Results from Bio Town Ag Co-digestion Experiment (Experiment 2)

Prepared by

Jennifer Rackliffe

Contents

Experimental Summary

Experiment 2 (E2): Experimental description

We conducted a co-digestion biomethane potential (BMP) test in September 2021. As manure contributes the greatest mass to the BTA digester, we combined the manure (F1) pairwise with some of the industrial feedstocks, specifically starch (F2), slaughterhouse waste (F3), soap stock (F5), and filter press slurry (F6). These combinations were made at two different ratios of the two feedstocks. The first set of treatments combined the manure and an additional substrate at a 1:1 ratio on a mass basis. The second set of treatments combined the feedstocks proportional to the amounts commonly used at BTA. Due to the extreme behavior of the starch and soap stock in the mono-digestion experiment, a final treatment pairing these two feedstocks at a 0.6:1 ratio was also included, which is proportional to the amounts used at BTA. The objective of the experiment was to evaluate potential synergism or antagonism from combinations of some of the feedstocks used in Experiment 1 (E1, conducted in April 2021). The experiment was conducted using 33 1000-mL lab-scale anaerobic digesters at mesophilic conditions of 101°F (38.3°C), using temperature-regulated water baths. One blank and a single mono-digestion digester for each feedstock was run to compare with the results from E1. Table 1 shows the treatment combinations of the feedstocks. The feedstocks are numbered the same as in E1 for consistency.

Table 1: Experiment 2 treatments.

Treatment	# digesters	S1 (g)	S2 (g)	I (g)	W (g)	S1/S2	I/S
Blank	1	--	--	1000	0	--	--
F1	1	101	--	399	500	--	0.5
F2	1	73	--	427	500	--	0.5
F3	1	111	--	389	500	--	0.5
F5	1	303	--	197	500	--	0.5
F6	1	94	--	406	500	--	0.5
F1+F2 Eq	3	42	42	415	500	1.0	0.5

F1+F3 Eq	3	53	53	394	500	1.0	0.5
F1+F5 Eq	3	76	76	349	500	1.0	0.5
F1+F6 Eq	3	49	49	403	500	1.0	0.5
F1+F2 Pr	3	83	13	404	500	6.3	0.5
F1+F3 Pr	3	85	18	397	500	4.7	0.5
F1+F5 Pr	3	92	24	383	500	3.8	0.5
F1+F6 Pr	3	91	9	401	500	10.6	0.5
F2+F5 Pr	3	52	88	360	500	0.6	0.5
Total:	33						

F1 = pad manure, F2 = starch, F3 = slaughterhouse waste, F5 = soap stock, F6 = filter press slurry, I = inoculum, W = water; S1 = substrate 1 (first substrate in list), S2 = substrate 2; S1/S2 = substrate 1 to substrate 2 ratio (by mass); I/S = inoculum to substrate (total) ratio in terms of *volatile solids*; TS = total solids of combined substrates and inoculum; Eq = treatment has equal amounts (mass basis) of both feedstocks; Pr = treatment amounts are proportional to the amounts used at BTA

In addition to the testing of feedstock and post-digestion digestate samples for physical and chemical characteristics, samples were taken directly from the digesters prior to digestion after the inoculum and feedstocks had been mixed and were tested for the same characteristics, as shown in Table 2. All vial test kits from the Hach company (Loveland, CO, USA) were measured using the Hach DR3900 Benchtop Spectrophotometer. Dilutions were done on a mass basis when needed for samples to be within an acceptable range for the chemical analysis. No samples were taken from the digesters during digestion, although dilution of the digesters was needed due to digester foaming. Post-digestion results are corrected accordingly.

Table 2: Physical and chemical characteristics measured in Experiment 1 and Experiment 2.

Characteristic	Experiment 2			Analysis method
	F	Pre	Post	
Carbohydrates	X	X	X	Anthrone method
Proteins	X	X	X	Modified Lowry method
Lipids	X	X	X	Bligh and Dyer method
Total solids (TS)	X	X	X	Standard Methods of the APHA (APHA, 1992)
Volatile solids (VS)	X	X	X	Standard Methods of the APHA (APHA, 1992)
Soluble chemical oxygen demand (SCOD)	X	X	X	Hach TNTplus Vial Test, Ultra high range (TNT823)
Total chemical oxygen demand (TCOD)	X	X	X	Hach (TNT823)

Total nitrogen	X	X	X	Hach Simplified TKN TNTplus Vial Test (TNT872)
Total Kjeldahl nitrogen (TKN)	X	X	X	Hach TNT872
Nitrate + nitrite nitrogen	X	X	X	Hach TNT872
Ammonia nitrogen	X	X	X	Hach Ammonia TNTplus Vial Test, High range (TNT832)
Volatile fatty acids (VFAs)	X	X	X	Hach Volatile Acids TNTplus Vial Test (TNT872)
Alkalinity	X	X	X	Hach Alkalinity (Total) TNTplus Vial Test (TNT870)

F = Feedstock; Pre = Pre-digestion sample, removed from the digester after mixing inoculum and feedstocks but prior to digestion; Post = Post-digestion sample, removed from the digester following the conclusion of the BMP test.

Macromolecular assays

We tested the Bio Town feedstocks from a mono-digestion biomethane test E1 and feedstocks and pre- and post-digestion samples from a co-digestion biomethane potential test to establish the validity of using the following methods for characterization of macromolecules:

- Anthrone method for carbohydrate characterization;
- Bligh and Dyer method for lipid characterization; and
- Modified Lowry method for protein characterization.

Each sample (8 feedstocks from E1; 5 feedstocks, 33 pre-digestion samples, and 33 post-digestion samples from E2) was tested in duplicate as described in Table 2.

Experimental Results

Variability between batches

In order to develop laboratory protocols for quantifying macromolecular composition of representative substrates used for agro-industrial anaerobic co-digestion, it was first necessary to verify whether the assays give replicable data. Accordingly, using the data acquired from the E2 samples, we calculated the coefficient of variation between sample replicates. The majority of the samples had a coefficient of variation <30% for each of the macromolecular assays.

We compared the results of the macromolecular characterizations of the feedstocks between E1 and E2 and found substantial differences between the two. Table 3 shows the percent difference between E1 and E2 for each of the macromolecular compositions. There does not appear to be any particular pattern to the differences other than the fact that a higher concentration of proteins and lipids was measured for all feedstocks, including the inoculum, in E1. However, the differences are so varied that there does not appear to be a single cause of this.

Table 3: Percent difference between E1 (April 2021) and E2 (September 2021) feedstocks. A negative number indicates an increase in concentration of the macromolecule from E1 to E2.

Feedstock	Carbohydrate	Lipid	Protein
Inoculum	-62%	53%	29%
F1	-7%	63%	25%
F2	25%	13%	22%
F3	66%	51%	74%
F5	-20%	25%	65%
F6	52%	62%	65%

If the feedstocks are regularly experiencing such large fluctuations, this could cause issues for BTA's digester, such as causing unanticipated fluctuations in gas production or inhibition. It would also make it difficult to establish a reliable method of recommending a feeding strategy to BTA as the composition of the feedstocks would need to be assessed more regularly. More research is needed to determine the magnitude of these fluctuations as part of developing laboratory protocols for measuring macromolecule composition for the purposes of informing BMP tests.

Gas production

Comparison of mono-digestion results between E1 and E2

Figure 1 compares the specific biogas production of the mono-digestion of the individual feedstocks from E1 and E2. Although in some cases the general shape of the methane production curves are similar, the slaughterhouse waste, soap stock, and filter press slurry (Figure 1D, E, and F respectively) all exhibit sufficient differences to require us to use the mono-digestion results from E2 only to make comparisons between co-digestion treatments, rather than being able to extrapolate from the results of E1. This is important as it establishes the necessity of these mono-digestion treatments in future experiments as well. However, further research is needed to determine the cause of these differences.

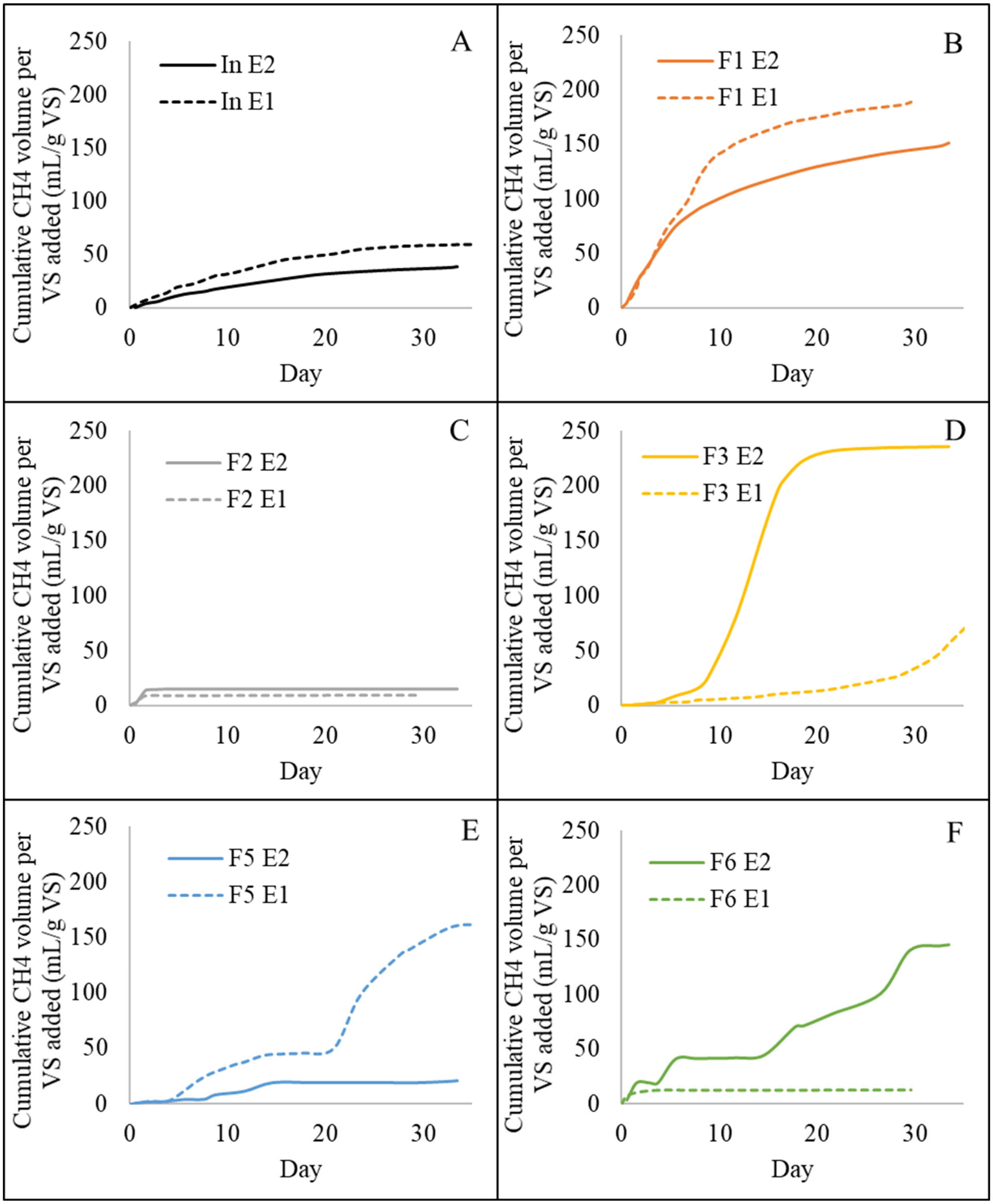

Figure 1: Comparison of mono-digestion results between E1 and E2.

Synergistic effects of co-digestion

During E2, we observed both total yield and kinetic synergy in all treatments. Only one digester (digester 19, one of the replicates from the starch and manure proportional treatment) produced substantially less (<30%) methane than would be expected for an additive effect for more than one day. An additive effect can be calculated as a weighted average between the mono-digestion specific methane potential curves at each time point. This effect can be seen in Figure 2, which shows the cumulative methane curves (corrected for inoculum contribution and averaged over the three replicates) of the mono-digestion digesters for manure and starch individually and the curves for both co-digestion treatments using both manure and starch.

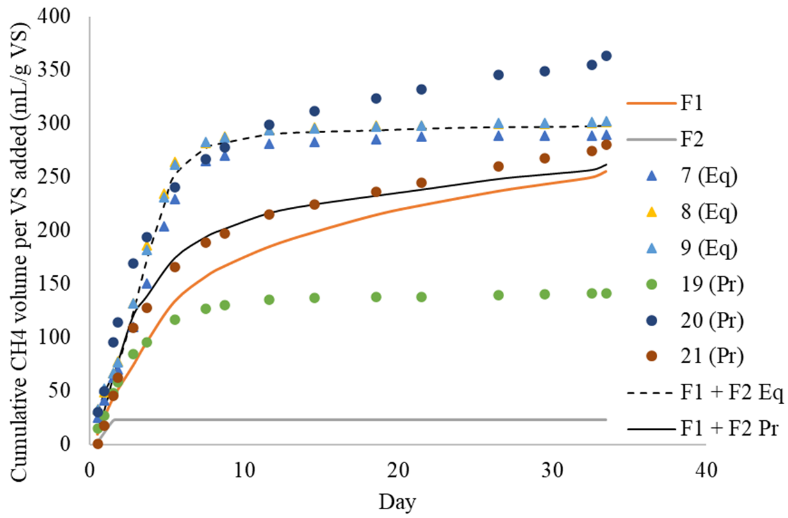

Figure 2: Cumulative specific methane production for manure (F1) and starch (F2). F1 + F2 Eq = 1:1 ratio of VS; F1 + F2 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate individual digesters (7-9 are Eq treatment, 19-21 are Pr treatment).

For each of the treatments, an additional figure will be included to show the average digester results (points) alongside the predicted co-digestion results based on the weighted average of the two mono-digestion results. Figure 3 shows that the equal parts manure and starch treatment significantly outperformed the prediction assuming no synergy for both kinetics and ultimate methane yield. The proportional treatment is not so definitive as one digester (Digester 19) underperformed the average significantly. Therefore, synergy cannot be assumed.

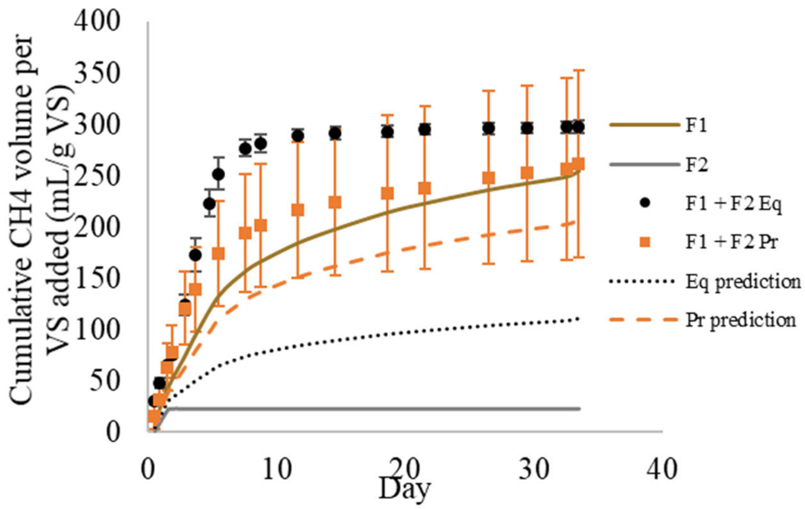

Figure 3: Co-digestion results for manure (F1) and starch (F2). F1 + F2 Eq = 1:1 ratio of VS; F1 + F2 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate treatment averages with error bars showing standard deviation. Dashed lines indicate treatment prediction assuming no synergy.

Figure 4 and Figure 5 show the same curves for the co-digestion of manure and slaughterhouse waste. These co-digestion treatments show that combining the feedstocks leads to a faster rate of methane production initially. They also show that co-digestion alleviates the lag phase experienced by the slaughterhouse waste.

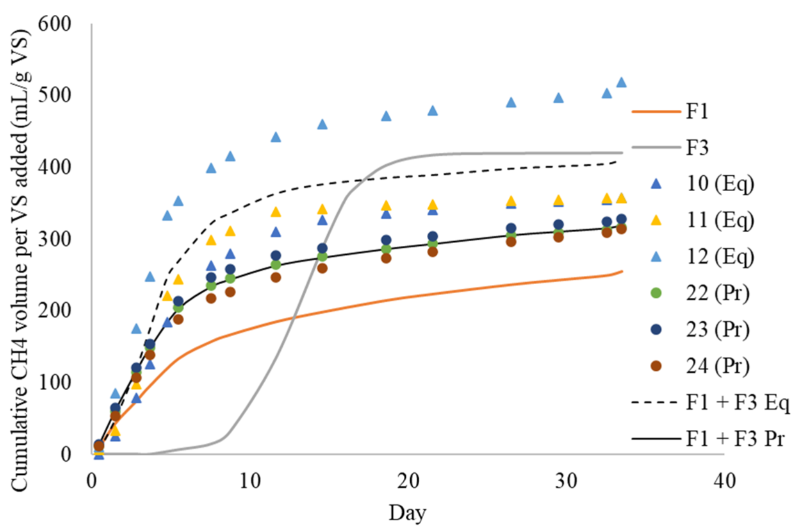

Figure 4: Cumulative specific methane production for manure (F1) and slaughterhouse waste (F3). F1 + F3 Eq = 1:1 ratio of VS; F1 + F3 Pr = ratio of VS is proportional to what full-scale digester receives.

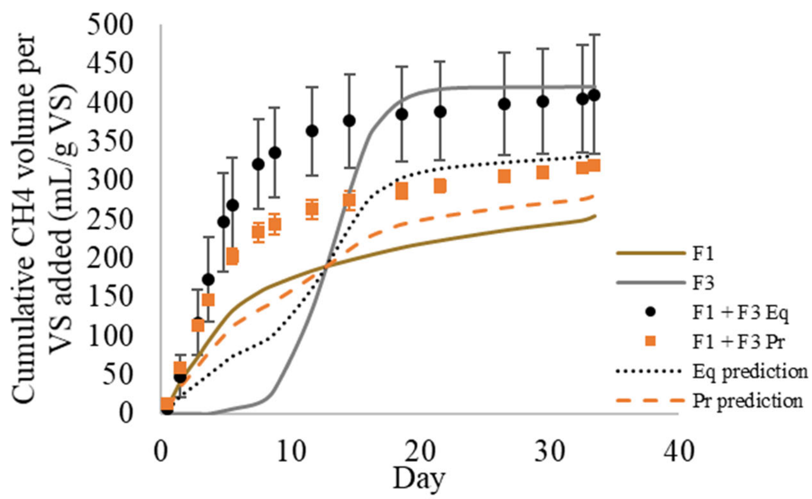

Figure 5: Co-digestion results for manure (F1) and slaughterhouse waste (F3). F1 + F3 Eq = 1:1 ratio of VS; F1 + F3 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate treatment averages with error bars showing standard deviation. Dashed lines indicate treatment prediction assuming no synergy.

Figure 6 and Figure 7 likewise show the same curves for manure and soap stock. Interestingly, while the same trend of improved kinetics and reduced lag phase holds true for these treatments, the F1 + F5 Eq treatment does experience a mild delay in methane production around day 3 as compared to the proportional treatment. This may indicate that the higher proportion of soap stock did have an impact on the methane production.

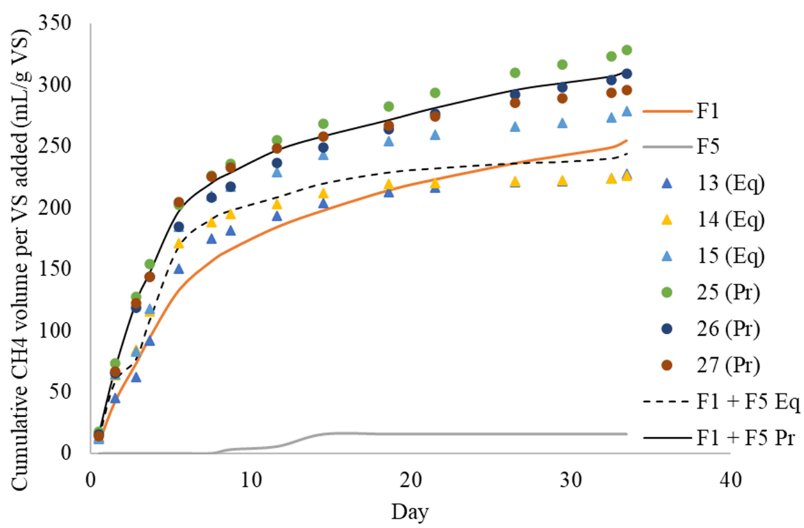

Figure 6: Cumulative specific methane production for manure (F1) and soap stock (F5). F1 + F5 Eq = 1:1 ratio of VS; F1 + F5 Pr = ratio of VS is proportional to what full-scale digester receives.

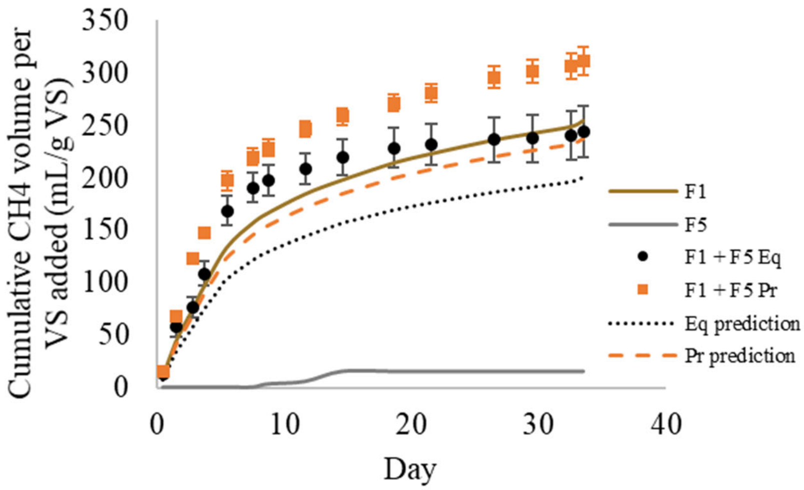

Figure 7: Co-digestion results for manure (F1) and soap stock (F5). F1 + F5 Eq = 1:1 ratio of VS; F1 + F5 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate treatment averages with error bars showing standard deviation. Dashed lines indicate treatment prediction assuming no synergy.

A similar phenomenon can be observed in Figure 8 and Figure 9, where the treatment with equal portions of manure and filter press slurry likewise experiences a slight delay in gas production.

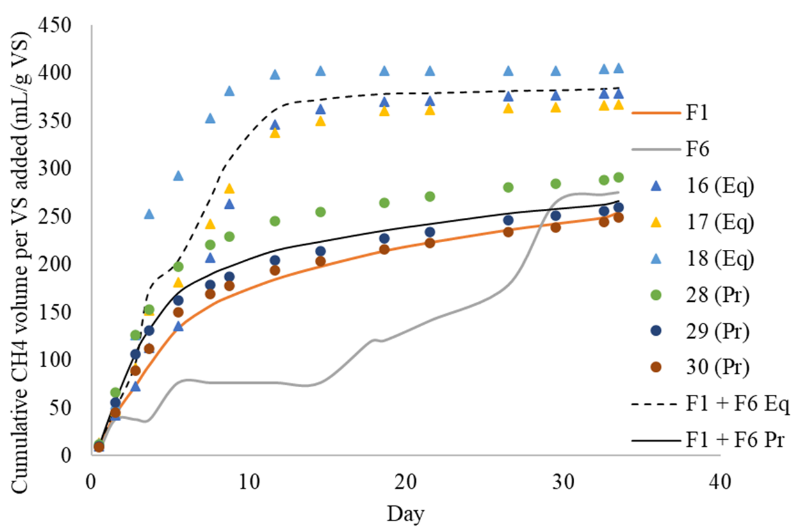

Figure 8: Cumulative specific methane production for manure (F1) and filter press slurry (F6). F1 + F6 Eq = 1:1 ratio of VS; F1 + F6 Pr = ratio of VS is proportional to what full-scale digester receives.

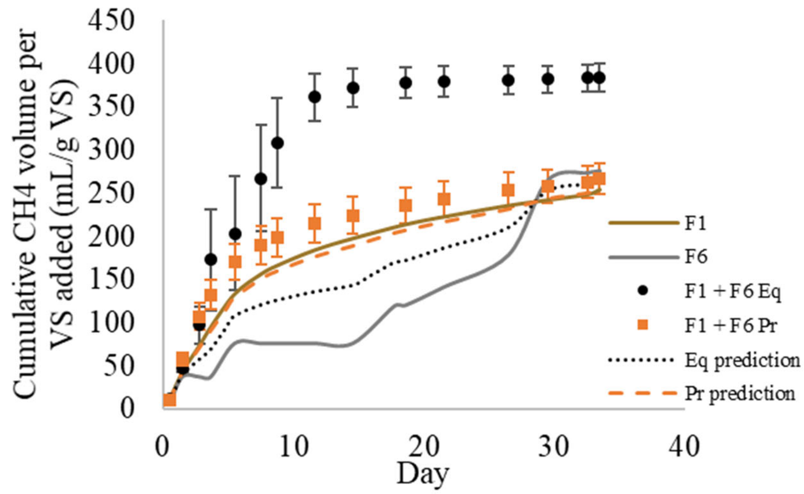

Figure 9: Co-digestion results for manure (F1) and filter press slurry (F6). F1 + F6 Eq = 1:1 ratio of VS; F1 + F6 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate treatment averages with error bars showing standard deviation. Dashed lines indicate treatment prediction assuming no synergy.

Finally, Figure 10 and Figure 11 show that despite the rapid cessation of gas production in the starch and soap stock combination, they still performed better together than separately. It is possible that for both the starch mono-digestion treatment and the co-digestion treatment rapid degradation of readily digestible carbohydrates led to accumulation of acids, inhibiting further gas production. This could be a possible cause for concern for Bio Town Ag as they consider adding readily available carbohydrates such as starch to their digester in large quantities.

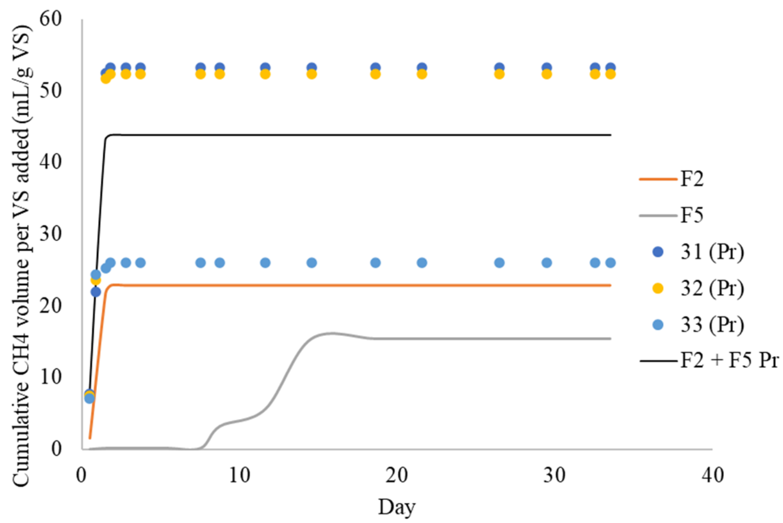

Figure 10: Cumulative specific methane production for starch (F2) and soap stock (F5). F2 + F5 Pr = ratio of VS is proportional to what full-scale digester receives.

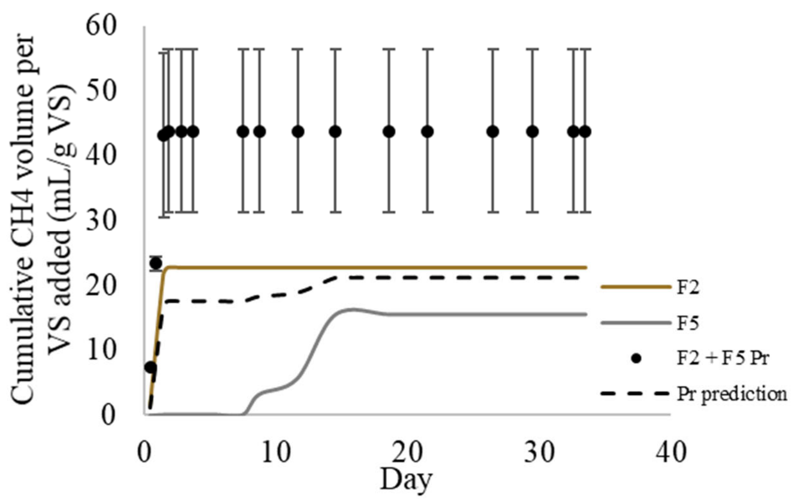

Figure 11: Co-digestion results for starch (F2) and soap stock (F5). F2 + F5 Eq = 1:1 ratio of VS; F2 + F5 Pr = ratio of VS is proportional to what full-scale digester receives. Points indicate treatment averages with error bars showing standard deviation. Dashed lines indicate treatment prediction assuming no synergy.

Next Steps

Experimental plan for testing feedstock variability

As described in Table 3, feedstocks from April 2021 and September 2021 exhibited substantial differences in macromolecular composition. We will repeat the assay measurements on the five feedstocks used in Experiment 2 on samples collected in March 2022 and again on samples collected in May and June 2022 to analyze the extent of variation between samples.

Experimental plan for synergy characterization

Follow-up experiments are needed to confirm the results of E2 and further explore the causes of the improvements in methane production. Combinations of more than two feedstocks is not common in literature, and most literature looks at amount or percentage or ratio of macromolecules to each other rather than comparing the results when the same proportions and amounts of these macromolecules are digested using different feedstocks. S. Astals et al. observed kinetic and yield synergy with a 17% carbohydrate, 66% lipid, 17% protein combination and a 33% split between the three (2014). Therefore, I propose conducting another co-digestion experiment (E3) as shown in Table 4. The purpose of this experiment would be three-fold. First, we will compare the effects of a three-way combination of feedstocks on yield and kinetic synergy to that of pairwise combinations to determine whether those effects continue to be relevant. Second, we will hold percentage of carbohydrates, lipids, and proteins constant for two sets of four treatments with different feedstocks each to establish the importance of feedstock on the results. Third, while holding percentages of macromolecules constant, we will also hold amount of macromolecules constant for at least three pairs of treatments while using different feedstocks (pairs will be 1 and 3, 5 and 7, and 6 and 8).

Table 4: Experiment 3 treatments with the mass of carbohydrates, lipids and proteins added to the digesters (not including inoculum).

Treat #	Treatment	n	S1 (wt%)	S2 (wt%)	S3 (wt%)	% C, L, P	C (g)	L (g)	P (g)
	Blank	1	--	--	--	--	--	--	--
	F1	1	100	--	--	--	30	4	31
	F2	1	100	--	--	--	241	2	4
	F3	1	100	--	--	--	5	47	25
	F5	1	100	--	--	--	1	18	6
	F6	1	100	--	--	--	17	63	4
1	F1+F2+F3	3	43	4	53	33, 33, 33	26	26	26
2	F1+F2+F5	3	30	2	69	33, 33, 33	13	13	13
3	F1+F3+F6	3	63	14	23	33, 33, 33	24	24	24
4	F1+F5+F6	3	50	34	16	33, 33, 33	18	18	18
5	F1+F3+F6	3	7	37	56	17, 66, 17	14	53	14
6	F1+F5+F6	3	8	65	27	17, 66, 17	7	29	7
7	F2+F3+F6	3	1	48	51	17, 66, 17	14	54	14
8	F2+F5+F6	3	1	87	12	17, 66, 17	6	23	6
	Total:	30							

F1 = pad manure, F2 = starch, F3 = slaughterhouse waste, F5 = soap stock, F6 = filter press slurry, Blank = inoculum only; S1 = substrate 1 (first substrate in list), S2 = substrate 2; S3 = substrate 3; C = carbohydrates; P = proteins; L = lipids.

A third co-digestion experiment (E4) will be carried out to follow up the results of the third co-digestion experiment and any other experiments with unusual results. This could include redoing the complete mixture digesters from E1 with all the feedstocks, retesting F3, F5, and F6 to determine the cause of the discrepancy between E1 and E2, and following up on any interesting and unusual results from E3.

References

Astals, S., Batstone, D. J., Mata-Alvarez, J., & Jensen, P. D. (2014). Identification of synergistic impacts during anaerobic co-digestion of organic wastes. *Bioresource Technology*, *169*, 421–427. https://doi.org/10.1016/j.biortech.2014.07.024