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Romain Cresson, Sébastien Pommier, Fabrice Béline, Théodore Bouchez, Claire Bougrier, Pierre Buffiere, Laurent Mazéas, André Pauss, Philippe Pouech, Sébastien Preys, et al.

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R. Cresson[a], S. Pommier[b], F. Béline[c], T. Bouchez[d], C. Bougrier[e], P. Buffière[f], L. Mazeas[d], A. Pauss[g], P. Pouech[h], S. Preys[i], T. Ribeiro[j], M. Rouez[k] and M. Torrijos[l]

[a] INRA Transfert Environnement, Avenue des Etangs, Narbonne, F-11100, France
(E-mail: *cresson@supagro.inra.fr)*
[b] CRITT Génie des Procédés et Technologies Environnementales, INSA Toulouse, 135 avenue de Rangueil, 31077 Toulouse Cedex 04, France
(E-mail: *spommier@insa-toulouse.fr)*
[c] IRSTEA, UR GERE, 17 avenue de Cucillé, CS 64427, F-35044 Rennes, France.
(E-mail: *Fabrice.Beline@irstea.fr)*
[d] IRSTEA, UR HBAN, 1 rue Pierre-Gilles de Gennes, CS 10030, 92761 Antony, France
(E-mail: *theodore.bouchez@irstea.fr; laurent.mazeas@irstea.fr)*
[e] VEOLIA Recherche et Innovation, Zone Portuaire, 291 Avenue Dreyfous Ducas, 78520 Limay, France
(E-mail: *claire.bougrier@veolia.com)*
[f] Université de Lyon, INSA-Lyon, Laboratoire de Génie Civil et d'Ingénierie Environnementale, 9, rue de la Physique, Villeurbanne cedex F-69621, France
(E-mail: *pierre.buffiere@insa-lyon.fr)*
[g] Sorbonne universités, Université de Technologie de Compiègne, TIMR EA 4297 UTC/ESCOM, Centre de Recherches de Royallieu, CS60 319, 60203 Compiègne cedex, France
(E-mail: *andre.pauss@utc.fr)*
[h] APESA, Hélioparc, 2 av. Pierre Angot, 64053 PAU, France
(E-mail: *philippe.pouech@apesa.fr)*
[i] Ondalys,4 rue Georges Besse, 34830, Clapiers, France
(E-mail: *SPreys@ondalys.fr)*
[j] Institut Polytechnique LaSalle Beauvais, Rue Pierre Waguet, BP 30313, 60026 Beauvais Cedex, France
(E-mail: *thierry.ribeiro@lasalle-beauvais.fr)*
[k] SUEZ Environnement, 38 Rue du President Wilson,78230, Le Pecq, France
(E-mail: *maxime.rouez@suez-env.com)*
[l] INRA, UR0050, Laboratoire de Biotechnologie de l'Environnement, Avenue des étangs 11 100 Narbonne
(E-mail: *torrijos@supagro.inra.fr*)

**Abstract**
A French inter-laboratory campaign on BMP assessment was realised in two phases: free protocol at first, then harmonized protocol. Results show good intra-laboratory repeatability and reproducibility, but in spite of an attempt in the harmonization of practices, the inter-laboratory reproducibility could not be improved and ranges in the magnitude of 20% RSD.

**Keywords**
BMP; methanogenic potential; inter-laboratory study

## INTRODUCTION

The determination of the methanogenic potential of substrates, also known as BMP (Biological Methane Potential) is a central and essential parameter for any anaerobic processes. Actually it is used for the technical and economic analysis of a project, for the design of treatment and recovery facilities and for the evaluation of the process performance. It is also a key indicator of the stability of solid wastes stored in landfills. While some publications mention methodologies for the determination of the methanogenic potential, the methods are often very different, especially for solid and heterogeneous substrates. This variability in analytical methods directly results from the lack of standardized protocol or adapted normative reference. It raises many questions about the quality of the results and limits their interpretation.

Some attempts for the standardization of the BMP protocols have been proposed. In particular, the

expert working group on anaerobic digestion (Anaerobic Digestion Specialist Group) of the International Water Association (IWA) has initiated a task group entitled "Task Group for the Harmonization of Anaerobic Biodegradation, Activity and Inhibition Assays". It resulted in the publication of a protocol for solid waste BMP determination (Angelidaki et al., 2009). This protocol relies on previous publications that had already attempted to make recommendations based on the analysis of the involved processes (Owens and Chynoweth, 1993,Angelidaki and Sanders, 2004, Hansen et al., 2004, Rozzi and Remigi, 2004), or on the application to very specific problems such as the substrate/inoculum ratio (Fernandez et al., 2001). However no further action has so far been proposed after this work and the task group recommendations are still only partially followed by the laboratories. As a consequence, the reliability of BMP measurements is in question. This was illustrated by recent inter-laboratory studies. The first one was reported by Raposo et al. (2011) and evaluated the results from 19 laboratories worldwide on simple model substrates (starch, cellulose, gelatine and mung beans). The second one was an Italian campaign reported by Proqueddu et al. (2013) and involved 19 laboratories which evaluated the BMP of freeze-dried samples of cheese whey, silage maize and biowaste. The findings of these studies support a wide dispersion of results with both a high number of outliers and poor reproducibility relative standard deviation.

The aim of the proposed French inter-laboratory campaign was to evaluate the current protocols used in the country for BMP evaluation and to establish their harmonization.

## EXPERIMENTAL

### Organization of the inter-laboratory study

Eleven French laboratories participated in this campaign. The study involved two experimental phases. For each of them, three samples were sent to each participating laboratory, which achieved 6 methanogenic potential measurements for each sample. During the first experimental phase each partner completed the methanogenic potential measurements according to its own protocol. During the second phase each partner made new measurements according to the common protocol defined after analysing the results of the first phase. For each of the experimental phases, the laboratories performed 2 sets of measurements in triplicates, the second set started at least 4 weeks after the first one. The purpose of these repetitions was to evaluate both the inter-laboratory reproducibility and the intra-laboratory reproducibility of the BMP measurement.

### Substrates

A sample of the substrates presented in Table 1 was sent to each laboratory. For the first phase, substrates SA, SA' (same substrate but dried and shredded) and SB were studied. For the second phase, substrates SA', SB and SC were studied.

**Table 1**. Composition of the substrates used in the inter-laboratory campaign.

| Substrate | Composition (%w.b) | | TS (%) | VS (%TS) | % VS in mixture |
|---|---|---|---|---|---|
| SA | Potato | 40% | 20% | 99% | 27% |
| | Grain maize | 20% | 27% | 99% | 18% |
| | Beef | 30% | 27% | 95% | 26% |
| | Wheat straw | 10% | 90% | 93% | 29% |
| | *total* | *100%* | *30%* | *95%* | *100%* |
| SA' | Idem SA | 100% | 97% | 95% | 100% |
| SB | Wheat Straw | 100% | 92% | 93% | 100% |
| SC | Mayonnaise | 100% | 75% | 99% | 100% |

**BMP Protocols**

For the first phase, participants applied their own typical BMP measuring protocol with the sole obligation to run the tests in two series of triplicates and to include blank samples. The identified factors differing from one laboratory to another were: the main method (manual or automated via AMPTS apparatus), the gas volume measurement technique, the mixing regime, the substrate/inoculum ratio (S/X), the pH buffer addition, the mineral medium complementation and the level of endogenous methane production.

For the second phase, which came after the analysis of results of the first phase, participants were asked to satisfy the following rules: use of a common mineral medium, use of 3 g/L $NaHCO_3$ as pH buffer and set the volatile solid substrate/inoculum ratio to 0.5.

**Statistical Analysis**

Calculations of the Relative Standard Deviation (RSD) for repeatability and reproducibility were performed according to the metrological NF ISO 5725-1.The influence of the protocol factors was assessed by ANOVA.

## RESULTS

**First phase: free protocol**

The first phase of the campaign was carried out in 2013. The dispersion of the obtained BMP values is synthetized in Table 2. For each laboratory, both repeatability among triplicates and reproducibility between two sets of tests were satisfactory. Nevertheless, the inter-laboratory reproducibility was quite poor, with a RSD around 20% whatever the substrate. ANOVA analysis did not show clear influence of the identified protocol factors, except for the mineral medium implementation which was found to raise the BMP value. For other factors like the assessment method (manual/automated), the S/X ratio and the buffer addition, the influence on the BMP value was found to be substrate-dependant. The solid substrate preparation before BMP assessment (fresh or freeze-dried) had no significant effect on the results.

**Table 2**. Synthesis for the first phase of the French inter-laboratory campaign.

| BMP (NmLCH4/gVS) | SA | SA’ | SB | SC |
|---|---|---|---|---|
| Mean | 425 | 403 | 267 | - |
| Median | 422 | 407 | 267 | - |
| Min | 289 | 250 | 175 | - |
| Max | 629 | 481 | 370 | - |
| Intra-laboratory repeatability RSD | 7% | 4% | 6% | - |
| Intra-laboratory reproducibility RSD | 9% | 6% | 8% | - |
| Inter-laboratory reproducibility RSD | 20% | 17% | 20% | - |

**Second phase: harmonized protocol**

After the first phase, discussions between the participants led to the proposal of a harmonized protocol with unified practices for the following factors: S/X ratio, pH buffer and mineral complementation (as described in the EXPERIMENTAL section). The subsequent second phase of the campaign was carried out in 2014 on substrates SA’ and SB (same as for the first phase), as well as on a new lipid substrate (SC). As shown in Table 3, despite the proposed harmonization, the

inter-laboratory reproducibility was not improved.

Efforts will now be made for the definition of a posteriori validation criterions related to the inoculum activity.

**Table 3**. Synthesis for the second phase of the French inter-laboratory campaign.

| BMP (NmLCH4/gVS) | SA | SA' | SB | SC |
|---|---|---|---|---|
| Mean | - | 405 | 277 | 848 |
| Median | - | 408 | 282 | 828 |
| Min | - | 260 | 195 | 660 |
| Max | - | 525 | 370 | 1026 |
| Intra-laboratory repeatability RSD | - | 4% | 4% | 4% |
| Intra-laboratory reproducibility RSD | - | 5% | 7% | 5% |
| Inter-laboratory reproducibility RSD | - | 19% | 21% | 13% |

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