Gravimetric BMP measurement*

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1 Introduction

By measuring BMP bottle mass loss due to biogas venting, along with biogas composition, methane (CH_4) production and biochemical methane potential (BMP) can be determined. This document describes the laboratory measurements needed for applying this "gravimetric method". Development and validation of the method is described in Justesen et al. [2019]. Comparisons with other methods can be found in Hafner and Astals [2019], Amodeo et al. [2020], and Hafner et al. [2020a]. For information on gravimetric calculations, see document 203 from the Standard BMP Methods website [Hafner et al., 2020d].

2 Protocol

2.1 Required equipment and supplies

- Electronic scale
- Syringes and needles
- Manometer (optional)
- Typical BMP bottles and septa
- Vials or other supplies needed for biogas analysis

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†For more information and other documents, visit https://www.dbfz.de/en/BMP. For document version history or to propose changes, visit https://github.com/sashahafner/BMP-methods.

- Incubator or heat block

The required accuracy of the scale will depend on the quantity of biogas produced. Generally, stated accuracy[1] of the scale should be 30 mg for every g of substrate volatile solids (VS) used, or better (smaller)[2]. As described below, the precision and stability of the scale is checked as part of the protocol.

A manometer can be useful to ensure that post-venting bottle headspace pressure is close to atmospheric, but is not necessary. If used, a simple closed U-tube manometer or an inexpensive electronic manometer is sufficient. A U-tube manometer can be made with some plastic tubing filled with water and a simple valve (made, e.g., by folding flexible tubing).

Biogas collection and analysis by e.g., gas chromatography, is required for this method. If a suitable instrument is not available, the gas density method can be used instead [Hafner et al., 2020b,c].

An incubator or temperature-controlled room is needed to keep bottles at the desired test temperature, e.g., 37°C. A water bath is not recommended because water on a bottle surface will affect its weight. Incubation of the bottles in heated air (or possibly in heat blocks) is preferable. Ideally, venting and weighing should be done inside a temperature-controlled room, so bottles are always at the incubation temperature and the headspace temperature–needed for calculations–is known. However, the effect of headspace temperature error on accuracy is very small, so this is not required.

2.2 Setup

During setup, inoculum and substrate are added to bottles, and the headspace of each bottle is flushed to remove O_2 and ensure anaerobic conditions. With the GD-BMP method, pure N_2 is preferred for flushing over mixtures containing CO_2.[3] Bottles are then weighed and placed in an incubator.

2.2.1 Step-by-step instructions

1. Carefully set up and level the scale on a stable surface (following manufacturer's instructions) and check its accuracy with a weight set. It is particularly important that the actual accuracy is close to reported accuracy when weighing an object with a mass close to the total mass of a BMP bottle and its contents. For a scale with a reported accuracy of 50 mg, for example, this could be checked by taring the scale with a full bottle or equivalent mass, and adding a 50 mg weight.

[1] Manufacturers often report accuracy as "linearity". Note that accuracy is not the same as "readability", which is the smallest value that can be read.

[2] For example, with 2 g of substrate VS added to each bottle, scale accuracy stated by the manufacturer must be 60 mg or better (e.g., 50 mg would be sufficient).

[3] Flushing gas results in a (generally small) error because its density may differ from produced biogas density (the density of N_2 is identical to a CH_4:CO_2 mixture with 58% CH_4 and 42% CO_2, and higher than a mixture with more CH_4) but this can be corrected in calculations [Justesen et al., 2019]. When flushing, be sure to avoid bubbling the gas through the inoculum/substrate mixture to minimize CO_2 stripping.

2. Add the required mass of inoculum, substrate, and other additions (e.g., a trace element solution) to each labeled bottle and seal with a septum and cover. Determination of the quantity of material added by mass difference is the recommended approach: tare scale with bottle, add approximately the desired quantity, wipe any material from near the mouth of the bottle, and finally determine the actual quantity from the scale reading. Note that the scale used here does not need to be the same scale used for determining mass loss (see "Incubation and sampling", below).

3. Flush the bottle headspace to remove O_2. A simple approach is to use a needle attached to a flow meter (e.g., a rotameter), a pressure regulator (to ensure low pressure), and a gas cylinder (generally with N_2) with plastic tubing, along with a separate needle for venting. Minimize CO_2 removal by flushing for only 3 to 4 headspace volume exchanges. Ensure that the flushing gas does not bubble through the liquid in the bottle (needle should not be submerged) to avoid CO_2 removal. Allow the pressure in each bottle's headspace to equilibrate with atmospheric pressure before removing the venting needle.

4. Make 3 "water control" bottles that contains only water. They should be the same size and weigh about as much as the other BMP bottles. These bottles should never be vented; they are used to check the stability of the scale and it is essential that they do not lose any mass.

5. Weigh each bottle and record as "initial mass". Repeat this initial weighing in order to minimize the chance of a recording error, because calculations of cumulative CH_4 production at all timepoints require an accurate initial mass measurement. If there is a discrepancy between these two initial measurements, weigh again to determine the correct mass. It is important that the only change in bottle mass after this time is due to biogas removal. Bottles should be kept clean, and labels should not be added after this time, for example.

6. Place bottles in incubator set at the test temperature.

2.3 Incubation and sampling

Bottles are removed from the incubator occasionally to vent, weigh, and take a biogas sample for analysis, in what is here referred to as a "sampling event". Biogas temperature affects water vapor loss and consequently the relationship between mass loss and standardised biogas volume (although less so than in volumetric and manometric meth- ods). As such, the time that bottles spend outside the incubator should be short, and the same procedure and timing should be followed for each sampling event. Ideally, venting and weighing should be done inside a temperature-controlled room, so bottles are always at the incubation temperature. However, the effects of headspace temperature on accuracy are small, so this is not required.

The accuracy of the gravimetric method is not affected by headspace pressure or leakage of biogas. However, for safety (to avoid bursting bottles) and to minimize possible effects of high CO_2 dissolution, total headspace pressure (absolute) should be kept below 3 bar. Bottle pressures can be estimated from headspace volume and calculated biogas production after measuring mass loss.[4]

2.3.1 Step-by-step instructions

1. Measure and record the room temperature and pressure at which biogas volume will be determined.

2. Remove the 3 water control bottles from the incubator and weigh them to confirm scale consistency. If the results are the same as the initial masses (within the expected accuracy) proceed, otherwise, identify and address the problem with the scale or replace the scale if necessary. If the problem cannot be resolved, proceed and later correct mass results for scale drift.[5]

3. Remove a single set of replicates from the incubator (e.g., the three replicates for cellulose).

4. Always starting with the same replicate (e.g., "1" or "a")[6] gently swirl the bottle for at least 10 s to mix the contents and encourage CO_2 equilibration between solution and headspace. During swirling, avoid contact between the liquid and the septum.[7]

5. Weigh the bottle and record the result as pre-venting mass.

6. Collect a biogas sample from the bottle using a syringe. Puncture the septum with a needle attached to a syringe, and allow the syringe to fill under pressure. Inject the required gas volume into a gas chromatograph for biogas composition analysis or into a gas sample container for later analysis.

7. Vent the bottle using a needle.[8]

[4] Or measured directly before venting if an electronic manometer is used. However, as with headspace temperature, the effect of uncertainty here is very small.

[5] Correction is done by subtracting the average apparent mass gain in the water control bottles from all mass measurements made during that particular sampling event. For example, if both water controls weighed 0.1 g more on day 4 than at the start, the measured masses of all bottles from day 4 should be adjusted downward by 0.1 g.

[6] If this is done the effect of gradual headspace cooling on measurement error (expected to be minor) can be confirmed by comparing BMP from individual replicates across all substrates.

[7] If the septum becomes contaminated with reacting material, a small amount may be pushed out during venting, which will result in error in the determination of mass loss. Generally it is easy to avoid this problem, but if it does occur, be sure to note the occurence to help with interpretation later. If the loss is small and there is no noticable difference among the replicates, the problem could be ignored. Otherwise, data from this replicate should be discarded.

[8] If desired, use the manometer to ensure that the pressure of the bottle headspace after venting pressure is close to atmospheric (gauge pressure = ±3 kPa).

8. Weigh the bottle after venting, and record the result as post-venting mass.

9. Proceed to the next replicate (e.g., "2" or "b") and repeat steps 3 - 7.

10. After all replicates have been mixed, weighed, vented, and weighed again, place the bottles back in the incubator.

11. Proceed to the next set of replicates (e.g., the three replicates for substrate "food waste A") and repeat steps 2 - 9.

3 Calculations

See document 203 from the Standard BMP Methods website [Hafner et al., 2020d] for a detailed description of calculations.

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