

Gas density-based BMP measurement*

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1 Introduction

By measuring BMP bottle mass loss and biogas volume, gas density can be determined, and from this, biogas composition can be calculated. With biogas volume measurements, methane (CH₄) production and biochemical methane potential (BMP) can be determined. This document describes the laboratory measurements needed for applying this “GD-BMP” (for **g**as **d**ensity) method. Development and validation of the method is described in Justesen et al. [2019]. For information on GD-BMP calculations, see document 204 from the Standard BMP Methods website [Hafner et al., 2020].

2 Protocol

2.1 Required equipment and supplies

- Electronic scale
- Syringes and needles
- Manometer
- Typical BMP bottles and septa
- Incubator or heat block

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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>.

The required accuracy of the scale will depend on the quantity of biogas produced. Generally, stated accuracy¹ 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 simple closed U-tube manometer or an inexpensive electronic manometer is sufficient for determining that post-venting headspace pressure is close to atmospheric. 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).

It is best to have several sizes of syringes, in order to have high relative precision in volume measurements when biogas production is both high and low. Ideally the largest syringe will be large enough to measure the largest volume of biogas produced in a single interval.³ But large syringes are expensive, and not necessary. Instead, a single small syringe can be used multiple times to remove the biogas from a single bottle in a single interval. But this approach requires that the manometer is directly connected to the syringe and there is a valve between the syringe and the bottle.

An incubator or temperature-controlled room is needed to keep bottles at the desired test temperature, e.g., 37°C. Although a water bath could be used, care must be taken to ensure that nothing is present on the surface of the bottle (e.g., water droplets) possibly affecting the bottle mass. Therefore 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 O₂ and ensure anaerobic conditions. With the GD-BMP method, pure N₂ is preferred for flushing over mixtures containing CO₂.⁴ Bottles are then weighed and placed in an incubator.

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

²For 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).

³Biogas production rate depends on substrate and inoculum characteristics, and is best estimated from previous experiments. For microcrystalline cellulose, typical production early in a test may be 200 to 300 mL biogas per g VS over one day. With 2 g substrate VS then, syringe volume would ideally be more than 600 mL (perhaps 1 L), but even a 150 mL syringe could be sufficient with multiple emptying cycles.

⁴Flushing gas results in a (generally small) error because its density may differ from produced biogas density (the density of N₂ is identical to a CH₄:CO₂ mixture with 58% CH₄ and 42% CO₂, and higher than a mixture with more CH₄) 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 CO₂ removal.

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.
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 2 “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 and weigh in what is here referred to as a “sampling event”. Biogas temperature affects water

vapor content. To minimize uncertainty in the headspace temperature used in calculations, the time that bottles spend outside the incubator should be short, and the same procedure and timing should be followed for each sampling event.

The accuracy of the GD-BMP 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 effects of high CO₂ dissolution, total headspace pressure (absolute) should be kept below 3 bar (2 bar gauge pressure). Bottle pressures can be estimated from headspace and vented biogas volume.⁵

Unlike headspace conditions, the temperature and pressure of biogas at the time of volume measurement is important to determine in order to standardize the volume. When using syringes, it is reasonable to assume that the syringe and gas inside are approximately at the ambient temperature. With a manometer, the pressure can be kept nearly identical to the ambient value. Therefore ambient pressure and temperature (room pressure and temperature) must be determined or, if necessary, estimated. Absolute pressure, can now be done using barometer apps on many smart phones. Alternatively, pressure can be determined from public meteorological data (from a close by weather station), with corrections for elevation if necessary.

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 both 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.⁶
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”)⁷ gently swirl the bottle for at least 10 s to mix the contents and encourage CO₂ equilibration between solution and headspace. During swirling, avoid contact between the liquid and the septum.⁸

⁵Or measured directly before venting if an electronic manometer is used. However, as with headspace temperature, the effect of uncertainty here is very small.

⁶Correction 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.

⁷If 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.

⁸If 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. Gen-

5. Weigh the bottle and record the result as pre-venting mass.
6. Vent the bottle using a syringe and measure biogas volume. Use the manometer to ensure that the pressure of both removed biogas and biogas remaining in the bottle headspace after venting pressure is close to atmospheric (gauge pressure = ± 3 kPa).
7. Weigh the bottle after venting, and record the result as post-venting mass.
8. Proceed to the next replicate (e.g., “2” or “b”) and repeat steps 3 - 7.
9. After all replicates have been mixed, weighed, vented, and weighed again, place the bottles back in the incubator.
10. 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 204 from the Standard BMP Methods website [Hafner et al., 2020] for a detailed description of calculations.

References

- S. D. Hafner, C. Justesen, R. Thorsen, S. Astals, C. Holliger, K. Koch, and S. Weinrich. Calculation of Methane Production from Gas Density-Based Measurements. Standard BMP Methods document 204, version 1.6., 2020. URL <https://www.dbfz.de/en/BMP>.
- C. G. Justesen, S. Astals, J. R. Mortensen, R. Thorsen, K. Koch, S. Weinrich, J. M. Triolo, and S. D. Hafner. Development and validation of a low-cost gas density method for measuring biochemical methane potential (bmp). *Water*, 11(12):2431, Dec. 2019. doi: 10.3390/w11122431. URL <https://www.mdpi.com/2073-4441/11/12/2431>.

erally 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 noticable difference among the replicates, the problem could be ignored. Otherwise, data from this replicate should be discarded.