
BMP - Biochemical Methane Potential - determination in Sludge with Respirometric Sensor System

Reference: **UNI/TS 11703** 2018 Method for the assessment of potential production of methane from anaerobic digestion in wet conditions - Matrix into foodstuffs

VDI 4630 - Fermentation of organic materials. Characterization of the substrate, sampling, collection of material data, fermentation tests

Tested with **Respirometric Sensor System 6 MAXI**, including **Wireless Databox™** (Code SA10200156) and **Incubator FOC 200E Connect** (Code F10300542)



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, sludge, organic waste, etc.

The possibility of using a substrate for anaerobic digestion is given by its biodegradability. The maximum methane production achievable by anaerobic degradation of a substance is defined as BMP, Biochemical Methane Potential. It gives an indication of the amount of energy that can be obtained by anaerobically from an organic substance, such as sludge.

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. CO₂ 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.

Sludge Sample

Sludge from municipal sewage plant, expected value between 70 - 450 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 (NaHCO₃), powder.

To correct the pH of the inocula:

- Sodium bicarbonate (NaHCO₃), powder.

Solutions of nutrients and trace elements

Solution A

- 2.7g Anhydrous potassium dihydrogen phosphate (KH₂PO₄)
- 11.2g Disodium hydrogen phosphate dodecahydrate Na₂HPO₄ • 12H₂O
- 5.3g Ammonium chloride (NH₄ 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 ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$),
- 1.0g Magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$),
- 0.2g Iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$)

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 ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$)
- 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 ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)
- 0.1g Cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$)
- 0.01g Nickel chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$)
- 0.005g Disodium selenite (Na_2SeO_3)

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

- Distilled water up to in 1 l of distilled water, using a volumetric flask

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

Microbial inocula and Sample Preparation

The inocula, mesophilic anaerobic sludge should be taken from a digester, and stored at 35°C under anaerobic conditions for 5 days. It always is advisable to reactivate the biomass present in the inoculum, placing the inoculum sample in a thermostat at the test temperature for 24 hours.

The sludge should be collected in a clean container, stored at a temperature below 4°C; before starting tests the sample should be kept at room temperature for 1h.

Analysis Preparation

Check the pH for the inocula and the sludge. If lower than 7.3, adjust it, for example with sodium bicarbonate (NaHCO_3). Prepare a single batch of water by running tap water for a few minutes
Prepare the nutrients solutions A, B and C.

Calculate the Volume for the analysis

In order to define the quantity of sample, sludge, inocula, 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)	40.1
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 ($\Delta p / p$) max	0.5
Vw Total volume of the bottle (ml)	1100
Concentration factor of solutions A+B+C (ml/ml)	11
Inoculum density	1
Sample density	1

	Blank	Sludge
M substrate [g]	-	32
M inocula [g]	145	145
V nutrients [mL]	Solution A 13.8	Solution A 13.8
	Solution B 13.89	Solution B 13.89
	Solution C 2.8	Solution C 2.8
V_H2O (mL)	99.8	67.8
V_Total (mL)	275	275

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 nutrient solutions by cylinder
Add the substrate, sludge, (except for the “blank” bottles where this volume is replaced by an equivalent volume of dilution water).

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).

6. Flush the headspace with nitrogen (or argon) deoxygenated gas: according to the flow for 5 to 10 minutes
7. Introduce about 20 g of KOH into the alkali collector, below the holes
8. Screw the RESPIROMETRIC Sensors onto each bottle and tighten.
9. Place the system in the thermostat at the desired temperature, without stirring
10. 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).
11. The system is ready to start the BMP measurement. Press START and start stirring.

Results of BMP determination in Sludge

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

Sample Name	P initial (hPa)	P final (hPa)	BMP [NmL/gSV]
Sludge	1013	1294	219
Sludge	1013	1351	266
Sludge	1013	1313	231
Sludge	1013	1297	218
		Average ± sd	233 ± 22%
		RSD%	9.6%
Expected value: 70- 450 NmL/gSV			

Conclusions

[RESPIROMETRIC Sensor System Maxi](#) is the innovative and extremely reliable solution for BMP analysis.

All the obtained results, are accurate and precise. The BMP value is 233 Nml / gSV \pm 22%, is in the expected range (70- 450 Nml / gSV).

The RSD is lower than 10% as requested by the UNI/TS 11703. A cellulose sample has been analyzed to verify the system. The obtained result is 365 Nml / gSV \pm 18% in accordance to the expected value in the UNI / TS 11703, value in the range 251 and 419 Nml / gSV.

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.

