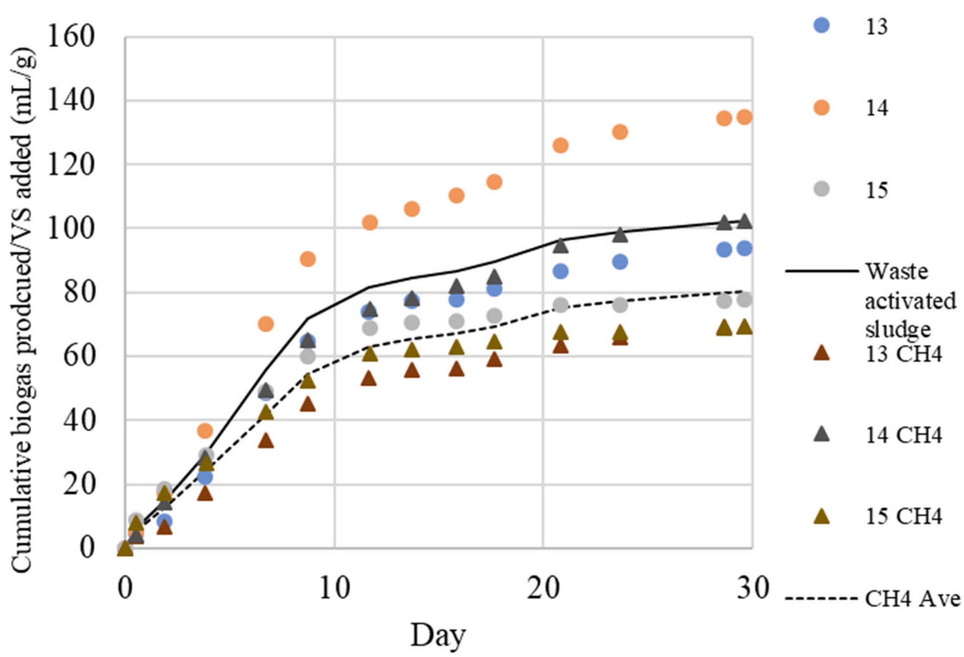


Figure 8: F4: Waste activated sludge cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F5: Soapstock

The soapstock digesters all followed an unusual pattern. Each reached a plateau of gas production approximately three different times. The final time, the digesters all started producing a significant amount of foam as well as more than double their previous biogas production. These digesters ran one week past the original 30 days in order to more fully observe this behavior. Similar to the slaughterhouse waste, the soapstock composition has low carbohydrates but high proportions of protein and lipids, which may contribute to this behavior.

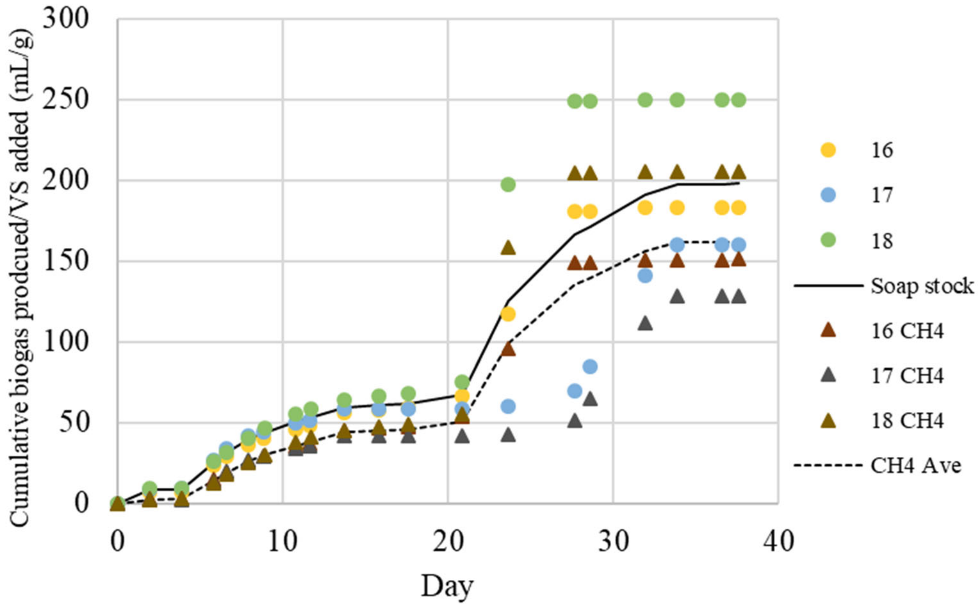


Figure 9: F5: Soapstock cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F6: Filter press slurry

The filter press slurry exhibited a very normal specific methane potential curve and all the digesters gave very consistent results. However, the BMP is one of the lowest exhibited.

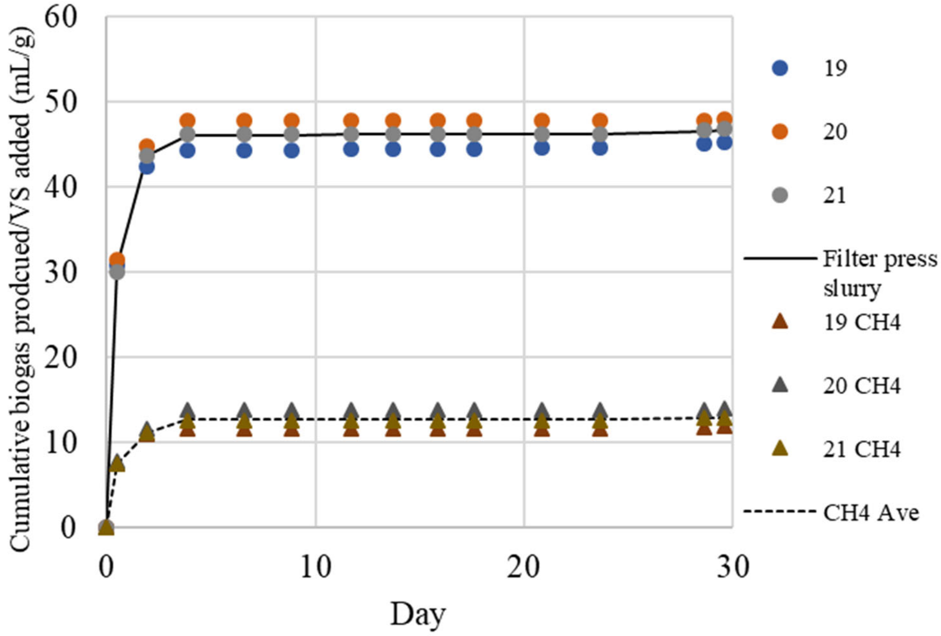


Figure 10: F6: Filter press slurry cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F7: Food waste DAF

The food waste DAF had one of the highest BMPs and exhibited fairly normal kinetics.

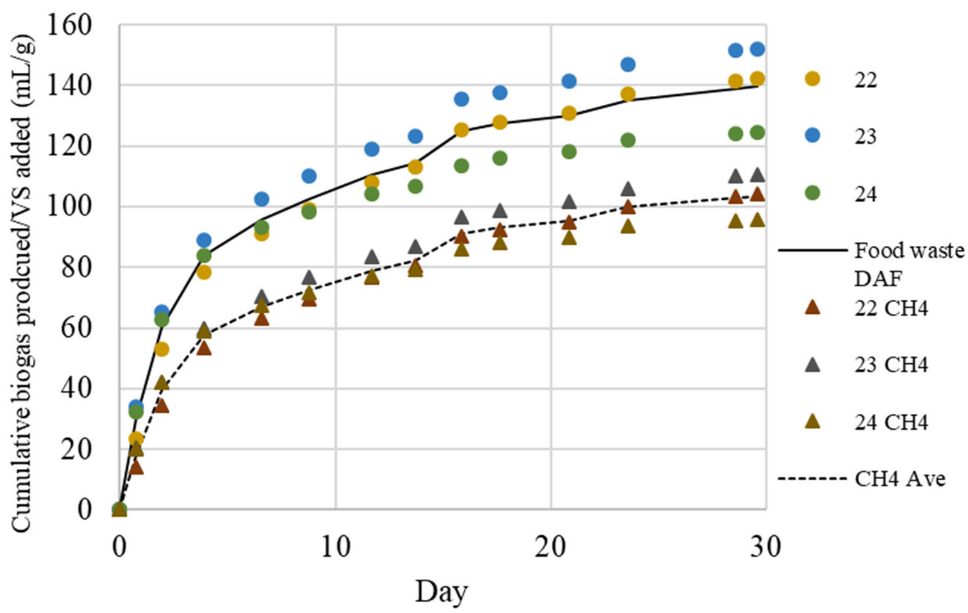


Figure 11: Food waste DAF cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

F8: AR effluent

The ammonia recovery effluent experienced a slight delay at the beginning of gas production, but its later gas production curve appears normal. However, it is also important to remember that biomethane potential is gas potential per gram of volatile solids added, and the AR effluent has the least volatile solids of all the feedstocks.

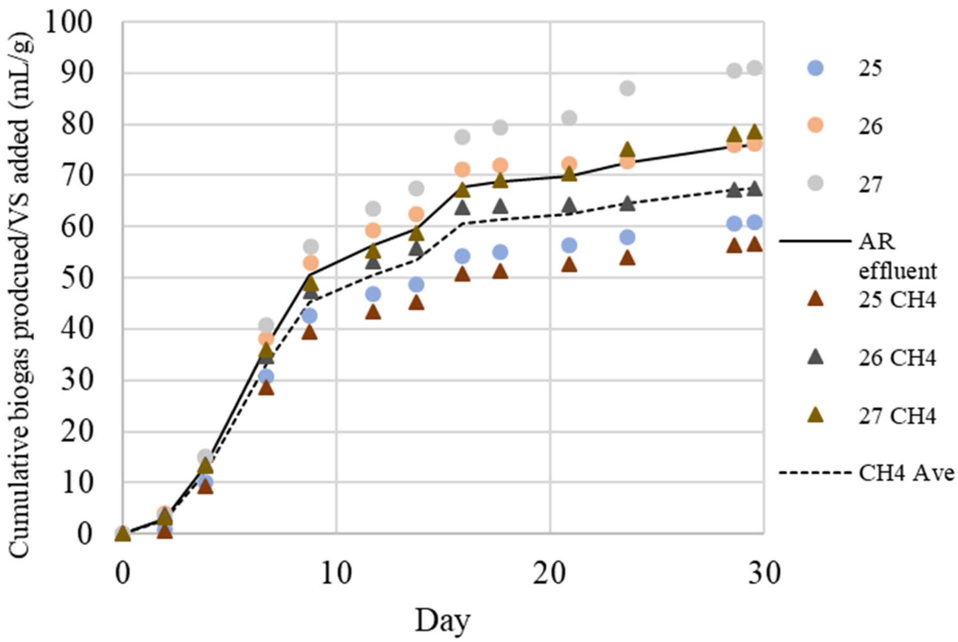


Figure 12: Ammonia recovery effluent cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

Mixture

The mixture digesters did not perform as anticipated, where the BMP of each individual feedstock can be added together to give a total expected BMP. In fact, as shown in Figure 14, two of the digesters did not produce as much gas as would be anticipated from the inoculum alone. It is possible that the dilution required due to overpressure of the digesters at the beginning may have had adverse effects on these digesters. Alternatively, during the dilution the digestate may have been insufficiently mixed, which could have resulted in less of certain feedstocks (such as the manure) than anticipated, which would also skew the results. A similar test should be tried again to determine the causes.

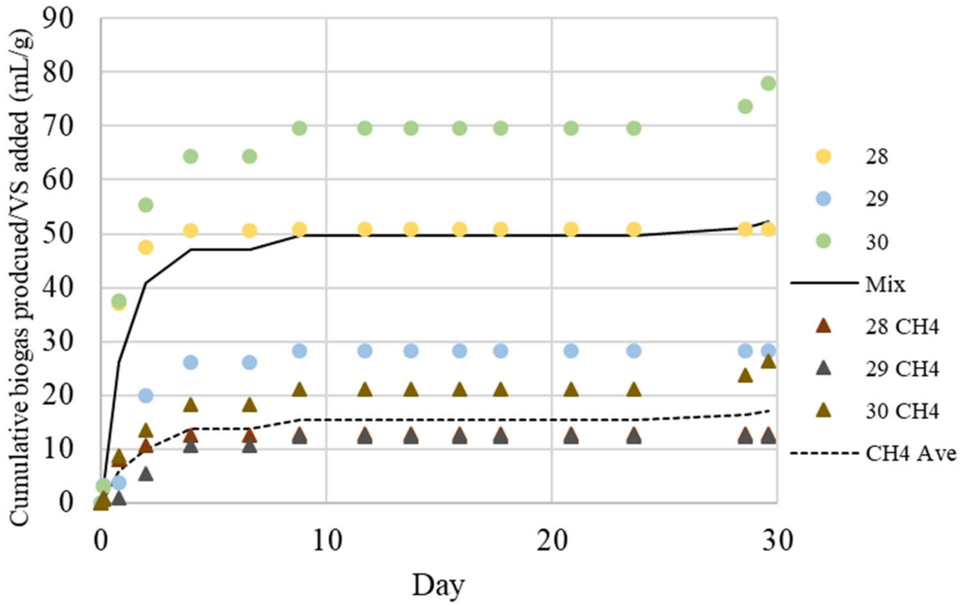


Figure 13: Mixture of all feedstocks proportional to their quantities in the Bio Town digester cumulative biogas and methane production. Solid line = biogas average, dotted line = methane average.

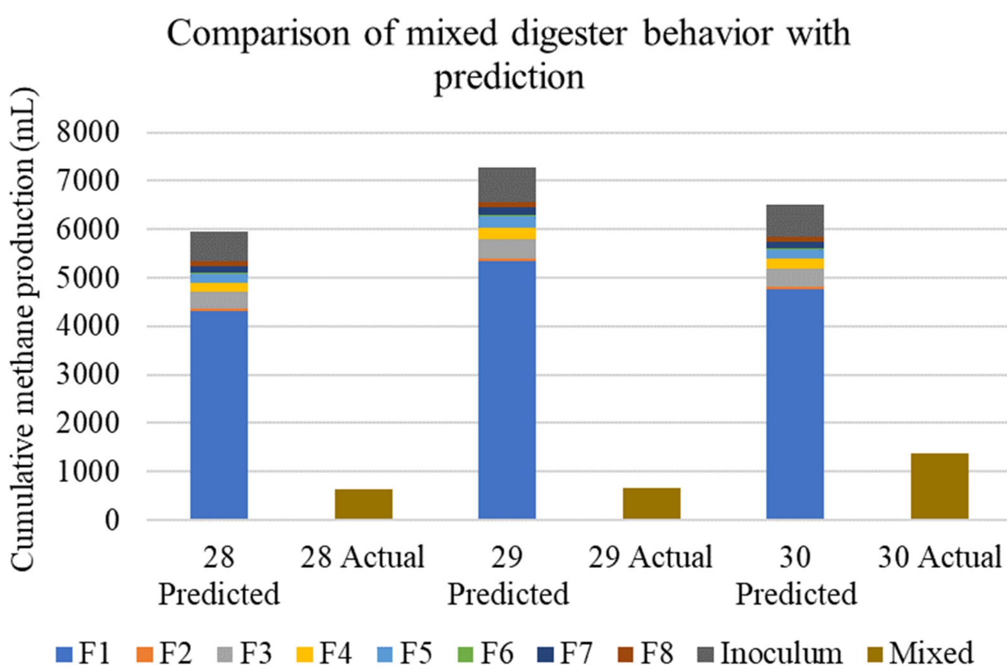


Figure 14: Comparison of mixed digester behavior with predicted methane production for digesters 28, 29, and 30.

Hydrogen Sulfide (H_2S) Concentrations

For simplicity, the maximum H_2S concentration from each digester and the average of each treatment is presented in the following table. Additional information regarding H_2S production over time can be provided.

Table 4: Maximum hydrogen sulfide concentration observed at any point during digestion, for individual digesters and treatment average.

Feedstock	Digester	Maximum hydrogen sulfide concentration (ppm)	
		Digester	Feedstock average
Blank	1	1	2
	2	2	
Cellulose	3	570	570
F1	4	2529	2712
	5	2536	
	6	3070	
F2	7	329	2307
	8	1591	
	9	5000	
F3	10	84	63
	11	92	
	12	12	
F4	13	341	229
	14	303	
	15	42	
F5	16	290	418
	17	467	
	18	496	
F6	19	301	337
	20	318	
	21	393	
F7	22	139	129
	23	137	
	24	110	
F8	25	3	8
	26	10	
	27	12	
Mix	28	515	565
	29	506	
	30	674	

Physical and Chemical Composition of Feedstocks and Digestate

Summary

Total Kjeldahl nitrogen, ammonia nitrogen, volatile fatty acids, total and soluble chemical oxygen demand, alkalinity, total and volatile solids, and concentrations of carbohydrates, proteins, and lipids were all measured for the feedstocks. All of these were measured for the digestate as well except for the carbohydrate, protein, and lipid concentrations. Additional details are available for any of these measurements, but only the most relevant information has been included in this report.

Volatile Fatty Acids

In most cases, VFAs were reduced from the original amounts contained in the feedstock. However, exceptionally high VFAs (>10 g/L) were found in the cellulose, starch (F2), filter press slurry (F6), and mixture digesters. In addition, VFAs increased from the initial feedstocks for those same digesters listed as well as the slaughterhouse waste and soapstock digesters, although VFAs for those remained on average 3.7 g/L and 1.7 g/L, respectively. The accumulation of VFAs in these digesters may have led to methanogenesis inhibition, which could account for the low BMPs of the cellulose, starch, filter press slurry, and mixture digesters. Co-digestion of these feedstocks could result in higher BMPs if inhibition was the cause of the low BMP. Additional research would be needed here, and an additional mono-digestion or co-digestion test with these feedstocks would be recommended.

Alkalinity

Alkalinity increased in all the digesters except cellulose, starch, and filter press slurry. These alkalinity results are consistent with the VFA results. Mixture change in alkalinity was not calculated, but the average mixture alkalinity (4.2 g/L) was only slightly higher than that of the feedstocks listed (averages: cellulose: 3.6 g/L; starch: 2.5 g/L; filter press slurry: 2.9 g/L).

Volatile Solids Reduction

Volatile solids were measured in duplicate for both feedstocks and digestate. On average, solids reduction (calculated to include inoculum contribution and take subsequent dilutions into account) were high (>60%) as shown in Table 5. The blank and AR effluent saw the lowest reductions, but also started with the lowest quantity of volatile solids (3.4 g/g and 2.9 g/g respectively).

Table 5: Average volatile solids reduction per feedstock.

Feedstock	Days of test	Average VS% Reduction
Blank	38	60%
Cellulose	30	83%
F1: Pad Manure	30	85%
F2: Starch	30	89%
F3: Slaughterhouse waste	38	80%
F4: Waste activated sludge	30	72%
F5: Soapstock	38	76%
F6: Filter press slurry	30	86%
F7: Food waste DAF	30	70%
F8: AR effluent	30	63%
Mixture	30	81%

Carbohydrates, Lipids, and Proteins

One of the future goals of this project is to determine whether carbohydrates, lipids, and proteins can be used, in conjunction with other feedstock properties, as metrics for choosing feedstocks and even predicting biomethane potential or kinetics. More work will be needed before this is possible. However, these initial results may give some insight into the BMP and kinetics results from this experiment. Figure 15 shows that the three feedstocks that were the fastest to finish gas production (as represented by the percentage of BMP produced after the first four days) also had the lowest protein content (filter press slurry (F6), starch (F2), and cellulose). Filter press slurry and starch also had the lowest BMPs when the contribution of inoculum was removed. In the case of starch and cellulose, the low BMP could have been the result of rapid acidification due to the fast degradation of the carbohydrates. The slowest in terms of gas production were slaughterhouse waste (F3) and soapstock (F5), and these had the lowest percentages of carbohydrates and almost the highest percentages of lipids. More analysis will be done in this area prior to the next experiment, but the initial results are promising. Co-digestion of some of these feedstocks may balance out some of the unwanted BMP and kinetic effects seen here, such as low BMP or exceptionally slow digestion.

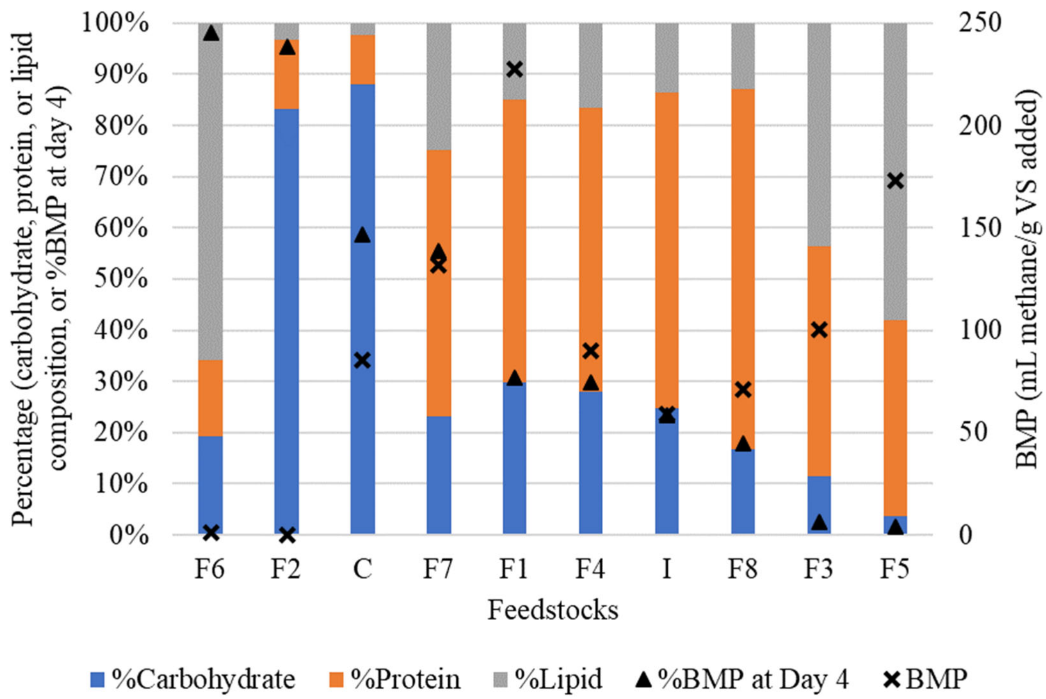


Figure 15: Comparison of carbohydrate, protein, and lipid compositions of digestate (averages for each treatment) using each feedstock, with BMP (averages).

Conclusions and recommendations

In conclusion, this was a promising initial experiment to study the mono-digestion characteristics of the feedstocks Bio Town Ag uses most frequently in their digester.

We observed that there is substantial biomethane potential left over in the BTA effluent. Some possible solutions for capturing this additional gas include the following:

- Increase hydraulic retention time by decreasing feedstock input or increasing digester working volume (adding additional digesters, removing built-up sludge in existing digesters).
- Recycle a larger portion of the digestate back into the digesters as opposed to the ammonia recovery effluent alone, which would require additional digester volume.
- Cover the digestate storage lagoons to recover methane.

Although the treatment containing all the feedstocks did not perform as expected, additional co-digestion experiments and further analysis may illuminate the root causes. Co-digestion may be important for some of these feedstocks to not experience inhibition or to digest more quickly. If feasible for BTA operations, some of the following co-digestion methods may be considered for additional laboratory-scale testing:

- Introduce feedstocks that require a shorter hydraulic retention time (such as starch) into the middle of the digesters. This would decrease the overall HRT while accommodating an appropriate HRT needed for complete digestion. However, research would be needed to ensure that no disruptions to the microbial community would occur.
- Manage digesters with different feedstocks and HRT. If possible for the BTA digester design, this could allow us to optimize the feedstock combinations so that each digester is operating with the best retention times for a given suite of feedstocks.

Appendix A: Additional experimental procedures.

Digester start-up procedure

Start up for the test (April 5, 2021) proceeded as follows:

- Empty digesters (square, 1-L Corning bottles) were weighed using a kitchen scale. This scale (error approximately ± 3 g) was used for all digester weight measurements, including tear-down and intermediate dilutions.
- Inoculum was mixed thoroughly and the specified mass was measured into each digester.
- Each substrate was mixed thoroughly from all 1-L sample containers provided by BTA for the collection of the feedstocks and the specified mass of each was added to each digester. Details of mass fraction added for each treatment are contained in Table 1.
- If additional volume was needed at this time, deionized water was added.
- A stir bar was added to each digester and the digester was mixed thoroughly using either a stir bar or manual stirring if the substrate was too thick. The stir bar remained in the digester until the experiment was completed so that the digesters could be stirred prior to any gas volume measurements. The final digester mass was measured.
- Each digester was wiped up to clean any spills, and capped.
- A gas bag was attached to each digester with tubing. Each gas bag had been evacuated previously and the gas bag valve was closed.
- All digesters were added to the water baths and the gas bag valves were opened.
- Some additional dilutions were needed for many of the digesters at various stages of the experiment due to excessive foaming or an explosion. Full details of these dilutions are provided below.

Digester tear-down procedure

- Measurements of final gas volume and composition were taken as done throughout the experiment. Date, time, atmospheric temperature and pressure, and pH were also recorded.
- The digester was dried from the water bath and the cap was removed. The kitchen scale was used to weigh the final digestate mass.
- The digesters were stirred thoroughly using both the stir bar and manually with a rod that could reach the bottom of the digester.
- A sample of the digestate was removed for physical/chemical analysis. At least 45 mL of digestate were stored in the refrigerator (4°C).
- Stir bar was removed and the digested material was poured into a secondary container for disposal.

Digester intermediate dilution procedure

During the experiment, excessive foaming, clogging, or extreme gas production resulted in explosions or near over-pressure events for many of the digesters. Following explosions and/or to prevent additional incidents, the digesters were diluted. Table 6 contains the reasons for the dilutions and the days the dilutions occurred. The following procedure describes how the digesters were diluted during these intermediate events.

- Gas bag was sealed and removed from the tubing for later measurement and analysis. Digester cap was removed.
- Digestate was stirred thoroughly to achieve as close to a homogeneous mixture as possible.
- If an explosion occurred, the outside of the digesters was cleaned prior to weighing the digester using the kitchen scale.
- Digestate was removed to a specific amount (determined based on the amount of foaming or the cause of the overpressure) and the digester mass was measured again. Most of the time, replicates were diluted at the same time and in as close to the same amount as possible.
- Deionized water was added to the digester to reach approximately 1000 mL of digester working volume. Mass of water added was also measured.
- Digesters were recapped and a fresh gas bag was added prior to putting the digesters back into the water baths.
- Note: These dilutions did impact the volatile solids content of the digesters over time. This was accounted for while calculating the specific biomethane potential of each feedstock.

Table 6: Date and time of each dilution event and amount of dilution based on mass.

Digester	Date	Time	Reason for dilution	% inoculum	% substrate	% water
1	4/5/2021	9:00 PM	Too foamy	72%	0%	28%
3	4/6/2021	7:30 AM	Clogged gas bags	71%	8%	22%
	4/8/2021	11:00 AM	Maxed out gas bags	67%	7%	25%
	4/8/2021	11:00 AM	Too foamy, clogged gas bags	56%	6%	38%
4	4/5/2021	7:00 PM	Too foamy	52%	32%	16%
	4/5/2021	9:00 PM	Too foamy	30%	18%	51%
5	4/5/2021	7:00 PM	Too foamy	55%	31%	14%
	4/5/2021	9:00 PM	Too foamy	31%	18%	51%
6	4/5/2021	7:00 PM	Too foamy	53%	32%	15%
	4/5/2021	9:00 PM	Too foamy	30%	19%	51%
7	4/7/2021	7:45 AM	Maxed out gas bags	50%	17%	33%
8	4/7/2021	7:45 AM	Maxed out gas bags	52%	17%	31%
9	4/7/2021	7:45 AM	Maxed out gas bags	51%	17%	32%
10	4/5/2021	7:00 PM	Too foamy	50%	30%	20%
	5/5/2021	7:30 AM	Foam, overflowed	40%	24%	36%
	5/8/2021	1:45 PM	Foam, overflowed	30%	18%	52%
	5/9/2021	2:45 PM	Foam, overflowed	19%	11%	70%
11	4/5/2021	7:00 PM	Too foamy	50%	30%	20%
	4/5/2021	9:00 PM	Too foamy	36%	21%	43%
	4/29/2021	8:30 AM	Foam	22%	13%	64%
	5/5/2021	7:30 AM	Foam, overflowed	18%	11%	71%
	5/8/2021	1:45 PM	Foam, overflowed	14%	8%	78%

Digester	Date	Time	Reason for dilution	% inoculum	% substrate	% water
12	4/5/2021	7:00 PM	Too foamy	51%	31%	18%
	4/5/2021	9:00 PM	Too foamy	37%	22%	40%
	5/7/2021	9:00 AM	Foam, overflowed	28%	17%	54%
	5/8/2021	1:45 PM	Foam, overflowed	21%	13%	66%
	5/9/2021	2:45 PM	Foam, overflowed	14%	8%	78%
13	4/6/2021	7:30 AM	Clogged gas bags	25%	31%	44%
14	4/6/2021	7:30 AM	Clogged gas bags	24%	22%	54%
15	4/6/2021	7:30 AM	Clogged gas bags	26%	23%	52%
16	4/28/2021	8:30 AM	Foam, overflowed	6%	65%	28%
	4/29/2021	8:30 AM	Foam	3%	35%	62%
17	4/14/2021	4:25 PM	Accidental overflow from closed gas bag	9%	91%	0%
	5/2/2021	3:00 PM	Foam, overflowed	5%	53%	42%
	5/4/2021	8:30 AM	Foam, overflowed	4%	42%	54%
18	4/27/2021	9:30 AM	Foam, overflowed	8%	84%	9%
	4/28/2021	8:30 AM	Foam, overflowed	6%	60%	34%
	4/29/2021	8:30 AM	Foam, overflowed	3%	30%	67%
	4/29/2021	4:30 PM	Foam, samples stored for later analysis	2%	18%	80%
19	4/6/2021	7:30 AM	Too much gas	37%	21%	41%
20	4/6/2021	7:30 AM	Too much gas	37%	22%	41%
21	4/6/2021	7:30 AM	Too much gas	36%	21%	43%
22	4/5/2021	11:15 PM	Too foamy	33%	24%	42%
	4/6/2021	5:20 PM	Getting too foamy	23%	17%	61%
23	4/5/2021	11:15 PM	Too foamy	33%	25%	42%
	4/6/2021	5:20 PM	Getting too foamy	23%	17%	60%
24	4/5/2021	11:15 PM	Too foamy	33%	25%	42%
	4/6/2021	5:20 PM	Getting too foamy	23%	17%	60%
28	4/5/2021	7:00 PM	Exploded/overflowed	40%	43%	17%
	4/5/2021	9:00 PM	Digestate in gas bags	23%	25%	52%
29	4/5/2021	7:00 PM	Exploded/overflowed	40%	43%	17%
	4/5/2021	9:00 PM	Digestate in gas bags	26%	27%	47%
30	4/5/2021	7:00 PM	Exploded/overflowed	40%	42%	18%
	4/5/2021	9:00 PM	Digestate in gas bags	25%	26%	48%

Appendix B: Photos

These photos have been included to give a general idea of the appearance of the digesters and specific feedstocks during digestion. Additional photos were taken during the experiment, particularly during set up and after explosion events, and can be provided upon request.

Digester 17 (soapstock) foaming excessively on May 4, 2021

Blank and cellulose digesters on April 16, 2021

F1: Pad manure digesters on April 16, 2021

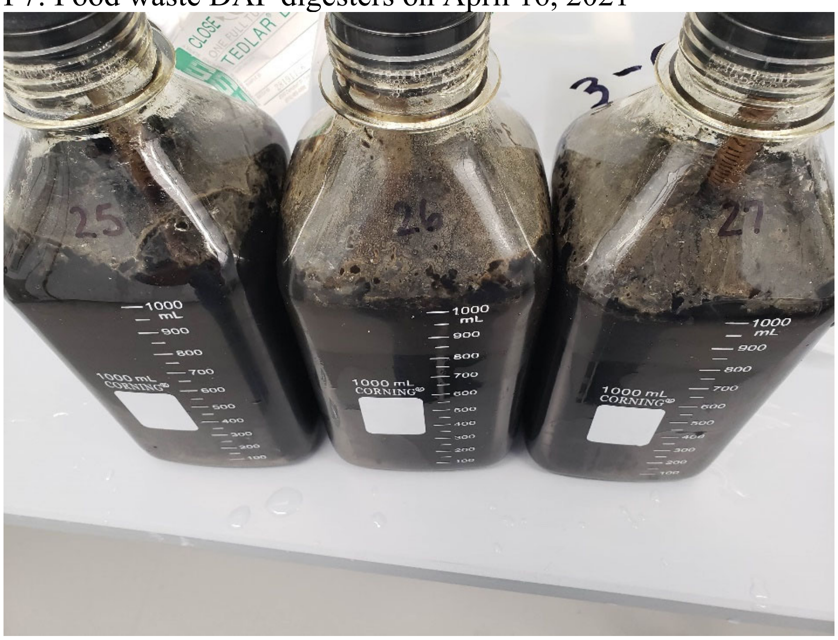
F2: Starch digesters on April 16, 2021

F3: Slaughterhouse waste digesters on April 16, 2021

F4: Waste activated sludge digesters on April 16, 2021

F5: Soapstock digesters on April 16, 2021

F6: Filter press slurry digesters on April 16, 2021

F7: Food waste DAF digesters on April 16, 2021


F8: AR effluent digesters on April 16, 2021

Mixed digesters on April 16, 2021