Using Biochemical Methane Potentials & Anaerobic Toxicity Assays

Lara Moody, P.E.

Iowa State University

To Be Discussed:

- Description of BMPs and ATAs
- Utilization of BMPs and ATAs in planning process
- Assay process
- Interpreting and utilizing results

BMP - Biochemical Methane Potential
ATA - Anaerobic Toxicity Assay

Controlled Anaerobic Digestion

- Careful planning and accurate design are necessary to optimize cost recovery
- Multiple data and information sources are available for initial planning
  - Using “book values” can lead to over or under-estimated performance
  - BMPs and ATAs can provide valuable information

Biochemical Methane Potential Assays (BMPs)

- Bench-scale test, generally 30 day duration
- Developed to determine anaerobic biodegradability of substrate
- Substrate is tested in a laboratory environment under optimal conditions

BMP Uses

1. Determine concentration of organics in a wastewater that can be anaerobically converted to CH_4

2. Evaluate potential efficiency of anaerobic process for a specific waste

3. Measure residual organic material amenable to further anaerobic treatment

Anaerobic Toxicity Assays (ATAs)

- Bench-scale test, 3-5 day duration
- Developed to determine the inhibition of CH_4 production by a given material
- Material is tested in a laboratory environment under optimal conditions

ATA Uses

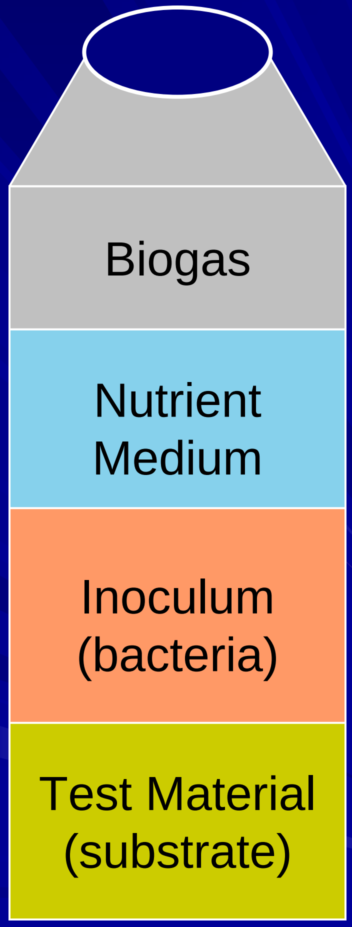
- Predicts likely effect of potential toxicant on biogas and CH_4 production
- Standard ATA does not show microorganism acclimation to toxicant or effect of toxicant build-up in the biomass
  - Continuous bench scale tests may be needed to study acclimation and toxicity due to compound build-up in the biomass

BMP and ATA References

- BMP
  - Owen et al., 1979; Water Research 13:485-492
  - Speece, 2008; Anaerobic Biotechnology & Odor/Corrosion Control
  - ASTM E2170-01
  - ISO 11734
- ATA
  - Owen et al., 1979; Water Research 13:485-492
  - ISO 13641-1

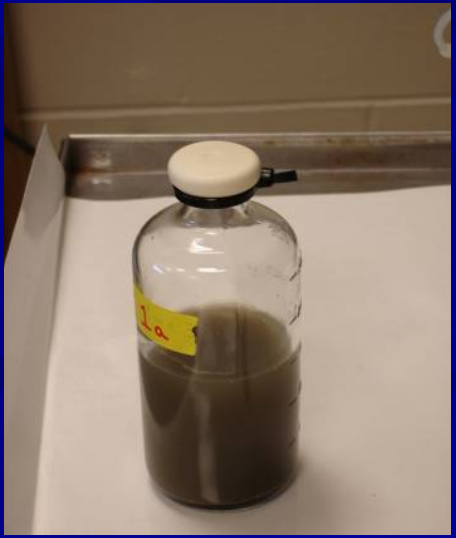
BMP General Principles

- Characterize substrate/test material:
  - pH, COD, TS, VS
- Place aliquot of test material in a serum bottle with inoculum & nutrient medium
- Prepare blank with inoculum & nutrient medium
- Prepare each assay in triplicate



BMP General Principles

- Purge bottles with 30% CO_2 / 70% N_2 gas, seal, & place on shaker at 35°C for 30 days



BMP General Principles

- Measure biogas production & CH_4 content (daily or as needed depending on production)

BMP General Principles

- Determine normalized biogas & CH_4 production (mL CH_4 / g substrate VS) and the extent of substrate anaerobic biodegradation (%)

Inoculum Bacteria

- Some labs get inoculum from local anaerobic treatment system and others maintain their own
  - Maintaining consistent source of inoculum removes some variability (Owen et al., 1979)
- Provide consistent food source to bacteria before assay

Quantity of Substrate and Inoculum to Add to BMP Bottle

1. Obtain measurable (but not excessive) CH_4 in BMP

   - Aim for at least 100-150 mL CH_4 / bottle, or 165-250 mL biogas @ 60% CH_4

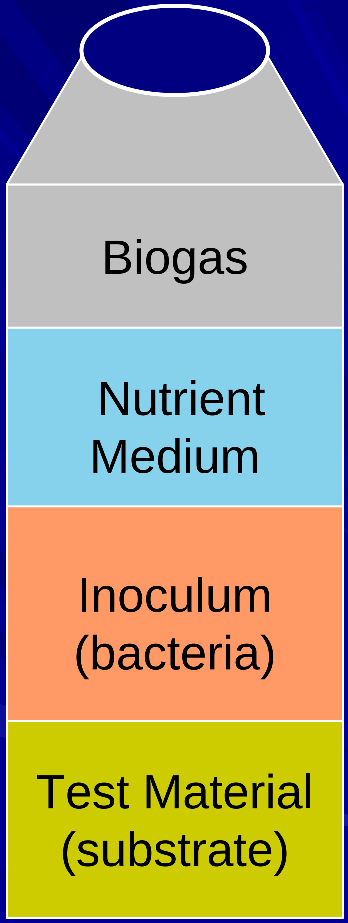
2. Use substrate [COD] to determine BMP substrate volume

   - 1 g COD reduction = 395 mL CH_4

mg COD required for 125 mL CH_4:

$$125 mLCH_4 \times \frac{1 mgCOD}{0.395 mLCH_4} \times 70\% = 221.5 mgCOD$$

* Assumes 70% COD conversion efficiency



Quantity of Substrate and Inoculum to Add to BMP Bottle

3. Calculate mass VS in BMP assay based on substrate volume

$$221.5\ mg\ COD \times \frac{1000\ mL}{X\ \ mg\ COD} = Y\ \ mL\ substrate$$

$$Y\ \ ml\ substrate \times \frac{Z\ \ mg\ VS}{1000\ mL} = A\ \ mg\ VS$$

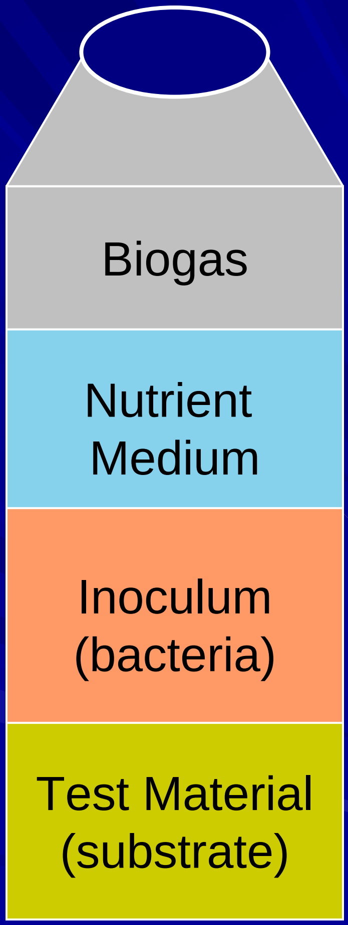
- Where:

X = COD concentration of substrate

Y = mL substrate to obtain necessary CH_4 production

Z = VS concentration of substrate

A = mg substrate to obtain necessary CH_4 production



Quantity of Substrate and Inoculum to Add to BMP Bottle

4. Calculate volume of inoculum to add to BMP bottle

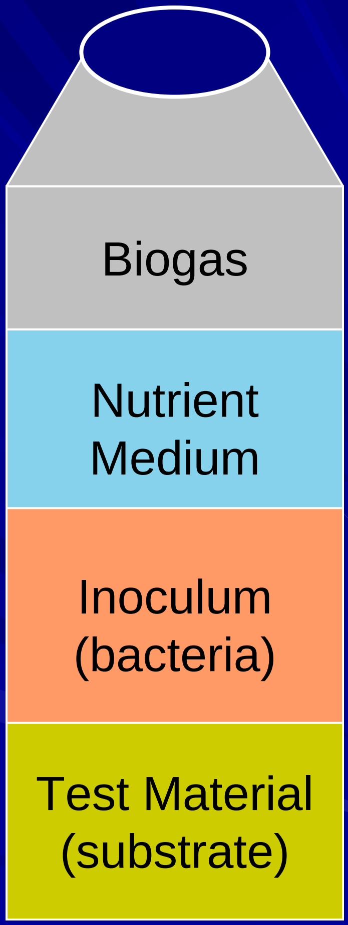
   - 1 g VS substrate / 1 g VS inoculum

$$innoculum\ (mL) = \frac{Y\ \ mL\ substrate * Z\ \ mg\ VS / L}{B\ \ mg\ VS / L} = C\ \ mL\ innoculum$$

$$C\ mL\ innoc \times \frac{B\ \ mg\ VS}{1000\ mL} = D\ mg\ VS\ innoculum$$

Where:

Y = mL substrate to obtain necessary CH_4 production
Z = VS concentration of substrate
B = VS concentration of inoculum
C = mL inoculum for 1:1 ratio
D = mass of inoculum for 1:1 ratio



5. Fill remainder of bottle with Nutrient Medium

BMP Results

- Calculate total CH_4 production using daily biogas volume and daily CH_4 content
  - Use blank to account for daily biogas and methane contributed by the inoculum
- Cumulative biogas and methane production between substrates can not be directly compared due to differences in assay loading rates for each material

BMP Results

- Normalize biogas and methane production to perform comparisons between substrates
- Normalize to mL / g substrate VS in assay

$$\text{Normalized } CH_4 \text{ production} \quad \frac{\text{ml } CH_4}{\text{g VS}} = \frac{\text{ml } CH_4 \text{ produced}}{\frac{\text{g VS substrate}}{\text{ml}} \times \text{ml substrate in bottle}}$$

Sample Type	TS (%)	VS (%)	COD (mg/L or mg/Kg)	Normalized CH_4 Yield (mL CH_4/g VS)	BMP Std. Dev.	Methane Yield (m^3 CH_4/mtonne)
Potato Chips	99.8	93.2	729,000	582	60	542.4
Food Grease	42.3	41.5	1,652,000	811	75.6	336.6
Dairy Manure	15.1	7.2	56,000	264	15.1	19.0

How do BMPs Compare to Full Scale Digesters?

vs.

How do BMPs Compare to Full Scale Digesters?

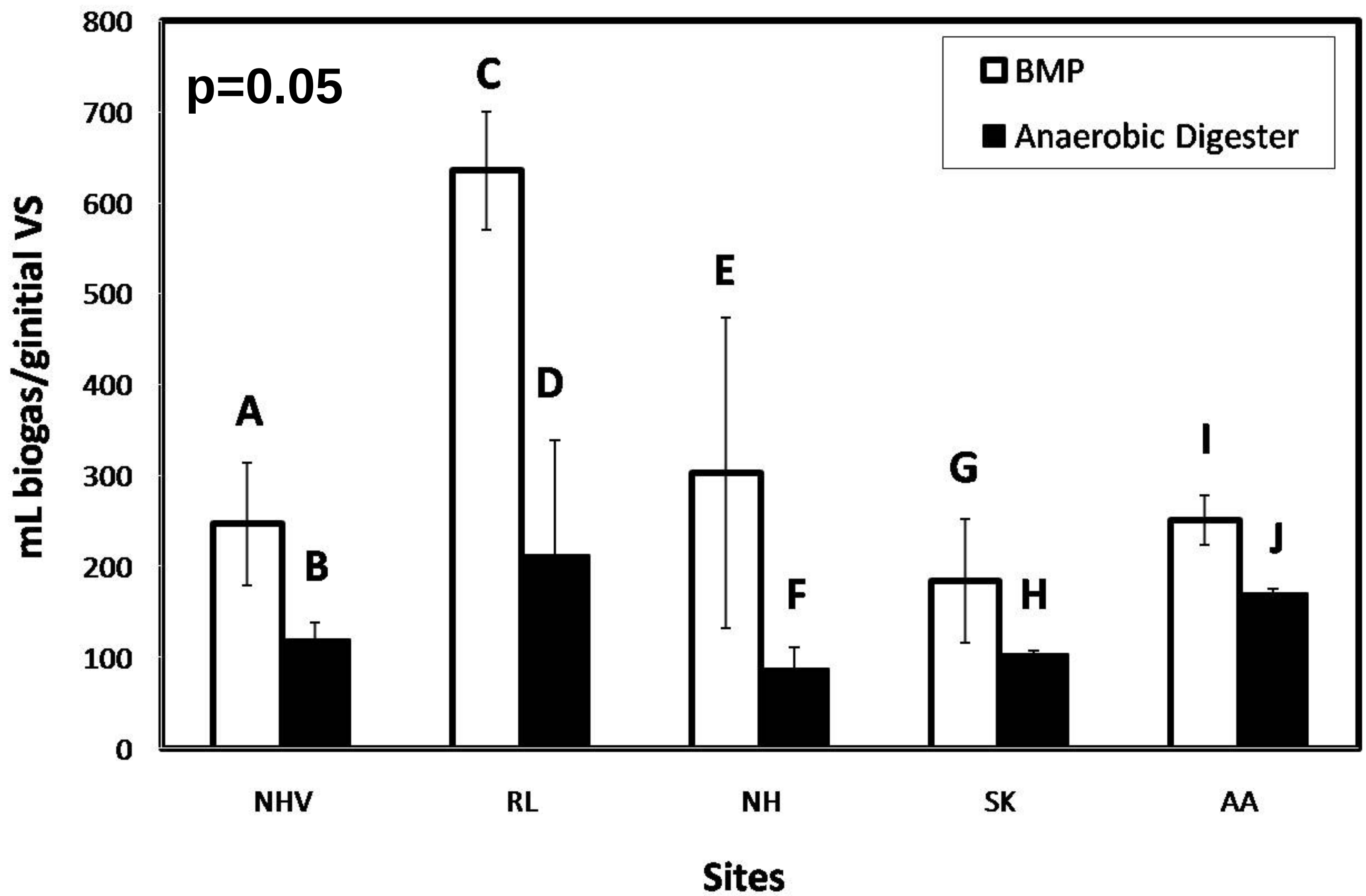
- ISU worked with Cornell University to compare BMP assay results to biogas and CH_4 production from full scale digesters
  - Cornell identified and obtained feedstock for 5 dairy digesters with biogas and CH_4 monitoring systems

How do BMPs Compare to Full Scale Digesters?

- ISU characterized the feedstocks and performed BMP assays on each
- Cornell simultaneously collected farm biogas production and CH_4 content data
- Data from the BMPs and full scale systems were normalized and compared

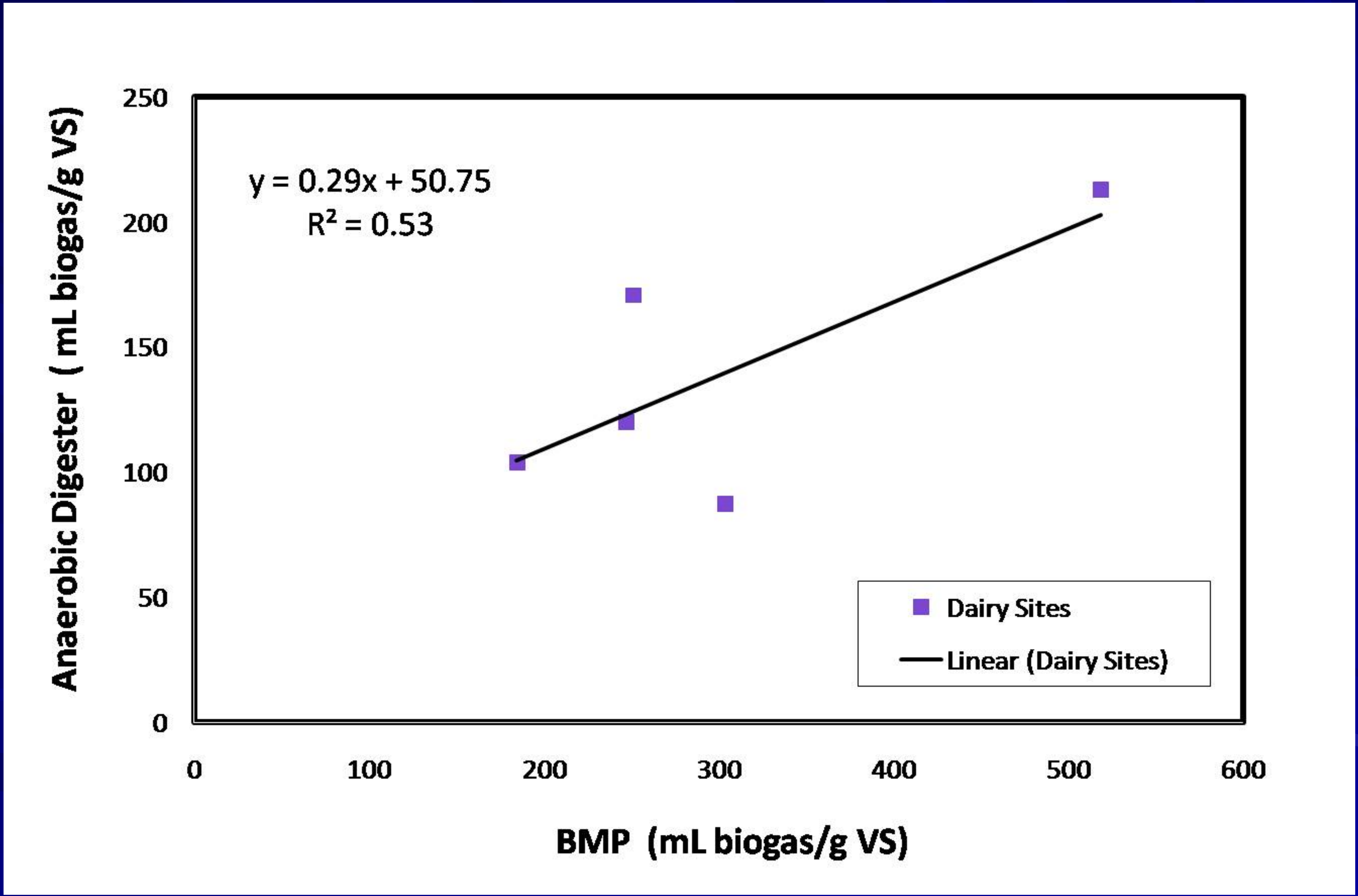
Bishop et al. 2009. Evaluation of Laboratory Biochemical Methane Potentials as a Predictor of Anaerobic Dairy Manure Digester Biogas ad Methane Production. Proceedings of the 2009 ASABE International Meeting. June 21-24, 2009. Reno, NV

Biogas Production Comparison



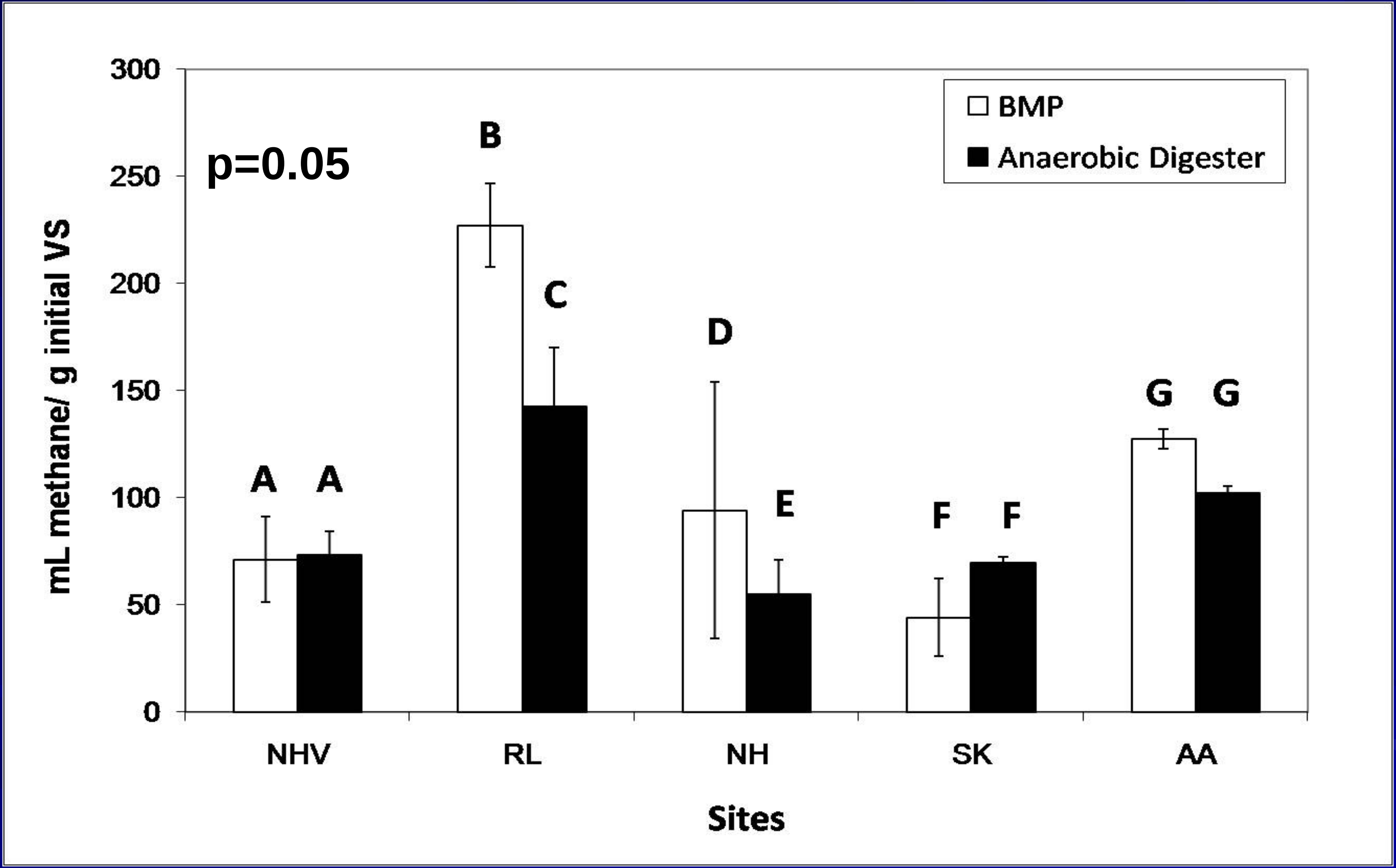
BMPs don’t seem to be a good predictor of biogas production in full scale digesters.

Regression of Biogas Production



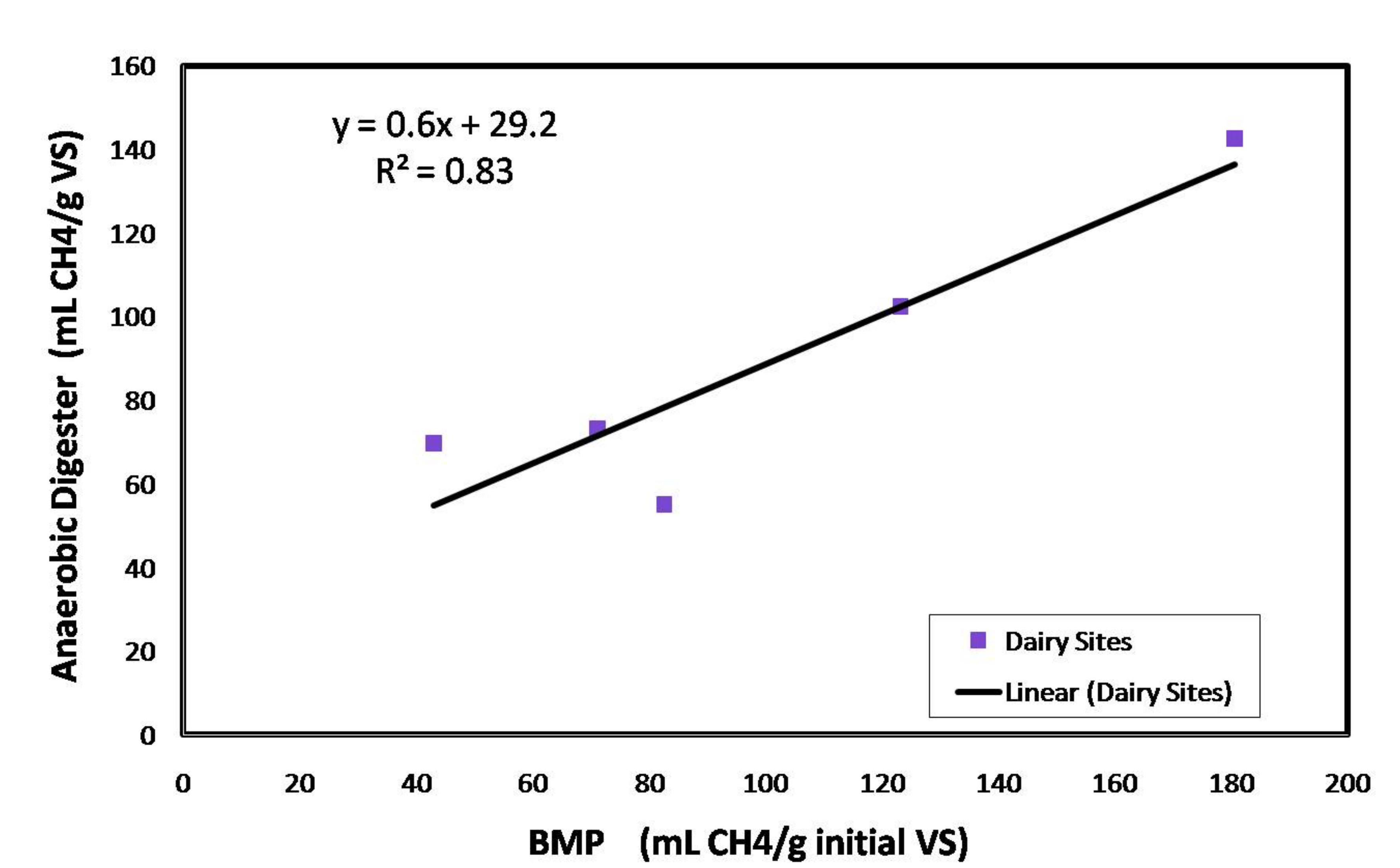
The R^2 value does not indicate a strong comparison between BMP and full scale biogas production.

Methane Production Comparison



BMPs provide a better prediction of methane production than biogas production in full scale digesters.

Regression of Methane Production



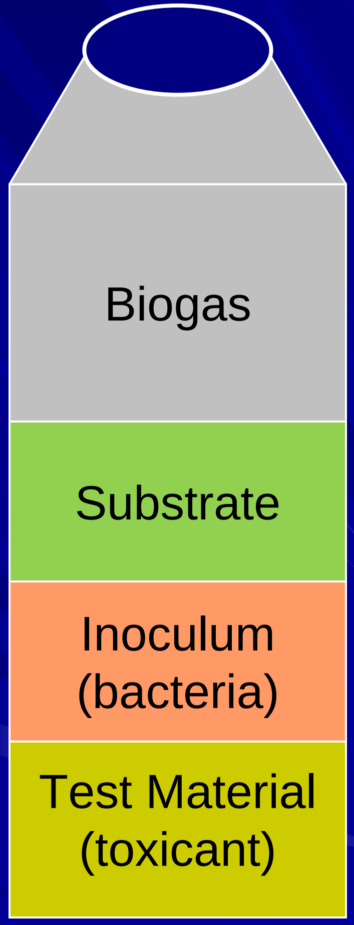
The R^2 value indicates a good correlation between BMP and full scale methane production.

Summary

- Biogas and methane production were over-predicted by BMPs
  - Average biogas production over predicted by 51.4%
  - Average methane production over predicted by 1.2%

ATA General Principles

- Characterize the test material/toxicant:
  - pH, COD, TS, VS
- Place aliquot of test material in serum bottle with substrate & inoculum
  - Prepare assays across range of test material concentrations
- Prepare control with substrate & inoculum
- Prepare each assay in triplicate



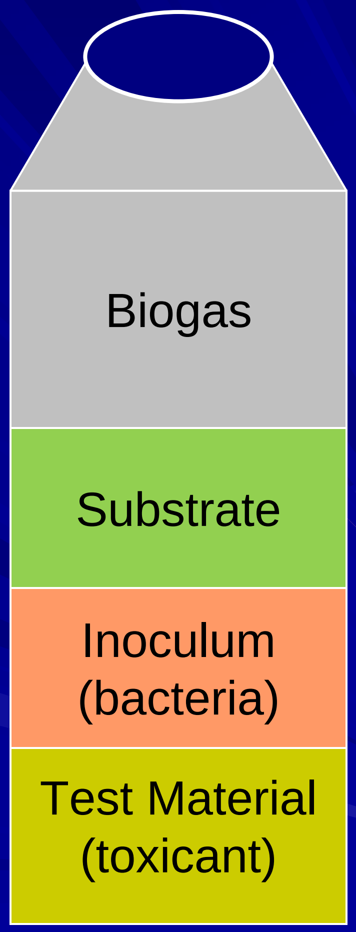
ATA General Principles

- Purge bottles with 30% CO_2 / 70% N_2 gas, seal, & place on shaker at 35°C for 3 - 5 days
- Measure daily biogas production & CH_4 content
- Develop inhibition curves, calculate percent inhibition, and determine the EC_{50}

EC_{50} - half maximal effective concentration – concentration of toxicant which induces a response halfway between the baseline and maximum response after a specified exposure time

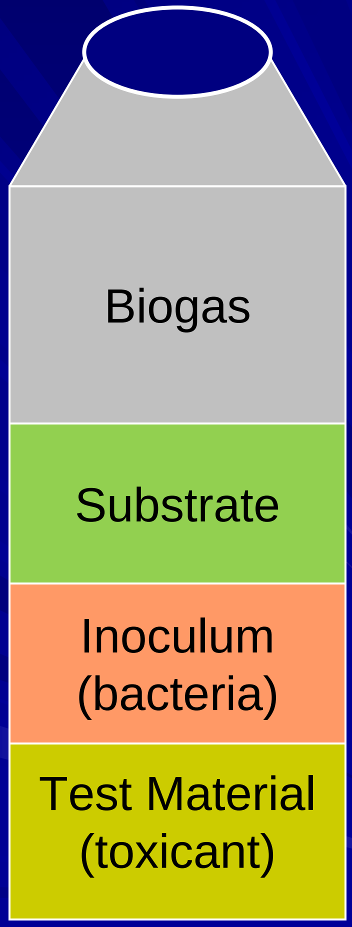
Quantity of Test Material, Substrate and Inoculum to Add to ATA Bottle

- Develop range of test material concentrations for the assay
  - Use known toxicity range for similar compounds
  - Perform preliminary range finding tests
  - Use range around expected loading rate
- Add test material to assay and use “make-up” water as necessary to achieve equal volumes



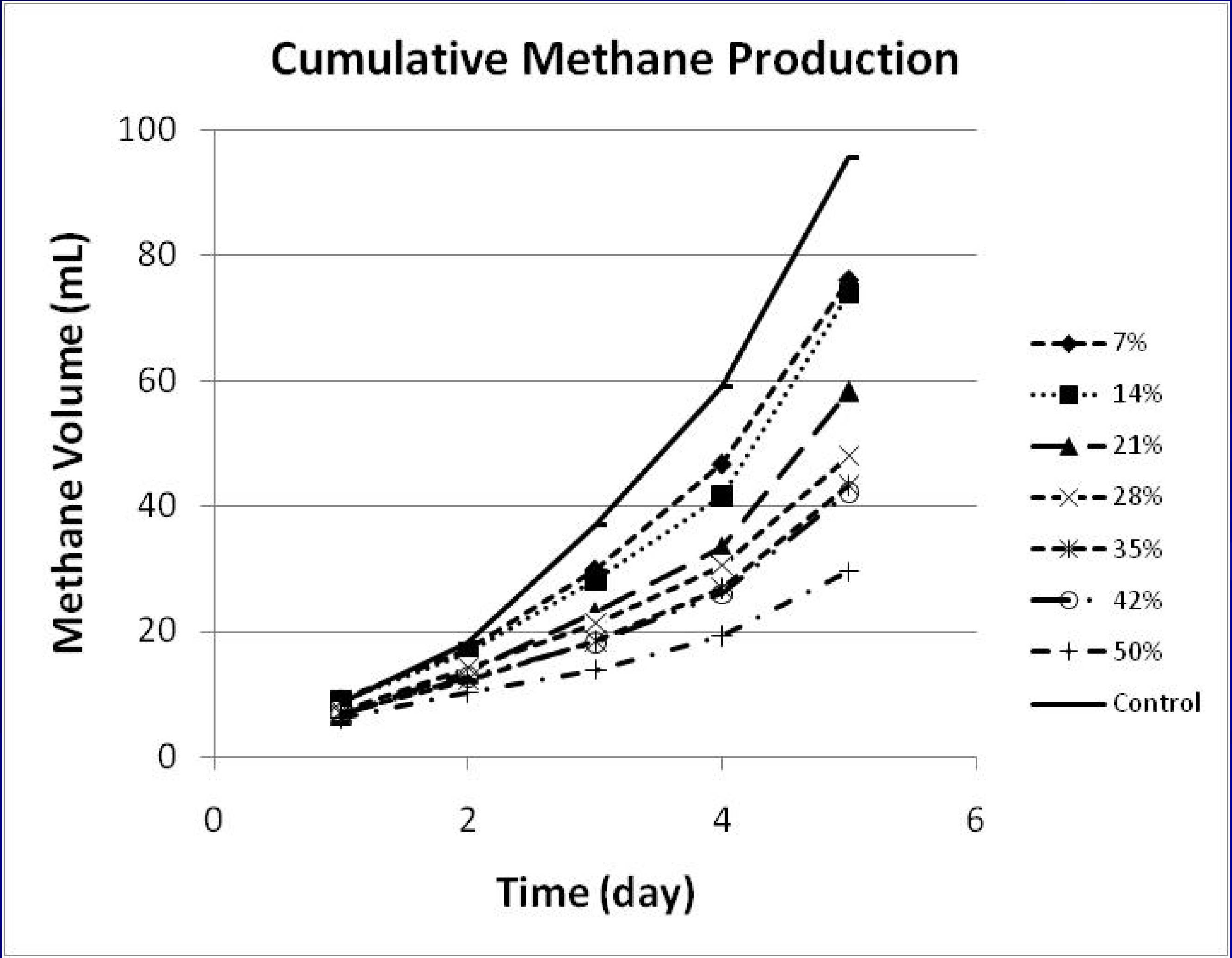
Quantity of Test Material, Substrate and Inoculum to Add to ATA Bottle

- Add 2 – 4 g of inoculum solids
  - 100 mL of solids with a concentration of 20-40 g/L
- Add 2 ml of substrate
  - Standard mix of nutrient broth, yeast extract, and D-glucose
- Prepare a control of substrate and inoculum for use in determining inhibition



ATA Results

- Graph cumulative CH_4 production against time for control and test material ranges



Data is from the ISU lab; this graph is the intermediate step to developing an inhibition curve.

ATA Results

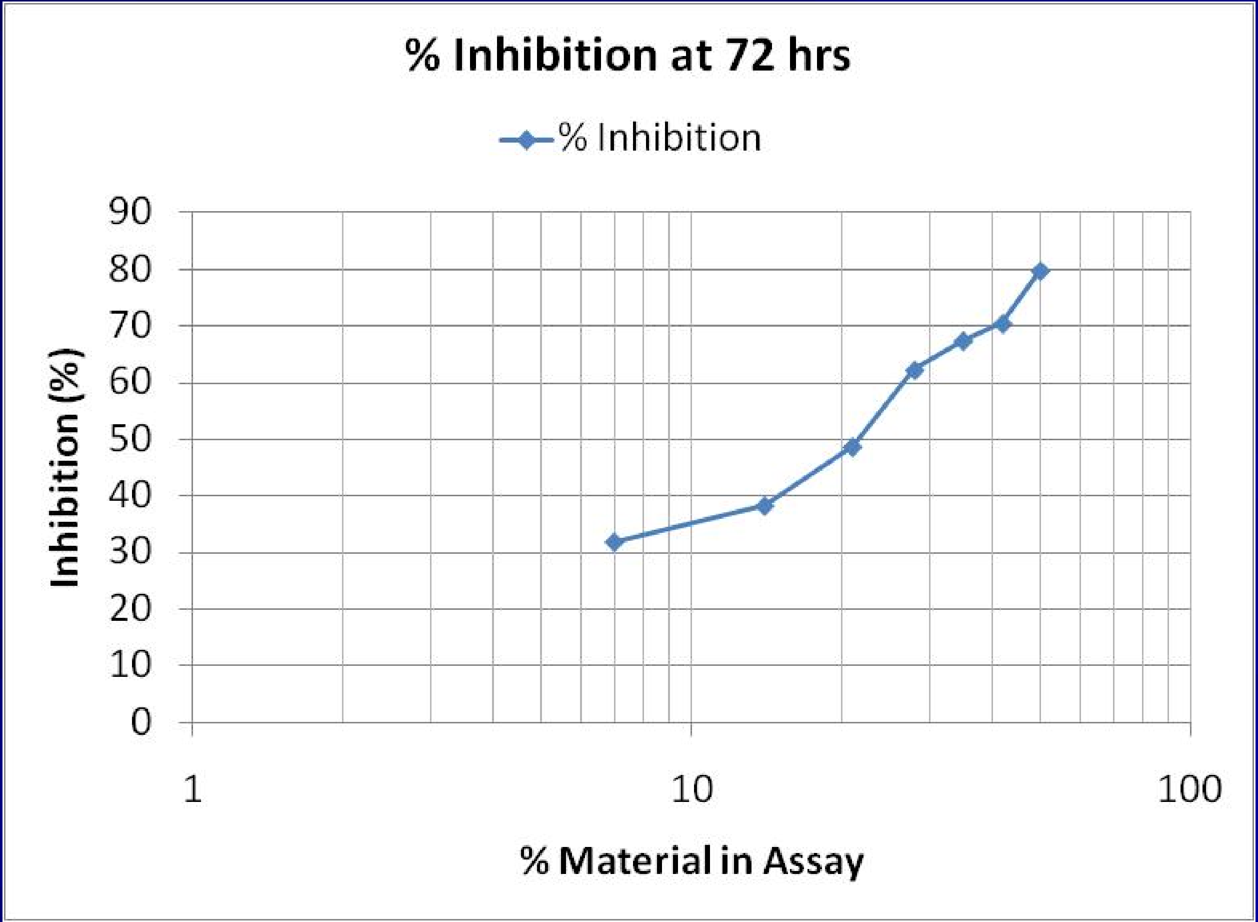
- Select time on linear part of curve (usually 48hrs) and calculate percent inhibition

$$I = (1 - \frac{CH4test}{CH4control}) * 100$$

Where:
I = percent inhibition
CH_4 test = volume CH_4 in test at a give time
CH_4 control = volume CH_4 in control at a given time

ATA Results

- Plot Percent Inhibition against the logarithm of the mass concentration of test material and determine EC_{50}



Data is from the ISU lab and represents a potential co-substrate for a manure digester that was determined to be toxic.

Use Caution When Applying Results

– BMP assays are diluted and can mask substrate toxicity

– BMPs are a batch loaded feed limited process; they are not continuously loaded processes and may not be representative of actual continuously fed digesters

– ATAs don't necessarily account for potential bacteria acclimation to a toxicant or build up of the material in the sludge to toxic levels

Digester Design

Lab Scale

- Can't design a digester with a BMP
- Bench & pilot digesters allow better understanding of how selected full scale process will operate
- BMPs & ATAs provide a "first-cut" evaluation when considering co-substrates

Bench Scale

Full Scale

The following university labs provide BMP & ATA testing services; these labs are listed for information purposes and ISU makes no endorsement of any lab:

- Iowa State University (Service Ended 4/15/2010)
  Agricultural Waste Management Lab
  Director: Robert Burns, rburns@iastate.edu
  515-294-4203

- Marquette University
  Water Quality Center
  Director: Dr. Dan Zitomer, daniel.zitomer@marquette.edu
  (414) 288- 5733

- Michigan State University
  Anaerobic Digestion Research & Education Center
  Manager: Dana Kirk, kirkdana@msu.edu
  517-432-6530

Questions?


New Brunswick Scientific
CLASSIC SERIES
C1 PLATFORM SHAKER
EDISON, NJ • U.S.A.
MaxQ
2000