Accepted for publication in Water Science and Technology on 23 June 2016

Towards a standardization of biomethane potential tests

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Abstract

Production of biogas from different organic materials is a most interesting source of renewable energy. The biomethane potential (BMP) of these materials has to be determined to get insight in design parameters for anaerobic digesters. Although several norms and guidelines for BMP tests exist, inter-laboratory tests regularly still show high variability of BMPs for the same substrate. A workshop was held in June 2015, in Leysin, Switzerland, with over 40 attendees from 30 laboratories around the world, to agree on common solutions to the conundrum of inconsistent BMP test results. This paper presents the consensus of the intense roundtable discussions and cross-comparison of methodologies used in respective laboratories. Compulsory elements for the validation of BMP results were defined. They include the minimal number of replicates, the request to carry out blank and positive control assays, a criterion for the test duration, details on BMP calculation, and last but not least criteria for rejection of the BMP tests. Finally, recommendations on items that strongly influence the outcome of BMP tests such as inoculum characteristics, substrate preparation, test setup, and data analysis are presented to increase the probability of obtaining validated and reproducible results.

Key words: Anaerobic digestion, batch assays, biomethane potential (BMP), organic materials, standardized protocol

Introduction

Biomethane potential (BMP) tests, although routinely applied to measure the ultimate methane production from different organic materials, both liquid and solid, are far from being trivial. Several norms aimed to standardization of BMP tests such as DIN 38414 TL8 (1985), ASTM D 5210 (1992), ASTM D 5511 (1994), ISO 11734 (1995), ISO 14853 (1998), and ISO 15985 (2004), have existed for many years, but their formulation of important parameters is often vague. This leaves too much freedom for interpretation and ultimately leads to the use of quite different test protocols in different laboratories. Recent international efforts for harmonization of anaerobic biodegradation tests have been undertaken by the "Task Group for the Anaerobic Biodegradation, Activity and Inhibition (ABAI-Group)" of the Anaerobic Digestion Specialist Group of the International Water Association (IWA). After the first workshop held in June 2002 at Lake Orta, Italy (Sanders and Angelidaki, 2004), several reviews were published that dealt with methods for microbial activity and inhibition assessment under anaerobic conditions (Rozzi and Remigi, 2004), the assessment of the anaerobic biodegradability of macropollutants (Angelidaki and Sanders, 2004), the equipment used for testing anaerobic biodegradability and activity (Guwy, 2004), and standardized methods for anaerobic biodegradability testing (Müller et al., 2004). Five years later, the same task group published a proposition for a protocol that defines the BMP of solid organic wastes and energy crops in batch assays (Angelidaki et al., 2009). These guidelines describe in detail aspects related to substrate, inoculum, medium, blanks, controls, replicates, mixing, experimental setup, data collection, data interpretation, and data reporting that must be addressed for a successful BMP test. Already in 2006, the Association of German Engineers published the first version of the detailed technical guideline VDI 4630 entitled "Fermentation of organic materials. Characterization of the substrate, sampling, collection of material data, fermentation tests" and very recently an updated version (VDI 4630, 2016).

Despite the fact that such detailed guidelines for BMP test protocols exist, recently published (Raposo et al., 2011; Cresson et al., 2015) and unpublished national and international inter-laboratory tests have shown that the outcome can vary significantly between laboratories, which indicates the need to further standardize the BMP test protocol. In order to agree on possible solutions to the conundrum of inconsistent BMP test results, a workshop was held from June 10-12, 2015, in Leysin, Switzerland. A group of forty researchers who routinely work with BMP tests, including members of the ABAI-Group of IWA, met to present results of different research projects that involved BMP and inter-laboratory tests and to identify possible avenues to make BMP tests more reliable and reproducible.

The inter-laboratory studies presented at the workshop showed quite different results. The best result was obtained in a German study led by KTBL/VDLUFA that involved thirty participating laboratories. After years of testing and adjusting, an inter-laboratory reproducibility with a relative standard deviation (RSD) of ± 14 % and a ratio between the maximum and minimum value of about 1.4 was achieved. On the other extreme, there was a case where the RSD was as high as ± 175 %. However, in most presented studies the RSD was around ± 20 % to ± 25 % with a ratio between the maximal and minimal value of about two, the latter of which was the more problematic study result. In technical and economic feasibility studies that are carried out prior to construction of an anaerobic digestion plant, energy income and size of fermenters and other equipment are often estimated from the BMP of the substrates. Since BMP tests are normally carried out by only one laboratory, these estimates can differ by a factor of two as well, depending upon the factors listed above, including which laboratory has carried out the BMP tests. For a further standardization of the BMP test protocol all involved components have to be considered such as choice, quality, and preparation of the inoculum;

substrate preparation and storage; test setup; data analysis and reporting; and last but not least criteria to validate or reject test results.

In the following, the outcome and consensus of the roundtable discussions are presented. The guideline is divided into two parts. The first part describes actions and criteria that are considered compulsory in order to accept and validate a BMP test result, while the second part presents recommendations concerning the inoculum, substrate, test setup, and data analysis and reporting in order to obtain test results that can be validated and reproduced. Although the kinetics of methane production is also an important issue, only the ultimate methane yield (BMP) is considered in the present guideline.

Compulsory elements for the validation of BMP test results

In order to validate BMP test results, the following elements must be fulfilled:

- all tests must be carried out at least in triplicate;
- besides the BMP of the substrate, blank assays (background methane production from the inoculum) and positive controls (e.g. microcrystalline cellulose, tributyrine) must be carried out;
- the duration of the BMP tests should not be fixed in advance, and tests should only be terminated when daily methane production during three consecutive days is < 1% of the accumulated volume of methane (i.e. $BMP_{1\%}$);
- the BMP is expressed as the volume of dry methane gas under standard conditions (273.15 K and 101.33 kPa) per mass of volatile solids (VS) added, with the unit $NL_{CH4}\ kg_{VS}^{-1}$;
- the BMP of the substrate and the positive control are determined by subtracting the methane production of the blanks from the gross methane production of the substrate/positive control assays;
- for the calculation of the BMP of the substrate and the positive control, the standard deviation (SD) of the blanks must be taken into account by using the formula:

  $BMP_{substrate\ /\ control} = BMP_{average,\ substrate\ /control} \pm \sqrt[2]{(SD_{blank})^2 + (SD_{substrate\ /\ control})^2}$;
- test results must be rejected if at least one of the following criteria is fulfilled:
  - if the RSD of the blank or the positive control is > 5%, even after applying a statistical test to eliminate a single outlier
  - if the RSD of a homogenous substrate is > 5%, even after applying a statistical test to eliminate a single outlier
  - if the RSD of a heterogeneous substrate is > 10 %, even after applying a statistical test to eliminate a single outlier
  - if the BMP of the positive control is <85% and >100% of the theoretical BMP (e.g. for cellulose: < 352 $NL_{CH4}\ kg_{VS}^{-1}$ and > 414 $NL_{CH4}\ kg_{VS}^{-1}$)

To eliminate a single outlier from triplicate measurements, the Dixon's test can be used. An easy to use tool to carry out such tests is available at http://contchart.com/outliers.aspx.

If substrate inhibition is suspected, tests with several inoculum-substrate ratios (ISRs) should be carried out in parallel.

Recommendations to obtain validated BMP test results

In this section, different items that strongly influence the outcome of BMP tests are presented. Recommendations are made that increase the probability of obtaining validated and reproducible BMP test results according to the compulsory elements mentioned above.

Inoculum

Origin

The inoculum should be taken from an active anaerobic digester that is digesting complex organic matter and is at steady-state at the time of sampling. This provides a highly diverse microbial community able to digest a large variety of organic molecules. The BMP assay temperature, mesophilic or thermophilic, is usually the same as the operating temperature of the inoculum digester. Anaerobic sludge from wastewater treatment plant digesters and digestate from agricultural plants treating manure as main feedstock are often used and can be recommended as sources of inoculum. An alternative to using a specific inoculum is to mix inocula from different sources to increase the diversity of the microbial community. It is not necessary that the inoculum be specifically adapted to the substrates to be tested, but if there is access to such an inoculum, it may shorten the test duration.

Quality criteria

As mentioned above, the inoculum should be taken from a well-functioning digester. Quality checks that can be carried out are analysis of pH, volatile fatty acids (VFA), ammonium, and alkalinity. The pH should always be measured before setting up a BMP test, and the other parameters can be analyzed on a less regular basis if the inoculum source is always the same. Indicative values for operational parameters of the digester most probably providing an inoculum of good quality are:

- pH: > 7.0 and < 8.5
- VFA: < 1.0 $g_{CH3COOH}$ L^{-1}
- NH_4^+: < 2.5 $g_{N\text{-}NH4}$ L^{-1}
- alkalinity: > 3 g_{CaCO3} L^{-1}

Another indicator of good inoculum quality is its activity with different standard substrates such as glucose, propionate, butyrate, acetate, and H_2/CO_2. These tests do not need to be completed routinely, but should be done for inocula that are not yet well known by the laboratory conducting the BMP tests. These activity tests are well described in Angelidaki et al. (2009).

Lastly, the inoculum should have a low endogenous methane production, meaning that little residual substrate that can still be transformed into biogas should be present. Endogenous methane production (blanks) should be below 20 % of total methane production (inoculum & substrate) and if it is too high, a pre-incubation for exhausting the easily biodegradable substrate in the inoculum might be needed. For substrates that have rather low BMPs, it can be difficult to fulfill this recommendation. In such cases, the inoculum should have very low endogenous methane production (~ 50 NL_{CH4} kg_{VS}^{-1}) and one should apply low ISRs.

Preparation and storage

The inoculum should, as much as possible, be used as sampled. However, some inocula may require preparation that can include sieving, dilution and pre-incubation. Coarser materials, if present, should be removed by sieving through a 1-5 mm mesh screen. Dilution should be avoided, but if the volatile solids (VS) content is too high (e.g. > 100 g L^{-1}), the inoculum can be diluted with nitrogen-flushed

deionized water. Inocula should not be washed, as sometimes proposed (ISO 14853, 1998), since such a treatment can remove soluble growth factors (e.g. macro- and micro-nutrients, trace elements, and vitamins) and extra-cellular enzymes necessary for good digestion performance.

Inocula with high endogenous methane production should be pre-incubated at the test temperature for one week. If high methane production still occurs after one week, other inocula should be sought after, as it seems that the digester from which the inoculum originated was not in steady-state. Inocula that do not require pre-incubation should be used as fresh as possible. These inocula should only be stored at test temperature, or at least ambient temperature (~ 20-25°C), for short periods of less than five days, e.g. until total solids (TS) and VS concentrations have been determined. Storage at low temperature (4°C) should be avoided, but is an option for certain inocula, such as anaerobic granular sludge from UASB reactors, which can be stored at 4°C for periods up to one month and even more.

Substrate

The substrate samples to be tested must be as representative as possible of the substrate to be digested at full scale. It goes beyond the scope of this document to recommend how sampling should be carried out in order to obtain representative samples. However, the samples to be tested should be well documented prior to any preparation, e.g. by taking pictures and describing the sampling procedure in detail. A detailed description of sampling procedures that are dependent on substrate properties is available in the German guideline VDI 4630 (2016). All recommendations made in the current document are based on the assumption that the available substrate samples adequately represent the organic matter to be digested at full scale.

Sample preparation and storage

As for the inoculum, substrate preparation should be minimal in order to avoid alteration of its properties and digestibility. Coarse inert materials such as gravel, sand and plastics, if present, should be removed. Shredding or grinding might be needed if the organic fraction particles are too large. Care must to be taken during these preparation steps, since they might heat the samples which would lead to loss of volatile organic compounds. An interesting option is to use cryogenic grinders or freezer mills for this purpose. All particles should be at most 10 mm in any dimension (diameter, length). To achieve this, the substrate sample can be sieved to separate the fraction > 10 mm, which can be ground and re-mixed with the fraction < 10 mm. The test report should describe all preparation steps in full details as well as the particle size distribution.

Substrate samples should be used as fresh as possible. They can be stored at 4°C, but in general only for two to five days. For longer storage, samples can be frozen and stored at - 20°C. Thawing of frozen samples should be done at 4°C followed by carefully increasing the sample temperature to ambient conditions before use in BMP tests. For certain substrates, such as waste sludge from wastewater treatment plants, freezing-thawing may significantly alter the BMP. Drying substrates for storage should be avoided. It is only an option for samples where loss by volatilization can be neglected or where it has been addressed by detailed analysis of volatile compounds (Kreuger et al, 2011).

Substrate analysis

TS and VS are compulsory parameters for substrate as well as inoculum analysis. Furthermore, substrate properties such as pH, VFA, total Kjeldahl nitrogen, ammonium, and alkalinity are worthwhile to be determined because they can be used to estimate potential inhibition problems during BMP tests. Another interesting parameter to analyze is the chemical oxygen demand (COD). Since total COD

can be difficult to analyze (Raposo et al., 2009), it is suggested to use special analytical procedures that address these issues (Raposo et al., 2008; Noguerol-Arias et al., 2012). Furthermore, COD should only be used as an indicative value to estimate the total gas production which can be of interest for the manometric measurements. Furthermore, total organic carbon (TOC) and elemental composition (CNHX) can be useful parameters as well.

Test setup

The recommendations made here concern the most commonly applied test setup with batch reactors and continuous or discontinuous measurement of gas production. Alternative setups that have been specifically developed for BMP tests are not considered here. Some of these latter setups are described in more detail in the German guideline VDI 4630 (2016) and by Guwy (2004).

Reactor vessels

The reactor vessels used for BMP tests should have a volume that is adapted to the homogeneity of the substrate, the expected volume of gas produced, and the sensitivity of the gas measurement technique. Smaller volumes (≈100 mL) can be used for homogenous substrates, whereas larger volumes (500 mL to 2000 mL) are more suitable for heterogeneous substrates. However, in order to increase reproducibility, a working volume of 400 mL to 500 mL is recommended, which means that vessels should have a total volume of approximately 500 mL and 1000 mL for volumetric and manometric gas measurement, respectively.

The vessels must either be closed with gastight butyl rubber septa that are thick enough to be pierced several times with a needle, especially in the case of manometric gas measurement, or be connected to the gas measurement device through gastight connectors and tubing that ensure no loss of produced biogas. Prior to incubation, a leakage test should be carried out in order to discard leaking bottles. Detection of leaks during the test automatically leads to rejection of the test results since no corrective measures can be taken.

Amendments

The fraction of inoculum in the overall test mixture is normally higher than the part of substrate added as discussed below when presenting the Inoculum-substrate ratio. Therefore, the inoculum is not only the source of the microbial community needed for anaerobic digestion but also an important source of macro- and micro-nutrients, trace elements and vitamins, as well as pH-buffering capacity. However, to avoid any deficiencies, trace elements and vitamins might be added according to the solutions proposed in Angelidaki et al. (2009). If alkalinity of the inoculum is below 3 g_{CaCO3} L^{-1}, sodium bicarbonate should be added to reach at least 3 g_{CaCO3} L^{-1}. Phosphate buffer should not be used.

Preparation of test batches

BMP test batches should be prepared such that there is minimal contact with air. A detailed batch preparation procedure description is available in Angelidaki et al. (2009). During test preparation, flushing should be done with a mixture of N_2 and CO_2 that contains a similar share of CO_2 as expected in the produced biogas (e.g. 20%-40% CO_2; rest as N_2; v/v) to avoid a disturbance of the carbonate balance (Koch et al., 2015). Flushing with pure N_2 should be carried out with care and only used for small head-space volume reactors.

VS content and Inoculum-substrate ratio (ISR)

A total VS concentration of 20 to 60 g_{VS} L^{-1} is recommended. The amount of VS added by the inoculum should be the same in all batches. Oxygen-free deionized water should be added to drier substrates (e.g. microcrystalline cellulose) to compensate for missing volume.

The ISR, the ratio of VS from the inoculum (partially due to actively degrading biomass) to VS from the substrate, is a key parameter of BMP tests. It is recommended that the portion of VS from the inoculum be greater than that from the substrate to minimize acidification or inhibition problems. Therefore, VS based ISRs should for most applications be between two and four. For easily degradable substrates where rapid accumulation of fermentation intermediates such as VFAs could lead to inhibition of anaerobic digestion, an ISR greater than or equal to four should be applied. For less degradable substrates, such as lignocellulosic organic matter, an ISR less than or equal to one can be applied.

For relatively unknown substrates or if there is a possible concern about inhibition due to the substrate, it is recommended to test several ISRs (e.g. three to four levels), and only if two ISRs lead to the same BMP, one can assume that there was no overload or inhibition.

Positive control

As already mentioned above under compulsory elements for the validation of BMP test results, positive controls have to be carried out. They allow validation of the inoculum activity with a standard substrate and compare it with its well-known nominal value. Positive controls also allow validation of the gas measurement procedure. Microcrystalline cellulose (CAS 9004-34-6) is often used as a standard substrate for several reasons. First, its composition is well-defined – it is composed of only glucose as the monomer, which allows the theoretical BMP to be easily calculated. Second, its polymer property involves all important AD degradation steps including hydrolysis. Lastly, it is easily manageable and storable, relatively cheap, and it can be easily purchased as a high-quality and high-purity product (e.g. from Sigma-Aldrich or Merck Millipore). Another less commonly used but also well-defined standard substrate is tributyrin. The use of a mixture of cellulose and tributyrin is an interesting option to validate inoculum activity towards more than only one class of biomolecules. For all positive controls substances it is important to confirm the TS and VS content of the product used in the tests.

Incubation conditions

BMP tests can be carried out under either mesophilic or thermophilic conditions since temperature does normally not influence the BMP; only the biogas production rates differ with temperature. BMP test vessels should be incubated in a temperature controlled environment with maximum variations of ± 2°C (incubator or water bath), and cooling during gas measurement must to be avoided as much as possible. Typical incubation temperatures for mesophilic and thermophilic tests are 37°C and 55°C, respectively. However, if the inoculum is obtained from a digester operated at another temperature, the BMP test should be carried out at this temperature as well.

Static incubation without any mixing should be avoided. However, if continuous mixing is applied, it should be gentle. Manual mixing once a day to avoid scum layer formation is in most cases sufficient, except if one wants also to determine kinetic parameters.

Gas measurement

Methane production can be measured by different techniques, e.g. by volumetric, manometric, and gas chromatography methods. With the first two techniques, gas composition must be measured on a

regular basis by gas chromatography with thermal conductivity detection. An exception for the volumetric techniques is where CO_2 is trapped in alkaline solution, then only the volume of CH_4 is determined. When measuring methane production manometrically, the pressure in the assay bottle should not exceed 300 kPa to avoid both excessive dissolution of CO_2 and accidents by explosion. Temperature and pressure at the measurement point should always be recorded in order to convert the measured gas volume to dry gas at standard conditions (273.15 K, 101.33 kPa; Strömberg et al. 2014). The gas measurement devices must be calibrated on a regular basis according to the suggestions provided by the manufacturer with a standard gases (e.g. mixture of CH_4 and CO_2; 50%/50%; v/v).

Data analysis and reporting

The first step in data analysis is the calculation of the volume of dry methane produced normalized to standard temperature and pressure conditions (273.15 K, 101.33 kPa). A useful, detailed description of the data analysis procedure is provided in the German guideline VDI 4630 (2016).

Data reporting should be as detailed as possible as emphasized in Angelidaki et al. (2009). The final BMP test report should include detailed descriptions of the inoculum and substrate with all of their physicochemical characteristics; the test conditions and setup; the graphs of gross methane production of the substrate batches, positive controls, and blanks; and the specific methane production that corresponds to the BMP of the substrate. This last is expressed as volume of dry methane gas produced per mass of VS of the substrate added ($NL_{CH4}\ kg_{VS}^{-1}$).

Inter-laboratory comparison

In order to verify the performance of the BMP test procedure, participation in round robin tests is recommended. A roundtable discussion after analysis of the results of such a test would enable the participants to compare their performance with other laboratories and to improve their own test protocols. The results of inter-laboratory studies could be published as a journal paper, as there is precedent (Raposo et al., 2011) and value in sharing such experiences with a broader community.

Conclusions

The presentations of the inter-laboratory tests during the workshop held in Leysin, Switzerland, clearly indicated the need for further standardization of the BMP tests. The major outcome of the roundtable discussions is the consensus on compulsory elements that must be fulfilled in order to validate BMP test results. This should allow obtaining BMP results with a high intra- as well as inter-laboratory reproducibility. All authors of these guidelines agreed that the recommendations given in the present document will facilitate obtaining BMP results that can be validated according to the compulsory elements.

Acknowledgements

The workshop in Leysin, Switzerland, has been financed by the Swiss Federal Office for Energy, and co-sponsored by Bioprocess Control Sweden AB, Lund, Sweden. The authors thank Alexandra Maria Murray for editing the English.

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