General protocol for measurement of biochemical methane potential (BMP)

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1 BMP-methods

File version 0.1. This file is from the GitHub repository BMP-methods. For more information, visit BMP-methods at `https://github.com/sashahafner/BMP-methods`.

2 Description

This document describes general requirements for measurement of biochemical methane potential (also called biomethane potential) in batch tests.

3 BMP test overview

To measure the BMP of a substrate, it is mixed with an anaerobic inoculum, added to sealed bottles, incubated, and 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").

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. Replication. All conditions in triplicate: at least 3 batches/bottles each for blanks (inoculum-only), cellulose (positive control), and each substrate.

2. Bottle size. Large enough to accept $\geq$ 1.0 g of substrate VS.

3. Inoculum-to-substrate ratio (ISR). On a VS basis, generally 2, 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[1].
   (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.

[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).

(a) Temperature controlled environment at mesophilic temperature (35-40°C) with ≤ 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 test as late as possible but not before 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). If different substrates are tested, each substrate can be terminated when the slowest batch (bottle) has reached the termination criterion. Blanks must be continued as long as any bottles with substrate.