General protocol for measurement of biochemical methane potential (BMP) (document no. 100)

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1 BMP Methods collection

Document number 100. File version 1.0. This file is from the DBFZ BMP Methods collection. For more information, visit the site at `https://www.dbfz.de/en/BMP`.

2 Description

This document describes requirements for measurement of biochemical methane potential (also called biomethane potential) (BMP) in batch tests. For details on the development of this protocol, see Holliger et al. [1] and Hafner et al. [2].

3 BMP test overview

To measure the BMP of a substrate, it is mixed with an anaerobic inoculum in bottles, which are then sealed and incubated. Methane CH_4 production is measured over time. Production of CH_4 from the inoculum is estimated by making similar measurements on bottles with only inoculum ("blanks"). For more details on the basic approach, see Owen et al. [4].

4 Protocol

4.1 Inoculum

1. Origin. Must be taken from mesophilic digester (35-40°C). Highly diverse methanogenic microbial community has to be present.
2. Treatment. Generally avoid. Sieving or other pre-treatment (grinding, dilution) acceptable if needed.
3. Analysis.

(a) Total solids (TS) by drying for at least 24 hours at 105°C in triplicate

(b) Volatile solids (VS) by combusting at 550°C for at least 2 hours in triplicate.

4. Quality check before use.
   Required:

   (a) pH between 7.0 and 8.5.

   (b) Alkalinity $\geq$ 3 g L^{-1} as $CaCO_3$.

   Optional:

   (a) Total ammonical nitrogen (TAN) $<$ 2.5 g L^{-1} as N

   (b) Total volatile fatty acids (VFAs) $<$ 1.0 g L^{-1} as acetic acid

5. Storage. Storage time between collection and setting up BMP tests $\leq$ 5 days at ambient (20-25°C) or mesophilic test temperature.

6. Methane production of the blank should be less than 40% of the methane production of cellulose.

4.2 Test setup

1. Samples and replication. All tests must include at least 3 batches (bottles) each with: inoculum only ("blanks"), a positive control substrate (microcrystaline cellulose recommended), and each substrate. All bottles must contain inoculum.

2. Substrate quantity and bottle size. At least 1.0 g of substrate VS must be added to each bottle. This affects bottle size, and care should be taken to avoid high pressure in manual methods (due to leaks or bottle breakage). Recommended maximum headspace pressure is 2 bar (gauge). Between 5-10 g substrate VS per L headspace volume is recommended as long as daily sampling is possible at the start.

3. Inoculum-to-substrate ratio (ISR). On a VS basis, ISR should generally be 2, but may be as low as 1 for slowly degradable substrates, and as high as 4 for easily degradable substrates.

4. Amendments.

   (a) Trace element and vitamin amendment is required.[1].

[1] Trace element solution (concentration in g L^{-1}): 2 $FeCl_2 \cdot {}_4H_2O$, 0.05 H_3BO_3, 0.05 $ZnCl_2$, 0.038 $CuCl_2 \cdot {}_2H_2O$, 0.05 $MnCl_2 \cdot {}_4H_2O$, 0.05 $(NH_4)_6Mo_7O_{24} \cdot {}_4H_2O$, 0.05 $AlCl_3$, 0.05 $CoCl_2 \cdot {}_6H_2O$, 0.092 $NiCl_2 \cdot {}_6H_2O$, 0.5 ethylenediaminetetraacetate, 1 mL concentrated HCl, 0.1 $Na_2SeO_3 \cdot {}_5H_2O$.
Vitamin mixture (concentration in mg L^{-1}): 2 Biotin, 2 folic acid, 10 pyridoxine acid, 5 riboflavin, 5 thiamine hydrochloride, 0.1 cyanocobalamine, 5 nicotinic acid, 5 P-aminobenzoic acid, 5 lipoic acid, X???? DL-pantothenic acid.
Add between 1 and 5 mL of each solution per 1 L of final slurry volume (typically 1 mL each per bottle).

(b) If alkalinity is too low, add $NaHCO_3$ to meet requirement.

5. Headspace flushing. Flush headspace prior to incubation to remove O_2. Use a mixture of N_2 and CO_2 (20-40% CO_2) or 100% N_2. Do not flush liquid phase with pure N_2. It is recommended to measure gas flow rate and to replace at least 3 headspace volumes.

6. Incubation.

   (a) Temperature controlled environment at mesophilic temperature (35-40°C) with $\leq$ 2°C variation during incubation. The temperature should match the temperature of the digester that was the source of inoculum.

   (b) Mixing is compulsory, if manually at least once a day.

7. Methane production measurement.

   (a) No restrictions on which system to use

   (b) If gas composition has to be analyzed, it has to be analyzed at each measuring point and for every single batch (bottle).

   (c) At each measuring point, ambient pressure and temperature has to be measured and recorded for use in gas volume standardization

8. Duration. Terminate BMP tests only after daily CH_4 production during 3 consecutive days is $< 0.5\%$ of the net accumulated volume of methane from the substrate (substrate minus average of blanks). This is refered to as the "1% net duration". If different substrates are tested, each substrate can be terminated when the slowest of the 3 batches (bottles) has reached the termination criterion. Blanks must be continued as long as the slowest (latest) batch (bottle) with substrate. Continuing tests beyond the 1% net duration is acceptable.

4.3 Calculations

Details on calculations can be found at `https://www.dbfz.de/en/BMP` both for specific methods and for BMP calculation from standardized gas volumes.

1. Data processing. Standardized CH_4 volume (dry, 0°C, 101.325 kPa) must be calculated from raw laboratory data (e.g., measured volume, pressure, mass, or concentration) using accepted methods, if available. Available methods can be found at `https://www.dbfz.de/en/BMP`. Checking calculations by comparison to available software is recommended.

2. BMP units. BMP should be expressed in standardized CH_4 volume per g of substrate VS added.

3. Calculation of BMP. BMP of all substrates (including positive control) must be calculated by subtracting inoculum CH_4 production (determined from blanks) from gross (total) CH_4 production from substrate with inoculum, and normalizing by substrate VS mass. Calculations should follow the accepted approach from `https://www.dbfz.de/en/BMP`. Checking calculations by comparison to available software is recommended.

4. Calculation of BMP standard deviation. The standard deviation associated with each mean ($n = 3$) BMP value must include variability from both blanks and batches (bottles) with substrate and inoculum, following the details given in the standard approach `https://www.dbfz.de/en/BMP`. Inclusion of variability from substrate VS determination is recommended.

4.4 Validation criteria

BMP results that meet *all* the following criteria should be considered "validated" by the standards of [2]. Otherwise, results are not validated, and tests should be repeated if possible, and 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 are met.

2. Mean cellulose BMP is between 340 and 395 NmL g^{-1} (standardized CH_4 volume (dry, 0°C, 101.325 kPa) per g substrate VS).

3. Cellulose relative standard deviation (including variability in both blanks and substrate bottles) is no more than 6%.

References

[1] Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., de Wilde, V., Ebertseder, F., Fernández, B., Ficara, E., Fotidis, I., Frigon, J.-C., Fruteau de Laclos, H., S. M. Ghasimi, D., Hack, G., Hartel, M., Heerenklage, J., Sarvari Horvath, I., Jenicek, P., Koch, K., Krautwald, J., Lizasoain, J., Liu, J., Mosberger, L., Nistor, M., Oechsner, H., Oliveira, J. V., Paterson, M., Pauss, A., Pommier, S., Porqueddu, I., Raposo, F., Ribeiro, T., Rüsch Pfund, F., Strömberg, S., Torrijos, M., van Eekert, M., van Lier, J., Wedwitschka, H., Wierinck, I. 2016, Towards a standardization of biomethane potential tests Water Science and Technology 74: 2515-2522

[2] Hafner, S.D., Fruteau de Laclos, H., Koch, K., Holliger, C. 2020, Improving inter-laboratory reproducibility in measurement of biochemical methane potential (BMP) Water

[3] Holliger, C., . . . 2020, General protocol for measurement of biochemical methane potential (BMP), https://github.com/sashahafner/BMP-methods

[4] Owen, W. F., Stuckey, D. C., Healy Jr, J. B., Young, L. Y., McCarty, P. L. 1979, Bioassay for monitoring biochemical methane potential and anaerobic toxicity Water Research 13: 485-492.