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## **Abstract**

Design and construction of full-scale anaerobic digesters that co-digest manure with various materials requires analysis of each substrate. Substrate combinations should be analyzed through a scale up procedure in which substrates are characterized, and then evaluated using biochemical methane potential assays (BMPs) and anaerobic toxicity assays (ATAs). The BMPs provide a preliminary indication of the biodegradability of a substrate and of its potential to produce methane via anaerobic digestion, while ATAs determine the degree to which a particular substrate inhibits methane production. Mixture combinations that perform well in BMPs and ATAs should be tested in laboratory-scale anaerobic digesters. Once proven in lab-scale reactors for at least three hydraulic retention times, the best mixture should be tested in a pilot-scale reactor. This paper focuses on the first steps in this process using BMPs and ATAs results to select mixtures for laboratory-scale digester testing. The baseline feedstock was beef manure obtained from concrete feedlot pens (open and covered) in eastern Iowa. Various bedding materials were available, including oat hulls, corn stover, and wood shavings. To provide additional energy production, industrial byproducts from cardboard manufacturing, enzyme production, and corn and soybean processing were also potential substrates. Substrates were characterized for TS, VS, COD, pH, alkalinity, and ammonia. Then BMPs were completed on all substrates and ATAs were performed as needed. The results reported here were used to develop mixtures for use in laboratory-scale anaerobic digester testing.

## **Keywords**

Anaerobic Digestion, Biochemical Methane Potential (BMP), Anaerobic Toxicity Assays (ATA), Beef Manure

## **Disciplines**

Agriculture | Bioresource and Agricultural Engineering

## **Comments**

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# APPROACHES FOR SELECTING ANAEROBIC DIGESTION CO-SUBSTRATES FOR A FULL-SCALE BEEF MANURE DIGESTER USING BIOCHEMICAL METHANE POTENTIALS AND ANAEROBIC TOXICITY ASSAYS

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## ABSTRACT

Design and construction of full-scale anaerobic digesters that co-digest manure with various materials requires analysis of each substrate. Substrate combinations should be analyzed through a scale up procedure in which substrates are characterized, and then evaluated using biochemical methane potential assays (BMPs) and anaerobic toxicity assays (ATAs). The BMPs provide a preliminary indication of the biodegradability of a substrate and of its potential to produce methane via anaerobic digestion, while ATAs determine the degree to which a particular substrate inhibits methane production. Mixture combinations that perform well in BMPs and ATAs should be tested in laboratory-scale anaerobic digesters. Once proven in lab-scale reactors for at least three hydraulic retention times, the best mixture should be tested in a pilot-scale reactor. This paper focuses on the first steps in this process using BMPs and ATAs results to select mixtures for laboratory-scale digester testing. The baseline feedstock was beef manure obtained from concrete feedlot pens (open and covered) in eastern Iowa. Various bedding materials were available, including oat hulls, corn stover, and wood shavings. To provide additional energy production, industrial byproducts from cardboard manufacturing, enzyme production, and corn and soybean processing were also potential substrates. Substrates were characterized for TS, VS, COD, pH, alkalinity, and ammonia. Then BMPs were completed on all substrates and ATAs were performed as needed. The results reported here were used to develop mixtures for use in laboratory-scale anaerobic digester testing.

**KEYWORDS.** Anaerobic Digestion, Biochemical Methane Potential (BMP), Anaerobic Toxicity Assays (ATA), Beef Manure

## INTRODUCTION

The push towards renewable energy and the reduction of green house gas emissions has prompted some farmers to consider installing anaerobic digestion (AD) systems. With low commercial energy prices in the US, operating such systems on manure alone requires large animal numbers to be economical. This has motivated the co-digestion of animal manure with industrial wastewaters or other sources of biodegradable materials for increased energy production (Braun and Wellinger, 2003). However, full-scale AD reliability has been low due to system design and management challenges (USDA – NRCS, 2007). Design and construction of a full-scale anaerobic digester should be validated by a scale-up procedure. Such a procedure should characterize hydraulic retention time (HRT), organic loading rate, and methane yield (Wilkie et al., 2004). The ideal process begins with laboratory characterization of potential substrates, and then uses biochemical methane potential assays (BMPs) and anaerobic toxicity assays (ATAs) to examine potential mixtures of substrates (Owen et al., 1979). High performing mixtures should be run in laboratory-

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scale anaerobic digesters to assess issues that may be masked in BMPs and ATAs. The resulting best mixture should be fed to a pilot-scale system to address materials handling issues (e.g., floating solids, clogging) and to provide data for an economic analysis based on realistic biogas production rates. This paper focuses on the first portion of the scale-up procedure in which laboratory-scale tests results of individual substrates were used to develop mixture ratios for three 100-L, plug-flow laboratory-scale anaerobic digesters.

The BMP is a powerful method of establishing baseline performance data for AD (Speece, 1996; Bishop et al., 2009). While BMPs provide information regarding the methane production of a substrate, they are typically highly diluted and may mask potential substrate toxicity (Moody et al., 2009). To overcome this issue, ATAs may be used. They determine how a particular substrate inhibits methane production by examining methane production from a mixture of a known degradable substrate and the test substrate. However, ATAs are feed-limited batch-loaded systems, and are therefore fundamentally different from typical large-scale anaerobic digesters, which are highly loaded, continuous flow devices. Although critical to early stage design, BMP and ATA results may be misleading when applied directly to full-scale operation due to their lack of information addressing HRT, substrate interaction, and continuous organic loading. Yet, scale-up of AD systems has not been widely reported. This paper provides guidelines for scale-up, and reports on the selection of preliminary substrate combinations based on BMP and ATA work.

## MATERIALS AND METHODS

Manure was obtained directly from confined concrete beef cattle feedlot pens (open and covered) in Eastern Iowa. The manure's estimated age was between 2 – 3 d, and the manure was selected from areas with minimal bedding mixed in. Bedding materials such as oat hulls, corn stover, wood shavings, short fiber cardboard waste, and reed canary grass were collected directly from farm stockpiles and were between 1 – 3 mo. of age. Enzyme production wastewater, food scrap waste, corn processing wastewater liquid, and corn processing wastewater solids were collected after delivery to the farm. Their estimated ages were < 1 d. Soybean processing wastewater was collected directly from the plant's wastewater discharge. Lagoon liquid was collected directly from the on-farm lagoon using a dipper. All samples were stored at 4°C and were analyzed within one week of collection.

Substrates were characterized for total solids, volatile solids, ammonia, alkalinity, and pH by the Iowa State University Agricultural Waste Management Laboratory. The total solids (TS) and volatile solids (VS) concentrations were measured using standard methods 2540 B and 2540 E, respectively. The pH measurements were taken with an Accumet Basic AB15 Plus pH meter and Accumet 13-620-285 pH probe. The chemical oxygen demand (COD) values were measured using Hach DR/890 Colorimeter Procedures Manual, Method 8000 and vials for COD 0-1500 ppm. Ammonia concentrations were measured using standard methods 4500-NH<sub>3</sub>-B Preliminary Distillation Step and 4500-NH<sub>3</sub>-C Titrimetric Method with 0.1N HCl as the titrant instead of sulfuric acid. Alkalinity was measured using standard methods 2320 B with 0.1 N HCl as the titrant (Standard Methods, 1995). A BMP assay was performed in triplicate for each of the substrate using a modified version of the International Standard ISO 11734:1995(E). The ATAs were performed in triplicate at seven dilution ratios on suspect substrates using a modified version of the International Standard ISO 13641-1.

Mixtures were designed to meet criteria including use of all available manure, keeping total solids <15% to facilitate pumping, maintaining pH between 6.5 and 8.2 for microbial ecology, providing high COD concentrations to maximize methane production, and achieving low ammonia to avoid toxicity (Speece, 1996).

Laboratory TS, VS, and COD results were used to calculate the sample size needed for a 250-mL BMP assay bottle. Sample sizes were calculated with a target of 125 mL CH<sub>4</sub> produced during a 30-day period, assuming 70% of COD converted to CH<sub>4</sub>, and 395 mL CH<sub>4</sub>/g COD reduced (Speece, 1996). This approach yielded average daily biogas volumes that were in a readily measurable range. The BMP reactors were seeded with an inoculum from a 60-L, mesophilic

(35°C), continuously stirred anaerobic reactor that was fed a mixture of high-protein dog food and nutrient medium. The BMP reactors were also seeded with nutrient medium containing supplemental inorganic nutrients and alkalinity (Speece, 1996). Inoculum was added for a 2:1 mass ratio between substrate and inoculum VS. Assay bottles were purged with 70% nitrogen and 30% carbon dioxide gas at ~0.5 L min<sup>-1</sup> for 5 min. Bottles were then capped with septa and zip tied, and incubated at 35°C on an orbital shaker at 150 rpm. Biogas production was measured daily by inserting a glass syringe into the septum and allowing the biogas pressure to displace the wetted barrel of the syringe. The volume was recorded, and the biogas was injected into an infrared gas analyzer (NDIR-CH<sub>4</sub> Gasanalyzer, University Kiel, Germany) to obtain the methane content (Bishop et al., 2009). A blank that included the inoculum source but no substrate was run so that each BMP could be corrected for the methane created by the inoculum source.

Materials of unknown toxicity were analyzed using ATAs. The known degradable substrate was a mixture of nutrient broth, yeast extract, and dextrose (D-glucose) in deionized water. Possible toxicants were combined with the degradable substrate and inoculum in seven mass concentrations (also referred to as % inclusions). The ATAs used the same inoculum and nutrient medium as the BMPs. Known-degradable-substrate controls defined the non-toxic methane production level. The control and all seven dilutions for each substrate were mixed in 250-mL serum bottles and were run in triplicate. Incubation conditions, biogas volume measurement, and methane content measurement were identical as for the BMPs. The methane yield during the linear portion of production was determined for all % inclusions and for the control. Toxicant effects were calculated by taking the ratio of the % inclusion yield to the control. Decreased methane production (inhibition) indicates toxicity, and inhibition generally increases as the ratio of test sample to degradable substrate increases. Higher (or equivalent) methane production indicates a non-toxic substrate.

## RESULTS AND DISCUSSION

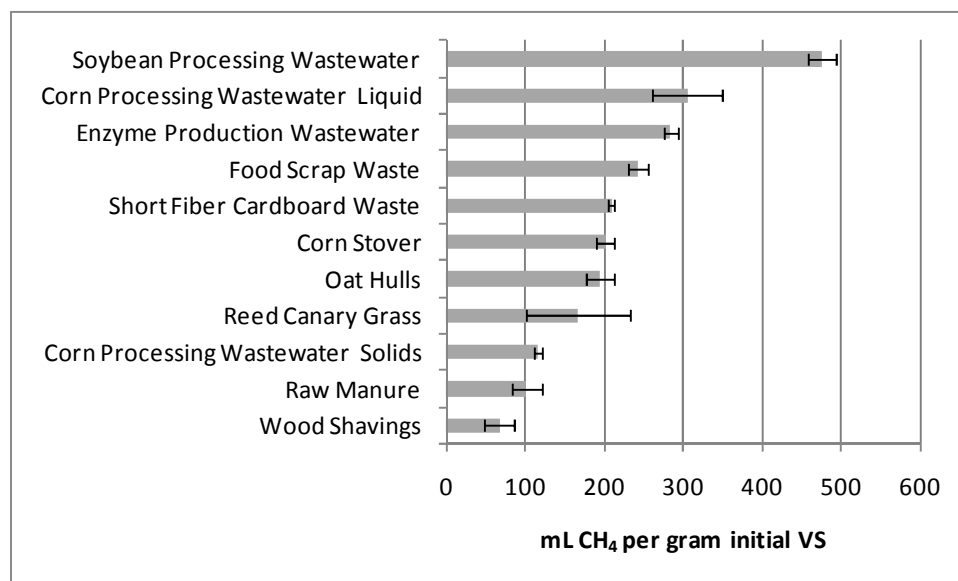
Substrate characteristics are shown in Table 1. Although variations are not shown, liquid samples were generally consistent while solid materials had high variations in some measured variables (e.g., 15 – 30% TS in manure samples). Subsample results listed in Table 1 reflect an average of stockpiles, and we used representative samples for the BMP and ATA assays.

**Table 1. Characterization of Available Substrates.**

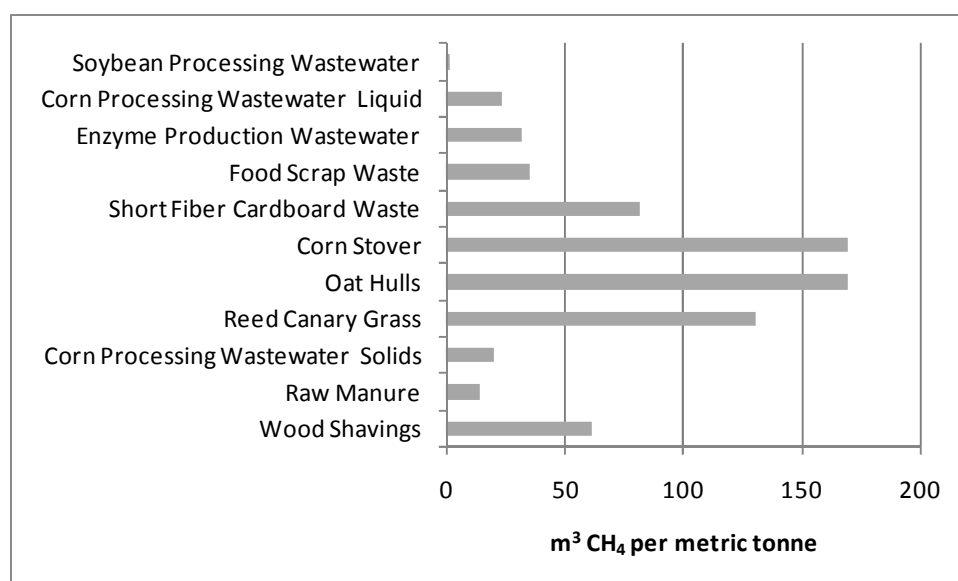
Substrate	TS (%)	VS (%)	pH	COD (mg/L or mg/g)	Ammonia (mg NH <sub>3</sub> -N/L)	Alkalinity (mg CaCO <sub>3</sub> /L)
<u>Off-Site Co-Substrates</u>						
Soybean Processing Wastewater	0.4	0.3	7.45	7,200*	0	300
Corn Processing Wastewater Liquid	8.3	7.6	4.02	107,600*	260	0
Enzyme Production Wastewater	12.8	11.3	5.05	162,300*	3,330	3,190
Food Scrap Waste	15.8	14.5	4.05	330	2,300	0
Corn Processing Wastewater Solids	18.1	17.5	-	208	-	-
Short Fiber Cardboard Waste	49.0	39.4	-	406	400	7,900
<u>On-Site Materials</u>						
Lagoon Liquid	1.3	0.9	7.06	22,500*	2,900	8,560
Raw Manure	17.0	14.0	6.60	156	1,980	6,000
<u>Bedding Materials</u>						
Reed Canary Grass	84.1	78.4	-	732	-	-
Corn Stover	90.3	84.0	-	870	-	-
Wood Shavings	91.8	91.6	-	170	-	-
Oat Hulls	92.1	87.4	-	750	-	-

\*COD reported in mg/L.

Figure 1 summarizes the BMP results on a methane volume per mass VS basis. On this basis, soybean processing wastewater appears to be an ideal source. However, the low VS concentration in this material means that the methane production per total mass of substrate is quite low. To address this, BMP results were also reported on a methane volume per total mass basis (Figure 2). This type of comparison is more meaningful for full-scale application since substrates will be loaded on a mass or volume basis. Figure 2 shows the attractiveness of energy-dense bedding materials.



**Figure 1. Comparison of substrate BMP results on a per gram of initial volatile solids basis. Error bars represent one standard deviation of the mean value.**



**Figure 2. Comparison of substrate BMP results on a cubic meter CH<sub>4</sub> per metric ton basis.**

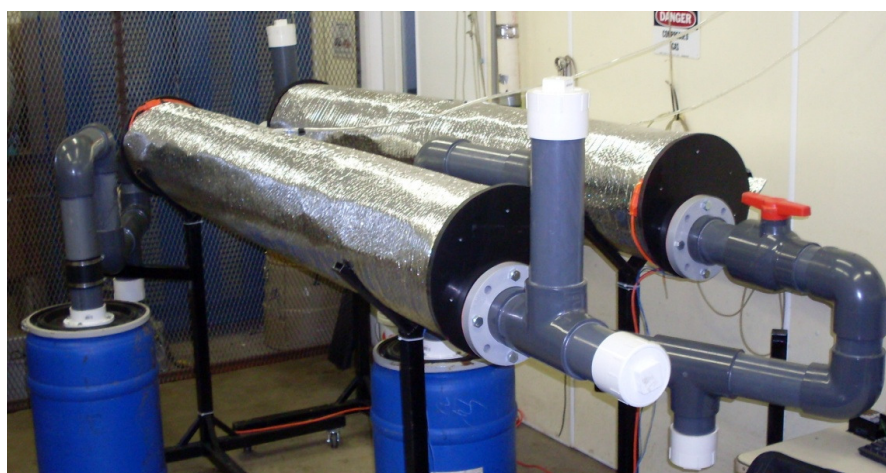
Short fiber cardboard waste, corn processing wastewater, and enzyme production wastewater all showed signs of toxicity in the ATAs, but at varying concentrations. Short fiber cardboard waste was toxic at inclusion rates above 15%. Enzyme production wastewater was toxic at all inclusion rates, perhaps due to its high ammonia levels. Due to the low pH of corn processing wastewater, 50/50 and 23/77 mixtures of manure/corn processing wastewater were examined (results not shown). The manure appeared to act as a buffer to the corn processing waste with negligible inhibition at inclusion rates less than 20%.

Mixture selection was based on material availability and on performance in BMPs and ATAs. Since bedding materials are a portion of the manure, they were not considered as a standalone substrate. Food scraps were available in limited amounts on an irregular basis and were eliminated on that basis. The low COD value and long trucking distance of the soybean processing wastewater caused its elimination, while the enzyme production wastewater was eliminated due to its toxicity. The corn processing wastewater pH was observed to drop rapidly, possibly hindering AD. However, the facility producing the corn processing wastewater was willing to adjust pH prior to delivery. Experiments were run to explore how mixing with manure would buffer this change. If the corn processing wastewater were adjusted to an initial pH of 8.5 with NaOH, a pH above 6.5 could be held for a week with a 10/90 wastewater/manure mixture.

Three mixtures were considered for further testing and evaluation within BMPs. The initial laboratory characterization of these mixtures is shown in Table 2. All mixtures listed in Table 2 use a highly dilute ingredient – either lagoon liquid, screened effluent liquid, or effluent liquid – to set TS at slightly below 10%. Each of these mixtures will be tested in the plug-flow laboratory-scale anaerobic digester depicted in Figure 3. These 100-L reactors have a design HRT of 21 days and will operate at 35°C. Mixtures will be allowed to stabilize over the course of three HRTs.

**Table 2. Mixture Laboratory Characterization**

Mixture Constituents	TS (%)	VS (%)	pH	COD (mg/L)	Ammonia (mg NH <sub>3</sub> -N/L)
<u>Mixture 1</u>					
22% Raw Manure					
15% Short Fiber Cardboard Waste					
16% Corn Processing Wastewater Liquid	9.2	7.2	6.50	80,200	2,150
48% Lagoon Liquid					
<u>Mixture 2</u>					
22% Raw Manure					
13% Short Fiber Cardboard Waste					
16% Corn Processing Wastewater Liquid	9.9	7.7	6.52	86,600	1,480
50% Screened Effluent Liquid					
<u>Mixture 3</u>					
22% Raw Manure					
13% Short Fiber Cardboard Waste					
16% Corn Processing Wastewater Liquid	9.8	7.7	6.53	94,200	1,060
50% Effluent Liquid					



**Figure 3. Two of three laboratory-scale 100-L, plug-flow anaerobic digesters. Reactors are aligned with flow counter to each other in this picture. Flow enters at stand pipes and exits through other side. Heat trace is wrapped around each reactor and covered with plastic insulation with a foil backing. Not shown is continuous temperature control is via a PC running LabView and continuous biogas monitoring via inverted tipping-bucket gas meters.**

## CONCLUSION

Design and construction of a full-scale anaerobic digester using multiple substrates requires careful selection of substrate mixtures. To select an appropriate mixture, a multi-step procedure is recommended. Initially, substrates are characterized, and then evaluated using BMPs and ATAs. Mixture combinations are then formed using criteria based on the site and data from the BMP and ATA work. Promising mixtures should be further analyzed via BMPs and ATAs, and best performers tested in laboratory-scale anaerobic digesters. This method allows for substrates to be selected and analyzed for any limitations in an anaerobic environment prior to full-scale application, so problems can be minimized. This paper reports results from the first steps of this process, involving characterization of potential substrates, analysis of methane production and possible toxicity, and selection of candidate mixtures. Further research will be performed on candidate mixtures using three 100-L laboratory-scale plug flow reactors. Performance of the mixtures in the 100-L reactors will be compared to that in BMPs to better understand how the BMP mixture results translate in a 500x scale-up (200 mL to 100 L).

## REFERENCES

1. Bishop, G. R., R. T. Burns, T. A. Shepherd, L. B. Moody, C. Gooch, R. Spajić, and J. Pronto. 2009. Evaluation of laboratory biochemical methane potentials as a predictor of anaerobic dairy manure digester biogas and methane production. In proceedings of The 2009 ASABE Annual International Meeting. Reno, Nevada.
2. Braun, R. and A. Wellinger. 2003. Potential of co-digestion. IEA Bioenergy. Task 37 – Energy from biogas and landfill gas.
3. Moody, L. R., R. T. Burns, W. Wu-Haan, and R. Spajić. 2009. Use of biochemical methane potential (BMP) assays for predicting and enhancing anaerobic digester performance. In proceedings of The 4<sup>th</sup> International and 44<sup>th</sup> Croatian Symposium of Agriculture. Optija, Croatia.
4. Owen, W. D., Stuckey, J., Healy Jr Young, and P. McCarty. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research*. 13:485-492.
5. Speece, R. 1996. *Anaerobic biotechnology for industrial wastewaters*. Archae Press. Nashville, Tennessee, U.S.A.
6. *Standard Methods for the Examination of Water and Wastewater*. (1995). American Public Health Association, 19<sup>th</sup> edition. Washington, D.C.
7. USDA - NRCS. (2007). *An analysis of energy production costs from anaerobic digestion systems on U.S. livestock production facilities*. Technical Note No. 1.
8. Wilkie, A. C., P. H. Smith, and F. M. Bordeaux. 2004. An economical bioreactor for evaluating biogas potential of particulate biomass. *Bioresource Technology* 92(2004): 103-109