

# Gas density-based measurement of biochemical methane potential (BMP)\*

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## 1 Introduction

The gas density BMP (GD-BMP) method is an approach that does not require expensive gas analysis equipment needed for other manual biochemical methane potential (BMP) methods. In this method, biogas volume and bottle mass loss are used together to determine biogas density, and from this, biogas composition is calculated.<sup>1</sup> Together with biogas volume measurements, methane (CH<sub>4</sub>) production and BMP can be determined. This document describes experimental procedures and laboratory measurements needed for applying the GD-BMP method. Development and validation of the method are described in Justesen et al. [2019]. For information on GD-BMP calculations, see document 204 from the Standard BMP Methods website [Hafner et al., 2020b].

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<sup>†</sup>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>.

<sup>1</sup>Composition can only be determined from density when the a mixture contains two gases, CH<sub>4</sub> and CO<sub>2</sub> here, although the effect of some residual flushing gas can be addressed as described in document 204 [Hafner et al., 2020b].

## 2 Protocol

### 2.1 Required equipment and supplies

For application of the GD-BMP method the following laboratory equipment and supplies are required:

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

The required accuracy of the scale will depend on the quantity of biogas produced. Generally, stated accuracy<sup>2</sup> of the scale should be at least 30 mg for every g of substrate volatile solids (VS) used.<sup>3</sup> As described below, the precision and stability of the scale is checked as part of the protocol.

A simple closed U-tube manometer<sup>4</sup> or an inexpensive electronic manometer is sufficient for determining that post-venting headspace pressure is close to atmospheric before measuring volume.

For the highest precision in volume measurements for low or high biogas production, it is best to have several sizes of syringes for different interval measurements. Ideally the largest syringe will be large enough to measure the largest volume of biogas produced in a single interval.<sup>5</sup> But large syringes are expensive. Instead, a single small syringe can be used multiple times to remove the biogas from a single bottle in a single interval. However, this approach requires a valve in addition to a manometer and it is slightly more complicated (see Section 2.5 for details).

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.

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<sup>2</sup>Manufacturers often report accuracy as “linearity”. Note that accuracy is not the same as “readability”, which is the smallest value that can be read.

<sup>3</sup>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).

<sup>4</sup>A closed U-tube manometer can be made with plastic tubing filled with water and a simple valve made by folding some flexible tubing. See Fig. 2 for an example.

<sup>5</sup>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 at about 200 mL g<sup>-1</sup> d<sup>-1</sup> (per g VS) within the first days, with values over 300 mL g<sup>-1</sup> d<sup>-1</sup> possible but rare (These values were taken from the data collected in the large IIS-BMP inter-laboratory study [Hafner et al., 2020a]). With 2 g substrate VS then, syringe volume would ideally be more than 600 mL (300 × 2, perhaps 1 L), but a 150 mL syringe could be sufficient with multiple emptying cycles.

However, the effect of headspace temperature error on accuracy is very small, so this is not required. Careful manual mixing at the time of sampling has been found to be sufficient (see Section 2.3).<sup>6</sup>

## 2.2 Setup

During setup, inoculum and substrate are added to bottles, and the headspace of each bottle is flushed to remove O<sub>2</sub> and ensure anaerobic conditions. 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 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<sup>7</sup>, 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. Note that the scale used here does not need to be the same scale used for determining mass loss (see “Incubation and sampling” in section 2.3).
3. Flush the bottle headspace to remove O<sub>2</sub>. 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 GD-BMP method, pure N<sub>2</sub> is preferred for flushing over N<sub>2</sub>/CO<sub>2</sub> mixtures.<sup>8</sup> Minimize CO<sub>2</sub> 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<sub>2</sub> stripping. Allow the pressure in

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<sup>6</sup>But gentle mechanical mixing could work, as long as the inside of the septum is kept clean.

<sup>7</sup>See Section 2.4. The “planning” tool in the web app OBA can be used to calculate these quantities: <https://biotransformers.shinyapps.io/oba1/>.

<sup>8</sup>Flushing gas results in an (generally small) error because its density may differ from produced biogas density (the density of N<sub>2</sub> is identical to a CH<sub>4</sub>:CO<sub>2</sub> mixture with 58% CH<sub>4</sub> and 42% CO<sub>2</sub>) but this can be corrected in calculations [Justesen et al., 2019].

each bottle's headspace to equilibrate with atmospheric pressure before removing the venting needle.

4. Make 3 “control” bottles to use 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  $\text{CH}_4$  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.

## 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”. Details on sampling frequency can be found in Section 2.4. 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 as short as possible, and the same procedure and timing should be followed for each sampling event.

Unlike headspace conditions, it is important to determine the temperature and pressure of biogas at the time of each measurement in order to standardize the volume. When using syringes, it is reasonable to assume that the syringe and gas inside are approximately at 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 measured using barometer apps on many smart phones, which can also be used for temperature measurement in conjunction with an external sensor. Alternatively, pressure can be determined from public meteorological data (from a nearby 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 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.<sup>9</sup>
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”),<sup>10</sup> gently swirl (not shake) the bottle for at least 10 s to mix the contents and encourage CO<sub>2</sub> equilibration between solution and headspace. During swirling, avoid contact between the liquid and the septum to prevent mass losses.<sup>11</sup>
5. Weigh the bottle and record the result as pre-venting mass.
6. Vent the bottle using a syringe and measure biogas volume (see Section 2.5). Prevent the needle from contacting any reacting material; keep it in the gas phase. Use the manometer to ensure that the pressure of both biogas in the syringe and biogas remaining in the bottle headspace after venting pressure is close to atmospheric (gauge pressure =  $0 \pm 3$  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 4 to 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 3 to 9.

This sequence of steps is shown in Fig. 1.

## 2.4 Quantities and sampling frequency

Selecting quantities, of inoculum, substrate, and total bottle volume, is typically based on experience. Some general guidance is given here; these suggested values can be adjusted depending on results and substrate characteristics.

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<sup>9</sup>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.

<sup>10</sup>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.

<sup>11</sup>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.

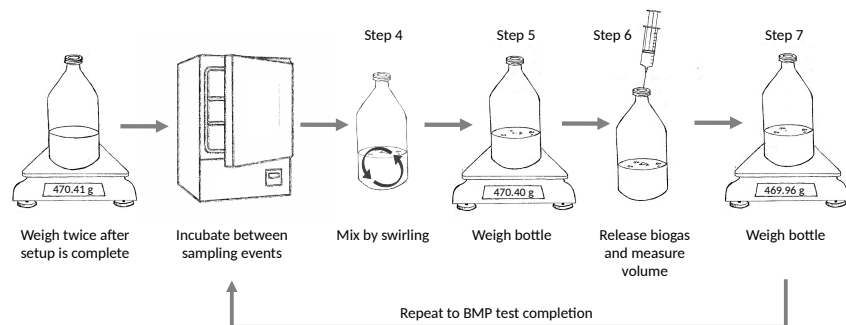


Figure 1: The data collection steps required for GD-BMP measurements. Step numbers match those listed in the text, and are repeated for each sampling event. For details on volume measurement (step 6) see Section 2.5.

The GD-BMP method requires determination of small mass losses from a heavy BMP bottle, so in general, maintaining high mass loss should be a goal. Substrate mass should generally be 1 g VS or higher. Starting with this value, 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.<sup>12</sup> With substrate and inoculum quantities, a bottle size can be selected.

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 (but perhaps unlikely) effects of high CO<sub>2</sub> dissolution, total headspace pressure (absolute) should be kept below 200 kPa (2 bar) gauge pressure, or better, 100 kPa [Hafner and Astals, 2019]. Bottle pressures can be estimated from headspace volume and expected (or measured) biogas volume. The headspace volume can be estimated by assuming a density of 1 mL g<sup>-1</sup> for the mixture. Additionally, to minimize the risk of foaming causing contamination of the septum, bottles are typically not filled beyond 50%.

Using the information given in note 5 for cellulose (maximum peak biogas production of 300 mL g<sup>-1</sup> d<sup>-1</sup>) and a goal of keeping headspace pressure below 100 kPa (gauge), a headspace of 300 mL per g substrate VS is generally sufficient. Larger headspace volume may be acceptable, but error from the flushing gas density increases with headspace volume, so 300 mL g<sup>-1</sup> is a good starting point, and can be adjusted based on expected degradation rate (slower, faster,

<sup>12</sup>See Holliger et al. [2016] for more discussion on this topic.

or similar to cellulose). An example that meets all these constraints would be: substrate VS 50% (percentage of fresh mass), inoculum VS 2%, ISR 2 (2:1), substrate VS mass 2 g, substrate fresh mass 4 g, inoculum fresh mass 200 g, total bottle volume 500 mL, approximate headspace 300 mL.

The “planning” tool in the web app OBA is helpful for calculating substrate and inoculum quantities and quickly meeting these and some other constraints through trial-and-error: <https://biotransformers.shinyapps.io/oba1/>.

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

- As long as the headspace:VS ratio is sufficiently high (see above in this section), there is no need to sample more than once per day
- Sampling frequency can change over time, and is generally highest at the start (1 d interval) and low at the end (perhaps 7 d interval)
- As long as biogas is not lost before or during measurement due to leakage, sampling frequency does not strongly affect accuracy or precision of the GD-BMP method

Based on this information, 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.

## 2.5 Volume measurement

Volume measurement in Section 2.3.1 should take place at ambient pressure, in order to later convert the measurement to standardized volume. This task can be done very simply with an inexpensive plastic syringe and a simple U-tube manometer, as long as the capacity of the syringe (maximum volume) is greater than the quantity of biogas produced in any sampling interval. Unfortunately, typical plastic syringes are generally not large enough ( $\leq 150$  mL), and large glass syringes (e.g., 1 L) are expensive. There are at least two solutions:

- Use multiple syringes to remove accumulated gas all at one time.
- Use a water manometer and a valve to measure accumulated gas in steps.

The first approach is straightforward, but handling three or more syringes with only two hands is challenging. The second requires a valve and a manometer, and one implementation is shown in Fig. 2. Its construction and use is described here. In this section the capital letters (labeled with A, B, C) and small Greek letters ( $\alpha$ ,  $\beta$ , ...) refer to the labels in Fig. 2. A manometer can be made from flexible plastic tubing, large enough to avoid problems due to

adhesion.<sup>13</sup> It is filled with water to the level shown by  $\beta$ , and clamped at location  $\alpha$  to create a *closed*-tube manometer. Clamping is essential; an open-tube manometer would lose its water under even moderate pressure. Smaller diameter tubing (at  $\gamma$ ) can be used to connect to the three-way valve (A, B, C in Fig. 2).<sup>14</sup> When in use, the system is connected to a BMP bottle via a needle ( $\delta$ ).<sup>15</sup> A syringe is connected through tubing to the valve ( $\epsilon$ ). This connection should be easy to disconnect, in order to vent biogas. With this system, the following steps are used for each bottle.

1. With syringe disconnected (around location  $\epsilon$ ), check that water levels at  $\beta$  are equal. Equilibrate closed end by removing and replacing clamp at  $\alpha$  if necessary.
2. Turn valve so connection B-C is open (A closed) and allow accumulated biogas to flow under pressure to no more than ca. 80 or 90% of syringe capacity.
3. Turn valve so connection A-C is open (B closed) and adjust syringe plunger position so the water levels at  $\beta$  are equal. Read and record volume.
4. Disconnect syringe at  $\epsilon$ , push out biogas, and return to step 1, repeating until all excess accumulated biogas has been removed, i.e., when headspace pressure is equal to atmospheric (determined by opening A-B, or even A-B-C if possible).

For an even simpler system, a regular (two-way) valve (possibly clamped tubing) could be placed between the syringe and bottle (needle), with a manometer attached directly to the syringe.

## 2.6 Testing

The GD-BMP method requires accurate determination of gas volume and bottle mass loss. It is important that any new setup is tested. Fortunately, ambient air can be used as a standard. To test the system, simply use a syringe to *carefully* force a volume (more than ca. 20% of a bottle headspace is difficult to add) into an empty bottle, and proceed with the measurement steps given above. Calculate apparent density based on bottle mass loss and volume of gas removed.<sup>16</sup> The value should be within 10% (or better, 5%) of the density for the known ambient pressure and temperature.<sup>17</sup>

<sup>13</sup>Clear polyvinyl chloride (PVC) tubing with a 10 mm (3/8 inch) inner diameter works well.

<sup>14</sup>Plastic valves sometimes called "disposable medical three-way stopcock valves" are inexpensive and work well for this task.

<sup>15</sup>21 gauge/0.8 mm works well.

<sup>16</sup>This volume may be smaller than the volume thought to be added because of leakage during addition or removal of the syringe.

<sup>17</sup>Air density can be calculated with <https://www.density.co.uk/calculators/density-of-air/> or similar tools. Humidity effects are small around room temperature (assuming temperature  $\leq 25^\circ\text{C}$ ). Density is  $1.20 \text{ mg mL}^{-1}$  for dry air at  $20^\circ\text{C}$  and  $101.3 \text{ kPa}$ , and  $1.19 \text{ mg mL}^{-1}$  when saturated with water.



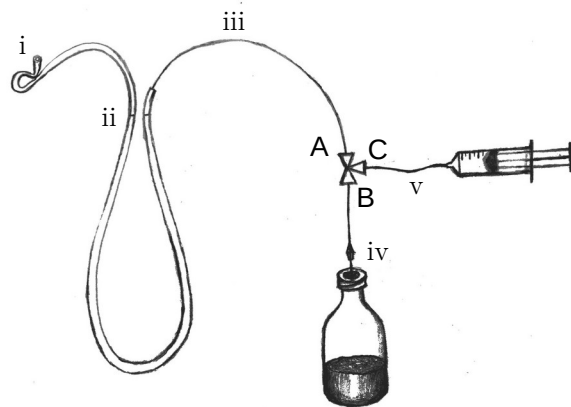


Figure 2: The U-tube manometer/3-way valve approach to measure biogas volume. The dashed line surrounds the u-tube manometer, which could be replaced with a digital model. See Section 2.5 for a detailed explanation.

### 3 Calculations

See document 204 from the Standard BMP Methods website [Hafner et al., 2020b] for a detailed description of calculations for the GD-BMP 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].

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