Inhibiting Constituent Biogas Assay Protocol

Michigan Department of Agriculture
"Byproducts Resulting from Anaerobically Digesting Blended Manure and Highly Processed Food Commodities Wastes" Project

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Introduction

There is substantial interest in anaerobically digesting agricultural residuals to both serve as a waste management technology and as a source of renewable energy – waste to energy. Anaerobic digestion of a complex organic waste to methane and carbon dioxide requires multiple microbial populations under anaerobic (low oxidation/reduction potential) conditions. Table 1 lists several parameters that should be optimized. More details can be found on the Alternative for Food Processor's Wastewater, Deliverables, Resource Recovery, internet web page (http://www.egr.msu.edu/~safferma/Research/Greeen/greeenprojecttasks.html).

Table 1. Parameters for Anaerobic Digestion

Analysis	Suggested Range
pH	6.5 to 8.2
VFA:TA	0.20 to 0.40
Alkalinity	2000 to 3000 mg/L $CaCO_3$
COD	> 4000 mg/L
Nutrients	Macro and Micro
C/N/P (by weight)	100/4.3/0.9
Soluble COD	> 3000 mg/L
Total Solids (TS)	< 15% for batch tests
Total Volatile Solids (TVS)	High Percent of Total Solids
Total Suspended Solids (TSS)	Relatively Low Percent of Total Solids
Total Volatile Suspended Solids (TVSS)	Relatively Low Percent of Total Solids

Sources: Speece, 1996, Bouallagui et al., 2004, and Carucci et al., 2005

To obtain anaerobic digester feed stocks that contain all necessary constituents for optimized microbial activity, and consequently biogas production and destruction of the waste, blending is often advantageous. For example, a waste material from a food processor or corn ethanol production facility may have a very high chemical oxygen demand (COD) but lack nutrients and buffering capacity. Blending such a waste with manure, that has a high level of nitrogen, phosphorus, and alkalinity, may result in an optimized feedstock.

A concern of blending food processing wastes results from the potential impact of amendments

- that pass through a treatment system and accumulate in the environment,
- disrupt the anaerobic treatment process reducing volatile solids removal and biogas formation, and
- synthesizes byproducts in the low oxidation/reduction environment that may be as toxic or more toxic than the original compounds.

The identification of these compounds is difficult in complex media such as blended anaerobic digestion feedstocks as concentrations may need to be measured at the parts per billion levels and is not amenable to routine standard methods.

Blending manure with fruit and vegetable waste material that has not undergone substantial processing and the addition of amendments is not likely to produce byproducts or inhibit

biogas production, with few exceptions. Likewise, blending manure with processed food waste (such as expired bakery or pasta waste) is also likely not of concern since it was intended for human consumption. However, blends of highly processed waste materials that may contain non-degradable contaminants used to condition water, control pests, and sanitize equipment may impact treatment efficiency and/or produce unintended byproducts.

Anaerobic Digestibility of Wastewater Constituents

General inhibitors in anaerobic digesters include ammonia, sulfide, light metal ions, heavy metals, and recalcitrant organic compounds. Each is discussed below.

Ammonia

A wastewater stream with high concentrations of ammonium, urea or proteins may cause inhibition. The mechanisms of toxicity include intracellular pH changes, increase of maintenance energy requirement or inhibition of a specific enzyme reactions (Whittmann et al., 1995). Inhibiting ammonia concentrations vary and are dependent on the microbial strain and environment (temperature, pH), and period of acclimation periods (Chen et al., 2008). However, low levels of ammonia, below 200 mg/L, are also inhibiting as it is essential nutrient for anaerobic microorganisms. A 50% reduction in methane production results when ammonia concentrations range from 1.7 to 14 g/L (Liu and Sung, 2002, Chen, et al., 2008). Technologies to reduce ammonia in the feedstock include air stripping and chemical precipitation. Impacts can also be lessened by the strategic design of the reactor system and modification of the process. Included is a high biomass retention time, use of inter material to immobilize the microorganisms (clay, activated carbon, zeolite), and adding antagonistic cations (Na^+, Mg^{2+} or Ca^{2+}) (Chen, et al., 2008).

Sulfur

During anaerobic digestion, sulfate reducing bacteria (SRB) reduce sulfate to sulfide. At high concentrations, sulfide is toxic and because SBR compete with methanoges for substrates, they suppresses methane production (Chen, et al., 2008). Parkin et al., 1990, reports that dissolved sulfide concentrations in the range of 100–800 mg/L and approximately 50–400 mg/L of undissociated H_2S is inhibitory. For optimal methanogen activity, sulfur is needed, at levels between 1 to 25 mg/L (Scherer and Sahm, 1981). Alternatives to reduce the sulfide concentration in an anaerobic treatment system are similar to those used to remove ammonia. Included are stripping and sorption/chemical precipitation. Aerobic biological conversions (partial oxidation to elemental sulfur) is also possible (Chen, et al., 2008).

Light Metals

Many light metal ions such as sodium, potassium, calcium, and magnesium are required for microbial growth. However, high levels can reduce the rate of growth and even can cause severe inhibition or toxicity (Soto et al., 1993). The half maximal inhibitory concentration (IC_{50}) of Mg^{2+}, Ca^{2+}, K^+ and Na^+ are reported as 1.92-1900 mg/L, 4.8-4800 mg/L, 5.85-4800 mg/L and 7.36-7400 mg/L, respectively (Speece, 1996). A microbial population can be acclimated to relatively high metal levels and both antagonistic and synergistic effects are reported (Chen, et al., 2008).

Heavy Metals

Several heavy metal ions in their soluble free form can have a substantial inhibiting impact, even at low concentrations. Included are chromium, iron, cobalt, copper, zinc, cadmium, and nickel (Swanwick et al., 1969). The effect results from the metal binding for an enzyme

resulting in the interruption of function (Vallee and Ulner, 1972). The order of potential toxicity is Cu > Zn > Cr > Cd > Ni > Pb and Cd > Cu > Cr > Zn > Pb > Ni (Lin, 1992, 1993). Metals can be removed by precipitation, sorption, and chelating by organic and inorganic ligands (Oleszkiewicz and Sharma, 1990).

Recalcitrant Organic Compounds

Organic compounds toxic to anaerobic digestion include alkyl benzenes, halogenated benzenes, nitrobenzenes, phenol, alkyl phenols, halogenated phenols, nitrophenols, alkanes, halogenated aliphatics, alcohols, halogenated alcohols, aldehydes, ethers, ketones, acrylates, carboxylic acids, amines, nitriles, amides, pyridine and its derivatives, some LCFAs, surfactants, and detergents (Chen, et al., 2008). Inhibition concentrations vary widely for specific toxicants, depending on their concentration, biomass concentration, exposure time, cell age, feeding pattern, acclimation, and temperature (Yang and Speece, 1986).

Anaerobic Digestibility of Feedstocks

Generalities concerning the source of feedstocks can be made, as discussed below.

Animal Agricultural Feedstocks

Animal waste contains significant amounts of protein and urea, often resulting in very high total ammonia nitrogen concentrations. Swine manure often contains high sulfate concentration because of a protein-rich diet (Chen, et al., 2008). Both can be at concentrations that are inhibitory. Feed additives also frequently contain antibiotics and chemotherapeutics, designed to improve food utilization and prevent infectious diseases. These are typically used at very low concentrations and consequently, concentrations in the waste stream are low, typically less than 30 ppm and do not inhibit anaerobic digestion (Hobson, 1991). However, an exception is Olaquindox - inhibitory at 1 mg/L (Hilpert et al., 1984). Pesticide and herbicide residues in crops that are consumed by the livestock may also be found in waste at low level and could reduce the rate of anaerobic digestion (Khalil et al., 1991; Chakraborty et al., 2002).

Food industry wastes

Wastes from crop-based commodity food processing are high in organic matter and are therefore ideal for anaerobic digestion. Amendments commonly found in food processing wastewater that may inhibit anaerobic digestion are generally associated with conditioning process water, processing water, wash water, and plant/equipment sanitizing/cleaning. General classes of materials included ligands, pesticides, sanitizers, and surfactants. More details on these chemicals can be found in the article Categorization of Chemical Additives to Food Processor Wastewater by Higginbotham, Saffron, Safferman, and Miller, located on the Alternative for Food Processor's Wastewater, Deliverables, Categorization internet web page (http://www.egr.msu.edu/~safferma/Research/Greeen/greeenprojecttasks.html). Seafood processing wastewaters may contain high concentrations of Na^+, Cl^-, and SO_4^{2-}. Dairy and meat industry wastewater can contain substantial amounts of ammonia and long chain fatty acids (Chen, et al., 2008).

Objective

The objective of this research is to develop a protocol (Inhibiting Constituent Biogas Assay Protocol) to determine if selected amendments found in fruit and vegetable food processing

wastewater, blended with manure, negatively impacts or synthesize new problem compounds when treated using anaerobic digestion.

Methods

The general method to develop a protocol to screen the potential inhibitory effect and byproduct formation of a waste constituent during anaerobic digestion entailed the following steps.

- Consult literature to find general trends that can be used to screen wastewater amendments that have a potential impact on anaerobic digestion.
- Use the existing MSU developed respirometer based biogas potential assay as a starting point for the Inhibiting Constituent Biogas Assay Protocol and incorporate potential modification based on literature and consultant with team members.
- Select target constituents that have potential to inhibit anaerobically digestion that may be found in plant commodity food processing wastewater.
- Conduct the draft Inhibiting Constituent Biogas Assay Protocol.
- Finalize the Inhibiting Constituent Biogas Assay Protocol and provide guidance including lessons learned.

A literature review was provided in the Introduction to this report. Further literature details and the existing MSU protocol can be found on the Alternative for Food Processor's Wastewater, Deliverables, Resource Recovery, internet web page (http://www.egr.msu.edu/~safferma/Research/Greeen/greeenprojecttasks.html). The remainder of this section focuses on the biogas assays used test/finalize the Inhibiting Constituent Biogas Assay Protocol.

Chemical Constituents

The waste amendments selected (endosulfan, buprofezin, benzalkonium, chloride2,4,6, trichloroanisole, and chlorothalonil). All are found in food processing wastewater and are semi-volatile, to simply experimentations and analyses. The constituents were reagent grade obtained from Fischer Scientific. The analytical method is greatly dependent on the specific compound and requires design and oversight by an experienced chemist. Such analysis are also very costly. Appendix A has the specific methods used to analyze these target compounds in this research.

Manure, Seed, and

Manure was provided by a dairy farm in Williamston, MI. Samples were collected and stored at approximately 4°C before use. Anaerobic digestion seed was obtained from a pilot-scale reactor operated by MSU.

Pre Respirometry Analysis

Table 2 contains the pre-respirometry analyses and method that were conducted for each sample.

Table 2. Pre Respirometry Analysis

Analysis	Method
pH	pH meter
Alkalinity*	Hach 8203
COD	Hach 8000 (EPA approved)
Soluble COD	HACH 8000 after Filtering with TSS Filter
Total Solids (TS)	Hach 8271 (EPA approved)
Total Volatile Solids (TVS)	Hach 8271 (EPA approved)
Total Suspended Solids (TSS)	Hach 8271 (EPA approved)
Total Volatile Suspended Solids (TVSS)	Hach 8158, 8164
Ammonia (mg/L N)	Hach
Phosphorus (mg/L P)	Hach

*Colorimetric test, may not be possible to analyze for all samples
All but the alkalinity will be duplicated to ensure quality.

Respirometry Set Up

For the first respirometry trial, 16 vessels were used for the assay, each with the content as shown in Table 3. Table 4 shows the content for the 8 flasks tested during the second trial. The values for each chemical amendment (Compound column in Tables 1 and 2) in trial 1 was selected based on an easily detected limit by the analytical chemistry methods. However, this resulted in a large amount of methanol that proved to be undesirable. Therefore, in trial 2, lower concentrations were selected to reduce the amount of methanol needed and to more closely align the compounds with the rough order of magnitude that may be present in wastewater, based on a literature review.

Total gas production and the gas production rate were continuously measured and percent methane and in the biogas were periodically determined. The same pre-respirometer analyses (Table 1) were measured after respirometry testing.

Table 3. Flask Composition, First Trial

Flask	Seed (ml)	Manure (ml)	Each Compound (mg)	Methanol (ml)	DI Water (ml)
Seed	59.8	0	0	0	590.2
Seed, Manure	59.8	83.2	0	0	507
Seed, Manure, Methanol	59.8	83.2	0	32.5	474.5
Seed, Methanol	59.8	0	0	32.5	557.7
Manure	0	83.2	0	0	566.8
Manure, Methanol	0	83.2	0	32.5	534.3
Seed, Manure, Methanol, X	59.8	83.2	3.25	32.5	474.5
Seed, Manure, Methanol, 0.8X	59.8	83.2	2.6	32.5	474.5
Seed, Manure, Methanol, 0.5X	59.8	83.2	1.63	32.5	474.5
Seed, Manure, Methanol, 0.2X	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, Endosulfan	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, Buprofezin	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, Benzalkonium Chloride	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, Chlorothalonil	59.8	83.2	0.65	32.5	474.5
Seed, Manure, Methanol, 0.5X Replicate	59.8	83.2	0.65	32.5	474.5

Table 4. Flask Composition, Second Trial

Flask	Seed (ml)	Manure (ml)	Each Compound (mg)	Methanol (ml)	DI Water (ml)
Seed, Manure	59.8	83.2	0	0	507
Seed, Manure, Methanol	59.8	83.2	0	0.5	506.5
Seed, Manure, Methanol, x	59.8	83.2	0.05	0.5	506.5
Seed, Methanol, Buprofezin	59.8	83.2	0.01	0.5	506.5
Seed, Manure, Methanol, Endosulfan	59.8	83.2	0.01	0.5	506.5
Seed, Manure, Methanol, Chlorothalonil	59.8	83.2	0.01	0.5	506.5
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	59.8	83.2	0.01	0.5	506.5
Seed, Manure, Methanol, Benzalkonium Chloride	59.8	83.2	0.01	0.5	506.5

Each vessel has a volume of 715 mL. Of this volume, 650 mL was occupied with the constituents and the balance serving was head space. Each vessel received seed based on

the recommended seed to waste ratio of 2:1, on a volatile solids basis (Chynoweth and Turick et al. 1993).

Once all constituents were in the respirometry vessels, the head space was flushed with nitrogen. The vessels were then sealed and placed atop the magnetic stirrer within the water bath.

Respirometry Operation

The temperature within the water bath was set at 35^0C. Both trials continued until clear trends were observed and the needed information to further develop the Inhibiting Constituent Biogas Assay Protocol was complete.

Gas Composition Analyses

Gas grab samples were periodically collected directly from each flask's head space and analyzed for carbon dioxide and methane. A Shimazdu GC8 gas chromatograph (GC) was used, equipped with a Supelco SCOTT MX 216 packed primary and reference column. The injection temperature was 140° and the oven temperature was run isothermally at 100°. Helium is the carrier gas. A 3 to 5 point standard curve was used to calibrate a linear regression curve. Hydrogen sulfide was also measured by collecting the gas in a gas bag and then pumping it through a Dragger tube.

Results and Discussion

Results for each trial are presented below, with an interpretation and discussion of the data.

Trial One

Figures 1 and 2 show the cumulative and rate of gas production, respectively. Figure 3 shows the percent methane in the gas. Tables 5 and 6 are the volume of biogas produced and amount of hydrogen sulfide. Clear inhibition is exhibited in all flasks that contained methanol and the compounds.

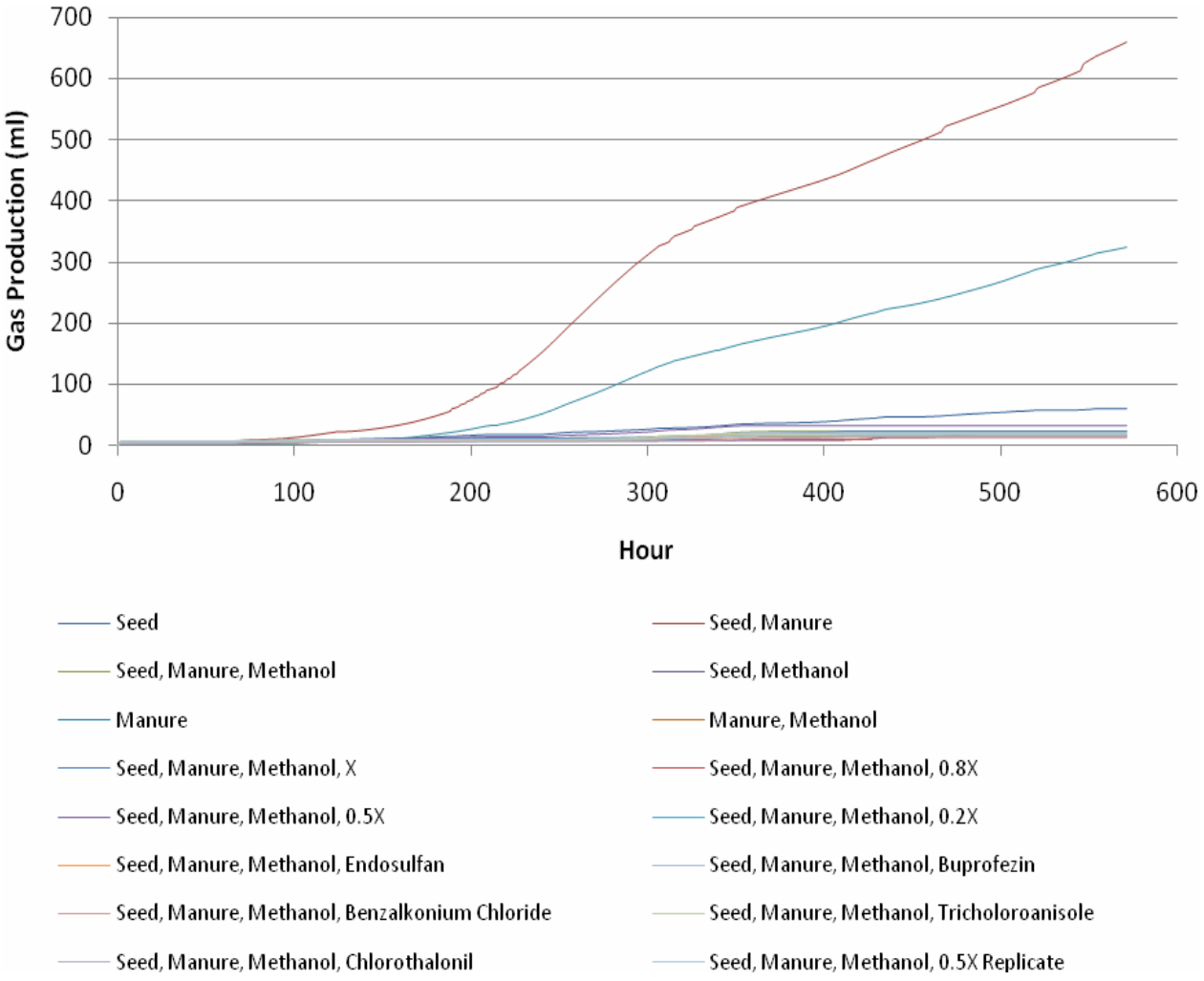

Figure 1: Total Gas Production, Trial One

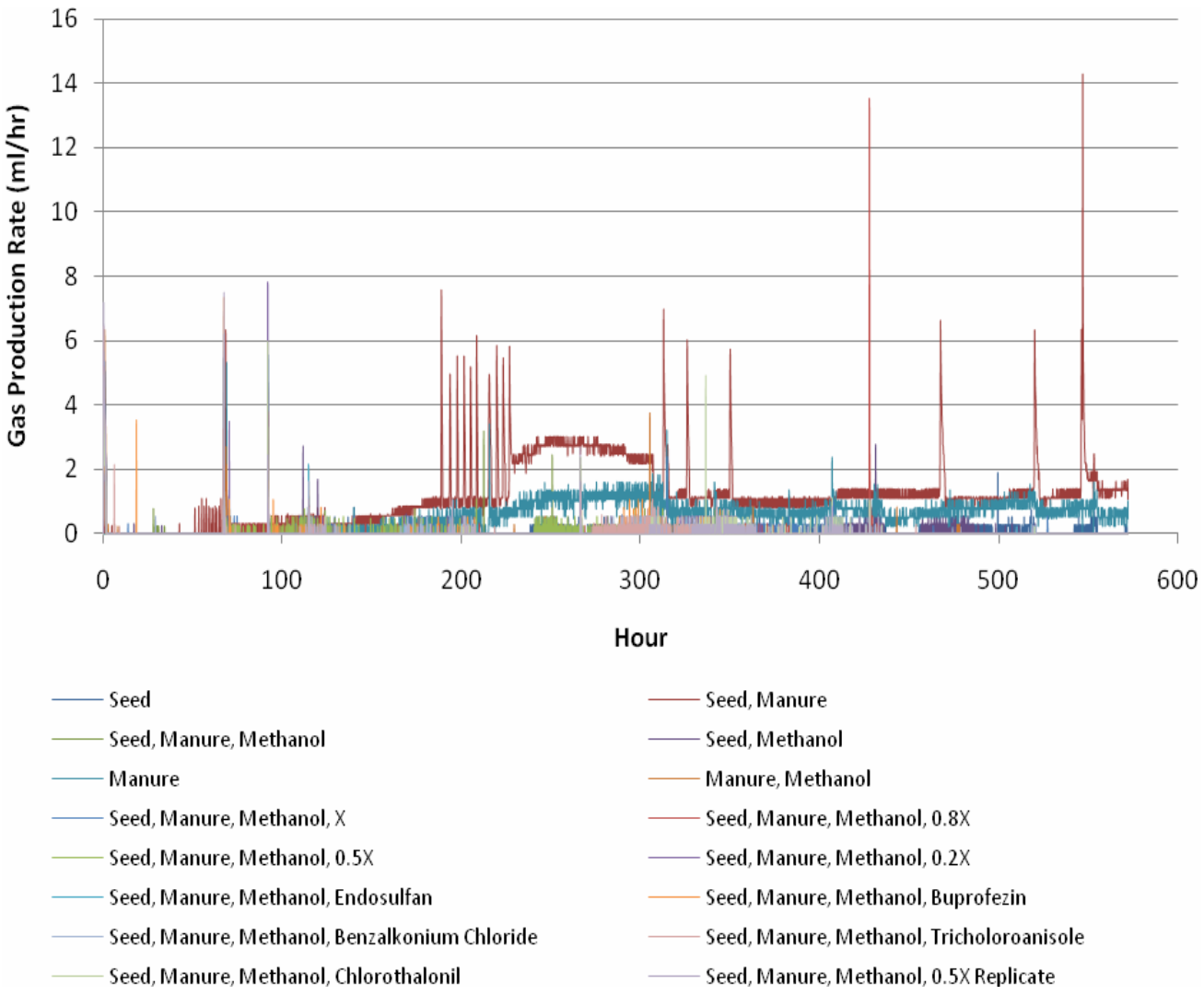

Figure 2. Gas Production Rate, Trial One

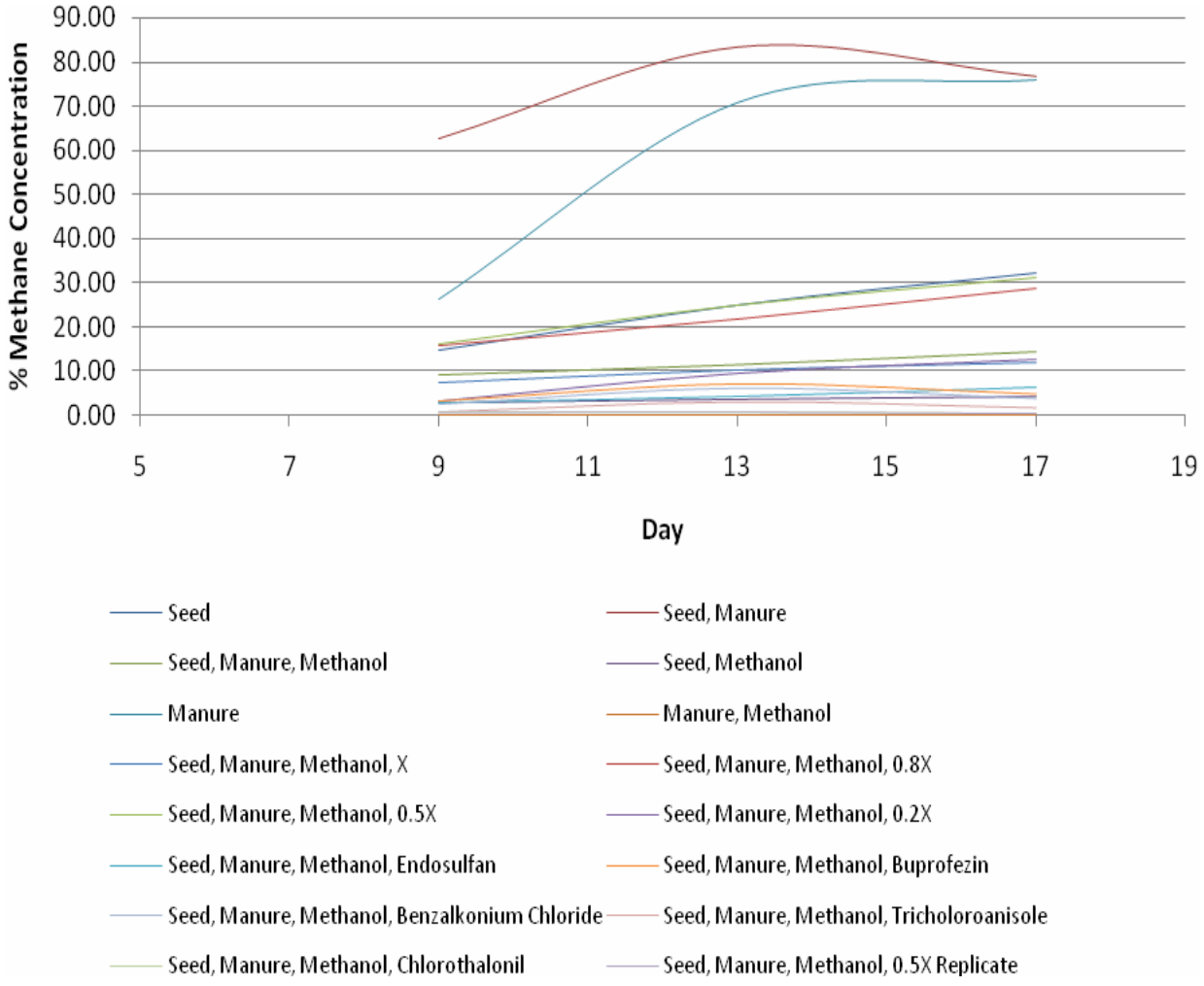

Figure 3. Methane Concentration, Trial One

Table 5. Total Gas Production, Trial One

Flask	(ml)
Seed	61
Seed, Manure	661
Seed, Manure, Methanol	24
Seed, Methanol	18
Manure	325
Manure, Methanol	15
Seed, Manure, Methanol, X	23
Seed, Manure, Methanol, 0.8X	13
Seed, Manure, Methanol, 0.5X	32
Seed, Manure, Methanol, 0.2X	19
Seed, Manure, Methanol, Endosulfan	20
Seed, Manure, Methanol, Buprofezin	18
Seed, Manure, Methanol, Benzalkonium Chloride	18
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	14
Seed, Manure, Methanol, Chlorothalonil	18
Seed, Manure, Methanol, 0.5X Replicate	61

Table 6. Hydrogen Sulfide Concentrations, Trial One

Flask	H2S (ppm)
Seed	*
Seed, Manure	1200
Seed, Manure, Methanol	*
Seed, Methanol	*
Manure	1500
Manure, Methanol	*
Seed, Manure, Methanol, X	*
Seed, Manure, Methanol, 0.8X	*
Seed, Manure, Methanol, 0.5X	*
Seed, Manure, Methanol, 0.2X	*
Seed, Manure, Methanol, Endosulfan	*
Seed, Manure, Methanol, Buprofezin	*
Seed, Manure, Methanol, Benzalkonium Chloride	*
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	*
Seed, Manure, Methanol, Chlorothalonil	*
Seed, Manure, Methanol, 0.5X Replicate	*

*Not sufficient gas production

Tables 7 and 8 shows the initial, final, and destroyed CODs and soluble CODs, respectively, for each flask

Table 7. COD, Trial One

Flask	Initial COD (mg)	Final COD (mg)	COD Destruction (mg)
Seed	2,756	2,379	377
Seed, Manure	5,067	3,328	1,739
Seed, Manure, Methanol	38,155	42,445	-4,290
Seed, Methanol	32,851	37,583	-4,732
Manure	2,509	2,002	507
Manure, Methanol	34,710	37,024	-2,314
Seed, Manure, Methanol, X	36,517	40,950	-4,433
Seed, Manure, Methanol, 0.8X	36,049	40,398	-4,349
Seed, Manure, Methanol, 0.5X	37,284	41,503	-4,219
Seed, Manure, Methanol, 0.2X	46,979	43,388	3,591
Seed, Manure, Methanol, Endosulfan	45,321	38,311	7,010
Seed, Manure, Methanol, Buprofezin	46,361	38,727	7,634
Seed, Manure, Methanol, Benzalkonium Chloride	45,175	42,250	2,925
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	34,801	35,399	-598
Seed, Manure, Methanol, Chlorothalonil	27,859	48,620	-20,761
Seed, Manure, Methanol, 0.5X Replicate	30,745	43,810	-13,065

Table 8. Soluble COD, Trial One

Flask	Initial SCOD (mg)	Final SCOD (mg)	SCOD Destruction (mg)
Seed	943	1,885	-943
Seed, Manure	2,269	1,918	351
Seed, Manure, Methanol	18,941	37,765	-18,824
Seed, Methanol	29,835	33,638	-3,803
Manure	1,489	1,365	124
Manure, Methanol	27,219	35,425	-8,206
Seed, Manure, Methanol, X	19,484	36,173	-16,689
Seed, Manure, Methanol, 0.8X	8,385	34,450	-26,065
Seed, Manure, Methanol, 0.5X	14,755	34,645	-19,890
Seed, Manure, Methanol, 0.2X	22,506	36,920	-14,414
Seed, Manure, Methanol, Endosulfan	28,210	35,620	-7,410
Seed, Manure, Methanol, Buprofezin	28,015	34,418	-6,403
Seed, Manure, Methanol, Benzalkonium Chloride	28,210	38,285	-10,075
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	24,993	32,273	-7,280
Seed, Manure, Methanol, Chlorothalonil	20,313	43,518	-23,205
Seed, Manure, Methanol, 0.5X Replicate	23,579	40,203	-16,624

Results are very variable but in general, all flasks with methanol and higher concentrations of the compounds had little to no COD removal, indicating an inhibitory environment. The variability in SCOD values makes it difficult for any conclusions to be reached.

Tables 9 and 10 are the results from the total and volatile solids, respectively.

Table 9. Total Solids, Trial One

Flask	Initial TS (mg)	Final TS (mg)	TS Destruction (mg)
Seed	2,901	2,538	363
Seed, Manure	4,447	3,877	570
Seed, Manure, Methanol	4,025	4,687	-662
Seed, Methanol	1,109	1,444	-335
Manure	2,051	1,649	402
Manure, Methanol	1,289	1,501	-211
Seed, Manure, Methanol, X	2,993	3,878	-885
Seed, Manure, Methanol, 0.8X	5,466	5,912	-446
Seed, Manure, Methanol, 0.5X	5,701	5,997	-296
Seed, Manure, Methanol, 0.2X	13,274	4,032	9,242
Seed, Manure, Methanol, Endosulfan	13,912	3,711	10,201
Seed, Manure, Methanol, Buprofezin	13,031	3,748	9,283
Seed, Manure, Methanol, Benzalkonium Chloride	11,348	3,723	7,625
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	3,874	2,716	1,159
Seed, Manure, Methanol, Chlorothalonil	5,477	2,787	2,690
Seed, Manure, Methanol, 0.5X Replicate	4,318	2,751	1,568

Table 10. Volatile Solids, Trial One

Flask	Initial VS (mg)	Final VS (mg)	VS Destruction (mg)
Seed	1,795	1,599	197
Seed, Manure	2,832	2,285	548
Seed, Manure, Methanol	2,589	3,249	-660
Seed, Methanol	561	935	-374
Manure	1,356	1,037	319
Manure, Methanol	950	1,090	-139
Seed, Manure, Methanol, X	1,795	2,683	-888
Seed, Manure, Methanol, 0.8X	3,523	4,122	-599
Seed, Manure, Methanol, 0.5X	3,712	4,113	-402
Seed, Manure, Methanol, 0.2X	8,839	2,854	5,985
Seed, Manure, Methanol, Endosulfan	9,242	2,556	6,687
Seed, Manure, Methanol, Buprofezin	8,721	2,637	6,084
Seed, Manure, Methanol, Benzalkonium Chloride	7,751	2,593	5,158
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	2,723	1,796	926
Seed, Manure, Methanol, Chlorothalonil	3,819	1,985	1,833
Seed, Manure, Methanol, 0.5X Replicate	2,987	1,893	1,093

Similar trends can be observed with the solids data in that total and volatile solids reduction was inhibited by high concentrations of the compounds and methanol.

Data for pH and alkalinity are shown in Tables 11 and 12, respectively.

Table 11. pH, Trial One

Flask	Initial pH	Final pH	pH Change
Seed	8.20	7.82	-0.38
Seed, Manure	7.96	7.32	-0.64
Seed, Manure, Methanol	8.00	5.79	-2.21
Seed, Methanol	8.16	5.50	-2.66
Manure	7.79	7.03	-0.76
Manure, Methanol	7.63	5.54	-2.09
Seed, Manure, Methanol, X	8.02	5.70	-2.32
Seed, Manure, Methanol, 0.8X	8.00	5.68	-2.32
Seed, Manure, Methanol, 0.5X	7.78	5.67	-2.11
Seed, Manure, Methanol, 0.2X	7.86	5.80	-2.06
Seed, Manure, Methanol, Endosulfan	7.88	5.81	-2.07
Seed, Manure, Methanol, Buprofezin	7.87	5.73	-2.14
Seed, Manure, Methanol, Benzalkonium Chloride	7.83	5.79	-2.04
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	7.83	5.69	-2.14
Seed, Manure, Methanol, Chlorothalonil	7.79	5.79	-2.00
Seed, Manure, Methanol, 0.5X Replicate	7.80	5.72	-2.08

Table 12. Alkalinity, Trial One

Flask	Initial Alkalinity (mg $CaCO_3$)	Final Alkalinity (mg $CaCO_3$)	Alkalinity Change (mg $CaCO_3$)
Seed	1,118	1,157	39
Seed, Manure	1,651	1,807	156
Seed, Manure, Methanol	1,502	884	-618
Seed, Methanol	715	351	-364
Manure	611	715	104
Manure, Methanol	436	312	-124
Seed, Manure, Methanol, X	1,352	780	-572
Seed, Manure, Methanol, 0.8X	1,775	1,053	-722
Seed, Manure, Methanol, 0.5X	1,092	1,066	-26
Seed, Manure, Methanol, 0.2X	2,314	819	-1,495
Seed, Manure, Methanol, Endosulfan	2,340	819	-1,521
Seed, Manure, Methanol, Buprofezin	2,301	819	-1,482
Seed, Manure, Methanol, Benzalkonium Chloride	1,781	793	-988
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	1,261	689	-572
Seed, Manure, Methanol, Chlorothalonil	1,378	650	-728
Seed, Manure, Methanol, 0.5X Replicate	1,378	702	-676

The initial pH and alkalinity were adequate for optimized anaerobic digestion. However, both dropped for several flasks indicating an imbalance with acid formers and methanogens. This condition would result in inhibition.

Tables 13 and 14 show the data for ammonia and phosphorous, respectively. Levels of ammonia are as expected and should not have caused inhibitory impacts. The cause of the large reductions in phosphorous levels for some flask is not clear and may be caused by an analytical error.

Table 13. Ammonia, Trial One

Flask	Initial Ammonia (mg NH_3)	Final Ammonia (mg NH_3)	Ammonia Change (mg NH_3)
Seed	119	158	38
Seed, Manure	177	215	37
Seed, Manure, Methanol	200	191	-9
Seed, Methanol	114	89	-25
Manure	64	105	41
Manure, Methanol	76	56	-20
Seed, Manure, Methanol, X	205	185	-20
Seed, Manure, Methanol, 0.8X	241	212	-28
Seed, Manure, Methanol, 0.5X	236	227	-9
Seed, Manure, Methanol, 0.2X	267	213	-54
Seed, Manure, Methanol, Endosulfan	275	221	-54
Seed, Manure, Methanol, Buprofezin	284	220	-64
Seed, Manure, Methanol, Benzalkonium Chloride	274	225	-49
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	219	201	-18
Seed, Manure, Methanol, Chlorothalonil	230	217	-13
Seed, Manure, Methanol, 0.5X Replicate	239	215	-24

Table 14. Total Phosphate, Trial One

Flask	Initial Phosphate (mg PO_4)	Final Phosphate (mg PO_4)	Phosphate Change (mg PO_4)
Seed	85	67	-18
Seed, Manure	137	135	-2
Seed, Manure, Methanol	254	263	10
Seed, Methanol	8	16	8
Manure	14	30	16
Manure, Methanol	10	24	15
Seed, Manure, Methanol, X	145	158	13
Seed, Manure, Methanol, 0.8X	375	363	-12
Seed, Manure, Methanol, 0.5X	416	370	-46
Seed, Manure, Methanol, 0.2X	802	176	-626
Seed, Manure, Methanol, Endosulfan	856	158	-698
Seed, Manure, Methanol, Buprofezin	830	150	-681
Seed, Manure, Methanol, Benzalkonium Chloride	796	155	-641
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	174	69	-105
Seed, Manure, Methanol, Chlorothalonil	262	67	-195
Seed, Manure, Methanol, 0.5X Replicate	209	70	-139

The draft analytical results for the compounds in each flask are presented in Appendix B. Clearly, there is much variability and trends cannot be found. This is an indication of the difficulty of extracting samples from a very dirty matrix and the need to more fully develop analytical methods beyond the time available during this research project.

Trial Two

Trial two was initiated due to the clear inhibitory conditions that resulted in trial one.

Figures 4 and 5 show the cumulative and rate of gas production, respectively. Table 15 is the cumulative gas production. A significant amount of gas production occurred initially and then leveled off before slightly increasing before the flasks had to be discontinued. Inhibitory effects are not evident.

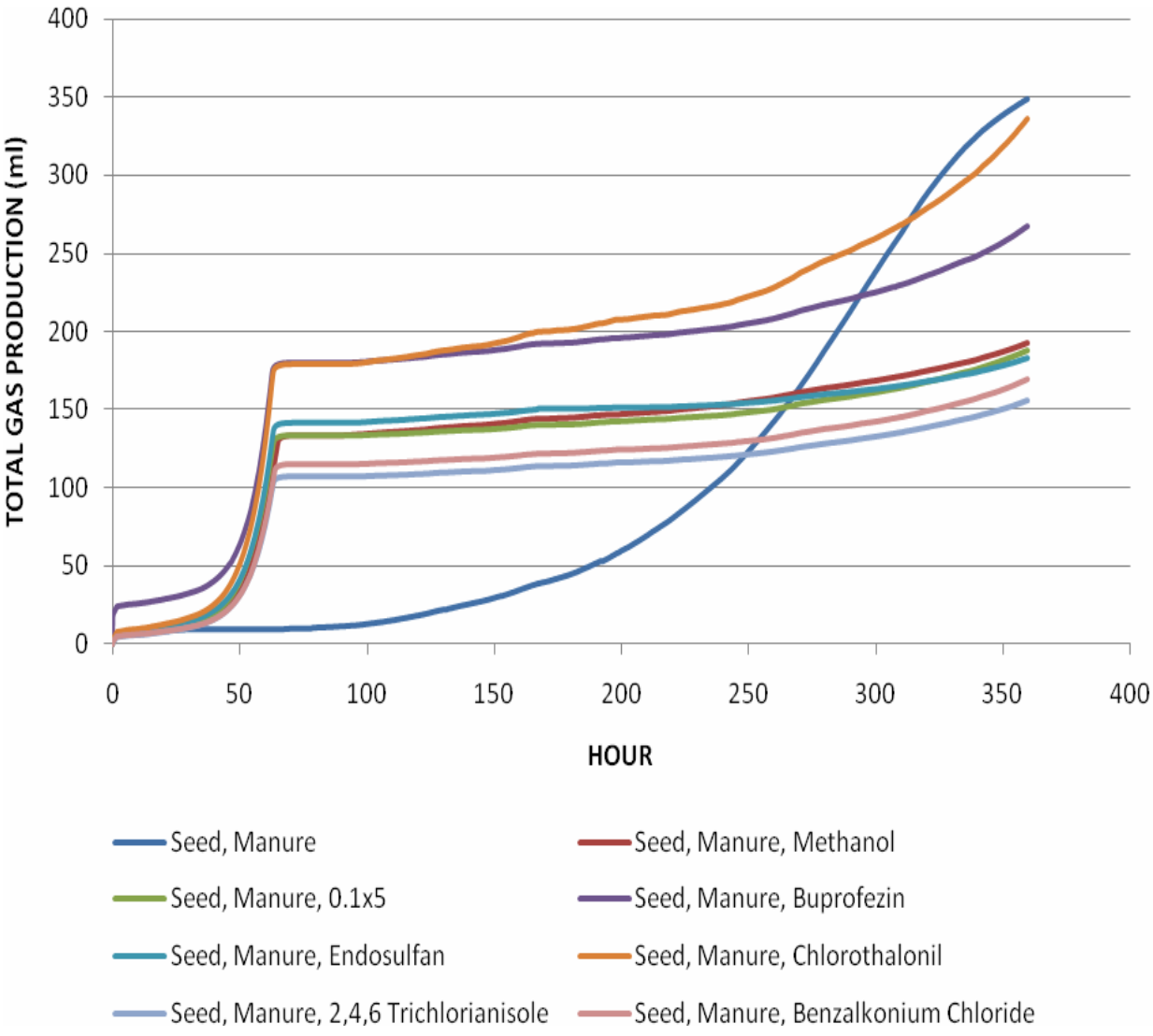

Figure 4: Total Gas Production, Trial Two

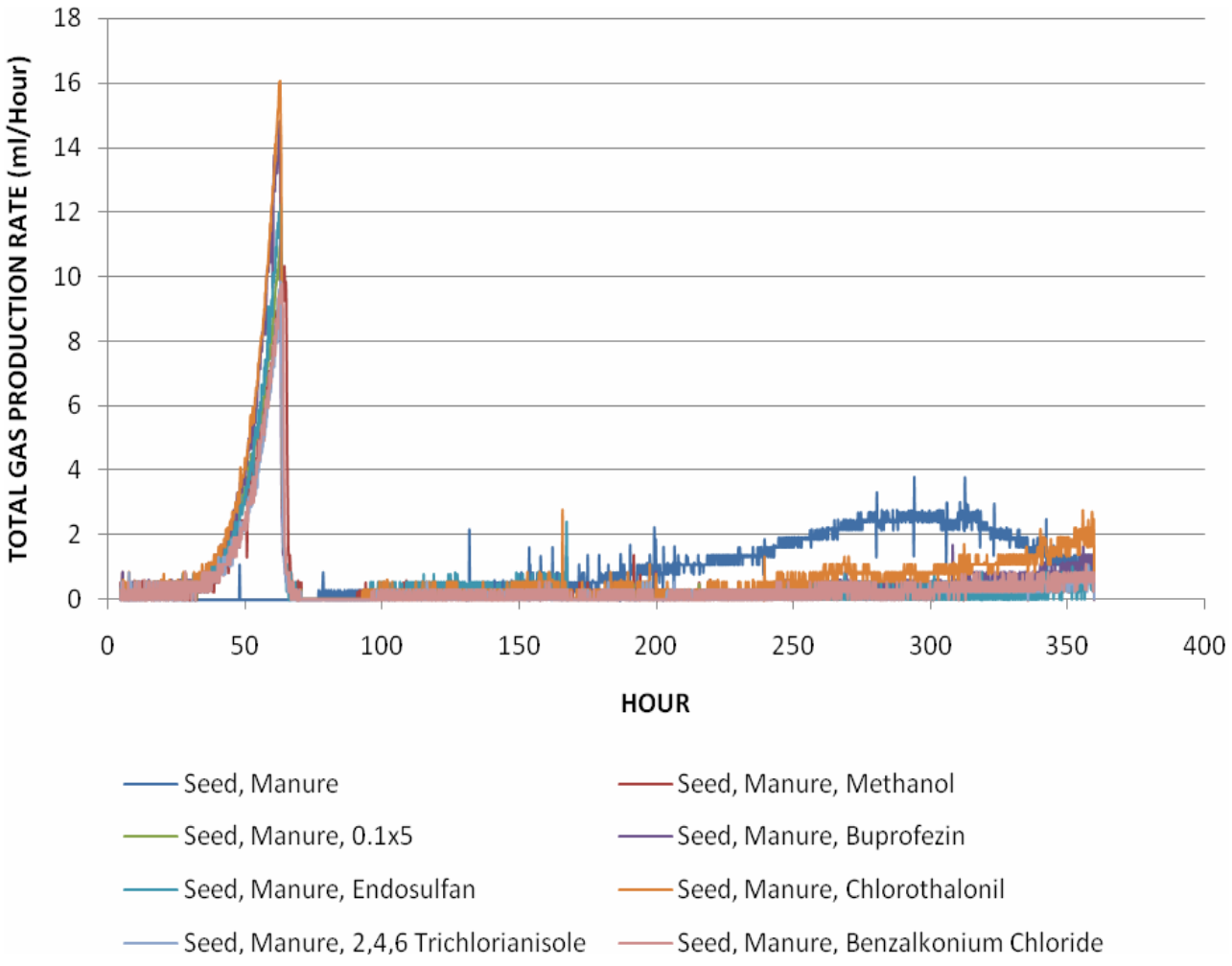

Figure 5: Total Gas Production Rate, Trial Two

Table 15. Total Gas Production, Trial 2

Flask	Total Gas Production (ml)
Seed, Manure	368
Seed, Manure, Methanol	349
Seed, Manure, Methanol, x	193
Seed, Methanol, Buprofezin	189
Seed, Manure, Methanol, Endosulfan	268
Seed, Manure, Methanol, Chlorothalonil	184
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	338
Seed, Manure, Methanol, Benzalkonium Chloride	157

Figure 6 shows the percent of methane in each flask and Table 16, the amount of hydrogen sulfide. Again, inhibitory conditions are not indicated.

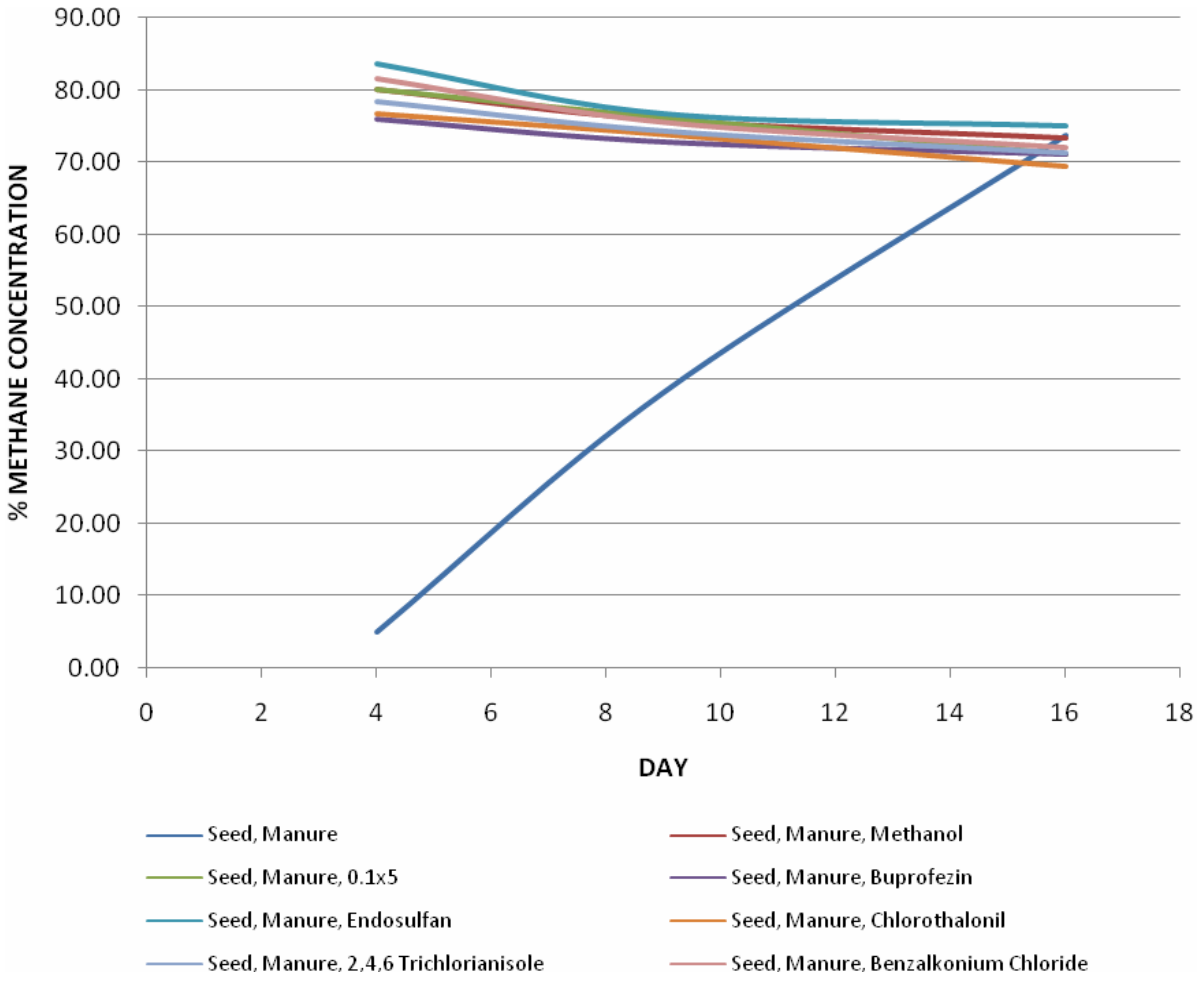

Figure 6. Methane Concentration, Trial Two

Table 16. Hydrogen Sulfide Concentrations, Trial Two

Flask	H_2S (ppm)
Seed, Manure	ND
Seed, Manure, Methanol	105
Seed, Manure, Methanol, x	50
Seed, Methanol, Buprofezin	45
Seed, Manure, Methanol, Endosulfan	45
Seed, Manure, Methanol, Chlorothalonil	25
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	ND
Seed, Manure, Methanol, Benzalkonium Chloride	45

ND: NOT DETECTED

Tables 17 and 18 are the influent, effluent and amount of COD and SCOD destroyed, respectively. Removals were much more consistent than in trial one but not significantly reduced. This may have resulted as trial two needed to be discontinued before gas production ceased.

Table 17. COD, Trial Two

Flask	Initial COD (mg)	Final COD (mg)	COD Destruction (mg)
Seed, Manure	5,769	4,347	1,422
Seed, Manure, Methanol	5,850	4,989	861
Seed, Manure, Methanol, x	5,769	5,054	715
Seed, Methanol, Buprofezin	6,102	5,159	943
Seed, Manure, Methanol, Endosulfan	6,029	4,932	1,097
Seed, Manure, Methanol, Chlorothalonil	6,013	5,021	992
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	6,175	4,973	1,203
Seed, Manure, Methanol, Benzalkonium Chloride	6,346	5,013	1,333

Table 18. Soluble COD, Trial 2

Flask	Initial SCOD (mg)	Final SCOD (mg)	SCOD Destruction (mg)
Seed, Manure	3,754	3,413	342
Seed, Manure, Methanol	4,485	3,916	569
Seed, Manure, Methanol, x	4,006	3,965	41
Seed, Methanol, Buprofezin	4,152	4,128	25
Seed, Manure, Methanol, Endosulfan	4,160	3,673	488
Seed, Manure, Methanol, Chlorothalonil	4,160	3,965	195
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	4,233	3,868	366
Seed, Manure, Methanol, Benzalkonium Chloride	4,241	4,103	138

Tables 19 and 20 are total and volatile solids. Consistent trends to the COD and soluble COD data is observed.

Table 19. Total Solids, Trial Two

Flask	Initial TS (mg)	Final TS (mg)	TS Destruction (mg)
Seed, Manure	5,518	4,483	1,035
Seed, Manure, Methanol	6,625	4,325	2,300
Seed, Manure, Methanol, x	5,547	4,631	916
Seed, Methanol, Buprofezin	5,536	4,850	686
Seed, Manure, Methanol, Endosulfan	5,761	4,585	1,176
Seed, Manure, Methanol, Chlorothalonil	5,403	4,792	611
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	5,384	4,732	652
Seed, Manure, Methanol, Benzalkonium Chloride	5,589	4,774	815

Table 20. Total Volatile Solids, Trial Two

Flask	Initial VS (mg)	Final VS (mg)	VS Destruction (mg)
Seed, Manure	3,653	2,759	894
Seed, Manure, Methanol	4,429	2,760	1,669
Seed, Manure, Methanol, x	3,928	2,984	944
Seed, Methanol, Buprofezin	3,830	3,121	709
Seed, Manure, Methanol, Endosulfan	3,905	2,910	995
Seed, Manure, Methanol, Chlorothalonil	3,666	3,102	564
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	3,669	3,054	615
Seed, Manure, Methanol, Benzalkonium Chloride	3,727	3,078	649

Unlike trial one, the pH and alkalinity in trial two (Tables 21 and 22, respectively) show acceptable values for all flasks.

Table 21. pH, Trial Two

Flask	Initial pH	Final pH	pH Change
Seed, Manure	7.98	7.46	-0.52
Seed, Manure, Methanol	8.10	7.17	-0.93
Seed, Manure, Methanol, x	8.15	7.16	-0.99
Seed, Methanol, Buprofezin	8.18	7.12	-1.06
Seed, Manure, Methanol, Endosulfan	8.23	7.15	-1.08
Seed, Manure, Methanol, Chlorothalonil	8.19	7.15	-1.04
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	8.20	7.17	-1.03
Seed, Manure, Methanol, Benzalkonium Chloride	8.20	7.17	-1.03

Table 22. Alkalinity, Trial Two

Flask	Initial Alkalinity (mg $CaCO_3$)	Final Alkalinity (mg $CaCO_3$)	Alkalinity Change (mg $CaCO_3$)
Seed, Manure	1,638	1,729	91
Seed, Manure, Methanol	1,612	1,521	-91
Seed, Manure, Methanol, x	1,508	1,560	52
Seed, Methanol, Buprofezin	1,625	1,612	-13
Seed, Manure, Methanol, Endosulfan	1,651	1,612	-39
Seed, Manure, Methanol, Chlorothalonil	1,573	1,872	299
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	1,547	1,573	26
Seed, Manure, Methanol, Benzalkonium Chloride	1,573	1,690	117

Tables 23 and 24, ammonia and phosphorus, respectively, show predictable values.

Table 23. Ammonia, Trial Two

Flask	Initial NH_3 (mg)	Final NH_3 (mg)	NH_3 Change (mg)
Seed, Manure	195	219	24
Seed, Manure, Methanol	203	249	46
Seed, Manure, Methanol, x	189	227	38
Seed, Methanol, Buprofezin	184	237	53
Seed, Manure, Methanol, Endosulfan	195	220	25
Seed, Manure, Methanol, Chlorothalonil	205	236	31
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	223	228	5
Seed, Manure, Methanol, Benzalkonium Chloride	220	232	12

Table 24. Total Phosphate, Trial Two

Flask	Initial PO_4 (mg)	Final PO_4 (mg)	PO_4 Change (mg)
Seed, Manure	183	152	-31
Seed, Manure, Methanol	223	153	-70
Seed, Manure, Methanol, x	188	160	-28
Seed, Methanol, Buprofezin	182	157	-25
Seed, Manure, Methanol, Endosulfan	181	159	-22
Seed, Manure, Methanol, Chlorothalonil	184	159	-25
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	184	166	-18
Seed, Manure, Methanol, Benzalkonium Chloride	186	166	-20

The draft analytical results for the compounds in each flask are presented in Appendix B. As observed in trial one, there is much variability and trends cannot be found. Further analyses of this draft data are needed.

Figures 7 and 8 are general total ion chromatograms of before and after treatment, respectively. Of interest is to compare the number and magnitude of the peaks. The reduction of the number of peaks and their mass after treatment is an indication of overall compound removal. Any new peak of significant mass may be a byproduct. This data does show an overall reduction.

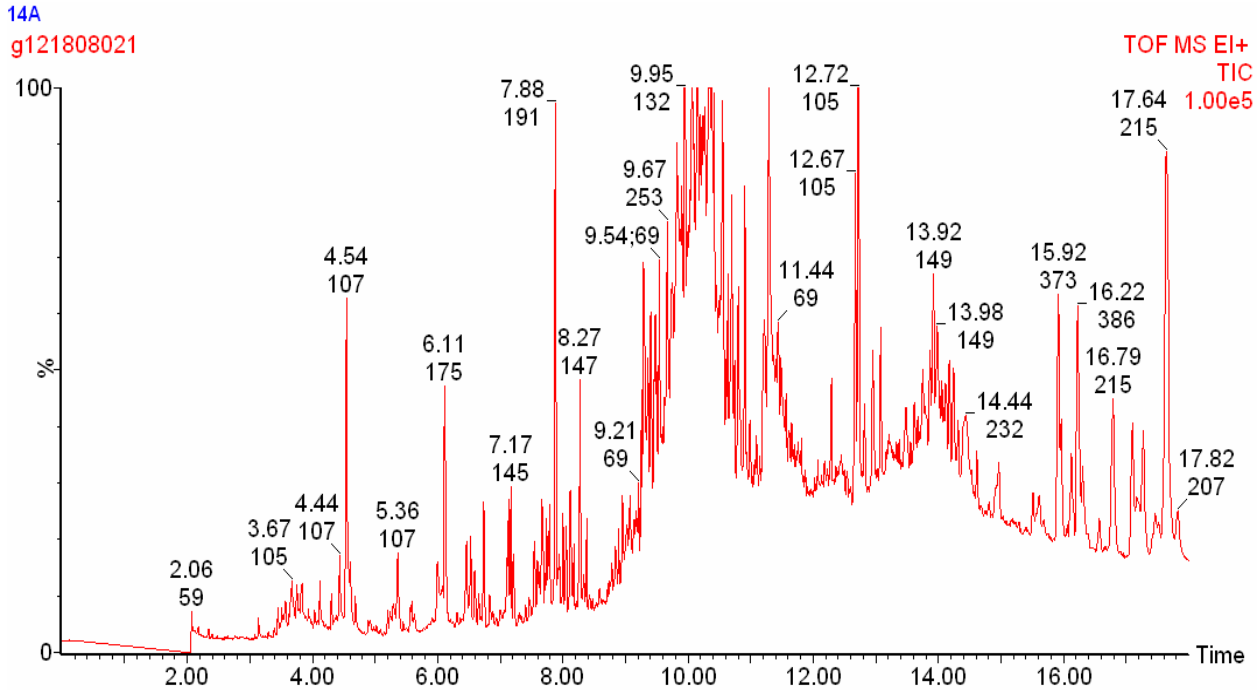

Figure 7. Total Ion Chromatogram Before Treatment

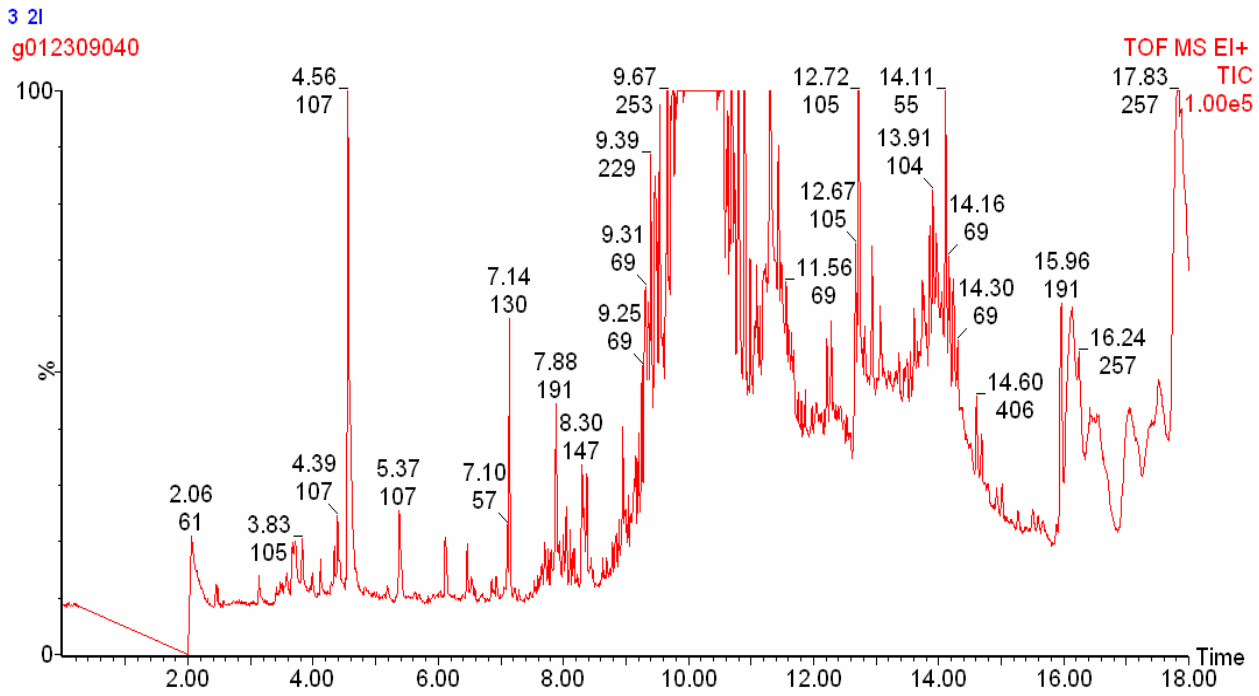

Figure 8. Total Ion Chromatogram After Treatment

Conclusions

This research indicates that the MSU respirometry based biogas assay (Appendix C) has the potential to also serve as the Inhibiting Constituent Biogas Assay Protocol. The conventional monitoring parameters (COD, SCOD, TS, VS, pH, alkalinity, ammonia, and phosphorus) clearly can indicate inhibiting conditions and the degree of treatment. Differences can be easily observed between treatments. The fate of potentially toxic compounds, however, was not easily determined using advanced analytical techniques. The very dirty matrix (manure) proved to be difficult to work with and much time and cost is required to enable the development and testing of the analytical methods needed to determine the fate of specific compounds. This determination will be more expensive and time consuming than the biogas assay itself and may never yield definitive results. A comparison of overall ion chromatograms is useful, however, to gain a generalized understanding of matrix changes after treatment.

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Appendix A. Compound Analytical Procedure

Extraction Protocol

1. A 50-ml polypropylene centrifuge tube was weighed using a 3-decimal place balance and the tube weight was recorded.
2. The reactor constituents were homogenized and 2g of the material was transferred to the weighed 50-ml centrifuge tube. The weight of the tube was recorded.
3. The internal standard 3, 4 dichloronitrobenzene was added.
4. 20ml of HPLC-grade dichloromethane was added; the tube was vortexed for 30 seconds and then stored at 4oC in the refrigerator for 2hours.
5. 15g of anhydrous sodium sulfate was added to a Buchner funnel and the funnel was set up above a receiving glass test tube.
6. The contents of the polypropylene centrifuge tube (dichloromethane extract) was poured into the Buchner funnel and the effluent was collected in the test tube.
7. 5ml of dichloromethane was added to the Buchner funnel and the eluting material was collected in the same test tube.

Evaporation Protocol

1. The test tubes with the eluted material were connected to the Nitrogen evaporator (The Meyer N-EVAP – analytical evaporator).
2. The samples were evaporated to complete dryness. A thin film was visible at the bottom of each tube.
3. 200µl of acetonitrile (CH3CN) was added to each test tube.
4. The samples were vortexed.
5. 200µl was extracted back out of the test tube and transferred into small vial bottles. The samples are now ready for the GC/MS procedure.

Analytical Methods

1. Compounds (other than benzalkonium) were analyzed by GC/MS using a Waters GCT Premier mass spectrometer coupled to an Agilent 6890A gas chromatograph with autosampler. The mass spectrometer was operated using 70 eV electron ionization (EI). Analytes were separated using a HP-5 MS capillary column (30 meters x 0.25 mm, 0.25 um film) using splitless injection of 1.0 uL and a temperature program from 80 C (initial, 2 min hold) to 300 C at 10 degrees/minute, with a final hold of 12 minutes. Quantitation was based on integrated peak areas of extracted ion chromatograms selective for each compound, calculated relative to the internal standard 3,4-dichloronitrobenzene.
2. Benzalkonium chloride was quantified by LC/MS/MS using either Quattro Premier XE or Quattro micro mass spectrometers (Waters, Milford, MA) operated in positive mode electrospray ionization. A Supelco Ascentis Express C18 column (2.1 x 50 mm, 2.7 um particles) was employed at a column temperature of 50 C, using a gradient based on solvent A = 50 mM ammonium acetate in water and solvent B = 90/10 (v/v) 2-propanol/water. The gradient was run from initial condition of 60%A/40%B (initial hold, 0.5 min) followed by a linear increase to 5%A/95%B at 1.5 minutes and a hold at these conditions until 3.5 minutes. Quantitation was based upon multiple reaction monitoring (MRM) transition of m/z 322.5>91, using a collision cell potential of 40 V.

In both cases, data processing was performed using Waters QuanLynx software.

Appendix B. Compound Analytical Tables

DRAFT Results - 1st Run: (units: ng/ml)

	Trichloroanisole Initial Tested	Trichloroanisole Initial Expected	Trichloroanisole Final Tested	Trichloroanisole Change
Seed	4.85E-03	0.00E+00	0.00E+00	-4.85E-03
Seed, Manure	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol	0.00E+00	0.00E+00	2.36E-03	2.36E-03
Seed, Methanol	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Manure	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Manure, Methanol	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol, X	1.18E+00	1.00E+04	1.70E-02	-1.16E+00
Seed, Manure, Methanol, 0.8X	3.19E+00	8.00E+03	1.13E-02	-3.18E+00
Seed, Manure, Methanol, 0.5X	5.98E-01	5.00E+03	0.00E+00	-5.98E-01
Seed, Manure, Methanol, 0.2X	1.69E+00	2.00E+03	2.05E-03	-1.69E+00
Seed, Manure, Methanol, Endosulfan	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol, Buprofezin	0.00E+00	0.00E+00	1.59E-02	1.59E-02
Seed, Manure, Methanol, Benzalkonium Chloride	5.66E-03	0.00E+00	0.00E+00	-5.66E-03
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	2.33E+00	1.00E+04	1.95E-02	-2.31E+00
Seed, Manure, Methanol, Chlorothalonil	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol, 0.5X Replicate	1.63E-01	5.00E+03	5.20E-03	-1.58E-01

	Endosulfan Initial Tested	Endosulfan Initial Expected	Endosulfan Final Tested	Endosulfan Change
Seed	0.00E+00	0.00E+00	1.22E-02	1.22E-02
Seed, Manure	0.00E+00	0.00E+00	1.95E-01	1.95E-01
Seed, Manure, Methanol	1.04E+01	0.00E+00	5.21E-02	-1.04E+01
Seed, Methanol	0.00E+00	0.00E+00	1.60E-01	1.60E-01
Manure	0.00E+00	0.00E+00	1.34E-01	1.34E-01
Manure, Methanol	0.00E+00	0.00E+00	4.34E-02	4.34E-02
Seed, Manure, Methanol, X	3.85E+02	1.00E+04	1.84E-01	-3.85E+02
Seed, Manure, Methanol, 0.8X	3.37E+01	8.00E+03	3.15E-01	-3.34E+01
Seed, Manure, Methanol, 0.5X	3.21E+01	5.00E+03	1.65E-01	-3.19E+01
Seed, Manure, Methanol, 0.2X	1.18E+01	2.00E+03	4.87E-02	-1.17E+01
Seed, Manure, Methanol, Endosulfan	2.05E+03	1.00E+04	1.46E-01	-2.05E+03
Seed, Manure, Methanol, Buprofezin	1.72E+01	0.00E+00	4.34E-02	-1.72E+01
Seed, Manure, Methanol, Benzalkonium Chloride	5.08E-02	0.00E+00	5.21E-03	-4.55E-02
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	0.00E+00	0.00E+00	1.56E-02	1.56E-02
Seed, Manure, Methanol, Chlorothalonil	1.02E-01	0.00E+00	0.00E+00	-1.02E-01
Seed, Manure, Methanol, 0.5X Replicate	1.72E+02	5.00E+03	2.78E-02	-1.72E+02

	Buprofezin Initial Tested	Buprofezin Initial Expected	Burporfezin Final Tested	Bufprofezin Change
Seed	2.23E-01	0.00E+00	3.17E-01	9.44E-02
Seed, Manure	6.53E+00	0.00E+00	1.19E+01	5.38E+00
Seed, Manure, Methanol	1.06E+01	0.00E+00	7.94E-01	-9.76E+00
Seed, Methanol	0.00E+00	0.00E+00	4.40E+01	4.40E+01
Manure	0.00E+00	0.00E+00	2.06E+00	2.06E+00
Manure, Methanol	5.87E-03	0.00E+00	1.13E+01	1.13E+01
Seed, Manure, Methanol, X	1.01E+02	1.00E+04	1.38E+03	1.28E+03
Seed, Manure, Methanol, 0.8X	1.22E+01	8.00E+03	3.14E+03	3.13E+03
Seed, Manure, Methanol, 0.5X	7.36E+00	5.00E+03	8.30E+02	8.23E+02
Seed, Manure, Methanol, 0.2X	2.71E+00	2.00E+03	1.30E+01	1.03E+01
Seed, Manure, Methanol, Endosulfan	4.93E-01	0.00E+00	1.05E+01	9.98E+00
Seed, Manure, Methanol, Buprofezin	2.84E+02	1.00E+04	3.02E+02	1.73E+01
Seed, Manure, Methanol, Benzalkonium Chloride	1.17E-02	0.00E+00	7.94E-01	7.82E-01
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol, Chlorothalonil	1.57E-02	0.00E+00	3.17E-01	3.02E-01
Seed, Manure, Methanol, 0.5X Replicate	4.20E+01	5.00E+03	3.83E+02	3.41E+02

	Chlorothalonil Initial	Chlorothalonil Expected	Chlorothalonil Final Tested	Chlorothalonil Change
Seed	0.00E+00	0.00E+00	2.29E-02	2.29E-02
Seed, Manure	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol	4.46E-02	0.00E+00	8.17E-02	3.71E-02
Seed, Methanol	0.00E+00	0.00E+00	9.50E-01	9.50E-01
Manure	0.00E+00	0.00E+00	2.29E-02	2.29E-02
Manure, Methanol	1.18E+00	0.00E+00	6.26E-01	-5.50E-01
Seed, Manure, Methanol, X	0.00E+00	1.00E+04	0.00E+00	0.00E+00
Seed, Manure, Methanol, 0.8X	0.00E+00	8.00E+03	3.60E-01	3.60E-01
Seed, Manure, Methanol, 0.5X	0.00E+00	5.00E+03	1.96E-01	1.96E-01
Seed, Manure, Methanol, 0.2X	0.00E+00	2.00E+03	0.00E+00	0.00E+00
Seed, Manure, Methanol, Endosulfan	0.00E+00	0.00E+00	9.32E-02	9.32E-02
Seed, Manure, Methanol, Buprofezin	0.00E+00	0.00E+00	6.37E-01	6.37E-01
Seed, Manure, Methanol, Benzalkonium Chloride	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	0.00E+00	0.00E+00	2.29E-02	2.29E-02
Seed, Manure, Methanol, Chlorothalonil	0.00E+00	1.00E+04	0.00E+00	0.00E+00
Seed, Manure, Methanol, 0.5X Replicate	0.00E+00	5.00E+03	2.29E-02	2.29E-02

	Benzalkonium Chloride Initial Tested	Benzalkonium Chloride Initial Expected	Benzalkonium Chloride Final	Benzalkonium Change
Seed	1.00E+02	0.00E+00	1.70E+03	1.60E+03
Seed, Manure	0.00E+00	0.00E+00	7.30E+03	7.30E+03
Seed, Manure, Methanol	0.00E+00	0.00E+00	5.50E+03	5.50E+03
Seed, Methanol	0.00E+00	0.00E+00	6.40E+03	6.40E+03
Manure	1.00E+02	0.00E+00	6.30E+03	6.20E+03
Manure, Methanol	0.00E+00	0.00E+00	3.40E+03	3.40E+03
Seed, Manure, Methanol, X	3.10E+03	1.00E+04	5.00E+04	4.69E+04
Seed, Manure, Methanol, 0.8X	1.70E+03	8.00E+03	6.15E+04	5.98E+04
Seed, Manure, Methanol, 0.5X	1.60E+03	5.00E+03	2.59E+04	2.43E+04
Seed, Manure, Methanol, 0.2X	6.00E+02	2.00E+03	1.33E+04	1.27E+04
Seed, Manure, Methanol, Endosulfan	0.00E+00	0.00E+00	1.00E+03	1.00E+03
Seed, Manure, Methanol, Buprofezin	1.00E+02	0.00E+00	1.60E+03	1.50E+03
Seed, Manure, Methanol, Benzalkonium Chloride	1.60E+03	1.00E+04	8.03E+04	7.87E+04
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	0.00E+00	0.00E+00	2.40E+03	2.40E+03
Seed, Manure, Methanol, Chlorothalonil	1.00E+02	0.00E+00	1.30E+03	1.20E+03
Seed, Manure, Methanol, 0.5X Replicate	1.20E+03	5.00E+03	4.51E+04	4.39E+04

DRAFT Results – 2nd Run: (units: ng/ml)

	Trichloroanisole Initial Tested	Trichloroanisole Initial Expected	Trichloroanisole Final Tested	Trichloroanisole Change
Seed, Manure	0.06	0	0.00	-0.06
Seed, Manure, Methanol	0	0	0.09	0.09
Seed, Manure, Methanol, x	3.15	100	0.17	-2.98
Seed, Methanol, Buprofezin	0.04	0	0.00	-0.04
Seed, Manure, Methanol, Endosulfan	0.02	0	0.00	-0.02
Seed, Manure, Methanol, Chlorothalonil	0.00	0	0.00	0.00
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	1.52	100	0.00	-1.52
Seed, Manure, Methanol, Benzalkonium Chloride	0.00	0	0.00	0.00

	Endosulfan Initial Tested	Endosulfan Initial Expected	Endosulfan Final Tested	Endosulfan Change
Seed, Manure	3.22	0	1.11	-2.11
Seed, Manure, Methanol	0.00	0	0.00	0.00
Seed, Manure, Methanol, x	46.88	100	0.00	-46.88
Seed, Methanol, Buprofezin	5.21	100	0.00	-5.21
Seed, Manure, Methanol, Endosulfan	6.94	0	0.00	-6.94
Seed, Manure, Methanol, Chlorothalonil	0.29	0	0.73	0.44
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	1.16	0	5.61	4.45
Seed, Manure, Methanol, Benzalkonium Chloride	0.00	0	0.00	0.00
	Buprofezin Initial Tested	**Buprofezin Initial Expected**	**Burprofezin Final Tested**	**Bufprofezin Change**

Seed, Manure	116.03	0	0.00	-116.03
Seed, Manure, Methanol	17237.14	0	88.41	-17148.73
Seed, Manure, Methanol, x	156.51	100	1614.13	1457.62
Seed, Methanol, Buprofezin	932.22	0	48.89	-883.33
Seed, Manure, Methanol, Endosulfan	152.70	0	0.00	-152.70
Seed, Manure, Methanol, Chlorothalonil	106.51	0	100.16	-6.35
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	13.81	100	0.00	-13.81
Seed, Manure, Methanol, Benzalkonium Chloride	155.08	0	0.00	-155.08

	Chlorothalonil Initial	**Chlorothalonil Expected**	**Chlorothalonil Final Tested**	**Chlorothalonil Change**
Seed, Manure	0.06	0	0.00	-0.06
Seed, Manure, Methanol	0.03	0	0.00	-0.03
Seed, Manure, Methanol, x	0.02	100	0.02	0.00
Seed, Methanol, Buprofezin	0.00	0	0.00	0.00
Seed, Manure, Methanol, Endosulfan	0.02	0	0.00	-0.02
Seed, Manure, Methanol, Chlorothalonil	0.00	100	0.00	0.00
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	0.02	0	0.02	0.00
Seed, Manure, Methanol, Benzalkonium Chloride	0.02	0	0.00	-0.02

	Benzalkonium Chloride Initial Tested	**Benzalkonium Chloride Initial Expected**	**Benzalkonium Chloride Final**	**Benzalkonium Change**
Seed, Manure	1600	0	1000	-600

Seed, Manure, Methanol	1500	0	1100	-400
Seed, Manure, Methanol, x	4000	100	2900	-1100
Seed, Methanol, Buprofezin	4200	0	800	-3400
Seed, Manure, Methanol, Endosulfan	700	0	400	-300
Seed, Manure, Methanol, Chlorothalonil	700	0	900	200
Seed, Manure, Methanol, 2,4,6 Trichloroanisole	2400	0	2400	0
Seed, Manure, Methanol, Benzalkonium Chloride	1400	100	2500	1100

Appendix C. MSU Respirometer Biogas Potential Assay

Introduction

Michigan State University, Department of Biosystems and Agricultural Engineering (BAE), will conduct anaerobic treatment screening assays on waste samples that may be feed stocks for anaerobic digesters. Sources may include manure, ethanol production byproducts, and food processing wastewater. The assays are designed simply to determine if further development studies are warranted in terms of the anaerobic biodegradability of the wastewater under the tested conditions. Results may also provide characteristics of the waste useful in such future studies. Assay conditions will not represent proposed on-site treatment techniques as the assay is strictly designed to serve as a screening tool.

Sample Collection and Shipment

Samples will be collected by Client (unless other arrangements are made for the University or another organization to collect the samples), immediately chilled, stored near 4°C, and shipped to Michigan State University (at a chilled temperature) with 24 hours. A total sample size of 2 L is required. The shipping information is provided below.

Michigan State University
Department of Biosystems and Agricultural Engineering
212 Farrall Hall
East Lansing, MI 48824

Attn: Steven Safferman
Phone: 517-432-0812 or 355-4720
Fax: 517-432-2892

Client will inform BAE by phone or in writing of the characteristics of the samples including the name of the facilities where the waste originated from (that will be kept confidential), the process(es) that generated the waste, special safety precautions, waste constituents that may inhibit anaerobic digestion, and any other relevant information.

Specific constituents of concern include the following.

- Oxidizing sanitizers
    - Hyperchlorites, chlorine, chloramines
    - Organic bromine
    - Iodine, alcohol-iodine, iodophors
    - Hydrogen peroxide, peroxy acids

- Biocides and non-oxidizing sanitizers
    - Organic acids (e.g. acetic acid, propionic acid, formic acid, carboxylic acids)
    - Acid anionic sanitizers
    - Acid-quat sanitizers
    - Quaternary ammonium compounds

- Polyphenols
- Long Chain Fatty Acids

Only samples that can be disposed of by flushing down the sink into the sanitary sewer can be accepted unless arrangements are made to return the samples back to the original facilities.

BAE will hold the samples at 4°C before processing, for up to 7 days, before initiating the respirometry assay.

If Client collects a sample from a facility that does not wish to be identified to the University, the identifying company information, such as name, location, and unique features that may identify the facility, can be excluded provided that Client provides the other needed information listed.

Pre Respirometry Analysis

Table 1 contains the pre-respirometry analysis that will be conducted for each sample, including the method and suggested ranges for idealized digestion, where applicable.

Table 1. Pre Respirometry Analysis

Analysis	Method	Suggested Range	Source
pH	pH meter	6.5 to 8.2	Speece, 1996
Alkalinity*	Hach 8203	2000 to 3000 mg/L $CaCO_3$	Speece, 1996
COD	Hach 8000 (EPA approved)	> 1000 mg/L COD	Speece, 1996
Soluble COD	HACH 8000 after Filtering with TSS Filter		
Total Solids (TS)	Hach 8271 (EPA approved)	< 10% for batch tests	Carucci et al., 2005
Total Volatile Solids (TVS)	Hach 8271 (EPA approved)	High Percent of Total Solids	
Total Suspended Solids (TSS)	Hach 8271 (EPA approved)	Relatively Low Percent of Total Solids	
Total Volatile Suspended Solids (TVSS)	Hach 8158, 8164	Relatively Low Percent of Total Solids	

*Colorimetric test, may not be possible to analyze for all samples
All but the alkalinity will be duplicated to ensure quality.

Respirometry Set Up

One respirometry unit, containing 8 vessels, will be used for each assay. Four conditions will be tested. For each condition, 2 vessels will be used. One will measure the total biogas produced and the other will measure biogas produced after carbon dioxide has been removed. Assuming that the biogas is composed of only carbon dioxide and methane, the carbon dioxide scrubbed biogas is an estimate of the amount of methane produced. The carbon dioxide will be removed by passing the biogas through an approximately 500 mg/L potassium hydroxide solution with a pH indicator.

Each vessel is 675 mL. Of this volume, 600 mL will be filled with liquid with the balance serving as head space based on recommendations by the respirometer manufacturer.

The four conditions (2 vessels for each condition) proposed for testing includes the following. However, plant-specific alternatives can be designed.

- Seed
- Seed/Nutrient
- Feed Stock/Seed
- Feed Stock/Nutrients/Seed (optimal)

Seed will be digester content obtained from a locally operational anaerobic digester (most likely digesting manure). This digester may be a bench-scale experimental unit or a field-scale system, depending on availability at the time analysis is run. Each vessel will receive 100 mL of seed, determined based on previous experience.

The volume of wastewater in each vessel will be based on the dilution expected in a theoretical plug flow reactor with a HRT of 15 days as the dilution would be equivalent to a batch reactor. Consequently, 40 mL will be used. However, the volume of substrate should never result in a total solids concentration greater than 10% for a typical assay.

Nutrients, where applicable, will be added as a solution containing the constituents shown in Table 2 (Shelton and Tiedje, 1984).

Table 2. Nutrient Solution

Parameter	mg/L
KH_2PO_4	270
K_2HPO_4	350 (adjusted to pH 7)
NH_4Cl	530
$CaCl_2 \cdot 2H_2O$	75
$MgCl_2$ 6 $\cdot H_2O$	100
$FeCl_2 \cdot 4\ H_2O$	20
$MnCl_2 \cdot 4H_2O$	0.5
H_3BO_3	0.05
$ZnCl_2$	0.05
$CuCl_2$	0.03
$Na_2Mo_4 \cdot 2H_2O$	0.01
$CoCl_2 \cdot 6H_2O$	0.5
$NiCl_2 \cdot 6H_2O$	0.05
Na_2SeO_3	0.05

The volume of the nutrient solution will be based on the COD of the feed stock. Specifically, a COD/Total Nitrogen (as added from the NH_4Cl) weight ratio of 100/4 will be used (Bouallagui et al., 2004). From this ratio, the mg of NH_4Cl will be calculated that can then be converted to a volume for the vessel. The other nutrients will be added in proportion. Because of the low concentration of the metals, a stock solution will be prepared and the appropriate amount transferred into each vessel. The media will be autoclaved for 5 minutes before use to drive off oxygen (Shelton and Tiedje, 19984). This level of nutrients assumes no nutrient value form the feed stock and seed although realistically, there would be a contribution. However, the excess should not have a negative influence and can be examined in the feed stock/seed vessel. Thus, the volume of the nutrient solution will be 6000 mL subtracted from the other applicable volumes (wastewater and/or seed).

Additionally, 1 mg/L of resazurin dye will be added to each vessel. Specifically, a 1,000 mg/L solution will be prepared and 0.6 mL will be added to each flask. If oxygen is present, the color of the solution will be bright pink. If the color is dark maroon, the condition is anaerobic.

Once all constituents are in the respirometry vessels, the head space will be flushed with nitrogen for 5 minutes. The vessels are then sealed and placed atop the magnetic stirrer within the water bath.

Respirometry Operation

The temperature within the water bath will be set at 35^0C, unless an alternative temperature is a variable to be tested. Gas production rates and cumulative gas volume are measured continuously and recorded every 3 hours.

To supplement the gas volume measurements with and without CO_2 scrubbing, gas grab samples will be collected directly from the head space of each treatment vessel and analyzed for methane and carbon dioxide using a gas chromatograph (GC). Specifically, a Supelco SCOTT MX 216 packed primary and reference column will be used in a Shimazdu GC8. The oven temperature is run isothermally at a temperature of 100^oC. Helium is the carrier gas. A 3 to 5 point standard curve will be used to calibrate a linear regression curve.

Once all of the vessels reach their maximum gas volume, as indicated by a horizontal cumulative biogas production curve, the assay will be considered complete and post respirometry analysis will be conducted.

Post Respirometry Analysis

The same parameters measured pre respirometry analysis will be repeated for each vessel. All analysis will be repeated for one randomly picked vessel to ensure quality.

Data Presentation

A letter report will provide a detailed summary of the operational protocol. Assay operational issues, if any, will also be reported.

A plot of the cumulative gas production with time and another with the gas production rate with time will be provided. Methane and total biogas potential of the wastewater over the course of the batch assay will be provided. These plots will be calculated by subtracting the optimal vessel results from the seed/nutrients vessel results and the feed stock/seed results from the seed vessel.

A table containing the ultimate methane and biogas volumes and all of the pre and post respirometry analysis for each vessel will be provided. Calculated values of the ultimate methane potential will be included by subtracting out the appropriate control. The methane potential will be normalized to the COD removed and to the volatile solids removed.

Based on the stoichiometry, 395 mL CH_4 at 35°C is produced per 1000 mg COD. The values obtained in the assays will be compared to this theoretical standard.

Results from the duplicates and GC analysis will be reported.

References

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