Towards a standardization of biomethane potential tests: a commentary

Christof Holliger, Sergi Astals, Hélène Fruteau de Laclos, Sasha D. Hafner, Konrad Koch and Sören Weinrich

ABSTRACT

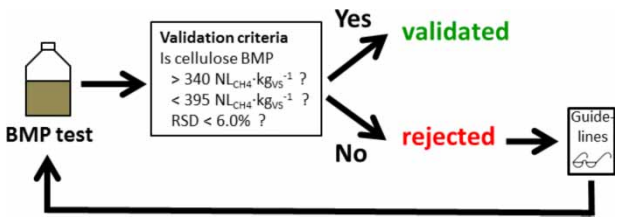
Inter-laboratory reproducibility of biomethane potential (BMP) is dismal, with differences in BMP values for the same sample exceeding a factor of two in some cases. A large group of BMP researchers directly addressed this problem during a workshop held in Leysin, Switzerland, in June 2015. The workshop resulted in a new set of guidelines for BMP tests published in 2016, which is the subject of the present commentary. The work has continued with two international inter-laboratory studies and one additional workshop held in Freising, Germany, in 2018. The dataset generated by the two inter-laboratory studies were used to refine the validation criteria for BMP tests. Based on these new results an update to the original guidelines is proposed here.

Key words | anaerobic digestion, BMP, biochemical methane potential, biogas technology, guideline, validation criteria

HIGHLIGHTS

- This commentary updates requirements and validation criteria defined previously in a *Water Science and Technology* paper published in 2016.

GRAPHICAL ABSTRACT



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Multiple national and international inter-laboratory studies show rather low reproducibility in measurement of biochemical methane potential (BMP), with differences for the same substrate exceeding a factor of two in some cases (Raposo *et al.* 2011; Ribeiro *et al.* 2020; Weinrich *et al.* 2018) despite the existence of well-known protocols (Angelidaki *et al.* 2009; VDI 4630). To help address this problem that affects anaerobic digestion (AD) research and industry, an international group of researchers met in Leysin, Switzerland, in June 2015 to discuss the standardization of BMP tests. The outcome of this workshop was the publication of a new set of BMP test guidelines where compulsory elements for the validation of BMP results were

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defined and recommendations on items that strongly influence the outcome of BMP tests, such as inoculum characteristics, substrate preparation, test setup, and data analysis, were presented (Holliger *et al.* 2016). The present commentary summarizes progress that has been made since the publication of this paper. Briefly, results from an additional workshop and two inter-laboratory studies led to the development of new validation criteria designed to improve BMP results, as described in detail in Hafner *et al.* (2020b). Here we propose that these validation criteria replace those given in Holliger *et al.* (2016), while the other requirements stand or are slightly modified. This combined set of requirements should be applied to all BMP tests from now on.

To assess the guidelines published in 2016 (Holliger *et al.* 2016), a first international inter-laboratory study (IIS) was conducted in 2016 and 2017 with 32 participating laboratories. Application of the 2016 validation criteria improved inter-laboratory reproducibility but only 16 of 62 BMP tests could be validated with these criteria. In order to continue the standardization and finally make BMP tests more reliable and reproducible, a second workshop was organized and a second IIS carried out. The workshop took place in April 2018 in Freising, Germany, and the second IIS concluded at the end of the same year. The two IIS generated a detailed dataset of BMP measurements made over a wide geographic range using several measurement methods (automated or manual volumetric, manual manometric, gravimetric, and direct GC). This dataset was used to quantify and identify sources of variability in BMP, and to test and refine validation criteria, and is publicly

Table 1 | Original and refined requirements and validation criteria for BMP measurements

Requirement/validation criterion	Original[a]	Refined[b]
Replicates	At least in triplicate	Same as original
Assays besides test substrate	Blanks and positive control (unspecified)	Blanks and microcrystalline cellulose positive control
Duration of BMP test	Terminate BMP test when daily methane production during three consecutive days is <1% of the accumulated volume of methane	Same as original but with clarification that methane production is net (inoculum contribution subtracted)
Expression of BMP	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} $kgvs^{-1}$	Same as original
Calculation of BMP	BMP is determined by subtracting the mean methane production of the blanks from the gross methane production of the substrate/positive control assays	Same as original but calculations now described in detail in Hafner *et al.* (2020a)
Calculation of standard deviation of BMP	For the calculation of the standard deviation of the BMP of the substrate and the positive control, the standard deviation (SD) of the blanks must be taken into account	Same as original but with addition of SD of VS measurement and clarification of calculations (Hafner *et al.* 2020a)
Validation criterion 1	Only if the RSD of the blank or the positive control is <5%, BMP results can be validated. It is possible to eliminate a single outlier after applying a statistical test	Only if the RSD of cellulose is <6%, BMP results can be validated. Rejection based on too high RSD of blanks is not recommended and elimination of outliers is only allowed if triplicates are still available at the end
Validation criterion 2	Only if the RSD of a homogeneous or heterogeneous substrate is <5% and <10%, respectively, BMP results can be validated. It is possible to eliminate a single outlier after applying a statistical test	No validation criterion recommended for substrate BMP, but RSD has to be provided for each substrate tested
Validation criterion 3	Only if the BMP of the positive control is between 85 and 100% of the theoretical BMP (e.g. for cellulose: between 352 NL_{CH4} $kgvs^{-1}$ and 414 NL_{CH4} $kgvs^{-1}$), BMP results can be validated	Only if the BMP of microcrystalline cellulose is between 340 NL_{CH4} $kgvs^{-1}$ and 395 NL_{CH4} $kgvs^{-1}$, BMP results can be validated

[a]Defined in Holliger *et al.* (2016).
[b]Defined in Hafner *et al.* (2020b).

available as supplementary material of Hafner *et al.* (2020b). Application of the new criteria to this large dataset showed that they can substantially reduce variability in BMP measurements and validate a higher percentage of the BMP test results. Whereas the relative standard deviations among laboratories (RSD_R, reproducibility) were below 8% and the relative ranges (RR) below 25% for all substrates and tests with the new validation criteria, RSD_R and RR were as high as 14 and 55%, respectively, with the original validation criteria defined in the guidelines of 2016. In addition, on average 73% of the test results were rejected using the original criteria, compared to 55% with the refined criteria. We therefore recommend applying the new validation criteria listed in Table 1 in all BMP tests. Most of the other requirements were changed slightly or not at all (Table 1).

By analyzing the large dataset, systematic biases were identified that were associated with both laboratories and tests within laboratories (Hafner *et al.* 2020b). Interestingly, errors in data processing or data entry were important sources of variability and there was evidence of negative biases in manual manometric and manual volumetric measurement methods. However, much of the observed variation in BMP values was not clearly related to any of these factors, and is probably the result of particular method details that vary among laboratories. Encouragingly, results show that it is possible to measure very similar BMP values with different measurement methods. Hence, in order to meet the validation criteria (Table 1), it is important that laboratories whose tests are rejected are willing to evaluate and make changes to their BMP methods.

To contribute to better standardization of BMP measurements, a new website was established to share detailed, up-to-date guidance on BMP measurement and data processing: https://www.dbfz.de/en/BMP. Users of this 'Standard BMP Methods' website can find the latest requirements and validation criteria described in detail (Holliger *et al.* 2020), documentation of calculations (e.g. Hafner *et al.* 2020a), links to standardized software for data processing (Hafner *et al.* 2018), and other guidance. We invite anyone interested in BMP tests to make use of the resource and to actively contribute to this ongoing project.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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