Results from Second Bio Town Ag Co-digestion Experiment

(Experiment 3)

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Experimental Summary

Experiment 3 (E3): Experimental description

The objective of the experiment was to continue evaluating potential synergism or antagonism from combinations of some of the feedstocks used in Experiment 1 (E1, conducted in April 2021). The results from the first co-digestion experiment (E2, conducted in September 2021) suggested that pairwise combinations of feedstocks resulted in synergy. This experiment was designed to evaluate whether balanced proportions of macromolecules impacted this synergy while using different combinations of feedstocks. Four treatments received approximately equal proportions of carbohydrates, proteins, and lipids in terms of mass added to the digester while varying the two of the three feedstocks. Three additional treatments received approximately the same proportions of lipids and proteins as each other but much more carbohydrates. A final treatment had similar proportions of macromolecules as the high carbohydrate treatment but restricted the amount of manure. Manure was used in all digesters due to its high prevalence in BTA waste streams and its favorable macromolecular characteristics.

We conducted the co-digestion biomethane potential (BMP) test in May 2022. 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 and E2. Table 1 shows the treatment combinations of the feedstocks. The feedstocks are numbered the same as in E1 and E2 for consistency. Each digester received a 2:1 substrate to inoculum ratio by volatile solids. Each digester was diluted by half to maintain total solids contents less than 7% to minimize foaming issues.

Table 1: Experiment 3 treatments.

Treatment	n	I (wt%)	S1 (wt%)	S2 (wt%)	S3 (wt%)	W (wt%)	%C/P/L (added)
Blank	1	50	--	--	--	50	--
F1	1	34	16	--	--	50	--
F2	1	40	10	--	--	50	--
F3	1	36	14	--	--	50	--
F5	1	34	17	--	--	50	--
F6	1	28	22	--	--	50	--
F1+F2+F3	3	37	5	2	6	50	33/33/33
F1+F3+F5	3	35	11	1	3	50	33/33/33
F1+F3+F6	3	34	9	3	4	50	33/33/33
F1+F5+F6	3	33	12	2	3	50	33/33/33
F1+F2+F3	3	38	3	6	3	50	66/17/17
F1+F2+F5	3	37	6	5	2	50	66/17/17
F1+F2+F6	3	35	7	3	5	50	66/17/17
F1+F2+F3	3	38	1	6	5	50	60/17/23
Total:	30						

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; S3 = substrate 3

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.

Experimental Results

Gas production

Comparison of mono-digestion results between E1, E2, and E3

Figure 1 compares the specific biogas production of the mono-digestion of the individual feedstocks from E1, E2, and E3. 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.

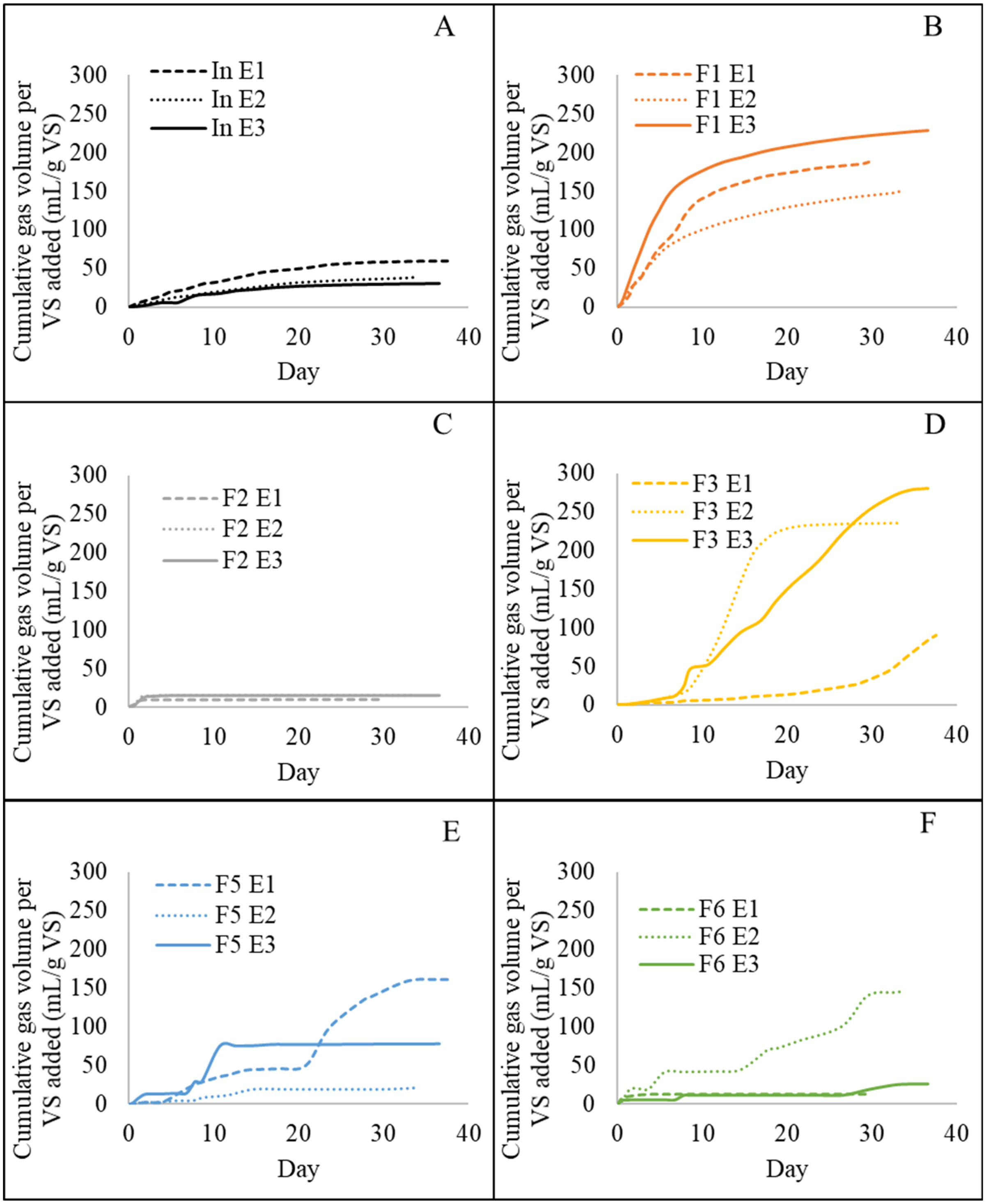

Figure 1: Comparison of mono-digestion results between E1, E2, and E3.

Synergistic effects of co-digestion

During E3, we observed some total yield and kinetic synergy in a few treatments, where synergy is defined as exceeding the gas production anticipated from the additive effect. An additive effect can be calculated as a weighted average between the mono-digestion specific methane potential curves at each time point. The mono-digestion results from this experiment were used to calculate the predicted methane production assuming the yield is additive.

Table 3 shows the methane yield results for each digester and overall treatment averages. Although there is some variability within each treatment, a clear pattern emerges overall. The digesters with a more balanced feed macromolecular composition either experienced overall yield synergy or at least (in the case of F1+F3+F6) approximately additive results. However, the high carbohydrate digesters all experienced rapid acidification, leading to digester collapse and total yield antagonism.

Table 3: Final cumulative biomethane potential of each digester in terms of volume produced per mass of volatile solids (mL CH_4/g VS). The estimated contribution of inoculum is subtracted from the results. Prediction of gas production is estimated based on the mono-digestion control results. A positive percent improvement over prediction indicates that more methane per amount volatile solids was produced than estimated based on the mono-digestion results.

Digester	Treatment	Methane produced (mL/g VS)	Treatment average (mL CH_4/g VS)	Percent improvement over prediction
1	Inoculum	0.0		
2	F1 (pad manure)	321.3		
3	F2 (starch)	18.2		
4	F3 (slaughterhouse waste)	252.9		
5	F5 (soap stock)	61.5		
6	F6 (filter press slurry)	14.3		
7	F1+F2+F3, 33% carbs added (33% protein, 33% lipid)	304.4	350.8	41%
8		393.5		82%
9		354.6		64%
10	F1+F2+F5, 33% carbs added	432.6	367.5	81%
11		332.3		39%
12		337.4		41%
13	F1+F3+F6, 33% carbs added	253.7	259.9	1%
14		284.9		13%
15		241.2		-4%
16	F1+F5+F6, 33% carbs added	234.7	314.3	-8%
17		340.8		34%
18		367.2		44%
19	F1+F2+F3, 66% carbs added (17% protein, 17% lipid)	16.8	17.5	-86%
20		16.0		-87%
21		19.8		-83%
22	F1+F2+F5, 66% carbs added	24.5	26.0	-82%
23		26.7		-81%
24		26.9		-80%
25	F1+F2+F6, 66% carbs added	14.8	17.5	-90%
26		20.8		-86%
27		17.1		-89%
28	F1+F2+F3, 60% carbs added (low manure)	10.9	10.0	-90%
29		9.1		-92%
30		9.9		-91%

In terms of kinetic behavior, the results are less clear. As Figure 2 shows, there was significant variability in the manure + starch + slaughterhouse waste treatment with 33%

carbohydrates. However, the overall treatment digested faster and produced more methane per mass volatile solids added than the additive prediction.

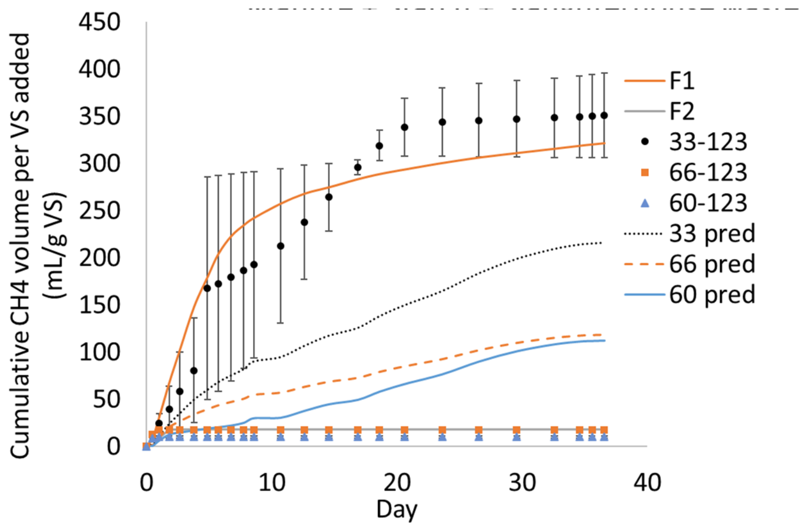

Figure 2: Cumulative methane production curves for the three manure, starch, and slaughterhouse waste (F1+F2+F3) treatments. Error bars indicate standard deviation and points represent treatment averages. Dotted lines indicate prediction curves based on weighted average of mono-digestion controls.

The manure + starch + soapstock with 33% carbohydrates treatment appeared more regular as shown in Figure 3. The early kinetic results were very close to the additive prediction.

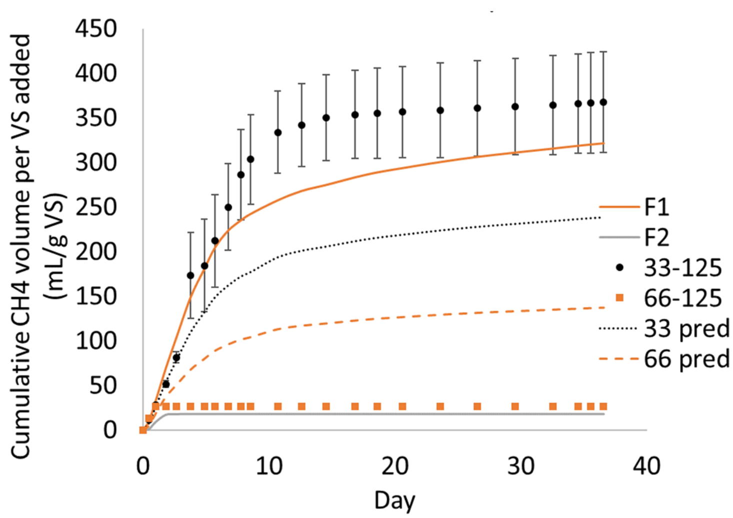

Figure 3: Cumulative methane production curves for the two manure, starch, and soap stock (F1+F2+F5) treatments. Error bars indicate standard deviation and points represent treatment averages. Dotted lines indicate prediction curves based on weighted average of mono-digestion controls.

Figure 4 shows that the manure, slaughterhouse waste, and filter press slurry treatment ultimately behaved quite similarly to the methane yield additive prediction, but there appears to be some sort of antagonistic effect that delayed the gas production kinetics.

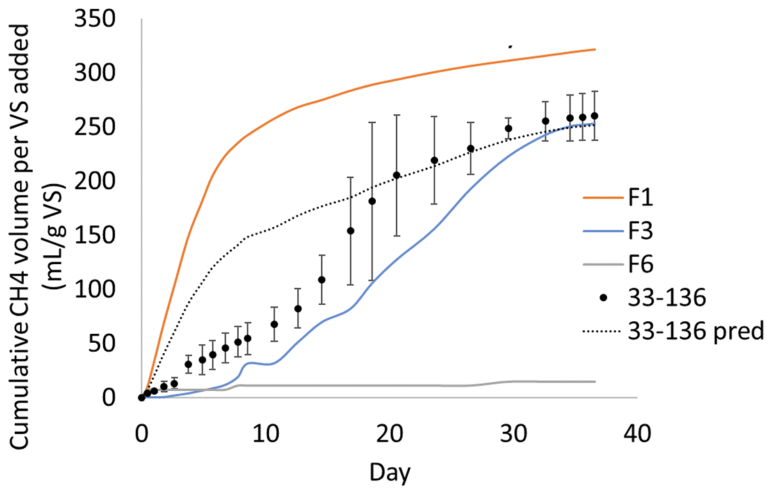

Figure 4: Cumulative methane production curves for the manure, slaughterhouse waste, and filter press slurry (F1+F3+F6) treatment. Error bars indicate standard deviation and points represent treatment averages. Dotted lines indicate prediction curves based on weighted average of mono-digestion controls.

Figure 5 shows that the combination of manure, soapstock, and filter press slurry generally outperformed the additive prediction, although with the variability between digesters in the treatment it is difficult to say that synergy is definitely occurring in this treatment.

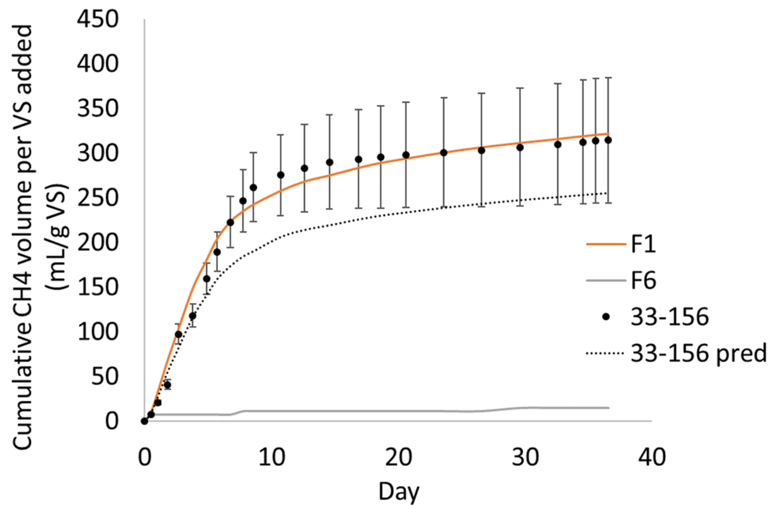

Figure 5: Cumulative methane production curve for the manure, soap stock, and filter press slurry (F1+F5+F6) treatment. Error bars indicate standard deviation and points represent treatment averages. Dotted line indicates prediction curve based on weighted average of mono-digestion controls.

Figure 6 shows that the high carbohydrate treatment was unsuccessful for manure, starch, and filter press slurry, similar to the other high carbohydrate treatments.

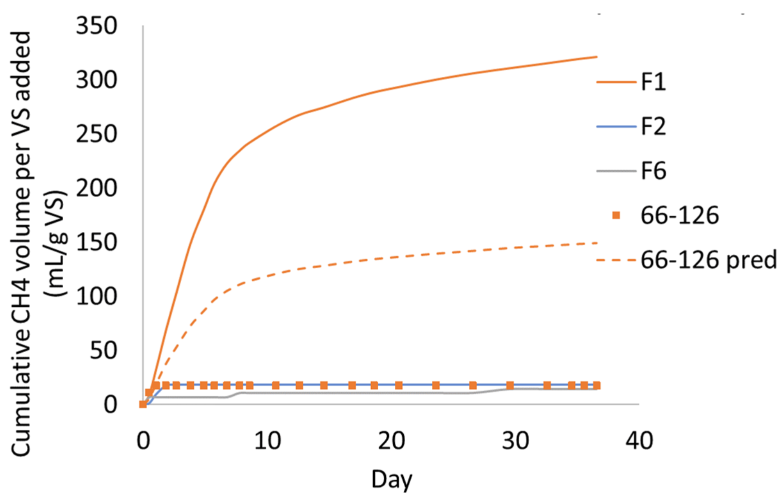

Figure 6: Cumulative methane production curve for the manure, starch, and filter press slurry (F1+F2+F6) treatment. Error bars indicate standard deviation and points represent treatment averages. Dotted line indicates prediction curve based on weighted average of mono-digestion controls.

Figure 7 compares the methane production curves for the low and high carbohydrate treatments. Three of the four low carbohydrate treatments have fairly similar curve shapes, and all four high carbohydrate treatments rapidly produced gas prior to quick failure. Upon closer inspection, it appears that both low carbohydrate treatments that included slaughterhouse waste experienced a lag phase. Although this lag phase was an improvement over that experienced by the slaughterhouse waste alone, it may be an indicator of some antagonism from substances in the slaughterhouse waste.

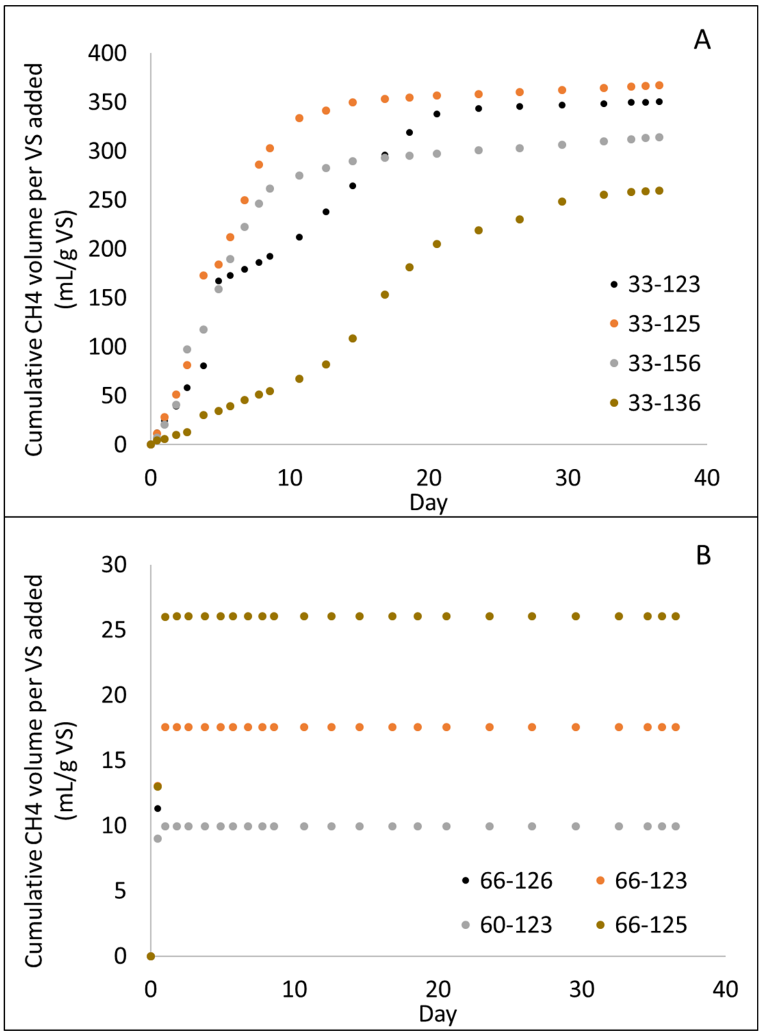

Figure 7: Cumulative methane production curves for the low (A) carbohydrate and high (B) carbohydrate treatments.

In conclusion, these results show that in this case, the balanced 33% carbohydrate, 33% protein, and 33% lipid mixtures generally performed at least as well as the prediction based on a weighted average of the results of the mono-digestion controls. However, the high carbohydrate digesters significantly underperformed expectations, likely due to acidification leading to rapid digester failure early in the digestion process. While a specific ratio of macromolecules does not guarantee a digester's kinetic performance, the 33% carbohydrate, 33% protein, and 33% lipid treatments performed relatively similarly to each other even with varied feedstocks. It is also clear that high carbohydrate amounts can cause problems for a digester.