



Final Report

Second international inter-laboratory study on biomethane potential (BMP) tests





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Zusammenfassung

Biomethanpotentialtests (BMP) sind ein Schlüsselinstrument in der anaeroben Vergärungsforschung, aber auch in der Praxis. Für letztere dienen sie dazu, die Produktion einer zukünftigen anaeroben Vergärungsanlage aus einem gegebenen Satz von Substraten vorherzusagen oder den ordnungsgemäßen Betrieb einer Vergärungsanlage zu validieren. Eine erste internationale Interlaborstudie (IIS-BMP) hat gezeigt, dass die in der kürzlich veröffentlichten Leitlinie vorgeschlagenen Validierungskriterien, die auf einem 2015 in der Schweiz organisierten Workshop mit zahlreichen auf internationaler Ebene in diesem Bereich tätigen Laboratorien beruhten, zu einem signifikanten Rückgang der Spannweite zwischen minimalem und maximalem Substrat-BMP geführt haben. Es konnten jedoch nur etwa 25% der BMP-Tests validiert werden. In einem zweiten Workshop in Freising wurde ein neuer Ansatz zur Bestimmung der Validierungskriterien vorgeschlagen, der in diesem zweiten IIS-BMP erprobt wurde. Insgesamt 38 Labors aus 14 verschiedenen Ländern nahmen freiwillig an dieser Studie teil. Ein bis zwei Tests wurden mit einem obligatorischen und einem optionalen Substrat plus einer Positivkontrolle (Cellulose) durchgeführt. Die Methodik, die Substrate, die Positivkontrolle, die Minerallösungen und eine Standardergebnisdatei wurden an jedes Labor geschickt. Jedes Labor berechnete den gemessenen BMP, übermittelte aber auch alle Rohdaten. Außerdem wurden in mehreren Labors gleichzeitig verschiedene Inokula getestet. Diese Auswertung zeigte, dass die Verwendung des gleichen Inokulums die Variabilität zwischen Laboratorien nicht verringern konnte. Der Vergleich gemeldeter BMPs mit berechneten BMPs aus Rohdaten ergab, dass die Berechnung eine zusätzliche Quelle für Variabilität darstellt, da sich viele gemeldete BMPs von BMPs unterschieden, die mit dem gleichen Ansatz berechnet wurden. Unter Verwendung nur berechneter BMPs wurde die höchste akzeptable relative Standardabweichung (RSD) für Cellulose-BMP mit 6% definiert, was > 80% aller Tests umfasste. Es konnte auch gezeigt werden, dass manuelle manometrische und volumetrische Methoden zur Messung der Methanproduktion dazu neigen, das produzierte Gasvolumen zu unterschätzen. Basierend auf diesen Beobachtungen wurde ein neuer Satz von Validierungskriterien definiert ($\text{RSD-Cellulose-BMP} \leq 6\%$, untere und obere Grenze für Cellulose-BMP: 340 und 390 $\text{ml}_{\text{CH}_4}/\text{g}_{\text{VS}}$) und angewendet. Durch diese neuen Validierungskriterien wurde die Spannweite der Substrat-BMPs auf etwa 20% gesenkt und hat die Validierung von 40-50% der Tests ermöglicht. Eine weitere Analyse aller Tests beider IIS-BMP, für die Rohdaten abgerufen werden können, wird hoffentlich die Nützlichkeit dieser neuen Validierungskriterien stärken.

Résumé

Les tests de potentiel de biométhane (BMP) sont un outil clé dans la recherche sur la digestion anaérobie (DA) mais aussi dans la pratique. Pour cette dernière, ils servent à prédire la production d'une future installation de DA à partir d'un ensemble de substrats s, ou à valider le bon fonctionnement d'une installation de DA. Une première étude inter-laboratoire internationale (IIS-BMP) a montré que les critères de validation proposés dans la directive récemment publiée, fondée sur un atelier organisé en Suisse en 2015 et impliquant de nombreux laboratoires actifs dans ce domaine au niveau international, ont permis de réduire de manière significative l'entendue entre le BMP minimum et maximum pour les substrats. Cependant, seuls environ 25% des tests BMP ont pu être validés. Au cours d'un deuxième atelier tenu à Freising, en Allemagne, une nouvelle approche pour déterminer les critères de validation a été proposée. Elle a été testée dans le cadre de ce deuxième IIS-BMP. Trente-huit laboratoires de 14 pays différents ont participé volontairement à cette étude. Un à deux tests ont été réalisés avec un substrat obligatoire et un substrat optionnel plus un contrôle positif (cellulose). La méthodologie, les substrats, le contrôle positif, les solutions minérales et un fichier de résultats standard ont été envoyés à chaque laboratoire. Chaque laboratoire a calculé le BMP mesuré mais a également soumis toutes les données brutes. De plus, différents inocula ont été testés simultanément dans plusieurs laboratoires. Cette évaluation a montré que l'utilisation du même inoculum ne pouvait pas diminuer la variabilité inter-laboratoire. La comparaison des BMP déclarés avec les BMP calculés à partir des données brutes a révélé que le calcul constituait une source supplémentaire de variabilité, car de nombreux BMP déclarés différaient des BMP



calculés avec une seule approche. En utilisant uniquement les BMP calculées, l'écart type relatif acceptable (RSD) le plus élevé pour les BMP de cellulose a été défini à 6%, ce qui inclut > 80% de tous les tests. On pouvait aussi montrer que les méthodes manométriques et volumétriques manuelles de mesure de la production de méthane ont la tendance à sous-estimer le volume de gaz produit. Sur la base de ces observations, un nouvel ensemble de critères de validation a été défini (RSD du BMP de cellulose $\leq 6\%$, limite inférieure et supérieure pour le BMP de cellulose: 340 et 390 mL_{CH4}/g_{VS}) et appliqué. Ce nouvel ensemble de critères de validation a ramené l'étendue du BMP du substrat à environ 20% et a permis de valider 40 à 50% des tests. Une analyse plus approfondie de tous les tests des deux IIS-BMP pour lesquels des données brutes peuvent être obtenues renforcera, espérons-le, l'utilité de ce nouvel ensemble de critères de validation.

Summary

Biomethane potential (BMP) tests are a key tool in anaerobic digestion research but also in practice. For the latter, they serve to predict the production of a future anaerobic digestion plant from a given set of substrates, or to validate the proper operation of an AD plant. A first international inter-laboratory study (IIS-BMP) has shown that the validation criteria proposed in the recently published guideline that was based on a workshop organized in Switzerland in 2015 involving numerous laboratories active in this field on the international level, enabled to decrease significantly the range between minimum and maximum BMPs for substrates. However, only about 25% of the BMP tests could be validated. During a second workshop held in Freising, Germany, a new approach for determining the validation criteria was proposed which was tested in this 2nd IIS-BMP. Thirty-eight laboratories from 14 different countries participated on a voluntary basis in this study. One to two tests were performed with one compulsory and one optional substrate plus a positive control (cellulose). The methodology, the substrates, the positive control, the mineral solutions and a standard result file were sent to each laboratory. Each laboratory calculated the measured BMP but also submitted all raw data. In addition, different inocula were simultaneously tested in several laboratories. This evaluation showed that using the same inoculum could not decrease the inter-laboratory variability. Comparing reported BMPs with calculated BMPs from raw data revealed that the calculation is an additional source of variability since many reported BMPs differed from BMPs calculated with one single approach. Using only calculated BMPs, the highest acceptable relative standard deviation (RSD) for cellulose BMP was defined to be 6% which included >80% of all tests. It could also be shown that manual manometric and volumetric methane production measurement methods has a tendency to underestimate the gas volume produced. Based on these observations, a new set of validation criteria was defined (RSD cellulose BMP $\leq 6\%$, lower and upper limit for cellulose BMP: 340 and 390 mL_{CH4}/g_{VS}) and applied. This new validation criteria set decreased the range of substrate BMPs to about 20% and allowed validating 40-50% of the tests. Further analysis of all tests of both IIS-BMP for which raw data can be obtained will hopefully strengthen the usefulness of this new validation criteria set.



Annexes

Annexe 1 : Liste of participating laboratories



Content

Zusammenfassung	3
Résumé	3
Summary	4
Annexes	5
Content	6
List of abbreviations.....	7
1 Introduction.....	8
2 Setup of 2 nd inter-laboratory study	9
2.1 Organisation.....	9
2.2 The test substrates.....	9
2.3 The protocol	9
2.3.1 Inoculum.....	9
2.3.2 Substrates	10
2.3.3 Set-up.....	10
2.3.4 Reporting of test results	10
2.3.5 Method of BMP calculation	11
2.4 Test of influence of inoculum	11
3 Results	12
3.1 Measurement of TS and VS.....	12
3.2 Global BMP test results of both IIS-BMP	12
3.3 Influence of inoculum on inter-laboratory variability.....	13
3.4 Comparison of reported and calculated BMPs	15
3.5 Optimization of test duration criteria	16
3.6 Highest acceptable relative standard deviation for cellulose BMP	16
3.7 Correlation between cellulose and substrate BMPs	17
3.8 Comparison of manual methane production measurement methods.....	18
3.9 Definition and application of new sets of validation criteria	18
3.10 Reasons for test exclusion due to cellulose BMP	21
4 General discussion.....	21
5 Concluding remarks	23
6 References	23
Annexe 1: List of participating laboratories	24



List of abbreviations

BMP	Biomethane Potential
TS	Total Solids
VS	Volatile Solids
ISR	Inoculum-Substrate Ratio
SD	Standard Deviation
RSD	Relative Standard Deviation



The biomethane potential (BMP) is a biological test used to determine the amount of methane that can be produced from a certain organic substrate. These tests are used in research and in practice. In research, they are used to investigate for example the influence of a certain pretreatment of the organic substrate. In practice, they are used in the following situations:

- The methane produced is the main, if not the only, source of revenues of an anaerobic digestion (AD) plant. It is therefore essential to be able to predict beforehand the methane production from the organic substrates to be digested in the future AD plant and to establish an economic feasibility study of the project. Since the profitability is often very limited, a project can get rapidly non-profitable with only 15% less revenue.
- The contracts of the AD plant constructors include more and more guarantees on the performance regarding methane production that have to be between 85-95% of the substrate BMP.
- Certain wastes that have a high BMP are no longer received for free but must be purchased, and it is important for AD plant planners and operators to evaluate the potential revenues in relation to the costs.

All these different uses of BMPs underline the importance of being capable to determine with high reliability and precision this important parameter of the substrates to be digested. However, inter-laboratory studies of the past have shown that BMP test results vary considerably between laboratories, but they have not been able to clearly identify the parameters responsible for the observed variability.

During an international workshop in Leysin, Switzerland, in June 2015 the problems with BMP tests have been discussed intensively and based on these discussions, a paper entitled « Towards a standardization of biomethane potential tests » has been published in WS&T, and it has been decided to carry out an inter-laboratory study based on the guidelines published in WS&T.

This first inter-laboratory study has shown that the application of validation criteria as defined in the guidelines considerably decreased the range of BMP test results. On the other hand, the range remained significant despite the application of a standard set of conditions and validation criteria. The obtained results would therefore not make it possible to use the BMP thus measured with the initial objectives to predict the production on an industrial installation from a set of substrates, or to use the BMP as a parameter to validate the proper functioning of an installation. Indeed the variability in BMP measurements is still much too large.

In order to continue the standardization and finally make the BMP tests reliable, it was decided that a second workshop should be organized and a second inter-laboratory study carried out. The workshop took place in April 2018 in Freising, Germany. The roundtable discussions indicated that the BMP test protocol has to be further standardized, that more suitable validation criteria had to be defined based on quantitative data, and that the influence of the inoculum has to be studied since each laboratory uses a different inoculum and it is only very little characterized.

A second inter-laboratory study (2nd IIS-BMP) was carried out in Fall and Winter 2018-19, and the results analyzed in Spring 2019. The outcome of this 2nd IIS-BMP is presented in this report. This study was led by a test management team consisting of H  l  ne Fruteau de Laclos, Sasha Hafner, Konrad Koch, and the author of this report.



2 Setup of 2nd inter-laboratory study

2.1 Organisation

Thirty-eight laboratories volunteered to participate in this inter-laboratory study (Annex 1). Two of the 1st IIS-BMP could not participate in the 2nd IIS-BMP, and seven new laboratories joined the discussions during the workshop in Freising, Germany, and also participated in the 2nd IIS-BMP.

One test series had to be carried with substrate C of the 1st IIS-BMP. Including a second substrate consisting of ground straw, a slowly degradable substrate with a low BMP, was optional.

The substrates, cellulose, a trace element solution, and a vitamin solution has been sent to each laboratory along with Excel templates for data reporting.

2.2 The test substrates

Two substrates have been chosen based on the following criteria :

- high stability to permit long distance transport by mail, hence as dry as possible
- homogeneity : if possible particle size already < 1 mm
- different composition with one being slowly degradable and having a low BMP.

a. Substrate C = Fodder called Probos

Substrate C contains 19% cod liver oil, 14% sunflower seed cake, 12% rapeseed cake, 12% soya cake, 10% flaxseed cake, 4% Ca₂PO₄, 3% wheat germs, 2.8% NaCl, 2% oat, 1% yeast, and the rest is fodder flour and bran.

- Elemental composition : C₁₈H₃₂O₈N
- Theoretical BMP : 608 NmL CH₄/g VS

b. Substrate D = ground wheat straw

Substrate D was to 100% composed of ground wheat straw. An elementary composition analysis was not carried out and therefore a theoretical BMP could not be calculated. The theoretical BMP was estimated to be between 350-400 NmL CH₄/gVS based on literature values.

2.3 The protocol

The protocol was again based on the WS&T paper entitled « Towards a standardization of biomethane potential tests ». Some parameters have been imposed. However, not all laboratories followed the protocol as requested.

2.3.1 Inoculum

The inoculum should be taken from an anaerobic digester with stable operation, fed with a complex substrate or a mixtures of substrate in order to contain a diverse microbial community. By preference, the inoculum should be taken from anaerobic digester of a wastewater treatment plant or an agricultural plant digesting manure.

The inoculum should undergo the least treatment as possible, e.g. no sieving if possible. The analysis of TS and VS in triplicate is compulsory and the VS content should be between 15 and 40 g/l.



The inoculum should have the following characteristics:

- pH between 7.0 and 8.5 (compulsory to analyze)
- VFA < 1 g/l HAc (optional to analyze with indication of method used)
- NH_4 < 2.5 g/l N- NH_4 (compulsory to analyze with indication of method used)
- Alkalinity > 3 g/l CaCO_3 (compulsory to analyze)

The inoculum should only be stored for a short period (2-5 days) at ambient temperature or at the temperature at which the BMP test will be carried out.

The inoculum should have a low endogenous methane production that is $\leq 40\%$ of the total production (inoculum+substrate). If expected to be too high, a pre-incubation of 1 week was recommended.

2.3.2 Substrates

The analysis of TS and VS in triplicate is compulsory. The substrates could be stored at 4-15°C in the dark and at a dry place.

2.3.3 Set-up

All tests and analyses had to be carried out at least in triplicates.

Each test bottle should contain at least 1 g of substrate.

The trace element and vitamin solution have to be added at 1ml/l each. If alkalinity is < 3 g/l CaCO_3 , bicarbonate has to be added to reach at least 3 g/l CaCO_3 .

The purge gas should be by preference N_2/CO_2 with 20-40% CO_2 but 100% N_2 is acceptable.

The total VS concentration should be between 20 – 60 g/l.

The ISR had to be 2 for substrate C and cellulose, and 1 for substrate D.

The incubation temperature had to be in the mesophilic range with a maximum variation of $\pm 2^\circ\text{C}$. Mixing was compulsory, at least once a day manually.

There were no restrictions on the way methane production is measured but if the method requires measurement of gas composition, this has to be done at each measuring point. In addition, the ambient temperature and atmospheric pressure has to be recorded.

The test duration was terminated at the point where daily methane production was <0.5% of the net cumulated methane production (substrate – blank) during 3 consecutive days, but the tests had to last at least 25 days. If different substrates were tested, test should be terminated when the slowest batch (bottle) from the slowest substrate has reached the termination criterion.

2.3.4 Reporting of test results

The BMP with SD and RSD had to be calculated with the laboratory's own calculation method by taking into account the termination criterion of < 0.5% of the net accumulated volume of methane per day during 3 consecutive days. The methane production by the blank that was subtracted from the gross methane production of the substrate/positive control was most probably different for the different substrates since the termination criterion was likely reached at different time points.

If RSD of cellulose was >10%, the test had to be rejected and repeated. If the cellulose BMP was <325 $\text{NmLCH}_4 \text{ gVS}^{-1}$ or >414 $\text{NmLCH}_4 \text{ gVS}^{-1}$, the test had also to be rejected and repeated.

The BMP had also to be calculated by using OBA (<https://biotransformers.shinyapps.io/oba1/>).



All data of inoculum and substrate analysis and individual batches had to be provided without further calculation in the Excel template provided by test management team. All raw data of methane production data of all batches had to be provided in one of the templates sent by the test management team

2.3.5 Method of BMP calculation

The net methane production per batch is equal to the total production of a batch minus the mean of the production of the 3 blanks. If not the same amount of inoculum was used in each bottle, the net methane production has be corrected accordingly. The BMP was then calculated as follows:

- BMP per batch = net methane production / VS mass of substrate
- Mean of the BMPs of the 3 replicates for a test and a substrate
- The standard deviation is calculated as follows :

$$SD_{BMP} = \sqrt{SD_{blank}^2 + SD_{substrate/control}^2} \quad \text{with}$$

SD_{blank} = standard deviation of the methane production of the blanks

- $SD_{substrate/control}$ = standard deviation of the methane production of the substrate/control

2.4 Test of influence of inoculum

Since it was decided to test the influence of the inoculum on BMP variability between laboratories the following two approaches have been applied:

- In Germany, France and Italy, one laboratory sent an inoculum to all other participating laboratories of the same country with an express transport company. This inoculum was then tested in all laboratories concerned at the same time.
- In Belgium, Switzerland, and The Netherlands, two participating laboratories exchanged their inoculum and they were tested at the same time.



3 Results

3.1 Measurement of TS and VS

The measurements of VS content per mass of wet substrate of all 38 laboratories are shown in Figure 1. Although the SDs between laboratories were quite low, there was surprisingly high variability in these measurements (Fig. 1). The VS content of cellulose varied from 94-102%, for substrate C from 74-84%, and for substrate D from 78-90%. In most cases, differences among laboratories were small, but for each substrate however, some laboratories (1-5) reported unusually low and sometimes too high values (Fig. 1). These differences finally also can contribute to the variation in BMPs among the different laboratories.

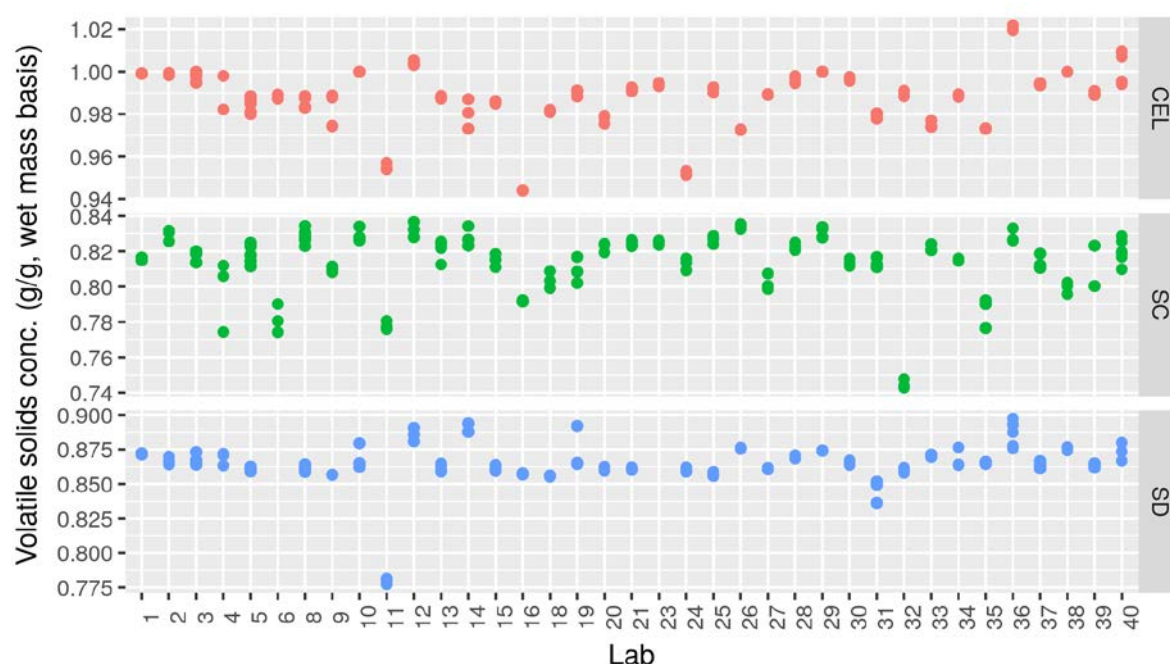


Figure 1. Variation of VS content of wet substrate mass determined for cellulose and substrates C and D.

Like in the 1st IIS-BMP, TS-VS determination was initially not considered as parameter that could influence the outcome of a BMP test. Based on the results obtained, this should be again reconsidered and discussed. It has to be decided on criteria for validation of these measurements, e.g. all in triplicate and including the measurement error in SD calculation. In order to eliminate the influence of this measurement on inter-laboratory variability, the median of VS per wet substrate mass was taken for calculations and not the laboratories own values.

3.2 Global BMP test results of both IIS-BMP

All BMP test results per lab and per study are depicted in Figure 2. Substrates A and B were only tested in the 1st-IIS-BMP whereas substrate D was only tested in the 2nd IIS-BMP. The BMPs of the 1st IIS-BMP are the means calculated from reported produced volumes of methane per bottle, volumes for dry methane gas and normalized for standard conditions (273.13 K and 101.33 kPa), and the mean VS content for each substrate. The BMPs of the 2nd IIS-BMP are the values reported by the laboratories.

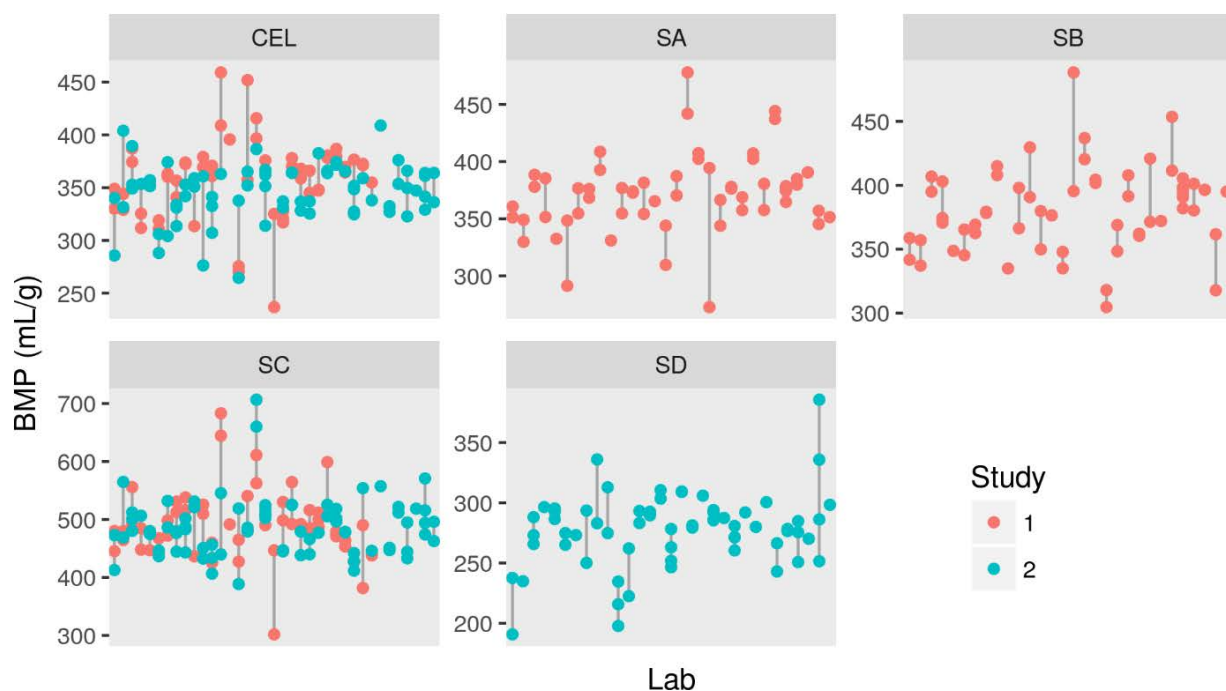


Figure 2. Reported BMPs per lab and per study for cellulose and substrates A, B, C and D.

For cellulose, the variability among the BMPs reported was lower in the 2nd IIS-BMP than in the 1st IIS-BMP. However, there were still quite many too low and a few somewhat too high values, values outside the range defined for the 2nd IIS-BMP. For substrate C, the range was still quite high, however with a higher minimum value. The variability of BMPs reported for substrate D, and compared to substrate A and B that were tested only in the 1st IIS-BMP, indicate that there was not a substantial improvement with regard to the range of BMPs reported.

3.3 Influence of inoculum on inter-laboratory variability

The influence of inoculum is often proposed as one major difference between laboratories since each laboratory carrying out BMP tests uses its own source of inoculum. In order to obtain indications whether the use of the same inoculum could reduce the variability among laboratories two approaches were applied.

The first approach consisted in sending one inoculum to all laboratories of the same country and test it simultaneously in all laboratories with minimum storage duration. This was done in France, Germany and Italy. The BMPs reported did not indicate that the variability among laboratories decreased due to the use of the same inoculum (Fig. 3). The variability among laboratories of the same country of BMPs obtained with the own inoculum or the shared inoculum was either quite similar or in some cases even higher (FR:CEL, IT:CEL, IT:SC).

The second approach consisting of exchanging inocula between two laboratories of the same country, showed no evidence of inoculum effects either (Fig. 4). Quite consistently if one laboratory determined lower BMPs with one inoculum, the BMPs were also lower with the second inoculum. But there was no evidence of consistent inoculum effects, based on statistical analysis.

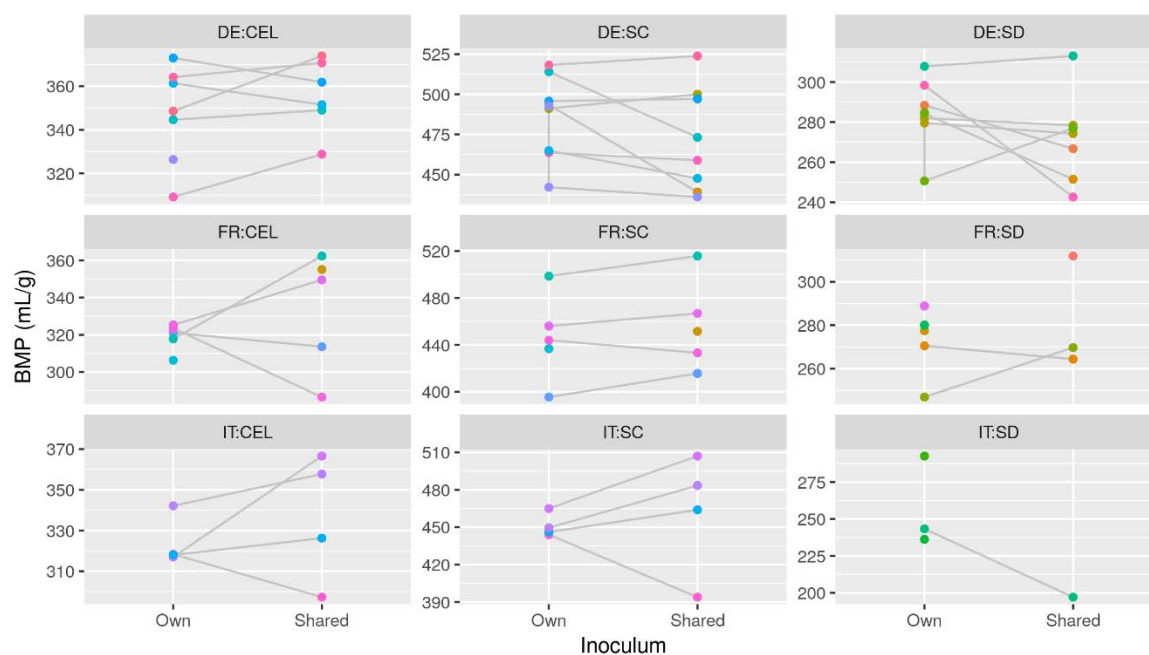


Figure 3. Calculated BMPs for cellulose and substrates C and D determined with either laboratory's own inoculum or a shared inoculum sent by one laboratory to partner laboratories of the same country. DE: Germany, FR: France, IT: Italy.

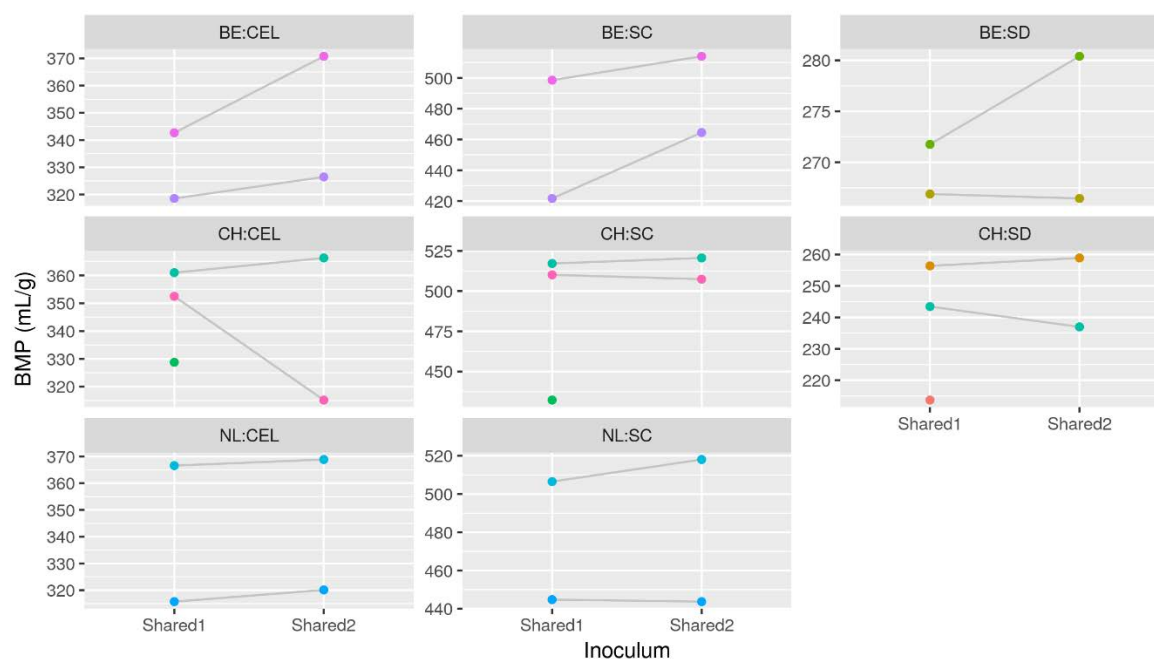


Figure 4. Calculated BMPs for cellulose and substrates C and D determined with two shared inocula by two laboratories of the same country. BE: Belgium, CH: Switzerland, NL: The Netherlands.

Hence, the variability among laboratories could not be decreased by using the same inoculum.



3.4 Comparison of reported and calculated BMPs

Data reporting between the two IIS-BMP differed significantly. For the first study laboratories corrected the methane volumes produced for dry gas and standard conditions with their own calculation method but they were not asked to calculate/report the BMPs. For the second study, BMPs had to be calculated and reported with their own calculation method and by using OBA. In addition, all raw data had to be submitted as well. By using the raw data, BMPs have been calculated with the study management team's own method and compared with reported BMPs (Fig. 5).

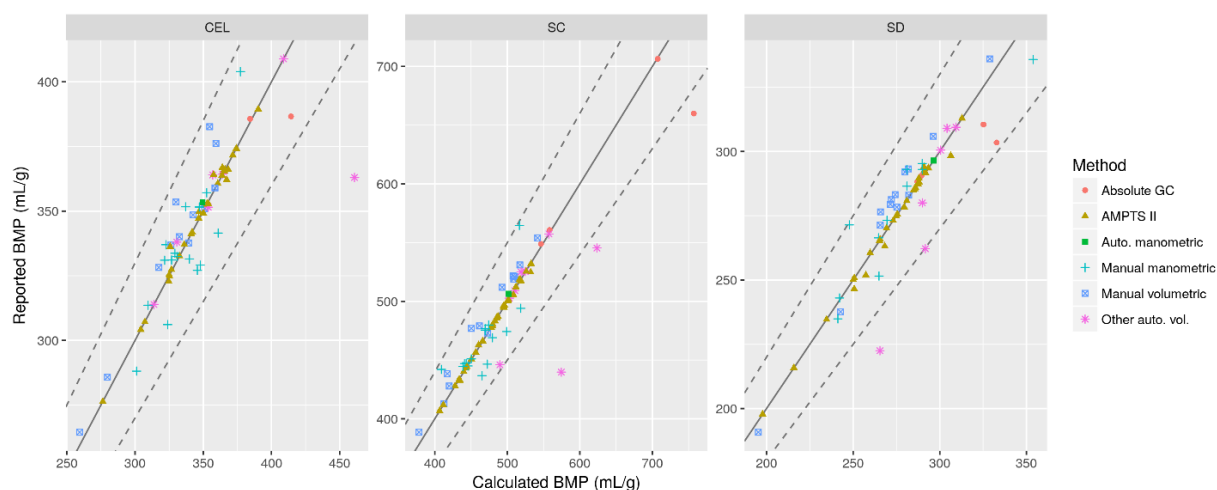


Figure 5. Comparison of BMPs calculated from raw data with BMPs reported by the participating laboratories. The dashed lines indicate 10% difference between calculated and reported BMPs.

This comparison showed clearly that the calculation methods used to determine from raw data the produced volume of dry methane gas under standard conditions were not the same and that this last step of BMP tests can also contribute to the variability among laboratories. The comparison was also done with the few raw data available from the 1st IIS-BMP and the result was the same. Considerable effort was undertaken to understand and identify the origin of these calculation differences but for several cases it was not possible to do so.

To continue the data analysis with a consistent data set, it was decided to work with only the BMPs calculated from raw data of both IIS-BMP (Fig. 6) which decreased available data from the 1st IIS-BMP to data from only 14 laboratories instead of 32. For substrate D, data from 32 instead of 38 laboratories were available since not all laboratories were obliged to test this substrate in the 2nd IIS-BMP.

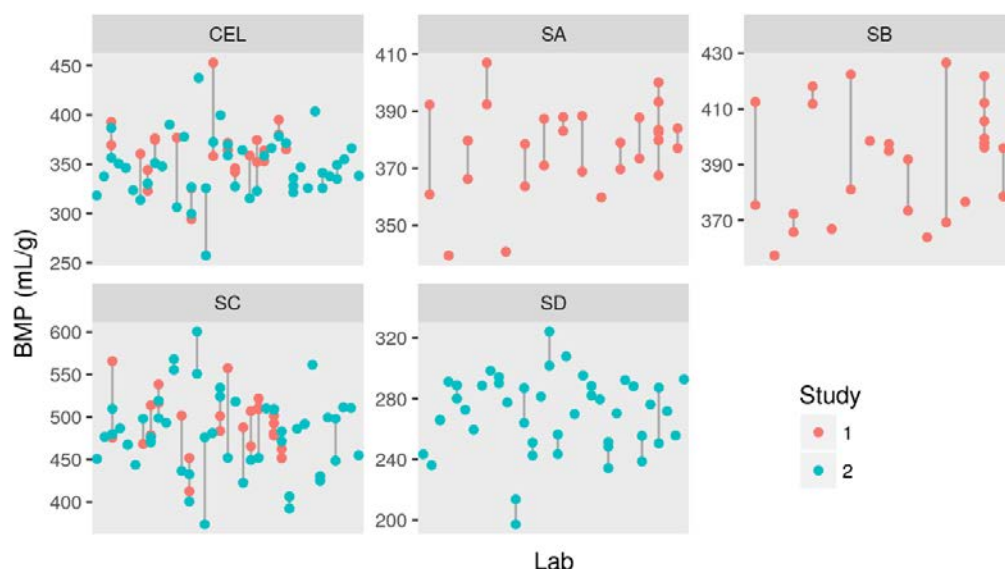


Figure 6. Calculated BMPs per lab and per study for cellulose and substrates A, B, C and D.



3.5 Optimization of test duration criteria

The test duration was one of the important parameters that needed to be optimized. In the original guideline, it was requested that the duration of the BMP tests should not be fixed in advance, and that tests should only be terminated when daily methane production during three consecutive days is $<1\%$ of the accumulated volume of methane. However, it was not specified whether the gross or net methane production (without or with subtraction of the blanks) is concerned. With data set available, several duration criteria were tested and compared, e.g. $<0.5\%$ or $<1\%$ during three consecutive days for gross or net methane production, or a fixed duration of 25 days.

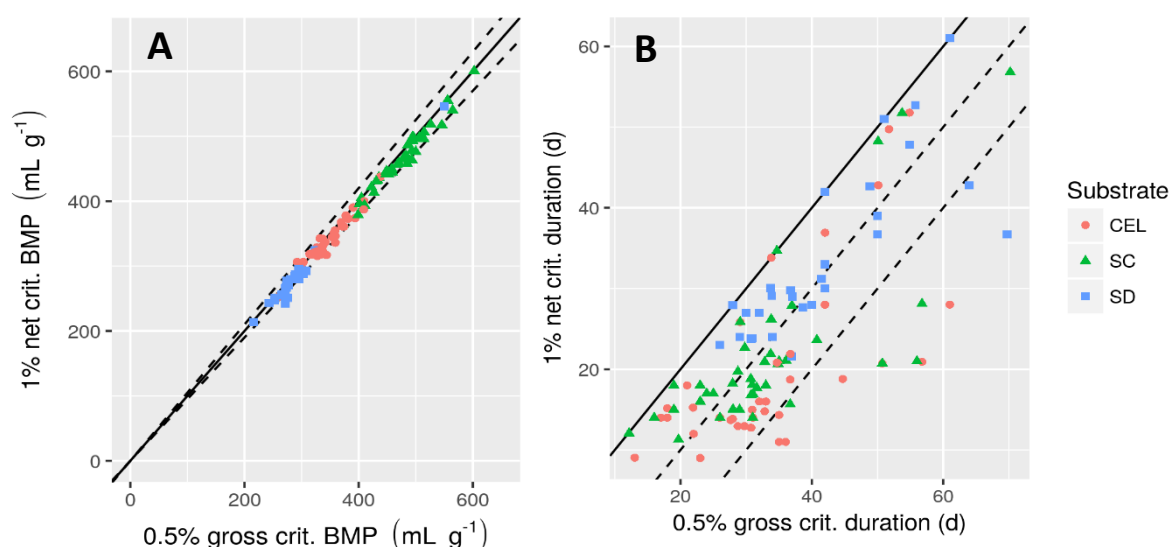


Figure 7. Comparison of BMPs (A) and duration (B) after application of two duration criteria. The dashed lines in (A) show $\pm 5\%$ and in (B) the difference of 10 and 20 days for test duration.

Obviously and generally speaking, gross methane production and $<0.5\%$ meant longer duration. The best approach seemed to be $<1\%$ net but with a minimum of 25 days. This had lower inter-lab variability than fixed durations (at least compared to 25 d), and was much shorter than $<0.5\%$ gross (Fig. 7B), but with only slightly lower BMPs (median $<1\%$ difference; Fig. 7A).

3.6 Highest acceptable relative standard deviation for cellulose BMP

The second parameter for which a more quantitative determination was carried out, was the relative standard deviation (RSD) for the cellulose BMP. Originally a relative standard deviation of 5% for the methane production of blanks was defined as validation criterion for BMP tests. The results of the 1st IIS-BMP have shown that this criterion does not really make sense. For blanks with a very low methane production, a RSD of 5% is easily exceeded. However, the absolute difference between blank replicates was very low and had little influence on the gross methane production. It was therefore decided during the second workshop in Freising that the standard deviation of the blanks has to be included in the calculation of the standard deviation of the net methane production of the substrates and positive control as defined in the guideline but should not be a validation criterion on its own. Since the RSDs defined in the guideline have only been based on experience and not really quantitative information, the RSDs of all tests were used to estimate the precision that can be reached in BMP tests with cellulose (Fig. 8). It showed that the majority of RSDs was $\leq 5\%$.

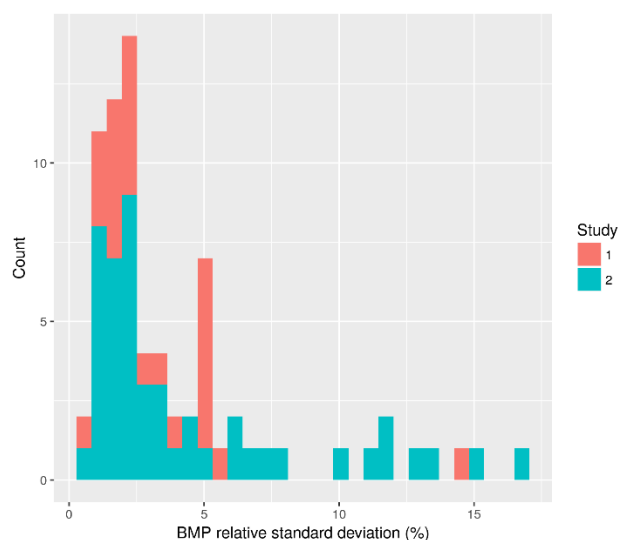


Figure 8. Distribution of the RSDs of the BMPs calculated from raw data of both IIS-BMP.

Calculating the percentiles for different RSDs indicated that 74% had a RSD of $\leq 5\%$ and that more than 80% of the tests could reach an RSD of 6% (Table 1). It was therefore propose to consider a RSD of 6% as highest acceptable value instead of 5%.

Table 1. RSDs for different percentiles for BMP tests with cellulose

Percentile	72%	74%	76%	78%	80%	82%	84%
RSD (%)	4.86	5.00	5.06	5.13	5.44	5.90	6.45

3.7 Correlation between cellulose and substrate BMPs

The BMPs of cellulose were compared with the BMPs of the substrates in order to obtain indications whether measuring a low cellulose BMP is linked to also measuring a low substrate BMP (Fig. 9).

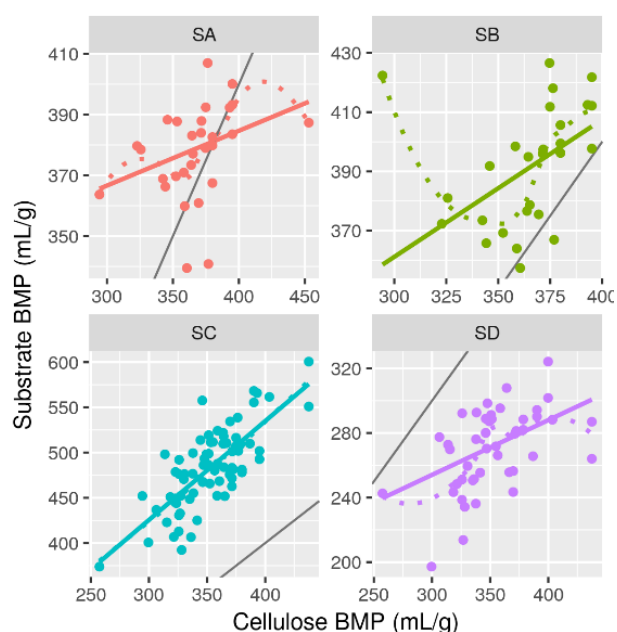


Figure 9. Comparison of cellulose BMPs with substrate BMPs. Black lines show 1:1 (for slope). Solids lines show a robust regression result in R. Dotted line is local polynomial.



This comparison shows that there was a rather good correlation between cellulose and substrate BMP with the clearest correlation for substrate C. This means that if a laboratory measures a low cellulose BMP, there is a quite high probability that it also measures a low BMP for substrates.

3.8 Comparison of manual methane production measurement methods

Some laboratories that used manual volumetric and manometric methods to measure methane production applied in addition the gravimetric determination of gas production. The comparison of the measured mass loss with the mass loss calculated from the measured volume of biogas produced showed that the produced gas volume was underestimated by using manual volumetric and manometric measurement methods in the majority of the bottles (Fig. 10). These results suggest that manual volumetric and manometric measurement methods can have an inherent systematic error and therefore low BMPs for cellulose should not be validated since they do not represent the true methane volumes produced in the bottles.

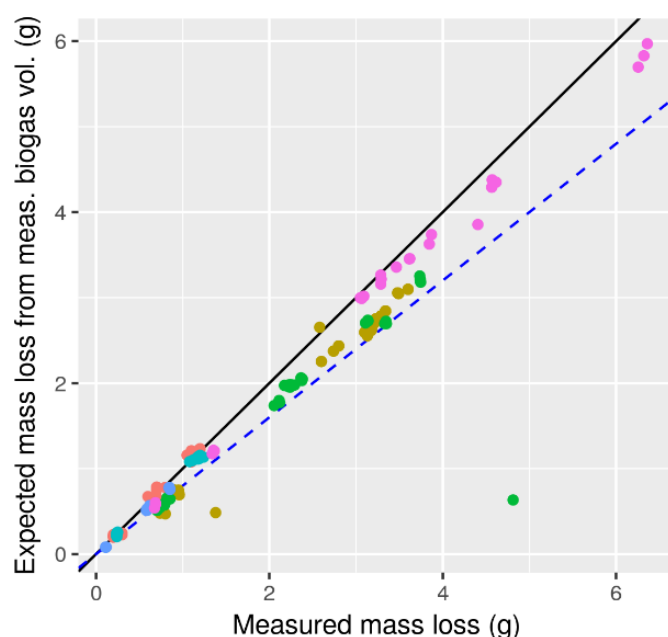


Figure 10. Comparison of measured mass loss and expected mass loss calculated from measured biogas volume. Dashed blue line is -20%.

3.9 Definition and application of new sets of validation criteria

Applying the original validation criteria reduced substantially the range between minimum and maximum BMPs measured by laboratories participating in the 1st IIS-BMP but it also led to the rejection of three-quarters of the tests. For the 2nd IIS-BMP a set of validation criteria was defined, revised set 1, where a lower limit for the cellulose BMP was acceptable and where the RSD criterion for blanks and substrates did not apply. In addition, an RSD of 10% for cellulose BMP was acceptable. This set of validation criteria should allow to get a data set with good quality, e.g. without too low cellulose BMPs, that should allow to calculate/estimate a lower cellulose BMP limit based on a large number of BMP tests.

As explained above, the RSD criterion was dropped and variability of blanks was included in the calculation of SD and RSD of cellulose and substrates. It is also proposed to drop RSD as validation criterion for substrates but SD and RSD of substrates have to be reported to indicate the uncertainty of the BMP measured.

Based on the observations that cellulose and substrate BMPs are correlated and that manual manometric and volumetric measurement methods have the tendency to underestimate the methane volume produced, new limits for cellulose BMP have been proposed. This led to the revised validation criteria



sets 2a – 2c with three different lower limits and one upper limit (Table 2). The upper limit is about 94% of the theoretical cellulose BMP and is proposed based on the fact that part of the cellulose is used for biomass growth and maintenance and therefore not transformed into biogas.

The highest acceptable RSD of the cellulose BMP is now 6% based on the analysis presented in 3.6.

Table 2. Original and revised sets of validation criteria

Validation criteria set	Lower limit cellulose BMP (mL _{CH₄} /g _{VS})	Upper limit cellulose BMP (mL _{CH₄} /g _{VS})	RSD cellulose BMP (%)	RSD substrate BMP (%)	RSD blank RSD (%)
Original	352	414	5	5	5
Revised set 1	325	414	5	n.a.	n.a.
Revised set 2a	335	390	6	n.a.	n.a.
Revised set 2b	340	390	6	n.a.	n.a.
Revised set 2c	345	390	6	n.a.	n.a.

n.a. : not applicable

The result of applying the different sets of validation criteria is depicted in Figure 11.

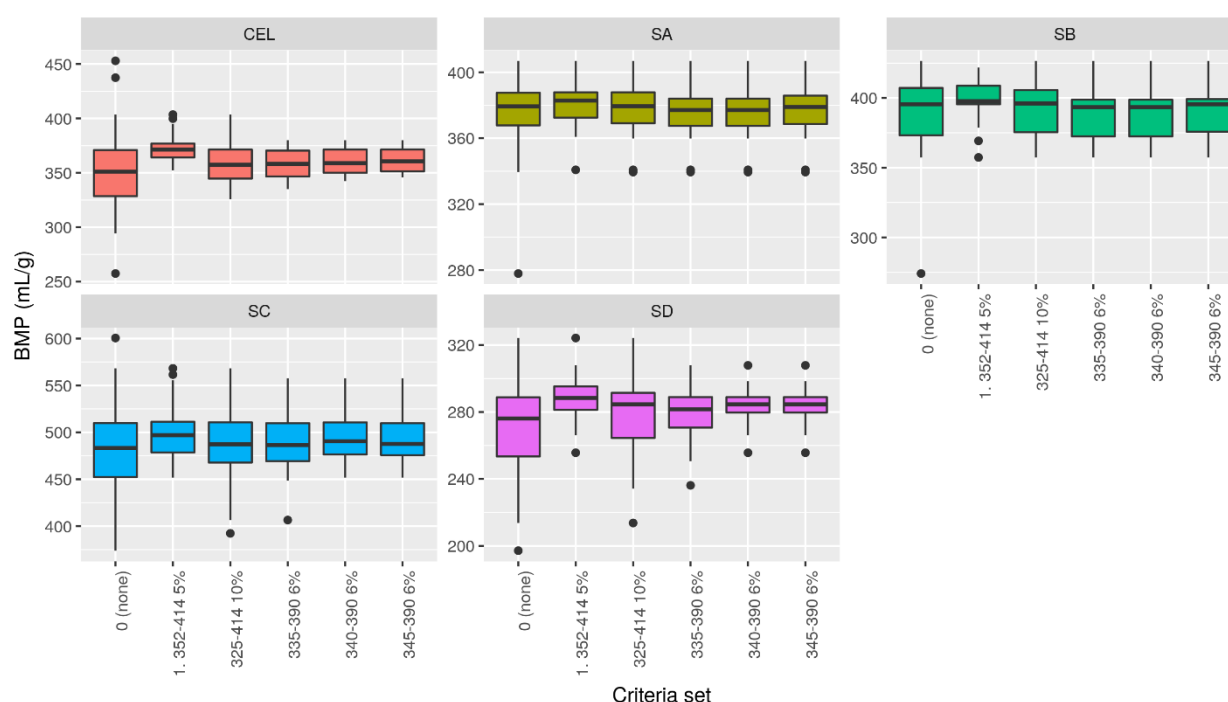


Figure 11. Comparison of variability of BMPs for cellulose and substrates A, B, C and D without and with application of different validation criteria sets.

The application of the new validation criteria sets 2a – 2c shows that set 2b and 2c has a similar effect as the original very strict validation criteria. Whereas the set 2a still included some extreme values, sets 2b and 2c had significantly reduced the interquartile range and excluded extreme values.



Whereas the application of the original validation criteria allowed to only validate 27-60% of the BMP tests, depending on the available data set for the different substrates, the application of the revised validation criteria set 2b allowed to validate 38-71% (Table 3). The validation rates for BMP tests was rather high for substrates A and B due to the limited available data set. Since only tests for which raw data were available, the number of tests used for this analysis is low. Furthermore, the raw data were mainly available for tests carried out with the automated gas production measurement equipment AMPTS II from Bioprocess Control which was shown in the 1st IIS-BMP to delivered the most reliable BMP test results. Hence, the available data set for substrates A and B is a biased one. For substrates C and D the data sets were less biased which is reflected by the low test validation rate of 34% and 27%, respectively, when the original validation criteria were applied.

For the biased as well as unbiased data sets, the relative range reached after application of the revised validation criteria set 2b was as low as 18-22%. This indicated that a much lower relative range would probably be very difficult to achieve. However, a relative range of about 20% is quite acceptable and would lead to minor differences in estimations of the methane production at full scale and the revenues linked to this methane production. The more severe original validation criteria did not result in really lower relative ranges and it was even higher for substrate D than the range obtained with validation criteria set 2b. The test validation rate however was always lower with the original validation criteria.

Table 3. Influence of validation criteria application on test validation rate and range of BMPs

Applied validation criteria set	# of BMP tests validated	% of BMP tests validated	Average BMP $\pm$ MAD ^a	Range (mL _{CH4} /g _{VS})	Range (%)
Substrate A					
None	30		374 $\pm$ 14	129	34
Original	18	60	380 $\pm$ 11	66	17
Revised 1	26	87	377 $\pm$ 14	68	18
Revised 2b	21	70	375 $\pm$ 14	68	18
Substrate B					
None	28		388 $\pm$ 27	152	39
Original	15	54	397 $\pm$ 12	64	16
Revised 1	25	89	392 $\pm$ 24	69	17
Revised 2b	20	71	388 $\pm$ 26	69	18
Substrate C					
None	77		483 $\pm$ 41	227	47
Original	26	34	499 $\pm$ 26	116	23
Revised 1	58	75	489 $\pm$ 33	176	36
Revised 2b	38	49	493 $\pm$ 29	106	22
Substrate D					
None	47		270 $\pm$ 24	127	46
Original	13	27	289 $\pm$ 11	69	24
Revised 1	32	68	277 $\pm$ 15	110	39
Revised 2b	18	38	283 $\pm$ 7	52	18

^a MAD = mean absolute deviation



3.10 Reasons for test exclusion due to cellulose BMP

Of the total of 182 tests included in the data set of BMPs calculated from raw data, 53% could be validated when applying the validation criteria set 2b (Fig. 12). The major reason for test rejection was a too low cellulose BMP (30% rejected tests) whereas a too high cellulose BMP resulted in 12% rejected tests. A total of 13% of the tests were rejected due to a too high RSD (>6%) and 8% of the tests were rejected due to two criteria.

The fact that the major rejection reason was a too low cellulose BMP together with the indication that manual manometric and volumetric methane production measurement methods have the tendency to underestimate the volume of methane produced, strengthens the argument that the lower limit for validation of the cellulose BMP should not be defined too low.

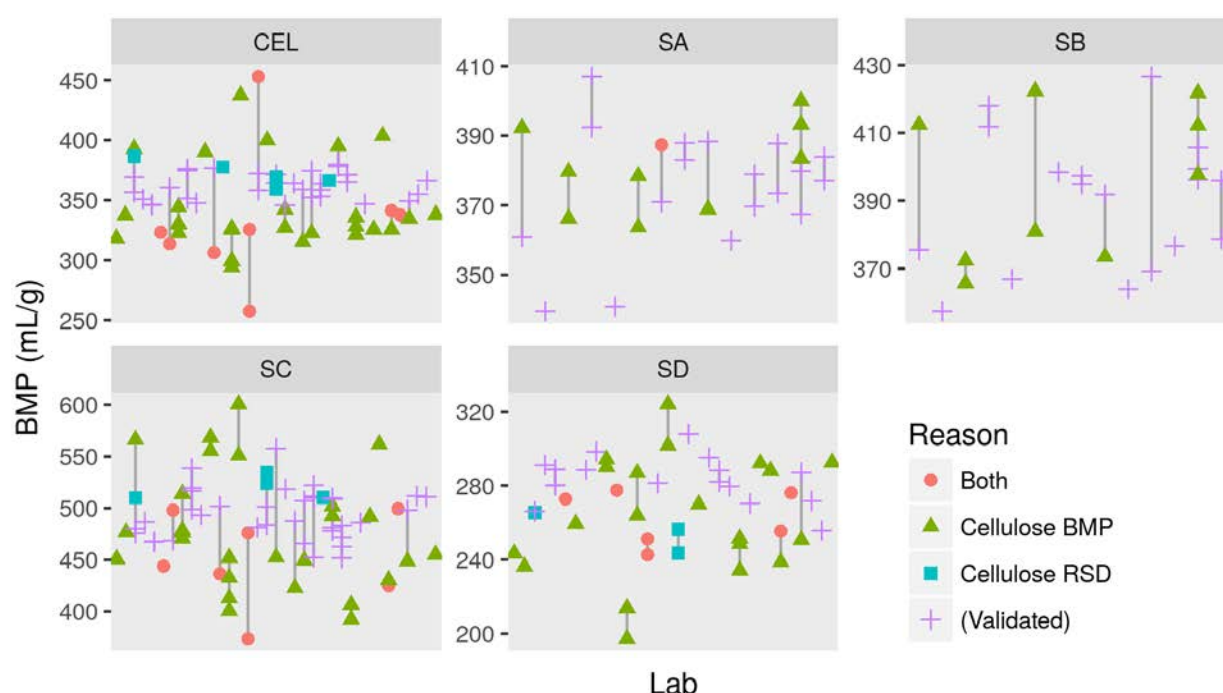


Figure 12. Reasons for rejection of BMP tests based on cellulose BMP and its consequences for validated and rejected BMPs for substrates A, B, C and D.

4 General discussion

The concept of data submission applied in this 2nd IIS-BMP consisting of laboratory's own BMP calculation together with corresponding raw data, allowed identifying another source of variability between laboratories, and a need for standardization. Transforming the measured volume of biogas or methane (in case carbon dioxide is trapped) into a volume of dry gas at standard conditions (273.13 K and 101.33 kPa) should not lead to different results and should be easy to standardize. For equipment such as the AMPTS II where this correction is done automatically by the software by taking the volume produced and the ambient temperature and pressured constantly measured into account, it cannot be verified whether this correction is done correctly or not. A discussion is currently on-going to find a consensus on the calculation method that finally could be published in a protocol and provided online. However, Hafner et al. (2018) already published an R package designed to help standardize biogas research and a web-based application called OBA (online biogas app) that provides access to the main functions (<https://biotransformers.shinyapps.io/oba1/>). The authors of this publication come from five laboratories that participated in the 2nd IIS-BMP. It remains now to achieve that also all other laboratories agree with the calculation method proposed.



The inoculum is often proposed as one of the most important BMP test parameters that lead to variability among laboratories. The purpose of the inoculum testing in this project was not to study the influence of the inoculum on the outcome of the BMP test as such but to elucidate whether the use of the same inoculum in different laboratories can decrease the variability among laboratories. The shipment and simultaneous use of an inoculum in several laboratories as well as the exchange of inocula between two laboratories did not indicate any influence of the inoculum on inter-laboratory variability. The variability was always as high or even higher when carrying out BMP tests with the same inoculum in two and more laboratories simultaneously.

For practice, the BMP test duration should be as short as possible in order to free analytical capacities as rapid as possible. The evaluation of different BMP test duration criteria showed that terminating the BMP test when the net methane production is <1% of the cumulated methane produced during three consecutive days is the best compromise between test duration and estimated ultimate methane production. At present, a minimum test duration of 25 days is however proposed since this reduced the inter-laboratory variability. Additional fixed test durations will be tested to evaluate whether 25 days is really needed or whether shorter fixed minimum duration have similar effects.

With regard to the precision of the methane production measurement, RSDs of all the tests included in the analysis showed that a quite high precision of determining the cellulose BMP is possible with >80% of the tests having an RSD of $\leq 6\%$. Whether a validation criterion based on RSD should also be applied for substrate BMPs is still a matter of debate. On the one hand there are people that propose that the RSD of the substrate BMP should only be reported but should not be used as validation criterion. This gives the people using the substrate BMP for their work an idea about the uncertainty of the BMP value but the test results can be used as such. However, there are also people that defend a reasonably high precision for the substrate BMP, especially due to the fact that people using BMPs will not take into consideration whether the RSD of the test results are low or high. These people propose to apply an RSD of at least 10% for substrate BMPs.

The results of the 1st IIS-BMP already indicated that the methane production measurement method has a strong influence on the inter-laboratory variability with the automated system AMPTS II being the method with the highest test validation rate and the lowest range between minimum and maximum BMP. These results also indicated that manual manometric and volumetric have the tendency to underestimate the gas volume produced. This tendency could be confirmed in this study with the comparison with the recently proposed gravimetric BMP method (Hafner et al., 2015). The measured mass loss was consistently higher than the mass loss calculated from manually measured gas volume produced, whether manometric or volumetric, and gas composition measurements. This is consistent with the earlier observed tendency to determine low BMPs with manual manometric and volumetric methods. It is therefore important to investigate the origin of this systematic error. It also substantiates the proposition that too low cellulose BMPs should not be validated since also the thus validated substrate BMPs are most probably too low.

Based on the data analysis, new validation criteria sets have been tested taking into account the minimum precision that can be reached as well as new lower and upper limits for cellulose BMP. Due to the limited number of raw data sets for the 1st IIS-BMP, the impact of the new validation criteria sets on the validation rates for tests and ranges for BMPs were somewhat biased for substrates A and B. It was less biased for substrate C since this was the main substrate of the 2nd IIS-BMP and also for substrate D since finally only 6 out of 38 participating laboratories did not test this optional substrate. For substrate C and D, the range decreased from 47% to about 20% when applying validation criteria set 2b and the test validation rate was about 40-50%. This indicates that the application of the new validation criteria allows to reach a situation where the risk to get a too low or too high BMP when sending a sample to one single laboratory is low, however only about half of the laboratories were able to produce BMP data that could be validated. The other half of the laboratories have to improve their way of carrying out the BMP tests in order to get reliable results.



5 Concluding remarks

Besides the important finding that calculation of BMPs from raw data has to be standardized, this 2nd IIS-BMP resulted in a new validation criteria that significantly lowered the range between minimum and maximum BMP of the laboratories for which the tests were validated. The validation rate of 40-50% of the BMP tests showed however also that a significant number of laboratories have to reconsider their way of determining BMPs.

As a follow-up of this inter-laboratory study, we decided to gather as much as possible the raw data of the 1st IIS-BMP. This will allow to calculate all BMPs with the same method which will eliminate the source of inter-laboratory variability due to different calculation approaches. Since many laboratories also carried out more than one test in the 2nd IIS-BMP when testing more than one inoculum, or more than one methane production measurement method, or both, we will include all tests and ignore that some laboratories did more tests than others.

Finally, the results of the two IIS-BMP will be published in an international open-access scientific journal and a new guideline with more details on different important elements of the protocol will be made available online, eventually together with demonstration videos of steps such as distribution of inoculum into test bottles for example.

6 References

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Annexe 1: List of participating laboratories

Preliminary remark: The laboratory numbering in the results section does not correspond to the alphabetical order presented here. Thus, the anonymity of the laboratories in connection with their results should be guaranteed.

- Advanced Water Management Center, The University of Queensland, Brisbane, Australia
- APESA, Plateau Technique, Cap Ecologia, Lescar, France
- Bavarian State Research Center for Agriculture, Freising, Germany
- Bioprocess Control AB, Lund, Sweden
- Centre of Biological Engineering, University of Minho, Braga, Portugal
- Chair of Urban Water Systems Engineering, Technical University of Munich, Garching, Germany
- CRPA - Research Centre on Animal Production, Reggio Emilia, Italy
- CREA - Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria, Modena, Italy
- Cranfield University, Bedfordshire, United Kingdom
- DBFZ Deutsches Biomasse Forschungs-Zentrum, Leipzig, Germany
- Delft University of Technology, Delft, The Netherlands
- Department of Biotechnology University of Verona, Verona - Italy
- Department of Chemical Engineering, Institute of Technology, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
- Dept. of Chemical Engineering, Environmental Technology and Biotechnology, University of Southern Denmark, Odense M, Denmark
- Dept. of Engineering, Aarhus University, Aarhus, Denmark
- ENAC IIE Laboratory for Environmental Biotechnology, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
- Hamburg University of Technology (TUHH), Institute of Environmental Technology and Energy Economics Harburger Hamburg, Germany
- HeigVD, Yverdon, Switzerland
- Innotech Alberta, Hwy 16A & 75 Street, Vegreville, Alberta, Canada
- INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Narbonne, France
- INSA – DEEP, INSA de Lyon, Villeurbanne Cedex France
- Institut Polytechnique LaSalle Beauvais, Département des Sciences et Techniques Agro-Industrielles, Beauvais, France
- Institute for Chemistry and Biotechnology, ZHAW School of Life Sciences and Facility Management, Wädenswil, Switzerland
- Instituto de la Grasa, Consejo Superior de Investigaciones Científicas, Seville, Spain



Second international inter-laboratory study on biomethane potential (BMP) tests

- IRTA, Barcelona, Spain
- Istituto di Ricerca sulle Acque (IRSA-CNR), Monterotondo st. (RM), Italy
- KU Leuven, Process and Environmental Technology Lab (PETLab), Sint-Katelijne-Wave, Belgium
- LeAF, Wageningen, The Netherlands
- LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
- Mälardalens högskola, Västerås, Sweden
- National Research Council Canada, Montréal, Canada
- OWS nv, Gent, Belgium
- Politecnico di Milano-DICA, Milano, Italy
- Sorbonne Universités, EA 4297 TIMR UTC/ESCOM, Compiègne, France
- State Institute of Agricultural Engineering and Bioenergy, University of Hohenheim, Stuttgart, Germany
- Technical University of Denmark, Department of Environmental Engineering, Lyngby, Denmark
- Universität der Bundeswehr München, Institut für Wasserwesen, Neubiberg, Germany
- University of Chemistry and Technology Prague, Prague, Czech Republic
- VERI (Veolia), Limay, France