APPLICATION NOTE

M-M-002-2021/A2

# BMP – Biochemical Methane Potential – determination in cellulose

## with RESPIROMETRIC Sensor System

Reference: **UNI/TS 11703** Method for the assessment of potential production of methane from anaerobic digestion in wet conditions - Matrix into foodstuffs.

Tested with **RESPIROMETRIC Sensor System MAXI,** including **Wireless Databox™** (Code SA10200156) and **Incubator FOC 200E** (Code F10300542)


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# BMP DETERMINATION IN CELLULOSE MANOMETRIC METHOD

## Introduction

Digestion or anaerobic degradation is a biochemical process which, in the absence of oxygen, leads to the degradation of complex organic substances with the production of gas, consisting of methane and carbon dioxide. This process is operated by specific microorganisms. This process, in addition to having a decisive role in the purification treatments, is also exploited to produce renewable energy, in plants called biogas plants. This biogas is produced from substrates of waste biomass such as, animal manure, cellulose, organic waste, etc.

Standardized protocols require inclusion of a positive control with a known BMP to check the experimental setup and execution, as well as the performance of the inoculum. Only if the BMP of the positive control is within the expected range is the entire test validated. It is advisable to periodically carry out control tests of the instrumentation and of the procedures adopted thanks to BMP tests on reference substrates such as microcrystalline cellulose.

## BMP Test using Respirometric Sensor System

VELP RESPIROMETRIC Sensor System offers an easy-to-use method for monitoring and quantifying the gases produced during the anaerobic degradation of organic material; processes widely applied both in the treatment of waste water and, more generally, for the production of renewable energy in the form of biogas.

The measurement of BMP (Biochemical Methane Potential) takes place by putting a known quantity of the substrate, with an inoculation of anaerobic microorganisms and constant conditions of temperature (i.e.: 35 °C) and stirring. The bottle is initially brought into anaerobic conditions thanks to the flushing of a mixture of anoxic inert gas. Once the test has started, as the degradation process proceeds, biogas will be produced in the bottle which can be quantified in terms of overpressure generated. $CO_2$ produced is adsorbed by Alkaly (KOH), thus the result is usually expressed in terms of mlCH4 @STP/gVS, which represents the volume of methane produced per unit of organic substance quantified in terms of volatile solids.

## Cellulose Sample

According to the reference norm UNI/TS 11703, the expected value of cellulose is between 251 - 419 NmLCH4/gVS. Recently, a protocol resulting from an international study on BMP tests requires mean cellulose BMP between 340 and 395 NmLCH4/gVS.

## Reagents required

Absorption of carbon dioxide:

- Potassium Hydroxide (KOH) in flakes, commercial grade or non-deliquescent soda lime, 1.0-1.7 mm granules.

To correct the pH of the mixture (inocula, substrate, water and nutrients):

- Hydrochloric acid (HCl), 1 N solution.
- Sodium hydroxide (NaOH), 1 N solution, or sodium bicarbonate ($NaHCO_3$), powder.

To correct the pH of the inocula:

- Sodium bicarbonate ($NaHCO_3$), powder.


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Solutions of nutrients and trace elements

*Solution A*

- 2.7g Anhydrous potassium dihydrogen phosphate ($KH_2PO_4$)
- 11.2g Disodium hydrogen phosphate dodecahydrate $Na_2HPO_4 \cdot 12H2O$
- 5.3g Ammonium chloride ($NH_4$ Cl)

Dissolve the above nutrients and trace elements in 0.5 L of distilled water, using a volumetric flask

*Solution B*

- 0.75g Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$),
- 1.0g Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$),
- 0.2g Iron (II) chloride tetrahydrate $FeCl_2 \cdot 4H_2O$

Dissolve the above nutrients and trace elements in 0.5 L of distilled water, using a volumetric flask

*Solution C*

- 0.05g Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)
- 0.005g Boric acid ($H_3BO_3$)
- 0.005g Zinc chloride ($ZnCl_2$)
- 0.003g Copper (II) chloride ($CuCl_2$)
- 0.001g Disodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$)
- 0.1g Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)
- 0.01g Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$)
- 0.005g Disodium selenite ($Na_2SeO_3$)

Dissolve the above nutrients and trace elements in 1 L of distilled water, using a volumetric flask

Deoxygenated gas (e.g., nitrogen or argon).

## Microbial inocula Preparation

The choice of the inocula is of fundamental importance for the success of the test and for the representativeness of the result. It can be taken from an operational anaerobic digester; for example, from a traditional mesophilic digester of the sludge line of a civil or cooperative wastewater purifier. It is also useful to provide a stabilization phase of the inocula, by incubation in endogenous conditions for a few days (usually 5-7), which guarantees the consumption of any organic residues that are not completely degraded.

The cellulose should be stored at room temperature and in a dry environment or a desiccator.

## Analysis Preparation

Prepare the nutrients solutions A, B and C. The Italian regulation UNI / TS 11703 - 2018 suggests the adoption of the following nutrient solutions to be added, in each test bottle of BMP:

- Solutions A and B in the ratio of 5% of the final test volume (working volume)
- Solution C in the ratio of 1% of the final test volume (working volume)

## Calculate the Volume for the analysis

In order to define the quantity of sample, cellulose, inoculum, tap water and nutrients, the software RESPIROSoft™ in Analytics / BMP Calculator, gives the indications for the request volumes, after insert the necessary parameters for the method:

| | |
|---|---|
| IS, ratio desired | 2 |
| Cs, Sample concentration (gSV / L) | 916.7 |
| Ci, Concentration of inoculated sludge (gSV / L) | 17.7 |
| Cmax, Maximum conc. of volatile solids in the final mix (gSV / L) | 14 |
| d, Expected degradability | 0.627 |
| COD / SV ratio of the sample (gCOD / gSV) r | 1.45 |
| % Methane expected p | 0.99 |
| Maximum overpressure at the end of the test (Δp / p) max | 0.5 |
| Vw Total volume of the bottle (ml) | 1100 |
| Concentration factor of sol. Trace elements | 11 |
| Inoculum density | 1 |
| Sample density | 1 |

| | Blank | Cellulose |
|---|---|---|
| M substrate [g] | - | 1.4 |
| M inocula [g] | 145 | 145 |
| V nutrients [mL] | Solution A 13.8<br>Solution B 13.89<br>Solution C 2.8 | Solution A 13.8<br>Solution B 13.89<br>Solution C 2.8 |
| V_H20 (mL) | 99.8 | 98.4 |
| V_Total (mL) | 275 | 275 |


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## Analysis Procedure

1. Set the incubator temperature to the desired value, e.g., 35°C.
2. Connect the Wireless DataBox™ and fill in the RESPIROSoft™ software database
3. Introduce a magnetic stir bar into each bottle
4. Weigh precise the mass of well mixed inoculum in a beaker
5. Add the nutrients solutions by cylinder
6. Add the substrate, cellulose, (except for the “blank” bottles where this volume is replaced by an equivalent volume of dilution water).
7. Check the pH of the mixture and, if lower than 7.3, adjust it (when removing the pH probe from the mix, be careful not to remove too much material from the bottle).
8. Flush the headspace with nitrogen (or argon) deoxygenated gas: according to the flow for 5 to 10 minutes
9. Introduce about 20 g of KOH into the alkali collector, below the holes.
10. Screw the RESPIROMETRIC Sensors onto each bottle and tighten.
11. Place the system in the thermostat at the desired temperature, without stirring.
12. Wait about an hour for the liquid mass present in the bottle to reach the test temperature, and then to develop the typical initial pressure increase, due to the vapor pressure. To remove this additional pressure, it is advisable, after one hour, to vent the test bottles to atmospheric pressure (by briefly loosening the ring nut).
13. The system is ready to start the BMP measurement. Press START and start stirring.

## Results of BMP determination in cellulose

The software RESPIROSoft™ automatically determine the BMP value, after the 30 days analysis.

| Sample Name | P initial (hPa) | P final (hPa) | BMP [NmL/gSV] |
|---|---|---|---|
| Cellulose | 1013 | 1680 | 368 |
| Cellulose | 1013 | 1671 | 363 |
| Cellulose | 1013 | 1666 | 358 |
| Cellulose | 1013 | 1673 | 362 |
| | | **Average ± sd** | **363 ± 4%** |
| | | **RSD%** | **1.2%** |
| Expected value: 251- 419 NmL/gSV | | | |


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## Conclusions

RESPIROMETRIC Sensor System Maxi is the innovative and extremely reliable solution for BMP analysis, as can be seen from the value of the RSD%, 1.2%, which is much less than the limit value declared by the regulations, which is 5%.

The obtained results, 363 Nml/gSV ± 4%, are in the expected range 251-419 Nml/gSV in accordance to the Italian Reference UNI / TS 11703.

Thanks to the innovative wireless technology, the sensor transmits the BMP value to the Wireless Databox™, based on the data transmission frequency set before starting the analysis.

Results are then displayed from the intuitive RESPIROSoft™ the optimal solution for data management and comparison of the results.

Connect the RESPIROMETRIC Sensor to the exclusive VELP Ermes Cloud Platform to improve your laboratory experience.


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