Final Technical Report for FY24 Research Projects

Funded by the North Dakota Soybean Council
Submitted by Iris Feng, 06/30/2024

a. Research Project Title, Principal and Co-Investigators

Title: Anaerobic Digestion of Defatted Soybean Meal for Biogas and Biofertilizer Productions

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b. Research Overview and Objectives

Background information: Renewable energy drive will boost soybeans production to approximately 5.3 billion by 2030 with over 390,000 metric tons of defatted soybean meal (DSM) annually in North Dakota. DSM contains valuable protein and essential micronutrients, but an anti-nutritional reagent employed to optimize soybean oil significantly restricts its food applications. Additionally, due to the small livestock size in ND, only 23% of projected DSM would be consumed annually for animal feed. Thus, there is a pressing demand to explore and expand new applications for DSM. Anaerobic digestion (AD) is a well-established technology that decomposes biomass in the absence of oxygen, generating biogas and biofertilizer which can be locally utilized for heating and plant operations, and as a substitute for commercial fertilizer. This innovative use of DSM offers ND producers a sustainable and cost-efficient alternative for renewable energy production and nutrient recovery.

Objectives: This proposal aims to assess the new use of DSM by investigating the effect of physical properties and chemical pretreatments of DSM for increasing biogas yields in AD, enhancing methane production and biofertilizer quality from DSM with co-digestion inclusion for energy sustainability and nutrient recovery. Specific objectives include 1). Evaluate the impact of feedstock to inoculum ratio, particle sizes, and temperatures on methane yield of DSM anaerobically digested in lab-scale reactors; 2) Investigate the effect of wet-state alkaline pretreatments of DSM on methane yield; 3) Enhance methane yield and biofertilizer quality of anaerobically digested alkaline pretreated DSM with co-digestion inclusion.

c. Materials and Methods

DSM samples were collected from the Northern Crops Institute at North Dakota State University (NDSU) in Fargo, ND. These samples underwent Scanning Electron Microscopy (SEM) analysis at NDSU's Microcore Lab and chemical properties analysis at NDSU's Animal Science Lab. Inoculum for the experiments was sourced from the Fargo Wastewater Treatment Plant. Manure used for co-digestion experiment was collected from the NDSU dairy research center.

The inoculum was mixed with DSM at two feedstock to inoculum (F:I) ratios of 1:2 and 4:1. For each ratio, the DSM samples were processed in a blender, then sieved into three particle sizes: fine (<0.48 mm), medium (0.48–0.70 mm), and large (0.72–1.00 mm). Each particle size group underwent anaerobic digestion in 500 mL bottles at both mesophilic (35°C) and thermophilic (55°C) temperatures. The experiment included six treatments plus one control (inoculum only), each replicated three times. Selection of particle size, temperature, and F:I ratio for further experiments was based on methane yield. Pretreatment of DSM involved 2% NaOH, 4% NaOH, 2% Urea, and 4% Urea. Co-digestion experiments with dairy manure were conducted at 1:1 and 1:2 mixing ratios, with and without the selected pretreatments. All treatments followed the established anaerobic digestion (AD) procedures. Biogas and slurry analyses adhered to previously described protocols.

Temperature control was managed using water baths. Daily biogas production was monitored using the biomethane potential (BMP) method in the lab. Biogas composition (CH_4, CO_2, and H_2S) was measured twice weekly using an SRI gas chromatograph (GC, SRI Instruments, California, USA) and a Jerome meter (Jerome 631X, Arizona Instrument LLC, Arizona, USA) sulfide analyzer. Pre-digested and post-digested samples from each treatment were collected for analysis of chemical properties and nutrients at NDSU's Animal Science Lab and AGVISE Lab, respectively. Slurry samples were also sent to LC Sciences (Houston, TX) for microbial analysis.

All treatments were performed in triplicate. Statistical analyses were conducted using Microsoft Excel and RStudio.

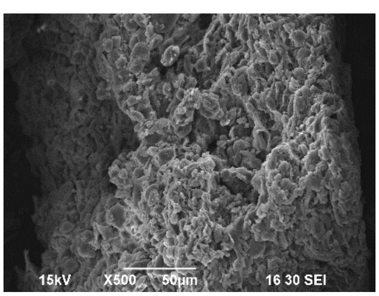
d. Research Results/Outcomes

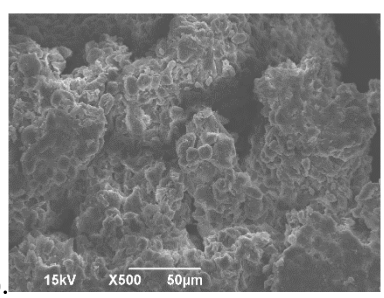
The raw DSM was analyzed for its chemical properties, including dry matter (DM), crude protein (CP), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, cellulose, ammonia, acetic acid, carbon (C), nitrogen (N), and sulfur (S) before the experiment (Table 1). The DM content was 92.65%, indicating that the raw DSM was quite dry. This high dry matter content suggests that the DSM might be difficult to digest at the beginning due to its low moisture content. The CP content was 52.82%, which is relatively high. This high protein content indicates that DSM is a rich source of nitrogen, making it a valuable feedstock for AD processes, as proteins can be broken down into biogas. Additionally, the C content was 44.45%, which is also high and beneficial for the AD process, as a higher C content can contribute to a higher biogas yield. Other components, such as hemicellulose (6.04%), cellulose (3.26%), ADF (6.22%), and NDF (9.47%), provide insights into the fibrous nature of the DSM. These fibrous components are crucial for understanding the breakdown process during AD, as they impact the rate and efficiency of microbial digestion.

The DSM was ground into three particle sizes for further experiments. The Scanning Electron Microscopy (SEM) analysis, shown in Figure 1, provides detailed images of the DSM structure, which helps in understanding how particle size affects the digestion process.

Table 1. Chemical properties and nutrient content of raw DSM samples.

Components	**Ammonia (mM)**	**Acetic (mM)**	**C (%)**	**N (%)**	**S (%)**	**DM (%)**
	1.00	3.91	44.45	8.46	0.44	92.65
Components	**Hemicellulose (%)**	**Cellulose (%)**	**CP (%)**	**NDF (%)**	**ADF (%)**	**ADL (%)**
	6.04	3.26	52.82	9.47	6.22	0.18

a). 

b). 

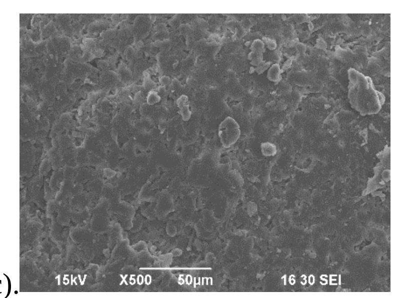
c). 

Figure 1. Scanning Electron Microscopy (SEM) analysis for soybean meal raw samples: a). Fine particle size; b). Medium particle size; c) Large particle size.

Effects of Feedstock to Inoculum Ratio, Temperature and Particle Size on Anerobic Digestion of DSM

All anaerobic digestion (AD) experiments were conducted over a 30-day period in water baths set to mesophilic (35°C) or thermophilic (55°C) temperatures. Due to spatial limitation of each water bath, these experiments were performed in two separate runs with triplicated controls and treatments, maintaining a minimum of three replicates per treatment.

i. Feedstock to Inoculum Ratio of 4:1 (F:I=4:1)

Cumulative biogas and methane yields for all treatments were evaluated under both temperature conditions and the results are presented in Figures 2 and 3. For the mesophilic condition, biogas production varied by particle size with the cumulative gas volume at day 30 of 1476 ± 41.1 mL for fine, 1568 ± 692.0 mL for medium, and 1827 ± 1042.6 mL for large, respectively (Figure 2). In contrast, the thermophilic condition resulted in lower yields which were 1075 ± 655.1 mL for fine, 889 ± 121.2 mL for medium, and 1319 ± 268.1 mL for large, respectively (Figure 2). The larger particles consistently produced more biogas than the medium and fine particles across both temperatures. Overall, the mesophilic condition had a significantly better performance on biogas production than the thermophilic condition of all three particle sizes ($P<0.05$).

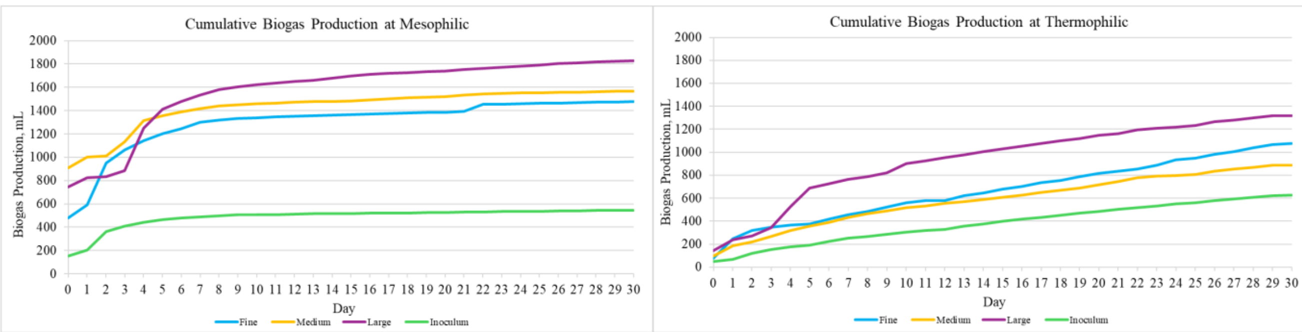


Figure 2. Cumulative biogas production of three particle sizes at mesophilic and thermophilic for F:I=4:1.

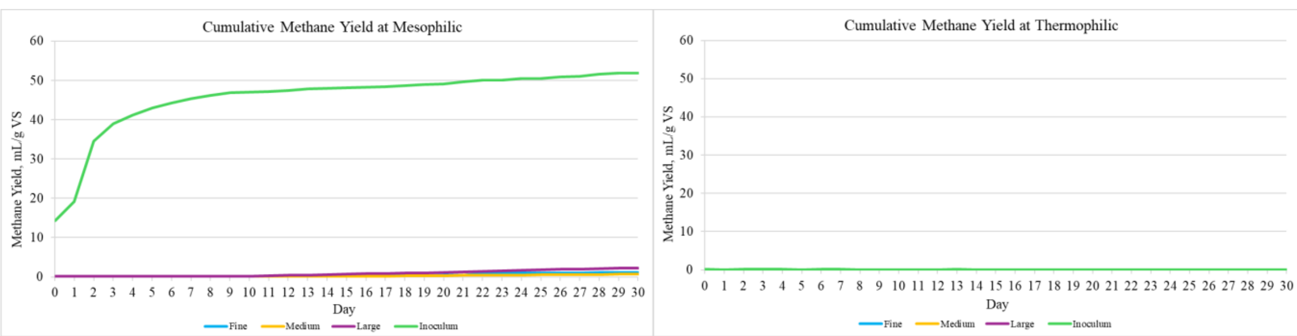


Figure 3. Cumulative methane yield of three particle sizes at mesophilic and thermophilic for F:I=4:1.

Methane concentrations within the biogas were generally low, with the inoculum producing the highest methane yields at both temperatures (Figure 3). At mesophilic conditions, cumulative methane yields were 52 mL/g VS for inoculum, 1.1 mL/g VS for fine, 0.7 mL/g VS for medium, and 2.3 mL/g VS for large, respectively (Figure 3). Thermophilic conditions resulted in negligible methane yields for all treatments, suggesting limited microbial activity at this higher temperature (Figure 3).

ii. Feedstock to Inoculum Ratio of 1:2 (F:I=1:2)

Feedstock to inoculum ratio of 1:2 was investigated, and the results of biogas production was shown in Figure 4. The inoculum produced more biogas at the higher temperature; however, the overall cumulative gas productions were greater at mesophilic temperatures for all particle sizes (Figure 4). A spike in biogas production observed on days 1-3 was attributed to missing data from the second run of the F:I=1:2 experiment, causing inconsistencies in the averaged results on those days. The medium particle size showed the highest biogas production at both temperatures which were 1637 ± 610.4 mL at mesophilic and 1516 ± 194.6 mL at thermophilic, respectively.

Methane yields, as shown in Figure 5, were consistently higher under mesophilic conditions than under thermophilic conditions for all particle sizes. The cumulative methane yields at mesophilic were 198 mL/g VS for fine, 229 mL/g VS for medium, and 141 mL/g VS for large, respectively. At thermophilic temperatures, the yields were 62 mL/g VS for fine, 111 mL/g VS for medium, and 51 mL/g VS for large particles, respectively, which were significantly lower than those at mesophilic. These results indicated a better methane yield at the lower temperature with the medium particle size achieving the highest yield.

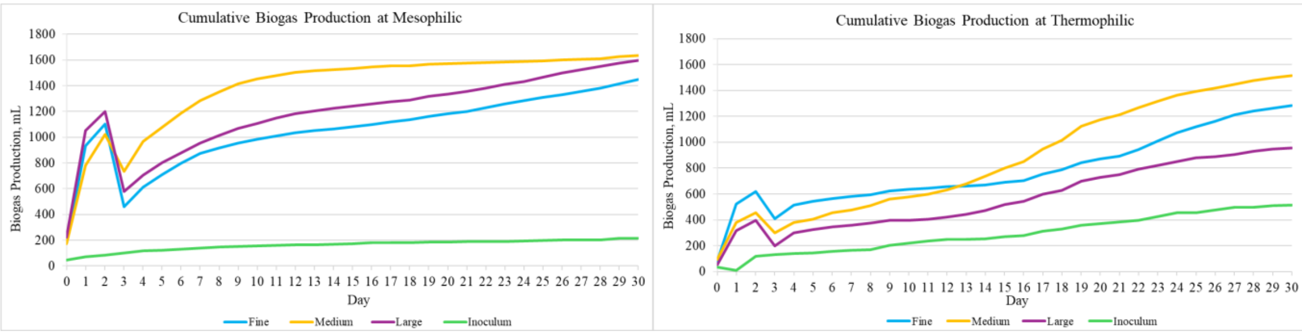


Figure 4. Cumulative biogas production of three particle sizes at mesophilic and thermophilic for F:I=1:2.

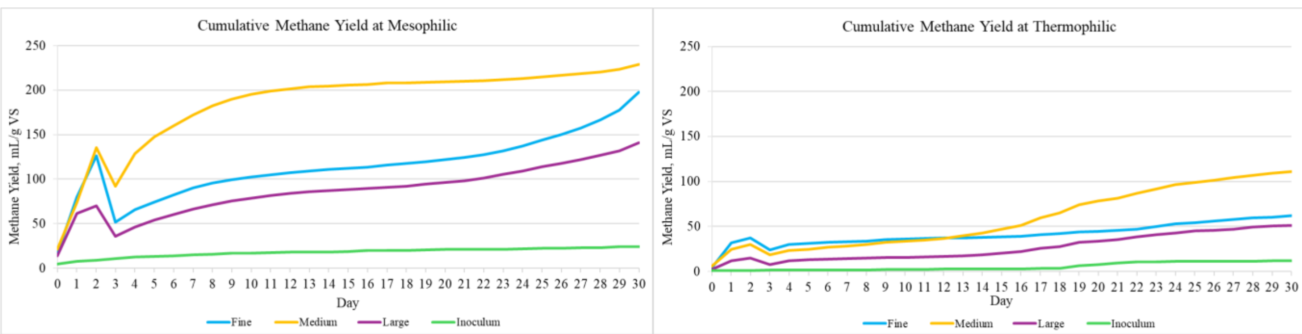


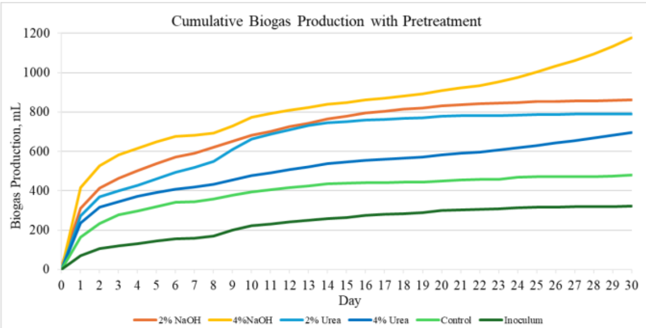
Figure 5. Cumulative methane yield of three particle sizes at mesophilic and thermophilic for F:I=1:2.

In summary, the biogas production for both the F:I=4:1 and F:I=1:2 ratios was comparable at both temperatures. However, biogas production was consistently higher under mesophilic conditions compared to thermophilic for both ratios. The methane yield for the F:I=1:2 ratio significantly higher than that of the F:I=4:1 ratio at both temperatures. Based on these results, the optimal conditions for methane production were identified as an F:I ratio of 1:2, using a medium particle size, under mesophilic conditions. The following experiments would be conducted using these parameters to further validate and expand upon these findings.

Effects of Alkali Pretreatment on Anaerobic Digestion of DSM

Two alkalis, NaOH and urea, were used at two concentrations (2% and 4%), resulting in four pretreatment groups. The pretreated DSM with a medium particle size were prepared in the lab four days prior to the experiment and mixed with inoculum at a F:I of 1:2. Non-pretreated DSM served as the control, and the blank treatment consisted only of inoculum. Thus, the experimental setup included four pretreatments: 2% NaOH, 4% NaOH, 2% Urea, and 4% Urea, along with one control and one blank, all processed in triplicates and anaerobically digested at 35°C for 30 days.

Cumulative biogas production and methane yield results are presented in Figures 6 and 7. DSM pretreated with NaOH produced more biogas, with yields of 1178 ± 357.7 mL for 4% NaOH and 791 ± 171.9 mL for 2% NaOH, compared to urea pretreatments which yielded 697 ± 369.0 mL for 4% urea and 591 ± 171.9 mL for 2% urea. All alkali pretreatments resulted in higher biogas production than the control (479 ± 34.9 mL), indicating the potential of alkali pretreatments to enhance biogas production from DSM in AD.



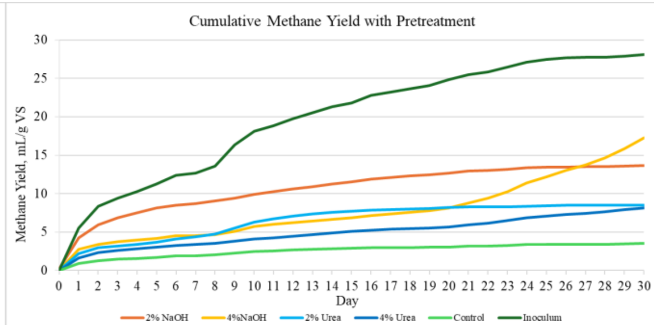


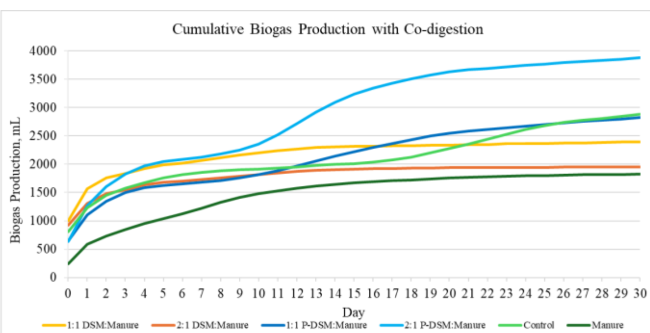
Figure 6. Cumulative biogas production of alkali pretreatments.

Figure 7. Cumulative methane yield of alkali pretreatments.

Methane yields were generally low across all pretreatments, as shown in Figure 7. NaOH pretreatments resulted in higher methane levels than urea, with yields of 17.2 mL/g VS for 4% NaOH and 13.6 mL/g VS for 2% NaOH, compared to 8.1 mL/g VS for 4% Urea and 8.5 mL/g VS for 2% Urea. However, the highest cumulative methane yield was observed in the blank inoculum treatment, achieving 28.1 mL/g VS—significantly higher than all the pretreated groups and the control, which yielded only 3.5 mL/g VS. This suggests that while alkali pretreatments may enhance biogas production, they do not significantly improve the methane concentration in the biogas produced during AD.

Effects of Manure Co-Digestion on Anaerobic Digestion of DSM

To enhance the biogas and methane yields from AD of DSM, co-digestion with manure was investigated under mesophilic conditions for 30 days. Two DSM to manure mixing ratios (1:1 and 2:1) were tested both with and without DSM pretreated with 4% NaOH, resulting in four treatment groups. The control group consisted of DSM without any pretreatment or co-digestion, while manure alone served as the blank group in this experiment.



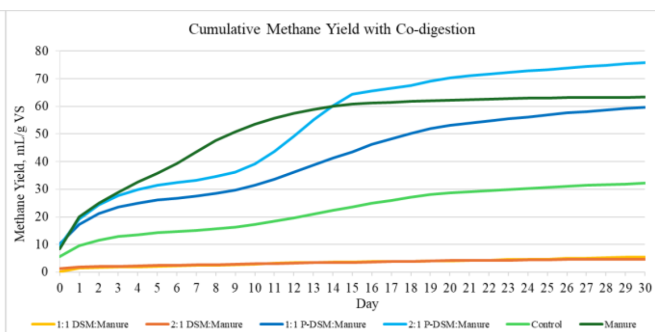


Figure 8. Cumulative biogas production of manure co-digestion.

Figure 9. Cumulative methane yield of manure co-digestion.

The inclusion of manure co-digestion significantly improved total biogas production compared to the results of previous experiments ($P<0.05$). Without NaOH pretreatment, the cumulative biogas productions for

DSM to manure ratios of 1:1 and 2:1 were 2391 ± 722.2 mL and 1954 ± 396.2 mL, respectively, as shown in Figure 8. With 4% NaOH pretreatment of DSM, biogas accumulations increased to 2824 ± 220.8 mL and 3877 ± 1562.6 mL for DSM to manure ratios of 1:1 and 2:1, respectively.

Methane yields are presented in Figure 9. The blank group, consisting of manure only, had a relatively high methane concentration, yielding 63.4 mL/g VS. This indicates that the manure sample contained useful and sufficient microbial and biosolid for methane production during AD. In contrast, the manure co-digestion groups without NaOH pretreatment produced significantly lower methane yields of 5.4 mL/g VS and 4.7 mL/g VS for the 1:1 and 2:1 ratios, respectively, compared to the control group's yield of 32.2 mL/g VS. However, the groups treated with 4% NaOH pretreatment exhibited significant improvements in methane yields, achieving 59.7 mL/g VS and 75.9 mL/g VS for the 1:1 and 2:1 ratio, respectively ($P<0.05$). This demonstrates the effectiveness of alkali pretreatment combined with manure co-digestion in enhancing the biogas and methane yields in AD.

e. Listings of any disclosures of inventions or plant varieties:

Not applicable for this project.

f. Discussion

Feedstock to Inoculum Ratio and Temperature

According to the results from the two F:I ratios, although the total biogas production for both ratios did not show significant differences, the methane content in the F:I=1:2 was much greater than that in the F:I=4:1 which might be attributed to the properties of the DSM. DSM is an extremely dry feedstock with high CP and C content, with less inoculum at the initial stage (as in the F:I=4:1), it might be challenging for the methanogens to adapt to the DSM or they may require a longer period in the AD process to produce methane efficiently. The higher inoculum content in the F:I=1:2 likely provided a more favorable environment for methanogen adaptation and activity, leading to higher methane content. However, the TS content in the AD process with the F:I=1:2 was very low, which were 2.9%, 2.4%, 3.9%, and 2.4% for the fine, medium, large, and inoculum, respectively, in the ingestate. This low TS content could pose a problem for scaling up the digester, as lower solids can impact the stability and efficiency of the digestion process on a larger scale. Moreover, for F:I=4:1, the pH dropped dramatically during the AD (Table2) and this significant acid accumulation suggests that there was substantial production of VFAs or other acidic compounds, which can inhibit methanogenic activity and thus adversely affect methane production.

Thermophilic digestion generally offers faster reaction rates and shorter retention times. Under thermophilic conditions, the hydrolysis of complex organic compounds can be enhanced, potentially benefiting methane yield. However, in this study, mesophilic conditions led to higher methane yields. This suggests that mesophilic conditions might be more stable and conducive to the growth of diverse methanogenic communities in the inoculum. Moreover, the specific properties of DSM might be more effectively utilized by the microbial communities thriving under mesophilic conditions, indicating that these microbes may have adapted better to the nutrient profile of DSM.

Pretreatment

Alkali pretreatment is widely recognized in the literature for its potential to improve methane yield in AD by breaking down complex lignocellulosic structures, thus enhancing the availability of substrates for microbial action. However, in this study, alkali pretreatment did not result in an enhanced methane yield for DSM at F:I=1:2. Considering the composition of DSM as shown in Table 1, the pretreatment may have had minimal impact due to the already accessible nature of DSM's composition. Additionally, the F:I=1:2 maintained an ideal pH range (Table 2), supporting efficient microbial activity and methane production without significant acid accumulation. The stable conditions and effective adaptation of the microbial community to DSM's nutrient profile likely diminished the perceived benefits of alkali pretreatment.

Table 2. pH of feedstock before and after AD for two F:I ratios under mesophilic and thermophilic conditions.

F:I=1:2	Before	After		F:I=4:1	Before	After	
		Meso	Thermo			Meso	Thermo
Fine	7.4 ± 0.07	7.9 ± 0.23	8.1 ± 0.19	Fine	7.1 ± 0.04	6.8 ± 0.07	5.0 ± 0.10
Medium	7.4 ± 0.05	8.1 ± 0.29	8.1 ± 0.25	Medium	7.0 ± 0.01	6.5 ± 0.34	4.8 ± 0.29
Large	7.6 ± 0.06	8.0 ± 0.32	7.9 ± 0.13	Large	7.0 ± 0.00	6.4 ± 0.32	4.7 ± 0.30
Inoculum	7.3 ± 0.04	7.4 ± 0.02	8.0 ± 0.02	Inoculum	7.8 ± 0.17	7.5 ± 0.31	8.0 ± 0.38

Table 3. pH of feedstock before and after AD for treatments of pretreatment and co-digestion.

Pretreatment	Before	After	Co-digestion	Before	After
2% NaOH	7.3 ± 0.10	6.5 ± 0.07	1:1 DSM: Manure	7.5 ± 0.18	6.6 ± 0.01
4% NaOH	8.8 ± 0.04	7.0 ± 0.54	2:1 DSM: Manure	7.6 ± 0.17	6.5 ± 0.04
2% Urea	7.7 ± 0.18	6.3 ± 0.03	1:1 PT_DSM:Manure	8.4 ± 0.08	8.0 ± 0.02
4% Urea	7.7 ± 0.18	6.7 ± 0.68	2:1 PT_DSM:Manure	8.5 ± 0.02	8.1 ± 0.04
Control	7.2 ± 0.03	6.2 ± 0.03	Control	7.3 ± 0.04	8.1 ± 0.13
Inoculum	7.1 ± 0.01	7.2 ± 0.03	Inoculum	7.2 ± 0.01	NA
			Manure	7.3 ± 0.03	NA

Manure Co-digestion

The integration of manure in the co-digestion process with DSM significantly enhanced biogas production and methane yield compared to pretreatment. This improvement underscores the synergistic effects of combining different types of substrates, where manure likely provided additional nutrients and balanced the C/N ratio, facilitating more efficient microbial activity. However, the overall methane yield across all treatments remained relatively low. This might be caused by the quality change of the inoculum for this experiment. The wastewater treatment plant, from which the inoculum was sourced, experienced technical issues that impacted the quality of the inoculum. Therefore, the inoculum collected for the pretreatment and co-digestion experiments differed significantly from that used in previous AD experiments. This variation in inoculum quality could explain the unexpectedly low methane concentrations observed in the co-digestion trials. The presence of a less effective inoculum likely hindered the microbial consortia's ability to efficiently break down the organic matter present in the DSM and manure mix, leading to reduced methane production. Future experiments should consider implementing controls to ensure the consistency of inoculum quality or explore alternative sources if recurrent issues are observed at the collection site.

Potential Usage of AD Slurry as Biofertilizer

After the 30-day AD period, slurry was collected and analyzed for its chemical properties and nutrient contents, as shown in Table 4. The C/N ratios of the treatments ranged from 7.6 to 11.9, which are relatively low, with nitrogen content between 2.9% and 5.8%. This indicates that the digestate has a high N content with more nitrogen relative to carbon, suggesting that N is readily available for plant uptake. This characteristic decreases the likelihood of N being immobilized by microbial activity, thereby enhancing the potential efficiency of the digestate as a fertilizer, particularly in terms of nitrogen delivery.

The moderate levels of NDF suggest that while some cellulosic material is present, it should not significantly impede the breakdown of organic matter nor the availability of nutrients to plants. The ADF content is also moderate, reflecting the presence of more resistant plant materials like lignin and cellulose. Although these tougher materials are present, their levels are not excessive, which supports a gradual nutrient release beneficial for sustaining plant growth over time without causing rapid nutrient leaching. The VFAs were high in the pretreatments and may pose concerns. While VFAs can serve as a carbon source for soil microbes, elevated levels can lead to soil acidification or phytotoxic effects that might inhibit plant growth. However, the VFA levels in other groups ranged from 20.9 to 91.4 mM, which are less likely to cause adverse effects.

Overall, the AD slurry exhibits promising characteristics as a biofertilizer due to its high N content and low C/N ratio. However, the suitability and quality of the biofertilizer should be further evaluated for different soil types and crops through pot testing and field application to ensure optimal benefits.

Table 4. Chemical properties and nutrient analysis of slurry residues in digestate.

Sample		C/N	NDF (%)	ADF (%)	VFA (mM)	S (%)	CP (%)	DM (%)
Meso	Fine	8.7	31.7	26.4	36.5	1.0	32.2	97.0
	Medium	8.5	31.3	26.9	20.9	1.0	32.8	97.0
	Large	9.3	32.4	26.5	46.7	1.0	31.4	96.7
	Inoculum	8.8	35.2	28.4	1.4	1.0	24.0	96.7
Thermo	Fine	11.1	33.9	27.9	91.4	1.1	21.0	95.8
	Medium	11.7	34.1	28.8	43.2	0.9	18.2	97.1
	Large	11.9	37.2	29.7	79.2	1.0	19.7	96.9
	Inoculum	10.4	33.2	29.9	6.8	1.0	21.0	97.1
Pretreatment	2% NaOH	7.9	26.1	18.9	295.2	1.2	36.0	84.0
	4% NaOH	8.8	28.5	20.2	307.4	1.1	32.4	84.3
	2% Urea	7.7	32.7	19.7	273.5	1.2	34.6	85.6
	4% Urea	8.0	34.2	22.0	298.6	1.2	36.1	87.5
	Control	7.6	28.6	19.4	245.2	1.3	36.0	86.2
	Inoculum	6.7	36.5	22.8	3.8	1.4	32.6	96.2

g. Conclusion/Benefits to the North Dakota Soybean Farmers and the Industry

The results of this project have demonstrated that DSM is a promising feedstock of AD for methane production. Results indicate that methane yield may be further improved through the co-digestion of DSM with manure. This approach not only offers a sustainable alternative energy source but also helps manage farm waste effectively in North Dakota. Additionally, the use of DSM in AD processes capitalizes on soybean by-products, potentially reducing waste and increasing profitability for farmers. The process also results in the production of potential high-quality biofertilizer, characterized by its high nitrogen content and low C/N ratio, which can improve soil health and crop yields. This contribution to sustainable agriculture practices not only supports the local farming community by providing a cost-effective use of soybean by-products but also promotes environmental sustainability within the industry.