Gravimetric Measurement of Biochemical Methane Potential (BMP)*

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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 Hafner et al. [2015]. 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., 2020c]. The gravimetric method is very similar to the gravimetric method described in Document 304 [Hafner et al., 2020b]. Much of the text is identical between the two documents.

2 Equipment and supplies

For application of the gravimetric method the following laboratory equipment and supplies are required:

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Or see https://www.dbfz.de/en/BMP for a BibTeX file that can be imported into citation management software.

†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.

- Electronic scale
- Gas sampling equipment (e.g., syringe and needles)
- Gas chromatograph or equivalent system for gas analysis
- Typical BMP bottles and septa
- Incubator

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

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 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. A mechanical mixer is not needed; careful manual mixing at the time of sampling has been found to be sufficient (see Section 4).[3]

The gravimetric method requires separate analysis of biogas composition. Generally, this will be done by gas chromatography. Details on operation of a gas chromatograph are not presented here.

3 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. Bottles are then weighed and placed in an incubator. Setup should follow the requirements listed in document 100 [Holliger et al., 2020], including the use of a positive control[4] and at least 3 replicates for each substrate.

3.1 Inoculum and substrate quantities and bottle size

Selecting quantities of inoculum and substrate, as well as bottle size (volume) is typically based on experience. Some general guidance is given here; these

[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] But gentle mechanical mixing could work, as long as the inside of the septum is kept clean.

[4] Microcrystalline cellulose is required currently, but if it is unavailable, other common substrates may be useful; see Koch et al. [2020] for suggestions. But note that BMP can not be validated without microcrystalline cellulose at this time [Holliger et al., 2020].

suggested values can be adjusted depending on results and substrate characteristics.

The gravimetric method requires determination of small mass losses from a heavy BMP bottle, so in general, maintaining high mass loss should be a goal. Therefore, substrate mass should generally be at least 1 g VS. Starting with this value as a minimum, inoculum quantity can be determined based on inoculum-to-substrate ratio (ISR), which is expressed on a VS basis and should generally be 2:1.[5]

With substrate and inoculum quantities, a bottle size can be selected. Alternatively, given a bottle size, the largest possible quantities can be selected. The accuracy of the gravimetric method is affected only slightly by variation in headspace pressure, and it is possible to correct for leakage of biogas. However, for safety (to avoid exploding bottles), for maximum precision, and to minimize possible (but perhaps unlikely) effects of high CO_2 dissolution, headspace pressure should be kept below 200 kPa (2 bar) gauge pressure, or even more cautiously, 100 kPa [Hafner and Astals, 2019]. Bottle pressures can be estimated from headspace volume and expected (or measured) biogas volume. With some experience, the bulging of septa can be used to identify excessive headspace pressure. The headspace volume can be estimated by assuming a density of 1 mL g^{-1} for the mixture. Additionally, to minimize the risk of foaming causing contamination of the septum, bottles are typically not filled beyond 50%.

Using information from earlier measurements [6] and the maximum recommended headspace pressures given above, headspace volume should between 100 and 300 mL per g substrate VS. This target can be adjusted based on expected degradation rate and biogas potential, i.e., slower, faster, or similar biogas production compared to cellulose. But error from initial headspace[7] increases with the ratio of headspace volume to total biogas production, and although a correction is available [Justesen et al., 2019], it is less accurate when residual flushing gas remains in the headspace. Therefore headspace volumes above 300 mL g^{-1} should be avoided if possible.

The "planning" tool in the web app OBA is helpful for quickly calculating substrate and inoculum quantities: `https://biotransformers.shinyapps.io/oba1/`. This tool also checks values against the recommendations given in Holliger et al. [2016]. Three examples for three different bottle sizes that meet the recommendations given in Holliger et al. [2016] in addition to the gravimetric recommendations given in this section are shown in Table 1 below.

[5] See Holliger et al. [2016] for more discussion on this topic.

[6] Biogas production rate depends on substrate and inoculum characteristics, and is best estimated from previous experiments. For microcrystalline cellulose plus inoculum, biogas production rate typically peaks around 200 mL g^{-1} d^{-1} (per g VS) within the first days, with values over 300 mL g^{-1} d^{-1} possible but rare (these values were taken from the data collected in the large IIS-BMP inter-laboratory study [Hafner et al., 2020a]).

[7] Due to a difference in density between the flushing gas and biogas.

Table 1: Example quantity and volume information for three bottle sizes for gravimetric method. Note that for example C, it would not be possible to meet all recommendations if the inoculum had a VS concentration below 3.4%.

Parameter	A	B	C
Total bottle volume (mL)	520	250	160
Inoculum VS (% FM)	2.0	2.0	4.0
Substrate VS (% FM)	99	99	99
ISR (VS basis)	2.0	2.0	2.0
Substrate VS (g)	1.50	1.0	1.0
Inoculum FM (g)	150	101	50
Substrate FM (g)	1.52	1.0	1.0
Mixture FM (g)	152	101	51
Headspace volume (mL)	368	149	109
Headspace:substrate VS (mL:g)	245	149	109

3.2 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 scale calibration weight. Problems with accuracy or stability over time could be related to air currents, and can generally be addressed by selecting a proper location or blocking air flow with, e.g., a cardboard box.

2. Add the required mass of inoculum and substrate (see Section 3.1), along with any other additions (e.g., a trace element solution [Holliger et al., 2016]) 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. When filling the bottles, the aim is not to achieve the determined value as closely as possible, but to work in a fast (in order to minimize possible separation of the sample) and careful way (to avoid spills, errors, or safety problems) and to then record the exact mass added. Note that the scale used here does not need to be the same scale used for determining mass loss (see Section 4).

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 with plastic tubing, along with a separate needle for venting. With the gravimetric method, pure N_2

is preferred for flushing over N_2/CO_2 mixtures.[8] Minimize CO_2 stripping 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 stripping. Allow the pressure in each bottle's headspace to equilibrate with atmospheric pressure before removing the venting needle.

4. Make 3 "control" bottles to use to to check the stability of the scale It is essential that they have constant mass throughout the experiment. Ideally they should be a similar size and weigh about as much as the other BMP bottles. This can be done by adding dry sand to a BMP bottle and sealing the top with a septum. Bottles filled with water have been used successfully, but in other cases have been found to lose a small amount of water. Calibration weights for scales could also be used.

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 the incubator set at the test temperature.

4 Incubation and sampling

Bottles are removed from the incubator occasionally to vent and weigh in what is here referred to as a "sampling event". Details on sampling frequency can be found in Section 4.1 and step-by-step instructions for each event in Section 4.2. Biogas temperature affects water vapor content. Although the effect is small, to minimize uncertainty in the headspace temperature used in calculations, the time that bottles spend outside the incubator should be as short as possible, and the same procedure and timing should be followed for each sampling event.

4.1 Sampling frequency

Determining when to sample bottles in any manual BMP method, i.e., when to intermittently remove and measure accumulated biogas, is typically based on experience. Some general considerations are:

- As long as the headspace:substrate VS ratio is sufficiently high (see Section 3.1), there is no need to sample more than once per day

[8] Flushing gas results in an (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) but this can be corrected in calculations [Justesen et al., 2019].

- Sampling frequency can change over time, and is generally highest at the start (1 d interval) and low at the end (maximum of perhaps a 7 d interval)
- As long as biogas is not lost before or during measurement due to leakage resulting from long incubation intervals, sampling frequency does not strongly affect accuracy or precision of the gravimetric method

Following these recommendations, a good approach is to sample daily from the start, and reduce the sampling frequency as biogas production slows, taking care to avoid high headspace pressure or too much accumulated biogas for the sampling system. As mentioned above (3.1), a bulging septum can be used as an indicator of excessive headspace pressure.

4.2 Step-by-step instructions

1. Measure and record the room temperature and pressure at which biogas volume will be determined.
2. Remove the 3 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.[9]
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"),[10] gently swirl (not shake) 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 to prevent mass losses.[11]
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.

[9] Correction is done by subtracting the average apparent mass gain in the control bottles from all mass measurements made during that particular sampling event. For example, if all three 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.

[10] 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.

[11] 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 occurrence to help with interpretation later. If the loss is small and there is no noticeable difference among the replicates, the problem could be ignored. Otherwise, data from this replicate should be discarded.

7. Vent the bottle using a needle.[12]
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 4 to 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 3 to 9.

This sequence of steps is shown in Fig. 1.[13]

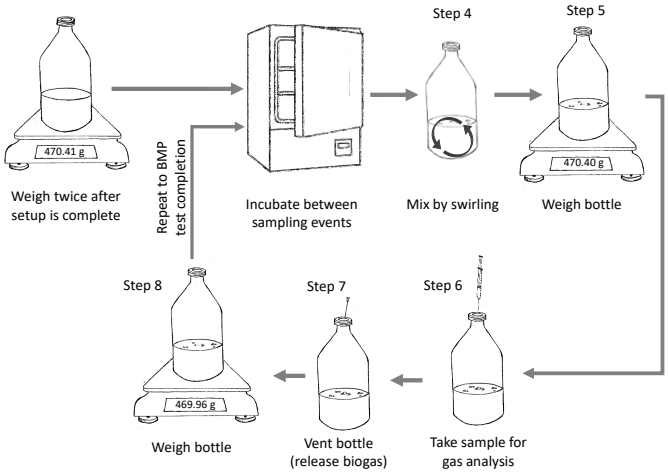

Figure 1: The data collection steps required for gravimetric measurements. Step numbers match those listed in the text, and are repeated for each sampling event.

5 Calculations

See document 203 from the Standard BMP Methods website [Hafner et al., 2020c] for a detailed description of calculations for the gravimetric method. Calculations can also be carried out using the free web app OBA (https://biotransformers.shinyapps.io/oba1/) or the biogas package in R (https://cran.r-project.org/package=biogas) [Hafner et al., 2018]. Final results should always be evaluated based on current validation criteria [Holliger et al., 2020].

[12] 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).

[13] It may be helpful to print a copy this figure and post it where measurements are made.

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