Michigan State University Abbreviated Anaerobic Digestion Protocol

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Introduction

Michigan State University Department of Biosystems and Agricultural Engineering (BAE) conducts anaerobic treatment screening assays on wastewater samples from food processors, farms, and municipalities. 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 which will be useful in future studies. The assay is strictly designed to serve as a screening tool as conditions do not represent actual treatment techniques.

Sample Collection and Shipment

Collected samples need to be immediately chilled, stored near $4^{o}C$, and shipped at a chilled temperature within 24 hours of collection. A total sample size of 2 L is required.

Characteristics of the samples, including the name of the plant where the wastewater originated (that will be kept confidential), the process(es) that generated the wastewater, special safety precautions, wastewater constituents that may inhibit anaerobic digestion, and any other relevant information must be provided with the samples. Specific constituents of concern that may negatively impact digestion 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 will be accepted.

Samples must be stored at 4^{o}C, for up to 3 days, before initiating the respirometry assay.

Pre Respirometry Analysis

Table 1 contains the pre-respirometry analysis that will be conducted for the sample and seed, 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 of filtrate through SS Filter	>50% of Total COD	-
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	-

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. Occasionally, another respirometry system will be available allowing for two simultaneous or overlapping assays.

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 a column of drierite and potassium hydroxide or through a sealed flask with potassium hydroxide with a color indicator before it enters the gas volume counter.

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 conditions proposed for testing include the following. However, plant-specific alternatives can be designed.

- Seed
- Seed/Nutrient
- Wastewater/Seed
- Wastewater/Nutrients/Seed (optimal)

Seed will be digester content obtained from a locally operated 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 by 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. This dilution is approximately 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%. Consequently, the volume of substrate will be reduced if this occurs. If a rapid analysis is required (less than 45 days), previous experience shows that the COD of the wastewater should not exceed 250 mg per flask.

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
$K_2H_2PO_4$	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 is based on the COD of the wastewater and/or seed, depending on the vessel. 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 are added in proportion to the NH_4Cl, as shown in Table 2. Because of the low concentration of the metals, a stock solution is prepared and the appropriate

amount transferred into each vessel. The media is autoclaved for 5 minutes before use to drive off oxygen (Shelton and Tiedje, 19984). This level of nutrients assumes no nutrient value from the wastewater and seed although realistically, there would be a contribution. However, the excess should not have a negative influence and can be examined in the wastewater/seed vessel. Thus, the volume of the nutrient solution will be 600 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 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 will then be 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. Gas production rates and cumulative gas volume will be measured continuously and recorded every 3 hours.

During the log growth phase of the cumulative gas volume curve, grab samples will be collected directly from the head space of each vessel and analyzed for methane and carbon dioxide using a gas chromatograph (GC). Specifically, a Supelco SCOTT MX 216 packed primary and reference column is used in a Shimazdu GC8. The oven temperature starts at 40^oC and rises to 110^oC at 20^oC per minute. Helium is the carrier gas. A 3 to 5 point standard curve is used to calibrate a linear regression curve.

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

Post Respirometry Analysis

The same parameters measured in the pre respirometry analysis will be repeated for each vessel. All analyses are 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 of 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 also be provided. These plots are formed by subtracting the optimal vessel results from the seed/nutrients vessel results and the wastewater/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 are compared to this theoretical standard.

Results from the duplicates and GC analysis will be reported.

References

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