Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study

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Abstract

BACKGROUND: This paper describes results obtained for different participating research groups in an interlaboratory study related to biochemical methane potential (BMP). In this research work, all experimental conditions influencing the test such as inoculum, substrate characteristics and experimental conditions were investigated. The study was performed using four substrates: three positive control substrates (starch, cellulose and gelatine), and one raw biomass material (mung bean) at two different inoculum to substrate ratios (ISR).

RESULTS: The average methane yields for starch, cellulose, gelatine and mung bean at ISR of 2 and 1 were 350 ± 33, 350 ± 29, 380 ± 42, 370 ± 36 and 370 ± 35 mL CH4 g−1 VSadded, respectively. The percentages of biotransformation of these substrates into methane were 85 ± 8, 85 ± 7, 88 ± 9, 85 ± 8 and 85 ± 8%, respectively. On the other hand, the first-order rate constants obtained from the experimental data were 0.24 ± 0.14, 0.23 ± 0.15, 0.27 ± 0.13, 0.31 ± 0.17 and 0.23 ± 0.13 d−1, respectively.

CONCLUSION: The influence of inocula and experimental factors was nearly insignificant with respect to the extents of the anaerobic biodegradation, while the rates differed significantly according to the experimental approaches.

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Keywords: anaerobic digestion; biodegradable; biomass; bioreactors; environmental biotechnology; reactor optimization

INTRODUCTION

Biochemical methane potential (BMP) is a procedure developed to determine the methane production of a given organic substrate during its anaerobic decomposition. The BMP assay has proved to be a relatively simple and reliable method to obtain the extent and rate of organic matter conversion to methane.1 The information provided by BMP is valuable when evaluating potential substrates and for optimizing the design and functioning of an anaerobic digester. Literature related to BMP assays is extensive, showing that this test has been used to evaluate a wide variety of substrates.2,3 Interest in recent years has increased as can be demonstrated by the wide range of research papers dealing with BMP assays. In addition, several batch methods have been utilized for measuring methane potentials, but unfortunately there is no standard protocol for carrying out the determination.4 Consequently, methane yields reported in the literature have limited comparability and cannot be precise because of possible differences in the experimental protocol used for the assay. There are many factors that may influence the anaerobic biodegradability of organic materials, and some of these factors are, at present, only poorly understood and frequently not described in the procedure. Recently a new...
A proposed protocol for BMP testing has been published, where some basic guidelines for a common procedure are given. On the other hand, very scarce information was found in the literature relating to similar research work. Only one interlaboratory study (in which 21 laboratories participated) has previously been published. Unfortunately, this interlaboratory study was designed from a more restricted point of view, using two organic substrates (palmitic acid and polyethylene glycol 400) as micro-pollutant (concentration 50 mg C L\(^{-1}\) and a complex gas measurement system (headspace pressure in conjunction with inorganic carbon determination).

Therefore, the purpose of this research work was to collect and compile results obtained in the BMP interlaboratory study using different solid organic substrates with the aim of providing an extensive database for BMP extent and rates in relation to the experimental conditions selected.

### EXPERIMENTAL

The approach of the BMP test is simple. An organic substrate is mixed with an anaerobic inoculum in defined operating conditions, and the gas evolved is quantified by a specific measurement system until gas production is virtually ceased. However, the protocols available in the literature are very different. The full description of factors influencing the results of the BMP test, such as inoculum, substrate and experimental conditions (Table 1), was considered as mandatory information to be reported by participating laboratories. For this interlaboratory study, as the substrates were the same for all participants, their effect can be disregarded as a source of uncertainty in the final results.

### Organization of the interlaboratory study

The interlaboratory study was organized by the Spanish National Research Council (CSIC) through the Instituto de la Grasa, specifically by the ‘Water and Wastewater Treatment’ group. The interlaboratory study coordinator and collaborators were responsible for designing the scheme, the preparation of test materials, the production and distribution of instructions and test material among the participating laboratories, the collection and statistical analysis of the data obtained, and feedback of the results to all participants (anonymously to guarantee confidentiality).

Each participating laboratory received a full set of samples, together with basic technical guidelines about how to proceed with the measurements; participating laboratories were free to select the inoculum and virtually free to choose the experimental conditions. In this interlaboratory study, 19 laboratories reported data, including two having results that were not appropriate for comparison purposes. The number assigned to each participating laboratory was given in random order to guarantee confidentiality of the results obtained.

### MATERIALS

#### Inocula

An important factor which cannot easily be standardized is the source of the sludge used as inoculum and its state of acclimation and adaptation to a test material. Given the microbial diversity typically encountered among most groups of microorganism forming the anaerobic inocula, the use of a standard inoculum is simply unrealistic. Most previous protocols have been promulgated using anaerobic sludge from municipal wastewater treatment plants (MWTP), owing to the metabolically active microbial assemblages and to the fact that it is easily available. In the present interlaboratory study no suggestions were made about the inoculum to be used. In addition, two participating laboratories (numbers 2 and 4) used three different sources of microbial biomass to carry out the BMP test. Table 2 summarizes the main characteristics of inocula used:

- **Origin/source:** different sludges from operating anaerobic reactors were selected as microbial biomass. MWTP was mainly used as inoculum source (12); followed by biowaste, manure and brewery sources (2), and finally sludges from the wastewater treatment of soft drink, potato, vinasses, paper mill and agrofood industries were selected in minor proportion (1).

#### Table 1. Factors affecting the BMP assays

<table>
<thead>
<tr>
<th>I. Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1. Origin</td>
</tr>
<tr>
<td>I.2. Characterization: pH, TS, VS, TSS, VSS</td>
</tr>
<tr>
<td>I.3. Amount (g) and concentration (g VS L(^{-1})) at start-up of the experiment</td>
</tr>
<tr>
<td>I.4. Activity</td>
</tr>
<tr>
<td>I.5. Time from sampling to starting test (days)</td>
</tr>
<tr>
<td>II. Substrate</td>
</tr>
<tr>
<td>II.1. Type (part and particle size)</td>
</tr>
<tr>
<td>II.2. Characterization: moisture, TS, VS, TKN, organic fraction composition, atomic or elemental composition, fiber composition</td>
</tr>
<tr>
<td>II.3. Amount (g) and concentration (g VS L(^{-1})) at start-up of the experiment</td>
</tr>
<tr>
<td>III. Experimental conditions</td>
</tr>
<tr>
<td>III.1. Quantification of gas</td>
</tr>
<tr>
<td>III.1.1. Measurement system (MS)</td>
</tr>
<tr>
<td>(a) Manometric (Man), by pressure (p)</td>
</tr>
<tr>
<td>(b) Volumetric (Vol), by water displacement (wd) or gas counter (gc)</td>
</tr>
<tr>
<td>(c) Gas chromatography (GC)</td>
</tr>
<tr>
<td>III.1.2. Type of gas (Type): Biogas (Bg) or Methane (Me)</td>
</tr>
<tr>
<td>III.1.3. Biogas composition (BgC): Yes, by GC analysis (Com)/No, CH(_4) directly (Di)</td>
</tr>
<tr>
<td>III.2. Operational conditions</td>
</tr>
<tr>
<td>III.2.1. Physicals</td>
</tr>
<tr>
<td>(a) Reactor capacity: Working volume (W(<em>{\text{vol}})) and Total volume (T(</em>{\text{vol}}))</td>
</tr>
<tr>
<td>(b) Temperature (T): Range: Mesophilic - 35(^{\circ})C/Thermophilic - 55(^{\circ})C</td>
</tr>
<tr>
<td>(c) Stirring (St): Manual (Ma)/Automatic (Au) and Continuous (C)/Batch (B)</td>
</tr>
<tr>
<td>If automatic: Magnetic bar (mb)/Shaker (sh) If batch: times/day (d)</td>
</tr>
<tr>
<td>Time (t): Pre-incubation (Pre-t) and test duration (TD-t) (e)</td>
</tr>
<tr>
<td>III.2.2. Chemicals</td>
</tr>
<tr>
<td>(a) Headspace gas (G(_{\text{hv}}))</td>
</tr>
<tr>
<td>(b) pH/alkalinity adjustment (pH/Alk Adj): If yes, chemical reagent and concentration at start-up of the experiment (c)</td>
</tr>
<tr>
<td>(c) Mineral medium (MM): If yes, chemical composition and concentration at start-up of the experiment (d)</td>
</tr>
<tr>
<td>III.2.3. Inoculum to substrate ratio (ISR)</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of the inocula used by participating laboratories

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Origin/Source</th>
<th>pH</th>
<th>TS (g L(^{-1}))</th>
<th>VS (g L(^{-1}))</th>
<th>VS/TS (%)</th>
<th>Co (g VS L(^{-1}))</th>
<th>Time from sampling (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Manure fed-Industry</td>
<td>7.8</td>
<td>57.9</td>
<td>37.8</td>
<td>65</td>
<td>37.8</td>
<td>30</td>
</tr>
<tr>
<td>2.1</td>
<td>Thermophilic biowaste (dry)</td>
<td>7.9</td>
<td>215.0</td>
<td>113.0</td>
<td>53</td>
<td>56.5</td>
<td>15</td>
</tr>
<tr>
<td>2.2</td>
<td>Thermophilic biowaste (wet)</td>
<td>8.0</td>
<td>66.9</td>
<td>39.3</td>
<td>59</td>
<td>39.3</td>
<td>8</td>
</tr>
<tr>
<td>2.3</td>
<td>MWTP</td>
<td>7.7</td>
<td>44.4</td>
<td>24.3</td>
<td>55</td>
<td>24.3</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>MWTP</td>
<td>7.8</td>
<td>21.6</td>
<td>12.4</td>
<td>57</td>
<td>10.4</td>
<td>19</td>
</tr>
<tr>
<td>4.1</td>
<td>Soft drink industry</td>
<td>7.4</td>
<td>30.0</td>
<td>25.0</td>
<td>84</td>
<td>15.0</td>
<td>4</td>
</tr>
<tr>
<td>4.2</td>
<td>Brewery industry</td>
<td>7.4</td>
<td>83.0</td>
<td>47.0</td>
<td>56</td>
<td>15.0</td>
<td>4</td>
</tr>
<tr>
<td>4.3</td>
<td>MWTP</td>
<td>7.6</td>
<td>43.0</td>
<td>20.0</td>
<td>48</td>
<td>15.0</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>MWTP</td>
<td>8.0</td>
<td>58.0</td>
<td>39.0</td>
<td>67</td>
<td>11.7</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Manure fed-Lab</td>
<td>7.8</td>
<td>15.0</td>
<td>6.3</td>
<td>42</td>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>Potato industry</td>
<td>7.8</td>
<td>21.6</td>
<td>13.8</td>
<td>55</td>
<td>13.7</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>MWTP</td>
<td>6.8</td>
<td>24.2</td>
<td>16.4</td>
<td>68</td>
<td>11.5</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>MWTP</td>
<td>6.8</td>
<td>25.0</td>
<td>13.8</td>
<td>55</td>
<td>13.7</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Distillery vinasses industry</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5.0</td>
<td>7–14</td>
</tr>
<tr>
<td>11</td>
<td>Brewery industry</td>
<td>7.3</td>
<td>39.4</td>
<td>33.9</td>
<td>86</td>
<td>10.0</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>MWTP</td>
<td>7.8</td>
<td>25.0</td>
<td>15.0</td>
<td>60</td>
<td>7.3</td>
<td>11</td>
</tr>
<tr>
<td>13</td>
<td>Paper mill industry</td>
<td>7.3</td>
<td>136.0</td>
<td>102.0</td>
<td>75</td>
<td>8.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>14</td>
<td>MWTP</td>
<td>7.3</td>
<td>24.2</td>
<td>13.5</td>
<td>56</td>
<td>3.1</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>Agrofood industry</td>
<td>8.2</td>
<td>117.0</td>
<td>97.0</td>
<td>83</td>
<td>20.0</td>
<td>150</td>
</tr>
<tr>
<td>16</td>
<td>MWTP</td>
<td>7.2</td>
<td>95.0</td>
<td>42.0</td>
<td>44</td>
<td>10.0</td>
<td>20</td>
</tr>
<tr>
<td>17</td>
<td>MWTP</td>
<td>7.4</td>
<td>50.0</td>
<td>30.7</td>
<td>61</td>
<td>30.0</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>MWTP</td>
<td>7.4</td>
<td>18.2</td>
<td>13.6</td>
<td>75</td>
<td>13.6</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>MWTP</td>
<td>7.4</td>
<td>27.5</td>
<td>16.2</td>
<td>59</td>
<td>8.1</td>
<td>1</td>
</tr>
</tbody>
</table>

MWTP: Municipal wastewater treatment plant.
ND: Not determined.

Table 3. Characterization of substrates used

<table>
<thead>
<tr>
<th>Starch/Cellulose</th>
<th>Gelatine</th>
<th>Mung bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>10/3</td>
<td>8</td>
</tr>
<tr>
<td>TS (%)</td>
<td>90/97</td>
<td>92</td>
</tr>
<tr>
<td>VS (%-TS)</td>
<td>99/100</td>
<td>100</td>
</tr>
<tr>
<td>Elemental (%-TS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 44.5*/44.0**</td>
<td>48.2</td>
<td>44.7</td>
</tr>
<tr>
<td>H 6.2*/6.0**</td>
<td>6.5</td>
<td>6.8</td>
</tr>
<tr>
<td>N –</td>
<td>18.4</td>
<td>4.4</td>
</tr>
<tr>
<td>S –</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>O 49.3*/50.0**</td>
<td>26.2</td>
<td>41.1</td>
</tr>
<tr>
<td>Empirical formulae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(<em>6)H(</em>{10})O(_5)*</td>
<td>C(<em>{366})H(</em>{595})O(_{313})**</td>
<td>C(<em>{402})H(</em>{648})O(<em>{164})N(</em>{131})S(_{2})</td>
</tr>
<tr>
<td>ThOD (g O(_2)/g TS)</td>
<td>1.184*/1.158**</td>
<td>1.236</td>
</tr>
<tr>
<td>COD (g O(_2)/g TS)</td>
<td>1.145*/1.164**</td>
<td>1.246</td>
</tr>
</tbody>
</table>

* Using theoretical values.
** Using experimental values.

- pH: the values ranged from 6.8 to 8.2, in all cases to achieve an initial pH value between 7.0 and 7.8.
- Total solids (TS), Volatile solids (VS) and VS/TS: the solid content ranged from 15.0 gTS L\(^{-1}\) to 215.0 gTS L\(^{-1}\), while the organic content ranged from 6.3 gVS L\(^{-1}\) to 113.0 gVS L\(^{-1}\). VS/TS ranged from 42% to 86%.
- Concentration in BMP test at the start-up of the experiment (C\(_o\)): the initial concentration of cellular biomass ranged from 3.1 gVS L\(^{-1}\) to 56.5 gVS L\(^{-1}\). The average value was 13.5 gVS L\(^{-1}\).
- Time elapsed from sampling: the range was also wide, ranging from 1 d to 150 d. The average value was 19 d.

Substrates

Substrates selected for this interlaboratory study were characterized according to their relevant substance-specific properties and suitability for biodegradability (Table 3). Two main groups of substrates have been used for this research:

(i) Positive control substrates
- Starch soluble from potato (Sigma-Aldrich) to measure the amylase activity.
- Avicel® PH-101 cellulose (Sigma Aldrich) to measure the cellulase activity.
Table 4. Summary of overall experimental conditions reported by laboratories participants∗

<table>
<thead>
<tr>
<th>LAB</th>
<th>MS</th>
<th>Capacity (L)</th>
<th>T (◦C)</th>
<th>Stirring</th>
<th>pH/Alk Adj</th>
<th>MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GC</td>
<td>0.025</td>
<td>38</td>
<td>TC</td>
<td>No</td>
<td>N2-O2</td>
</tr>
<tr>
<td>2</td>
<td>Vol-wd</td>
<td>0.500</td>
<td>52</td>
<td>TC</td>
<td>No</td>
<td>N2-CO2</td>
</tr>
<tr>
<td>3</td>
<td>Vol-wd</td>
<td>0.080</td>
<td>36</td>
<td>TC</td>
<td>Ma-B</td>
<td>N2</td>
</tr>
<tr>
<td>4</td>
<td>Vol-wd</td>
<td>0.250</td>
<td>37</td>
<td>TWB</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>5</td>
<td>GC</td>
<td>0.500</td>
<td>37</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>6</td>
<td>Man-p</td>
<td>0.100</td>
<td>38</td>
<td>TC</td>
<td>Ma-B</td>
<td>N2</td>
</tr>
<tr>
<td>7</td>
<td>Vol-wd</td>
<td>0.250</td>
<td>35</td>
<td>TC</td>
<td>Ma-B</td>
<td>N2</td>
</tr>
<tr>
<td>8</td>
<td>Vol-wd</td>
<td>0.700</td>
<td>35</td>
<td>TWB</td>
<td>Ma-B</td>
<td>N2</td>
</tr>
<tr>
<td>9</td>
<td>Man-p</td>
<td>0.500</td>
<td>35</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>10</td>
<td>Vol-wd</td>
<td>0.400</td>
<td>35</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>11</td>
<td>Man-p</td>
<td>0.375</td>
<td>35</td>
<td>TWB</td>
<td>Au-C</td>
<td>N2</td>
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<tr>
<td>12</td>
<td>Vol-wd</td>
<td>0.700</td>
<td>35</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>13</td>
<td>Man-p</td>
<td>0.200</td>
<td>35</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
</tr>
<tr>
<td>14</td>
<td>Man-p</td>
<td>0.400</td>
<td>35</td>
<td>TC</td>
<td>Au-C</td>
<td>N2</td>
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<tr>
<td>15</td>
<td>Vol-wd</td>
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<td>35</td>
<td>TWB</td>
<td>Au-C</td>
<td>N2</td>
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<td>16</td>
<td>Vol-wd</td>
<td>0.150</td>
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<td>N2</td>
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<tr>
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<td>TC</td>
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<td>N2</td>
</tr>
<tr>
<td>18∗∗</td>
<td>Vol-gc</td>
<td>0.100</td>
<td>37</td>
<td>TWB</td>
<td>Ma-B</td>
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<tr>
<td>19∗∗</td>
<td>Vol-wd</td>
<td>0.750</td>
<td>35</td>
<td>TC</td>
<td>Ma-B</td>
<td>N2</td>
</tr>
</tbody>
</table>

* The information about terminology selected is included in Table 1.

** Data not considered for comparative purpose.

- Gelatine to bacteriological use (Panreac) as protein substrate to measure the proteinase activity.

(ii) Biomass material

The seed of the plant *Vigna radiata* known as mung bean (MB) was selected as biomass sample owing to its biodegradable nature and the novelty, because it had not previously been used (according to the literature) as substrate for BMP assays. The seeds were ground and sieved and used in powder form. The particle size of the material used in this assay ranged from 0.125 mm to 0.500 mm. Its organic composition (dry basis) includes mainly carbohydrates (72.4%) and protein (23.1%), with a low content of fat (1.5%). In addition the substrate presented low fiber content (5% of neutral detergent fiber-NDF and 4% of acid detergent fiber-ADF) and no lignin.

**Experimental conditions**

For this interlaboratory study, full details of experimental procedures such as gas measurement systems and operational conditions (physical, chemical and inoculum to substrate ratio – ISR) were reported by the participating laboratories and are compiled in Table 4.

**Gas measurement systems**

Gasometrical methods are the ones most frequently used for determining anaerobic biodegradability. In such methods, biogas/methane production can be quantified either manometrically or volumetrically. Also a gas chromatography (GC) technique can be used for this purpose.

For this interlaboratory study volumetric methods were used most (63%), followed by manometric methods (26.3%) and finally by GC methods (10.5%). Furthermore, all the participants based their biogas composition on GC analysis, except one laboratory (number 4) which measured the methane directly after CO2 removal by flushing the biogas through NaOH 2N solution.

**Physical operational conditions**

- Reactor capacity: a wide range of working volumes (W_VOL) was used, varying from 25 mL to 750 mL. The most often used capacities were 100 mL and 500 mL (three times each).
- Temperature: most participants used the mesophilic range, with temperature ranging from 35 °C to 41 °C. Exceptionally, one participant (lab number 2) also used a thermophilic temperature range (52 °C).
- Stirring: agitation of digesters can be carried out in a number of ways including manual shaking, magnetic stirrers, orbital...
shaking, etc. Also, the main factors affecting mixing strategy are the intensity and the duration. In this interlaboratory study, three participants used a static system, seven participants mixed manually and nine participants mixed using automatic devices.

- Time: the duration of the BMP ranged from 13 d to 87 d, with average value was 32 d.

**Chemical experimental conditions**

- Headspace gas (G<sub>0</sub>): different gases were reported as components of the headspace, such as N<sub>2</sub>, N<sub>2</sub>–CO<sub>2</sub>, mixtures, air (N<sub>2</sub>–O<sub>2</sub>) and He. In this interlaboratory study, pure N<sub>2</sub> was the most widely used headspace gas (63%).
- pH/alkalinity adjustment (pH/Alk Adj): batch tests must be carried out at pH values ranging from 7.0 to 7.8. The alkalinity controls the capacity of the system to neutralize acids and provides resistance to significant and rapid changes in pH; it is also known as 'buffering capacity'. A value of 2500 mg CaCO<sub>3</sub> L<sup>−1</sup> is considered to be normal for sewage sludge. A more desirable range of 2500–5000 mg CaCO<sub>3</sub> L<sup>−1</sup> provides a buffering capacity for which a much larger increase in VFA can be accommodated with a minimum drop in pH.<sup>9</sup>

In this interlaboratory study, 7 of 17 participants (41%) that reported appropriate data used different concentrations of NaHCO<sub>3</sub> to increase the buffer capacity of the system.

- Mineral medium (MM): it is well documented that all microbial-mediated processes require nutrients and trace elements (metals and vitamins) during organic biodegradation.<sup>9</sup> However, it is not clear if under the normal conditions of a BMP test sufficient nutrients are available from the sludge and/or organic substrate, or if additional supplements are necessary. In fact, 12 participants (71%) that reported appropriate data used different MM solutions to increase the performance of the test. Full details about the different minerals and concentrations were provided by participating laboratories, although these are not included in this manuscript.

**Inoculum to substrate ratio (ISR)**

Chudoba et al. clearly stated that ISR is one of the most important parameters in batch tests.<sup>19</sup> Unfortunately, many research papers do not report the ISR used in the experimental design. In addition, the units used (TS, VS or COD basis) must be clearly stated. In this interlaboratory study this parameter was considered crucial and it was fixed in advance by the interlaboratory study coordinator (VS basis). BMP determinations were established by highlighting the importance of using an adequate ISR to control the biodegradation process. The ISR can be low or high. Previous research work suggested the use of high ISR, >2.<sup>1,11,12</sup> Following the earlier suggested value, in this interlaboratory study an ISR of 2 was used for starch and cellulose. Taking into account that ammonia is an inhibitor of the anaerobic digestion process, the organic load for pure protein substrate (gelatine) was decreased to achieve an ISR of 3. For MB, two ISRs (2 and 1) were used to study the influence of this parameter on the BMP results.

**Operational procedure**

The operational procedure used in this interlaboratory study included six runs; three runs to evaluate the activity of the different inocula used and as quality control of the BMP tests; and two runs to determine the methane potential of mung bean, including the influence of ISR on the results. In addition, a blank control run was mandatory to consider the influence of background biogas production. Following the recommendations of various protocols related to BMP, triplicate determinations were carried out to evaluate the BMP tests. This is because the assay is a biological determination using inoculum from different sources (varying quality) and because the test material should also be relatively heterogeneous.

**Theoretical methane potential (BMP<sub>Th</sub>)**

The theoretical methane potential is widely used to predict the methane production of a specific organic substrate. It is frequently expressed as ml CH<sub>4</sub> at standard temperature and pressure (STP) conditions per amount of organic material added (VS or COD basis), although it can also be expressed per organic material removed. In the present research, the selected units used for expressing the methane potential were mainly ml CH<sub>4</sub> g<sup>−1</sup> VS<sub>sad</sub>. There are different ways to calculate this parameter:

(i) Traditionally BMP<sub>Th</sub> has been calculated when the atomic (AtC) or the organic fraction compositions (OFC) are known.<sup>9</sup>

- BMP<sub>ThAtC</sub> or BMP<sub>ThOFC</sub>: Empirical formulae (C<sub>a</sub>H<sub>b</sub>O<sub>c</sub>N<sub>d</sub>) can be designed from elemental analysis determination. Assuming the total stoichiometric conversion of the organic matter to methane and carbon dioxide using Buswell’s equation the methane yield can be calculated:<sup>13</sup>

\[
B_{\text{O} \rightarrow \text{ThAtC}} = \frac{[(a/2) + (b/8) - (c/4)] \cdot 22400}{12a + 16b + 16c} \quad (1)
\]

However, when proteins are present, ammonia and H<sub>2</sub>S are released and must be taken into consideration using Boyle’s equation:<sup>14</sup>

\[
B_{\text{O} \rightarrow \text{ThOFC}} = \frac{[(a/2) + (b/8) - (c/4) - (3d/8) - (e/4)] \cdot 22400}{12a + 16b + 16c + 14d + 32e} \quad (2)
\]

- BMP<sub>ThAtC</sub> or BMP<sub>ThOFC</sub>. If the organic fraction composition (lipids, proteins, and carbohydrates) is known, methane yield can be estimated using the following general equation:

\[
B_{\text{O} \rightarrow \text{ThOFC}} = 415\ \%\text{Carbohydrates} + 496\ \%\text{Proteins} + 1014\ \%\text{Lipid} \quad (3)
\]

where the different fractions must be quantified by analytical composition measurements of the organic matter. The coefficients in this equation are derived from stoichiometric conversion of model compounds representing average formulae for carbohydrates (C<sub>a</sub>H<sub>b</sub>O<sub>c</sub>N<sub>d</sub>S<sub>e</sub>), proteins (C<sub>f</sub>H<sub>g</sub>O<sub>j</sub>N<sub>k</sub>) and lipids (C<sub>L</sub>H<sub>l</sub>O<sub>m</sub>N<sub>n</sub>S<sub>p</sub>).

Recently, some authors have proposed more sophisticated multiple regression models to predict the methane yield of organic matter from their chemical composition.<sup>15–17</sup> (ii) COD analysis permits the calculation of BMP<sub>Th</sub>-<sub>AtC</sub>. Theoretically, 0.350 L of methane at STP or 0.395 L at 35 °C and 1 atm can be obtained from 1 g COD removed (COD<sub>rem</sub>).

- BMP<sub>ThCOD</sub> or BMP<sub>ThOFC</sub>. Unfortunately, directly measuring the COD of a solid waste is often thought to produce erroneous results.<sup>18</sup> However, a new recent interlaboratory test showed that the participation in proficiency tests hugely improved the precision and truth of results obtained.<sup>19</sup> Moreover, COD is necessary for real reactor design, helping to normalize the results independently of
VS fraction composition. To calculate the methane yield, the following equation can be applied:

\[ B_{o-\text{ThCOD}} = \text{VS}_{\text{added}} \cdot (g \text{ COD} / g \text{ VS}) \cdot 350 \]  

---

### BMPExpKIN or Bo

- **BMP**<sub>ThOD</sub> or **B**<sub>O-ThOD</sub>. The calculation of the theoretical oxygen demand (ThOD) based on atomic composition provides an attractive and easy alternative for obtaining the organic strength of some solid substrates. The empirical formula can also be used to calculate the estimated organic content, applying the following simple equation:

\[ \text{ThOD}(g \text{ O}_2 \cdot g^{-1} \text{VS}) = \frac{[(2a) + (b/2) - c - (3d/2)] \cdot 16}{(12a + b + 16c + 14d)} \]  

However, in this work ThOD has been calculated following the procedure suggested by ISO/DIS 10 707. Independently of how ThOD is calculated, the methane yield can be obtained by applying:

\[ B_{0-\text{ThOD}} = \text{VS}_{\text{added}} \cdot (g \text{ ThOD} / g \text{ VS}) \cdot 350 \]  

---

### Experimental methane potential (BMPExp)

The major disadvantage of the BMP test is the duration of the assays and the fact that it does not provide short-term results. Because of the time necessary to perform a BMP test, it would be better if methane yield could be predicted by any of the earlier proposed methods. However, experimental assays are necessary to accurately check the real methane potential of the organic materials. Two experimental methane potentials can be used:

1. **BMPExpCAL** or **B**<sub>o-Exp</sub>. This value is calculated (CAL) by dividing the net methane production under STP conditions by the weight of the sample added (VS or COD basis).
2. **BMPExpKIN** or **B**<sub>O</sub>. This derived value is defined as the ultimate methane yield or maximum value at infinite digestion time. It can be calculated by applying one of the different forms of the first-order kinetic (KIN) model, which is a simple and useful model that has been frequently applied to anaerobic digestion systems. However, this model does not predict the conditions for maximum biological activity and system failure. The basic equation is:

\[ \frac{dS}{dt} = -k \cdot S \]  

where \( k \) is the first-order kinetic constant (time \(^{-1}\)), \( t \) is the digestion time and \( S \) represents the biodegradable substrate concentration. As \( S \) is a difficult parameter to measure, it is preferable to derive the model by using the measurement of gas, which is much easier to determine:

\[ B = B_0 \cdot [1 - \exp(-k \cdot t)] \]  

where \( B \) (mLCH\(_4\) g\(^{-1}\) VS) is the cumulative methane yield, \( B_0 \) (mLCH\(_4\) g\(^{-1}\) VS) is the maximum or ultimate methane yield of the substrate, \( k \) (days\(^{-1}\)) is the first-order rate constant and \( t \) (d) is the time.

The results from the experimental methane yields can be fitted to monophasic or biphasic curves. The former have been recommended because only when the accumulation of intermediary compounds during anaerobic digestion is negligible can methane production be related to hydrolysis rate. The model is usually used to determine the extent and rate of biodegradation.

It is important to note that in the present research work \( B_o \) was not used in further analysis; however, when \( B_o \) differs from \( B_{o-Exp} \) by more than 10%, the kinetic model cannot be used to explain the data obtained because then, experimental data does not fit the proposed model (Equation (8)), and \( k \) is not valid.

### Biodegradability based on methane yield (BDCH4)

The experimental methane yield can be used to calculate the level of anaerobic biodegradability under the defined test conditions in comparison with its theoretical value, as follows:

\[ \text{BDCH4} (\%) = \frac{B_{o-Exp} / B_{o-Th}}{100} \]  

When the anaerobic biodegradability of the organic material is calculated from the methane conversion efficiency according to the above equation, it can be considered that the main organic matter removed is converted into methane, but some defined amount of the organic matter is used for growth of the microorganisms and to maintain cellular metabolism. This amount cannot be measured directly but needs to be estimated. It is known from practical experience that about 5–15% of the organic matter removed is consumed in the generation of new microbial biomass. However, Scherer et al. obtained a lower value (3%) in batch assays of spent grains from breweries by measuring DNA. This means that to find the real degree of biodegradation, the value obtained from experimental data should be increased by the value of this cellular yield.

On the other hand, considering the biodegradability nature of the substrates utilized for this interlaboratory test, the results reported with BDCH4 < 70% (methane production basis) were considered as outliers or not valid data.

### Analytical methods

Standard environmental and feedstuff analytical procedures were used to characterize the inocula and substrates. These analyses were performed in duplicate or triplicate and included the following parameters:

- Moisture, TS-dry matter and VS-organic matter were determined according to the APHA Standard Methods 2540B and 2540E.
- Total chemical oxygen demand (COD) was determined using the reported method proposed by Raposo et al.
- The fat content was extracted from a dried sample with hexane, using a Soxhlet system.
- The total protein content was determined by multiplying the value of this cellular yield. This means that to find the real degree of biodegradation, the value obtained from experimental data should be increased by the value of this cellular yield.
- The total carbohydrate content was determined by multiplying the difference between total Kjeldahl nitrogen (TKN) and ammonia by 5.5. To determine TKN the procedure reported by Raposo et al. was used. Ammonia was determined according to the APHA Standard Methods 4500B and E.
- The total protein content was determined by multiplying the difference between total Kjeldahl nitrogen (TKN) and ammonia by 5.5. To determine TKN the procedure reported by Raposo et al. was used.
- Ammonia was determined according to the APHA Standard Methods 4500B and E.
- The total carbohydrate content was determined by multiplying the difference between total Kjeldahl nitrogen (TKN) and ammonia by 5.5. To determine TKN the procedure reported by Raposo et al. was used.
- Ammonia was determined according to the APHA Standard Methods 4500B and E.
- The total protein content was determined by multiplying the difference between total Kjeldahl nitrogen (TKN) and ammonia by 5.5. To determine TKN the procedure reported by Raposo et al. was used.
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- Ammonia was determined according to the APHA Standard Methods 4500B and E.
RESULTS AND DISCUSSION
BMP extent: specific methane yield and biodegradability
Figures 1 and 2 show the data reported by participating laboratories, including detailed information about theoretical values and valid data excluding outliers. Table 5 summarizes the results of methane production obtained during the course of experiments for each substrate, including methane yield, and the associate methane conversion efficiency or anaerobic biodegradability. This table can be evaluated considering two approaches: all the data or only data without outliers. Results excluding outliers improved the performance of the test. As a general trend, the results from valid data proportioned higher values of methane yield, precision (lower reproducibility relative standard deviation – RSDR) and anaerobic biodegradability. It is important to note that the average precision for all the substrates assayed was around 10%. This is better than the 25% reported by the previous interlaboratory test.6

Starch
The theoretical methane yield ($B_0$−ThO) calculated from the elemental composition was 414 mLCH$_4$ g$^{-1}$ VS. The experimental methane yields ($B_{0−Exp}$) reported at the end of assays were substantially different, ranging from 126 ± 6 mLCH$_4$ g$^{-1}$ VS$_{added}$ to 417 ± 15 mLCH$_4$ g$^{-1}$ VS$_{added}$, with an average value of 320 ± 77 mLCH$_4$ g$^{-1}$ VS$_{added}$. When this value is compared with the stoichiometric methane yield, the BDCH$_4$ was 77 ± 19%. However, when outliers (four) were deleted, the reported value was more precise. The $B_{0−Exp}$ value ranged from 293 ± 6 mLCH$_4$ g$^{-1}$ VS$_{added}$ to 417 ± 15 mLCH$_4$ g$^{-1}$ VS$_{added}$, with an average value of 350 ± 33 mLCH$_4$ g$^{-1}$ VS$_{added}$, which assumed higher values of precision (RSDR 9%) and BDCH$_4$ (85 ± 8%). Assuming that this substrate can be fully degraded, the average amount of organic matter used for the growth of new cells and for cell metabolism calculated by subtraction was around 15%.

Literature data related to anaerobic biodegradability of starch is scarce. Hansen et al.3 studied the repeatability and reproducibility of BMP tests on the basis of seven series of triplicates using a thermophilic sludge treating mainly manure mixed with other

Figure 1. Methane yield reported by participants using solid positive substrates: (a) starch; (b) cellulose; (c) gelatine.

Figure 2. Methane yield reported by participants using mung bean as substrate: (a) Mung bean (ISR of 2); (b) Mung bean (ISR of 1).
organic wastes. They reported a similar methane yield value of 348 mL CH₄ g⁻¹ VSadded.

Cellulose

The value of BDCH₄ for this carbohydrate was of the same order of magnitude as that calculated for starch. The experimental data reported were also similar for both carbohydrates. The B₀–Exp values reported at the end of assays were more precise although also substantially different, ranging from 175 ± 8 mL CH₄ g⁻¹ VSadded to 412 ± 8 mL CH₄ g⁻¹ VSadded, with an average value of 340 ± 52 mL CH₄ g⁻¹ VSadded. When this value is compared with the stoichiometric methane yield, the BDCH₄ was 82 ± 13%. However, when outliers (three) were deleted the values of B₀–Exp ranged from 303–412 mL CH₄ g⁻¹ VSadded, with an average value of 350 ± 29 mL CH₄ g⁻¹ VSadded, which assumed a higher precision (RSD 8%) and BDCH₄ (85 ± 7%). Similarly to the earlier substrate, the average amount of organic matter used to form new cells and cell metabolism was also around 15%.

Cellulose has frequently been used as a BMP reference substrate, and similar methane yields have been reported.1,3,25,31,32

Gelatine

The value of BDCH₄ for this proteinaceous substrate calculated from the elemental composition was 433 mL CH₄ g⁻¹ VS. The B₀–Exp values reported at the end of assays were varied, ranging from 124 ± 3 mL CH₄ g⁻¹ VSadded to 480 ± 19 mL CH₄ g⁻¹ VSadded, with an average value of 300 ± 110 mL CH₄ g⁻¹ VSadded. When this value is compared with the stoichiometric methane yield, the BDCH₄ is 69±26%. This low biodegradability can be explained considering that degradation of the protein should be inhibited due to the accumulation of intermediates (VFA and free ammonia).9 Hansen et al.3 reported the same problem of inhibition when gelatine was selected as proteinaceous substrate for anaerobic digestion. However, when outliers (nine) were deleted, the reported value was more precise (RSD 11%), ranging from 310 ± 6 mL CH₄ g⁻¹ VSadded to 433 ± 17 mL CH₄ g⁻¹ VSadded, with an average value of 380 ± 42 mL CH₄ g⁻¹ VSadded, which assumed higher BDCH₄ (88 ± 9%).

In this case, the average amount of organic matter used to form new cells and cell metabolism should be around 12%.

Mung bean

The theoretical methane yield values for MB using both methods (THOD and THOFC) ranged from 434 mL CH₄ g⁻¹ VSadded to 443 mL CH₄ g⁻¹ VSadded, respectively. Results from B₀–THOFC deviated more from the rest of the theoretical values, as was previously reported.1,3 In this interlaboratory study and for comparison purposes, the value of B₀–THOD was considered to be the theoretical methane yield.

The B₀–Exp values reported at the end of assays for ISR 2 and 1 ranged from 189 ± 23 mL CH₄ g⁻¹ VSadded to 447 ± 13 mL CH₄ g⁻¹ VSadded and from 170 ± 6 mL CH₄ g⁻¹ VSadded to 437 ± 17 mL CH₄ g⁻¹ VSadded, with average values of 340 ± 63 mL CH₄ g⁻¹ VSadded and 330 ± 78 mL CH₄ g⁻¹ VSadded, respectively. When these average values are compared with the stoichiometric methane yield, the BDCH₄ for ISR 2 and 1 were 78 ± 15% and 76 ± 18%, respectively. However, when outliers (five and six) were deleted, the B₀–Exp for ISR of 2 and 1 ranged from 322 ± 9 mL CH₄ g⁻¹ VSadded to 447 ± 11 mL CH₄ g⁻¹ VSadded and from 330 ± 12 mL CH₄ g⁻¹ VSadded to 437 ± 11 mL CH₄ g⁻¹ VSadded, with average values of 370 ± 36 mL CH₄ g⁻¹ VSadded and 370 ± 35 mL CH₄ g⁻¹ VSadded, respectively. These similar average values proportioned the same value of BDCH₄ (85 ± 8%). Following the same criterion of fully biodegradable substrates, the average amount of organic matter used to form new cells and cell metabolism was around 15%.

For this substrate it is important to note that:

- The experimental values of BMP were similar for both ISRs, and therefore, the methane yield was not at all dependent on the ISR.
- The results of methane and cellular yields were in agreement with the expected values, considering, on one hand the previous values reported for carbohydrates and proteinaceous substrates, and on the other hand the organic fraction composition of MB in terms of carbohydrates and protein and no lignin content.
Table 6. BMP rate: summary of overall results obtained by participating labs

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total data</th>
<th>Selected data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K (d⁻¹)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSDₚ (%)</td>
<td></td>
</tr>
<tr>
<td>Starch (ISR 2)</td>
<td>0.24 ± 0.15</td>
<td>63</td>
</tr>
<tr>
<td>Cellulose (ISR 2)</td>
<td>0.21 ± 0.14</td>
<td>67</td>
</tr>
<tr>
<td>Gelatine (ISR 3)</td>
<td>0.34 ± 0.23</td>
<td>68</td>
</tr>
<tr>
<td>Mung Bean (ISR 2)</td>
<td>0.30 ± 0.17</td>
<td>57</td>
</tr>
<tr>
<td>Mung Bean (ISR 1)</td>
<td>0.21 ± 0.13</td>
<td>62</td>
</tr>
</tbody>
</table>

RSDₚ: Reproducibility Relative Standard Deviation.

**BMP rate: first-order rate constant (k)**

Kinetic studies are also useful to understand the mechanism of anaerobic biodegradation, including inhibition of the process. Conventionally, the rate of the anaerobic digestion process can be evaluated using the methane production values from BMP data.

Table 6 shows the values corresponding to k. As a general trend, this parameter showed very low precision (RSDₚ of 55–70%), and this parameter was only slightly affected by deletion of invalid data. Regarding the outliers, two conditions (BDₕₗₜ ≥70% and 0.9–1.1 Bₒ ≈ Bₒ·Exp) were considered as criteria to select valid data. The number of full outliers was 5, 5, 10, 7 and 8 for starch, cellulose, gelatine, MB 2 and MB 1, respectively.

The highest rates of methane production were reported by the participating laboratory which used thermophilic sludges. The kinetic constant of methane production from selected substrates ranged from 0.2–0.3 d⁻¹. The data obtained in this study were higher than the values of 0.016–0.125 d⁻¹ reported by Gunaseelan, using more than fifty fruits and vegetable wastes as substrates.²

**Starch, cellulose and gelatine**

The use of the raw experimental data for starch, cellulose and gelatine proportioned average rate constants of 0.24 ± 0.15 d⁻¹, 0.21 ± 0.14 d⁻¹ and 0.34 ± 0.23 d⁻¹, respectively. When using only the selected experimental data (removing outliers) the values were 0.24 ± 0.14 d⁻¹, 0.23 ± 0.15 d⁻¹ and 0.27 ± 0.13 d⁻¹, respectively. As expected, the average values for both carbohydrates were very similar. On the other hand, the average value for gelatine was slightly higher, probably due to the higher ISR selected for this substrate to avoid inhibition by accumulation of intermediate compounds.

Previous research work carried out using cellulose as reference substrate proportioned a wide range of values: 0.14–0.18 ± 0.02 d⁻¹, 0.039 ± 0.04 d⁻¹, 0.247 ± 0.020 d⁻¹ and 0.090–0.145 ± 0.015 d⁻¹.²⁻⁵⁻¹³⁻¹¹

**Mung bean**

The use of the raw experimental data for ISR 2 and 1 proportioned two different average rate constant values of 0.30 ± 0.17 d⁻¹ and 0.21 ± 0.13 d⁻¹, respectively. When using only the selected experimental data, the values were 0.31 ± 0.17 d⁻¹ and 0.23 ± 0.13 d⁻¹, respectively. As can be seen, for this substrate the rate constant was affected by the ISR. The lower ISR showed an inhibition phenomenon with increase in the substrate concentration, achieving a decrease in rate constant of around 26%. It can be concluded that for future harmonization of results working at high ISR is the way to obtain reproducible kinetic constants.

**BMP results: influence of different factors**

In this first BMP interlaboratory study, it was not possible for all the experiments to be designed by factorial planning to enable further analysis of the results obtained. Therefore, the main objective of this interlaboratory test was not to evaluate the influence of experimental factors on the BMP results. However, the results reported have been assessed in a way enabling a qualitative description of the different experimental factors affecting the anaerobic biodegradability and the final results obtained.

**Influence of inoculum**

Theoretically, this factor is one of the most important for the BMP test, with a clear influence on the results obtained. The results reported were analysed in terms of three different characteristics of the inocula utilized: concentration, time from sampling and source.

1. **Concentration.** Practical experience has demonstrated that the level of inoculum concentration affects the rate of biodegradation. Normally, the higher the inoculum concentration, the faster the anaerobic conversion of the substrate will occur, and the quicker the test will be completed. However, in this interlaboratory study the concentration of microorganisms was adjusted considering the concentration of the organic substrates until the desired ISR was reached. Below this ISR, the extents and rates of BMP reported by different participants showed high variability, which were totally independent of the inoculum concentration.

2. **Time elapsed from sampling.** The effect of sludge storage on the BMP test is not well reported in the literature. For micro-pollutant compounds, sludge storage had no significant effect on
the extent of degradation, but the duration of lag times could be affected, and, therefore, substrates could be degraded more slowly.\(^{24}\)

Based on reported data no clear statements can be made about the influence of this factor on BMP test extent and rate.

(3) Source. Different sources of inoculum could lead to different biodegradability extent and rate values as a consequence of the different levels of microbial population and diversity.\(^ {25,36}\)

To evaluate this factor, the results reported for the different participants in the interlaboratory study were classified into two sets of data, one from MWTPs and one from other sources. There was no significant difference in either of the parameters evaluated, the extent and the rate of the BMP test.

Influence of experimental factors

The results were also analysed considering the physical and chemical operating conditions selected.

(4) Working volume. The total volume of the reactor used for batch tests is inversely related to the number of replicate samples that could be tested at the same time using a fixed amount of sludge and substrate. The nature of the substrate can also influence the selection of the ideal volume, because the more homogeneous the material, the smaller the volume of reactor required to determine methane potential more accurately.

In this interlaboratory study, the influence of working volume on BMP extent and rate was totally insignificant.

(5) Temperature. Methane can be formed over a wide range of temperatures; however, anaerobic digestion processes depend strongly on temperature. The majority of data in the literature related to BMP assays refers to experiments performed at mesophilic temperatures, with only a few at thermophilic temperatures.

To study the influence of this parameter, the results reported by the participating laboratory using mesophilic and thermophilic sludges were utilized. The methane yields obtained were not significantly different between thermophilic wet and mesophilic sludges, while the values from the thermophilic dry sludge were slightly higher. In contrast, the rate constants of thermophilic sludges were very similar and both differed significantly from the rate constants of mesophilic sludges.

Previously, Veeken and Hamelers studied the anaerobic biodegradability of six selected components of biowaste as a function of temperatures in the mesophilic range (20 °C, 30 °C and 40 °C). They reported that the extent of anaerobic biodegradability did not depend on temperature, while the rate constants increased at higher temperatures.\(^ {22}\)

(6) Stirring. The influence of mixing on the BMP test has not been reported previously. The stirring process is essential for the rate of gas production, whereas it is independent of the extent of degradation.\(^ {57}\)

The results reported for the different participants were classified into two sets of data, one for continuous automatic stirring and one for the rest (static and manual stirring). Methane yields achieved in this interlaboratory study were comparable independently of the mixing. On the other hand, values of rate constant for the substrates selected were inconsistent, sometimes equal, sometimes higher in a stirred system and sometimes higher in static and manually stirred systems. The same lack of concrete relationship between mixing and anaerobic biodegradability was reported previously when using livestock wastes as substrate.\(^ {38}\)

(7) Headspace gas. No previous research work has been carried out to study the influence of headspace gas on anaerobic biodegradation in batch mode. The experimental results obtained using pure N\(_2\) were not significantly different from those obtained with other gases.

(8) pH/Alkalinity adjustment and MM used. Results reported can be evaluated only from a restricted point of view of additional buffer/MM addition or no addition, and methane yields and rates of methane production were very similar. To analyse the influence of these factors with total accuracy, the initial pH and total alkalinity concentration, and the composition and concentration of nutrients existing throughout the BMP test system, must be obtained and reported by participating laboratories.

CONCLUSIONS

The results obtained during this interlaboratory study enabled the following conclusions to be drawn regarding the BMP test:

- Most of the BMP yield results reported by the participants were satisfactory, with a low number of outliers except for gelatine.
- The influence of inocula and experimental factors on the extents of anaerobic biodegradation were almost insignificant, while the rates differed significantly according to the experimental approaches.
- The precision (RSD\(_3\)) of the data reported for BMP extents and rates were around 10% and 55–70%, respectively.
- The ISR is a critical factor for the BMP test, with crucial influence on the kinetics, and variable influence on the yield of the BMP test depending on the biodegradable nature of the substrate.

ACKNOWLEDGEMENTS

The coordinator of this first BMP interlaboratory study gratefully acknowledges the interest shown by all the participating laboratories and their excellent contribution. The extra time-consuming tasks undertaken by the different researchers who participated in the interlaboratory study is also highly appreciated. Without their efforts this article would not have been possible.

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