

Requirements for measurement and validation of biochemical methane potential (BMP)*

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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>.

1 Introduction

This document presents the minimal requirements for measurement and validation of biochemical methane potential (also called biomethane potential) (BMP) in batch tests, based on the consensus of more than 40 biogas researchers. The list of requirements is based on [Holliger et al. \[2016\]](#), with some recent modifications of validation criteria as described in [Hafner et al. \[In preparation\]](#) and additional details on calculation standardization. For details and many additional recommendations, see these papers [[Holliger et al., 2016](#), [Hafner et al., In preparation](#)].

2 Requirements for BMP measurement

2.1 Analysis of substrate and inoculum

Volatile solids (VS) content of inoculum and substrate is needed to determine quantities for a selected inoculum-to-substrate ratio (ISR) and for calculation of BMP. Details on measurements of TS and VS can be found elsewhere (including a detailed free document from the US EPA [[EPA, 2001](#)], as well as [Strach \[2016\]](#) and [Baird et al. \[2017\]](#)).

1. Total solids (TS). Measure for the inoculum and all substrates, by drying until constant weight at 105°C in triplicate.
2. Volatile solids (VS). Measure for the inoculum and all substrates by combusting the dried sample at 550°C until constant weight in triplicate.

2.2 Test setup and duration

1. Samples. All BMP trials must include three types of samples: batches with only inoculum (“blanks”), with inoculum and microcrystalline cellulose as a positive control¹, and with inoculum and substrate.
2. Replication. All tests must include at least 3 batches (bottles) for each condition². The minimum number of batches used in a BMP test with one substrate is therefore 9 (3 blanks, 3 cellulose, 3 substrate).
3. Duration. Terminate BMP tests only after daily CH₄ production from individual batches during 3 consecutive days is < 1.0% of the net accumulated volume of methane from the substrate (substrate batch minus average of blanks). For manual or other methods where measurements

¹ Other positive control substrates could be used in the future, but only cellulose has had extensive testing that was used to develop the validation criteria described below in Section 2.4 [[Hafner et al., In preparation](#)].

² If a bottle is lost through, e.g., breakage, resulting in $n = 2$ for any condition, results cannot be validated. Therefore it is prudent to include 4 replicates, especially for blanks. Outliers can be eliminated if there is good reason to suspect there was an error in measurement (e.g., leakage) but the remaining number of replicates must be at least 3.

are not made every day, termination can take place at the end of the first measurement interval of at least 3 days where the rate of production drops below the 1% maximum (or two or more intervals that sum to at least 3 days, all with rates below the 1% maximum). If different substrates are tested, each substrate can be terminated when the slowest of the 3 replicate batches has reached the termination criterion. Blanks must be continued as long as the slowest (latest) batch with substrate. Continuing tests beyond this 1% net duration is acceptable and can help ensure that validation criteria are met (Section 2.4).

2.3 Calculations

1. Data processing. Standardized CH₄ volume (dry, 0°C, 101.325 kPa) is calculated from laboratory data using standardized methods.³
2. BMP units. BMP should be expressed in standardized CH₄ volume (dry, 0°C, 101.325 kPa, referred to as “normal” volume) per unit mass of substrate organic matter added (typically VS but sometimes chemical oxygen demand (COD)) (often written as NmL_{CH₄} g_{VS}⁻¹).
3. Calculation of BMP. BMP of all substrates (including cellulose) is calculated by subtracting inoculum CH₄ production (determined from blanks) from gross (total) CH₄ production from substrate with inoculum, and normalizing by substrate VS mass. Any differences in inoculum or substrate mass among batches must be reflected in calculations. Calculations must follow a standardized approach⁴.
4. Calculation of BMP standard deviation. The standard deviation associated with each mean ($n = 3$) BMP value must include variability from at least both blanks and batches (bottles) with substrate and inoculum⁵.

2.4 Validation criteria

BMP results that meet *all* the following criteria can be described as “validated”. Otherwise, results are not validated, and tests should be repeated. Otherwise, the lack of validation should be made clear in any reporting of the results.

1. All required components of the BMP measurement protocol listed above (Sections 2.1 through 2.3) are met.
2. Mean cellulose BMP is between 340 and 395 NmL_{CH₄} g_{VS}⁻¹.

³ Detailed descriptions of calculations are available for the following measurement methods in the Standard BMP Methods collection (<https://www.dbfz.de/en/BMP>): volumetric (document 201) [Hafner and Løjborg, 2019], manometric (document 202) [Hafner et al., 2019], gravimetric (document 203) [Hafner et al., 2020], and gas density (document 204).

⁴ Calculation of BMP is described in detail in document 200 from the Standard BMP Methods collection (<https://www.dbfz.de/en/BMP>).

⁵ See document 200. Inclusion of variability from substrate VS determination is optional.

3. Relative standard deviation for cellulose BMP (standard deviation, including variability in both blanks and substrate bottles, divided by mean BMP) is no more than 6%.

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