Standard Operating Procedure for Using, Managing, and Analyzing Anaerobic Digestion Reactors

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Document Overview

Purpose of This Guide	The Standard Operating Procedure for Using, Managing, and Analyzing Anaerobic Digestion Reactors provides reference information related to anaerobic digestion in the BIOTAR lab. This guide describes the process of building, maintaining, and studying anaerobic digestion.
Audience	This guide is designed for novice and experienced researchers.
Assumptions	This document assumes you are a current researcher in the BIOTAR lab. This guide also assumes that you have fundamental knowledge of anaerobic digestion.
Text Conventions	This guide uses the following conventions: • **<u>Bold and underlined</u>** indicates important safety information Always wear **<u>gloves</u>** when completing work in the laboratory. • *Italics* indicate new or important words *Always* wash dishes after completing lab work
Related Documentation	Please see the Analytical Methods and Lists of Solvents and Reagents in the Biological Treatment of Residues and Water Laboratory to assist you in your research.
How to Obtain Support	Reach out to Professor Tania Forester-Carneiro in the case of any question. If you need emergency assistance, please call 1-6000 for campus police.

Chapter 1: Preparation

This chapter reviews the initial set up of an anaerobic digestion reactor. This set up only needs to be completed once, at the very start of the experiment. This chapter includes the necessary steps to successfully install a reactor for anaerobic digestion.
This process includes:

1. Preparing the Digestate
2. Assembling the Reactor
3. Cleaning the Workspace

1.1 Preparing the Digestate

This section details how to prepare the contents of the reactor. The digestate is made of water, organic material, and inoculum. Inoculum houses the microorganisms which will undergo anaerobic digestion of the organic material. The inoculum in the lab is sourced from the AmBev Brewing company and is not parthenogenic. The digestate should be prepared **under the fume hood**, while wearing a **lab coat**, **breathing mask**, and **gloves**. Preform the following steps:

Prerequisite:

- 3 medium beakers
- 1 Large Plastic Beaker
- Inoculum Pot from the Incubator
- Organic Feed Material (Ex. Orange peels, barley bagasse)

1. Calculate the correct proportions for your reactor, which can be viewed on Page 5 in Figure 1
2. Consult Professor Tania to make sure your proportions are correct
3. Mix the water and inoculum together in the Large Plastic Beaker until they are well integrated
4. Split the organic material into three to five smaller batches
5. Mix each portion of the organic material into the inoculum and water mixture one at a time

NOTE: ALL THE ORGANIC MATERIAL MIGHT NOT INTEGRATE INTO THE MIXTURE, KEEP IT ASIDE AND WAIT UNTIL THE DIGESTATE BECOMES LIQUID TO COMBINE ALL PARTS

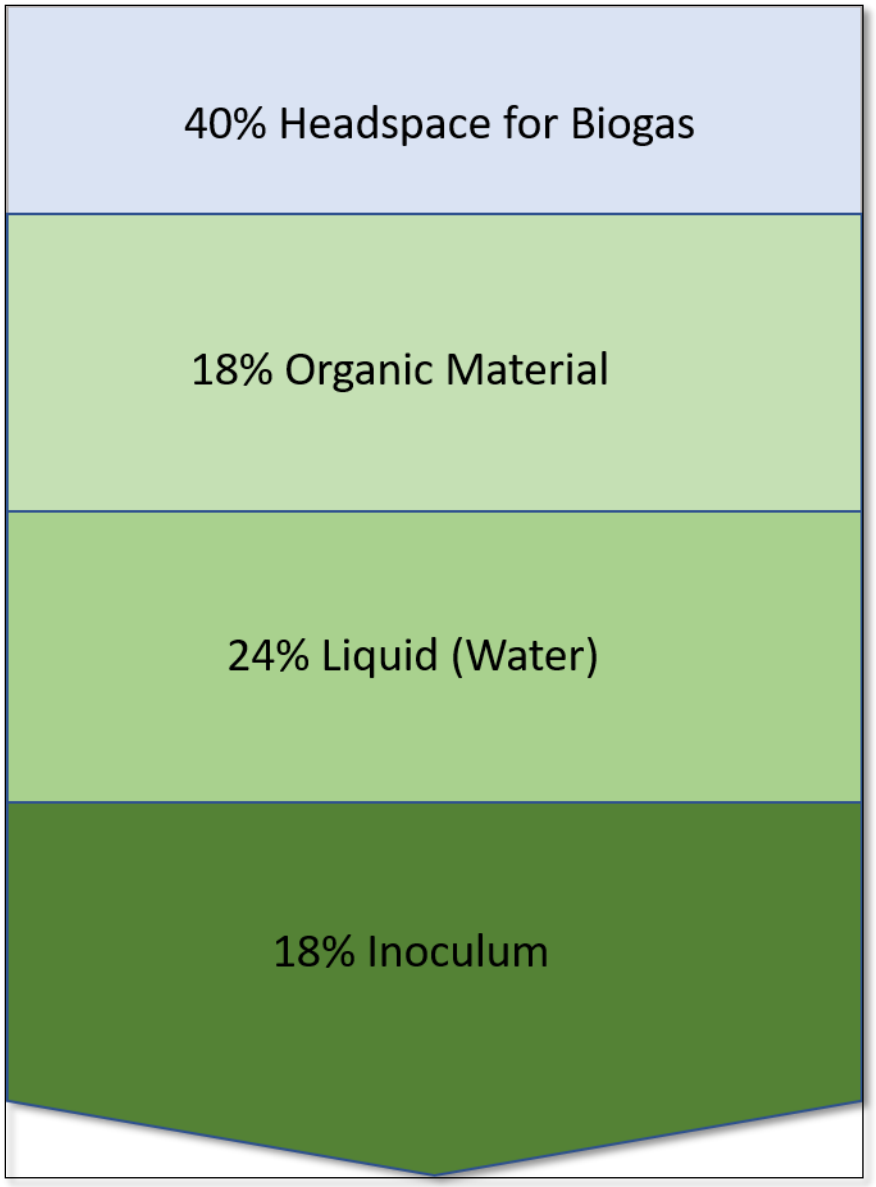

***Figure 1*: Typical Proportions of Materials in an Anaerobic Reactor**

1.2 Reactor Unit Assembly

This section details how to assemble the reactor unit. The reactor unit is comprised of the reactor, cooling system, and collection bag. The reactor unit must be assembled properly to successfully conduct anaerobic digestion. The reactor unit should be assembled while wearing a **lab coat** and **gloves**. Preform the following steps:

1. Measure the Reactor Volume
2. Construct the Reactor Unit

Procedure 1: Measure the Reactor Volume

This procedure describes how to measure the volume of the reactor if it is unknown. The reactor volume is essential for calculating the correct proportions for the components of the digestate.

Prerequisite:

- 1 medium beaker

1. Fill beaker with a known volume of water
2. Dispense water into reactor
3. Record volume of water dispenses into reactor
4. Repeat Steps 1-4 until reactor is full
5. Sum the recorded water volumes to obtain the total volume of the reactor
6. Empty the water out of the reactor
7. Use a paper towel or cloth to dry the reactor

Procedure 2: Constructing the Reactor Unit

This procedure describes how to construct the reactor unit.

1. Remove any dust or particulates from the inside and outside surfaces of the reactor with a cloth
2. Attach the inlet and exit cooling water tubes around the reactor
3. Tape a mylar collection bag onto the reactor apparatus
4. Attach a plastic tube to the opening of the collection bag
5. Mark the closing point of the knob by drawing a point on the bag with a marker
6. Connect a T-joint to the plastic tube on the end of the bag
7. Use plastic tubing to connect one end of the T-joint to the reactor
8. Place a septum on the open end of the T-joint

NOTE: THE SEPTUM SHOULD BE SECURE AND EASILY ACCESSIBLE

1.3 Cleaning the Workspace

This section describes how to properly clean instruments and beakers used in assembling and managing an anaerobic digestion reactor. Adequate sanitization of equipment is essential in making sure there is no cross contamination between experiments. Preform the following steps:

1. Wash Dishes
2. Decontaminate Instruments Used with Inoculum
3. Wash Writing from Beakers
4. Dispose of Hazardous Materials

BROKEN GLASSWARE SHOULD BE PUT IN THE LARGE WHITE BIN ON THE SHELF TO THE RIGHT OF THE SINK.

Procedure 1: Washing Dishes

This procedure describes how to clean beakers, spoons, and implements in the laboratory. This procedure describes the basic steps you should take to clean the things you use.

1. Throw any solid material in the trash before washing
2. Place dishes in the sink
3. Rinse with water
4. Scrub with a sponge or brush to get rid of any residues
5. Place on one of the drying racks to the side of the sink

Procedure 2: Contaminated with Inoculum

This procedure describes how to properly clean beakers, spoons, and implements used with inoculum. Inoculum should be properly cleaned to avoid contamination and health hazards. You should complete the steps in Washing Dishes (General) before moving onto to this section.

1. Follow steps 1-4 from Washing Dishes (General)
2. Rinse with Alcohol from a squirt Bottle
3. Flush with water again to wash off any remaining residues
4. Place the beaker on one of the drying racks to the side of the sink
5. Rinse the sink, counter, and surrounding area with alcohol

Procedure 3: With Writing

This procedure describes how to wash dishes with writing on them. If you write on your beakers to label them as yours, please wash off the writing before putting them to dry.

1. Follow steps 1-4 from Washing Dishes (General)
2. Rinse the outside of the beaker with alcohol from a squirt bottle until the writing disappears
3. Place on one of the drying racks to the side of the sink

Procedure 4: Hazardous Material (COD Testing)

This procedure describes how to clean things used with hazardous materials, like the test tubes used in COD Testing. It is important to not wash hazardous materials in the sink.

Gather the following:

- COD Waste Container
- Vortex Mixer
- Squirt Bottle with Deionized Water

Note: You may need to use an adapter or an extension cord to plug in the vortex mixer

1. Open your test tube and pour the contents into the COD waste container
2. Squirt a few milliliters of deionized water into the test tube
3. Cap and place the test tube on the vortex
4. Hold the test tube on the vortex for approximately 15 seconds
5. Pour the contents of the test tube into the COD waste container
6. Repeat steps 4-7
7. Place the uncapped test tube on a test tube rack upside down
8. Place the test tube rack in the oven at 105℃

Chapter 2: Reactor Management

This chapter includes the necessary procedures for reactor management. The procedures in this chapter should be done every Monday, Wednesday, and Friday. This chapter includes instructions to properly manage your reactor. This process includes:

1. Continuously Feeding the Reactor
2. Collecting Samples from the Reactor

2.1 Continuously Feeding the Reactor

This section describes how to feed the reactor; the section only pertains to experiments where the reactor must be continuously feed. If the reactor requires a feed, the exact volume should be noted. The reactor should be fed after the measurement of biogas composition, biogas volume, and daily samples. This procedure should be completed while wearing **a mask, gloves, and a lab coat.** Preform the following steps:

1. Open the reactor by using the sample removal hatch, noted in Figure 2 on Page 10
2. Place and secure a funnel in the reactor opening

Note: Ask for help if another pair of hands is necessary

3. *Slowly* pour the feed into the funnel
4. Scrape the sides of the funnel with a spoon to help it enter the reactor
5. Continue pouring and scraping until of the feed has been used
6. Scrape the funnel with a spoon to get any excess feed into the reactor
7. Remove the funnel and set aside for cleaning
8. *Immediately* close the reactor to stop any air from entering

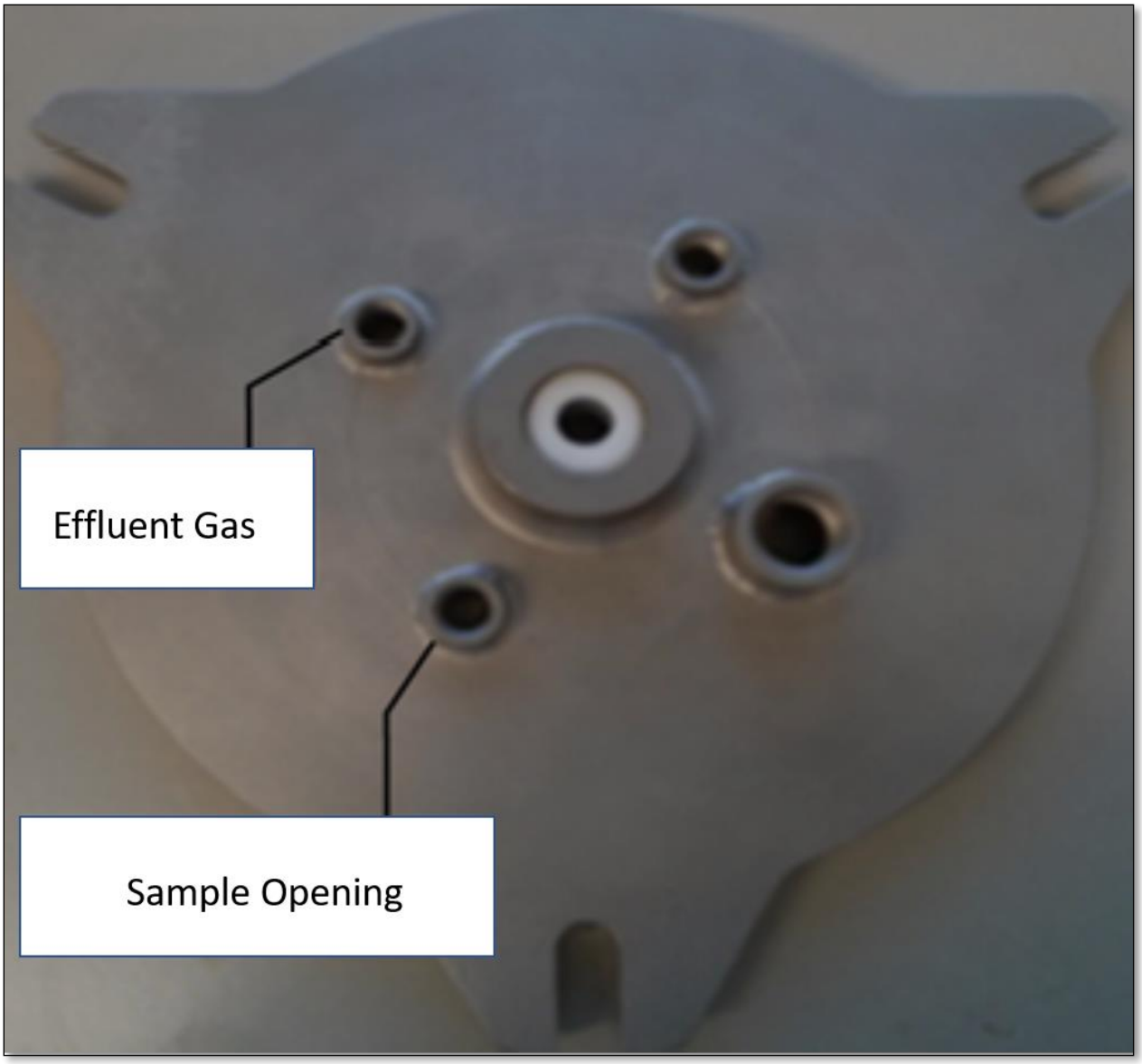

***Figure 2*: The Effluent Gas and Sample Opening holes on an Anaerobic Reactor**

2.2 Collecting Samples from the Reactor

This section describes how to collect samples from the reactor and test the pH of the reactor. These procedures should be followed every Monday, Wednesday, and Friday. These processes should be completed while wearing a **lab coat, gloves, and a mask**.

Preform the following steps:

1. Collecting Gas Phase Samples
2. Collecting Liquid and Solid Samples
3. Testing the pH

Procedure 1: Collecting Gas Phase Samples

This procedure describes how to properly collect gas phase samples. Gas phase samples should be collected to analyze the composition of biogas via gas chromatography. Exercise caution when collecting gas phase samples to not introduce oxygen into the reactor or damage the collection needle.

1. Collect the long thin needle located next to the gas chromatograph
2. Locate the septum on the reactor

Note: *Do Not* Poke the Bag or Tube with A Needle, It Will Cause A Leak

3. Slide the needle into the septum and gently pull the plunger to collect a gas phase sample
4. Transfer the gas sample into the gas chromatograph unit immediately
5. Wait for the results in the gas chromatograph unit to appear
6. Return the needle to the container next to the gas chromatograph

Procedure 2: Collecting Liquid and Solid Samples

This procedure describes how to collect liquid and solid samples from the reactor. Liquid and solid samples are necessary for testing the pH of the reactor, analyzing for moisture content, and preparing samples for analysis. Always wear a **lab coat, gloves, and mask** when collecting these samples.

1. Find a plastic beaker and use a marker to indicate the volume you intend to collect from the reactor
2. Open the reactor by unscrewing one of the cap on the top
3. Put the beaker into a bin and keep it close to the reactor
4. Use a long-nosed syringe to collect samples from the reactor and deposit them into the marked beaker
5. Shake the beaker regularly to make sure there are no air bubbles present in the sample
6. Put the sample under the fume hood once you have the desired quantity
7. Seal the reactor and begin to wipe down the area with a paper towel immediately
8. Wash the beaker, bin, long-nosed syringe, and any other tool you used to collect a sample

Procedure 3: Testing the pH

This procedure describes how to test the pH of an anaerobic digestion reactor. This task should be done after obtaining a liquid and solid sample from the reactor (refer to Procedure 2 in Section 2.2). The pH is an important test to make sure the environment in the reactor is optimal for producing methane. This procedure should be completed wearing **a lab coat, mask, and gloves.**

Gather the following:

- 50 mL plastic beaker
- 50 mL glass beaker
- Pipette
- Squirt Bottle with Deionized Water
- pH meter

Note: The pH probe should *always* be submersed in liquid

1. Put the probe into the sample from the reactor and **r**ead the results

STORING SAMPLES

GAS SAMPLES SHOULD NOT BE STORED, THEY SHOULD IMMIEDIATELY BE RELEASED INTO THE GAS CHROMATOGRAPH OR THE ATMOSPHERE.

LIQUID AND SOLID SAMPLES SHOULD BE TRANSFERRED INTO A FALCON TUBE AND FROZEN UNTIL THEY ARE READY FOR ANALYSIS

Only follow the remaining steps if the pH of the sample is higher or lower than the desired value

2. Transfer a known volume of the sample into the plastic beaker
3. Fill the glass beaker with NaOH solution (to raise the pH) or H2SO4 solution (to lower the pH)
4. Add and count drops of the solution into the sample
5. Gently mix the beaker to make sure the solution is incorporated
6. Put the probe into the adjusted sample and read the results
7. Rinse the probe with deionized water
8. Repeat steps 5-10 until you read the desired results
9. Calculate and add the proportion of solution needed to correct the entire reactor

Chapter 3: Analysis

This chapter reviews methods for keeping up with the analysis of an anaerobic digestion reactor. These procedures should be completed daily, throughout the lifetime of the reactor. This chapter includes the necessary steps to successfully record data associate with an anaerobic digestion reactor.
This process includes:

1. Keeping Records of Data
2. Displaying Data Graphically

3.1 Keeping Records of Data

This section details how to keep records of the data associated with an anaerobic reactor. Data associated with an anaerobic digestion reactor includes moisture content, biogas volume & composition, and the results of chemical oxygen demand (COD) ammonia, and alkalinity testing. A notebook and an excel sheet should be used to keep track of data. The notebook should be updated regularly and kept clean and dry. Preform the following steps:

1. On the first page of the notebook, draw a diagram of the reactor and include the total volume, percent of headspace for gas, and the weights of the components of the feed
2. On the second page of the notebook, record the daily pH of the reactor. Leave space for the data, sample identification, pH, and temperature.
3. On the third page, record the data associated with the solid analyzes of the reactor by saving space for the date, sample identification, P0, P1, P2, and P3.
4. On the fourth page, record the data from the COD testing by saving space for the date, sample label, absorption, and COD results.
5. On the fifth page, record the data from the ammonia testing by saving space for the date, sample identification, initial pH, number of drops, final pH, and volume of sulfuric acid.
6. On the sixth page, record the data from the volume of biogas by saving space for the data, description of the sample (reactor or collection bag), and volume collected.
7. On the seventh page, record the data associated with alkalinity testing by saving space for the date, sample identification, initial pH, volume used, and the final pH.
8. On the eighth page, reserve space for the biogas composition data by creating columns for the data, H2, CH4, CO2, and O2 data.

Each of the different types of analysis reveal important information about the anaerobic reactor.

Method	*Purpose*
Total Solids	Measure the moisture content in the reactor
Volatile Solids	Measure the amount of biodegradable organic matter in the reactor
Alkalinity	Measures the basicity and buffering capacity of the digestate
Chemical Oxygen Demand	Measures the potential of the microorganisms to convert organic material into methane
Ammoniacal Nitrogen	Measures the amount of ammoniacal nitrogen present in the reactor environment