Suggested method for mesophilic inoculum acclimation to BMP assay

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Abstract: The biochemical methane potential (BMP) index from agricultural, agroindustrial and livestock wastes it is essential for bioreactor's design and to enable the biogas plants. For BMP standard procedure is necessary to use an inoculum stable, acclimated to the test conditions and adapted to the substrate. In general, inoculum seed from co-digestion biogas plant is used, but this is not practicable in countries like Brazil. This work reports a laboratory case study to produce and maintain a mesophilic anaerobic inoculum for BMP test according VDI 4630. This manuscript suggests a mesophilic acclimation methodology of a mixture made with different inoculum seeds (fresh dairy manure, UASB sludge from swine manure treatment and UASB sludge from food industry). The laboratory acclimated inoculum show efficient biogas yield for cellulose standard. However, in an interlaboratory study, show very similar results for biogas yield and BMP for two reference samples tested.

Keywords: acclimation, agricultural waste, BMP, inoculum, mesophilic, VDI 4630.

Introduction

Brazil has a high potential of substrates to be used for biogas production due to the huge amounts of wastes originating from agriculture, livestock and food industry (SCGAS, 2010). For this potential use, first of all, it is necessary to know some data for the engineering of biogas plants, such as: the waste amount, substrate quality and the quantity of biogas or methane yield are essential to bioreactor's design and calculation of profitability to enable the biogas plants (Deublein and Steinhauser, 2011).

However, the kinetic information about degradation and the specific methane recovery for some Brazilians residues is poor available. Data base from other countries may differ due to different waste characteristics and different process production.

Besides, the data available for Biochemical Methane Potential (BMP) of Brazilian wastes don't use a standardized methodology (i.e. biogas or methane yield not expressed in normalized volume or expressed in different basis [eg. COD, TS or VS], or not expressed in a specific basis) which makes the data use or inter comparison quite difficult. In the international literature there are standard procedures to obtain the specific BMP where the anaerobic digestion is performed under controlled and monitored conditions. In the literature could found protocol strategies for BMP protocol (Angelidaki et al., 2009). Some normative standard methods for BMP assay are described in DIN 38414-8 (1985), ISO 11734 (1995), ASTM E2170-01(2013) and VDI 4630 (2014). In all methods is necessary to use an inoculum acclimated to the test conditions or adapted to the substrate which will be evaluated. Unsufficient activity or quality of the inoculum can lead to wrong results. Therefore is important to improve BMP methods. Usually, inoculum from co-digestion is preferred because it may contain a good variety of microorganisms or better ecology. In Brazil there is no large availability of facilities practicing co-digestion and this means a barrier to obtain a profitable inoculum for BMP batch test.

This work reports a laboratory case study to produce and maintain a mesophilic anaerobic inoculum for BMP test according VDI 4630. This manuscript suggests a mesophilic acclimation methodology of a mixture made with different inoculum seeds and its verification by intra and interlaboratory study.

Material and Methods

Inoculum production: At the Embrapa's Laboratory of Biogas Studies10 litre of fresh dairy manure, 10 litre of UASB inoculum from Embrapa's swine manure treatment plant and 10 litre of UASB inoculum from wastewater treatment plant of a local protein industry were mixed in the laboratory. The mixture was conducted in a 40 litre capacity PVC CSTR, under 37 °C, and fed during 7 days with loading rate of 0.3 $kg_{VS}(m^3 \cdot day)^{-1}$ with substrate mixture according Table 1 and kept without feeding for next 7 days until start the BMP batch test.

Table 1 – Substrate proportion used to feed the inoculum.

Substrate	**% ($w \cdot w^{-1}$) of VS loading rate**
Dried grass	30
Milk powder	25
Swine feed (maize basis)	30
Vegetable oil	15

Inoculum analysis: Once a week a sample of laboratory inoculum was collected to verify the solid content (total solids [TS] and volatile solids [VS]) by gravimetry, pH by potentiometry and acidity/alkalinity ratio by titration (Buchauer, 1998; APHA, 2012).

Biogas analysis: The biogas was collected from the eudiometer tube using bag samplers (plastic/aluminum foil, Hermann Nawrot AG, Germany) and analyzed by photoacustic gas analyzer INNOVA 1412 (LummaSense Technologies Inc., USA).

BMP assay: The BMP test was performed according the VDI 4630 (2006) in a 250 milliliter glass reactor coupled to an eudiometer tube. The gas production was read daily by displacement of the level in the sealant liquid (DIN 38414-8, 1985) in the eudiometer tube and the dried biogas volume was corrected to 273 K and 1013 hPa. The reactors were incubated with 180 to 200 gram of inoculum and 1 to 2 gram of sample, depend on the VS content (respected proportion $VS_{sample}/VS_{inoculum} \leq 0.5$). The system was sealed and stored at 37 °C until the daily gas production rate was lower than 1% of the total amount already produced.

Intra and interlabortory verification: Microcrystalline cellulose standard (Sigma-Aldrich, 20 µ size) was used as substrate for performance evaluation. Complementarily, also two reference samples, maize silage and dried distilled grain with soluble (DDGS) provided by the German Biomass Research Center (DBFZ), were also tested. Each treatment was performed in triplicate.

Statistical analysis: Was used statistical t-test ($\rho = 0{,}05$) to verify accordance between the results of biogas and methane yield from the reference samples in the interlaboratories batch tests. For this statistical evaluation was used the computer software Microsoft Office Excel (2010).

Results

During all period of inoculum monitoring the pH was stable between 7 and 8 and the acid/alkalinity ratio was lower than 0.25. After the first acclimation period, the TS in inoculum mixture were higher than 11% ($w \cdot w^{-1}$). According to the normative recommendation (ISO 11734, 1995; VDI 4630, 2006) the fermentation batch should contain 1.5 to 2% ($w \cdot w^{-1}$) of VS from the seeding sludge. For this reason was checked 3 different inoculum treatment: a) *in natura* (no pre-treatment), b) screened in 2 mm sieve (to remove coarse solids) and c) screened in 2 mm and diluted to 10% ($w \cdot v^{-1}$) with ISO

11734 buffer-micronutrient solution. The table 2 shows the biogas yield for cellulose standard in the first batch test.

Table 2 – Specific biogas yield for standard cellulose using different inoculum pre-treatment.

Inoculum treatment	**TS** (% - $w.w^{-1}$)	**Average** ($mL_N.g_{VS}^{-1}$)	**SD** ($mL_N.g_{VS}^{-1}$)	**Recovery*** (%)
a	11.4	620	145	82.7
b	9.9	768	64	102.4
c	1.09	549	16	73.2

* Recovery level based on 740-760 $mL_N.g_{VS}^{-1}$ for cellulose standard.

According VDI, it was considered satisfactory biogas recovery above 600 $mL_N.g_{VS}^{-1}$ (≥ 80%). The *in natura* inoculum shown satisfactory recovery, but the relative standard deviation (RSD) was 23.4% and considered high variability between replicates. The screened inoculum shown the best recovery factor and satisfactory variability (RSD = 8.3%). The dilution of inoculum according to ISO recommendation did not show satisfactory recovery factor.

To reduce the solid content in the inoculum mixture, the reactor was kept without feeding for 3 months. After this the TS was decreased and stabilized between 4 and 6% ($w·w^{-1}$). After that, new batch tests with cellulose were carried monthly. Two weeks before each test, about 1/4 of the inoculum volume was replenished using fresh original seeds.

The specific cumulative biogas productions for the sequential 6 batch test are shown in Figure 1 and the specific biogas yield are demonstrated in graphical control in the Figure 2. In the test nº 2 no satisfactory biogas recovery was observed due to problems caused by leaks and room temperature variations. These problems were rectified before the conduction of test nº 3. The sequential batch tests (nº 3 to 6) have shown satisfactory results (> 600 $mL_N·g_{VS}^{-1}$).

For all tests the biogas production started on 2nd or 3rd day after incubation and was stabilized before the 25th day. The delay (lag-phase) observed at the beginning of the batch test is reported in the literature like a necessary time to promote the cellulose hydrolysis. This phenomena is generally related as the slow degradation of β-(1,4)-glycosidic bonds (Bochmann et al., 2007; Fernandes et al., 2009).

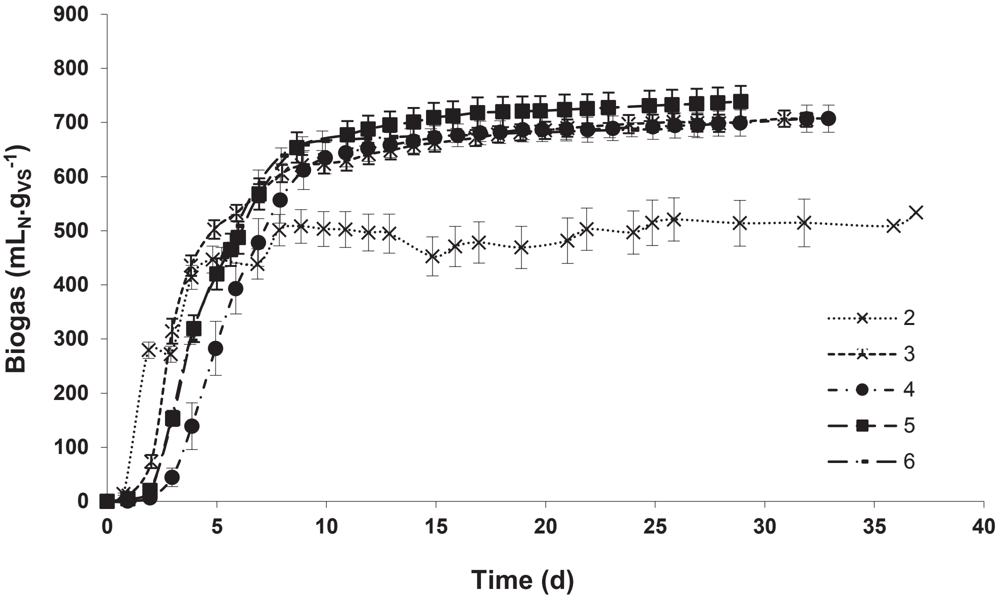

Figure 1 – Specific cumulative biogas yield for cellulose standard (batch test nº 2 to 6).

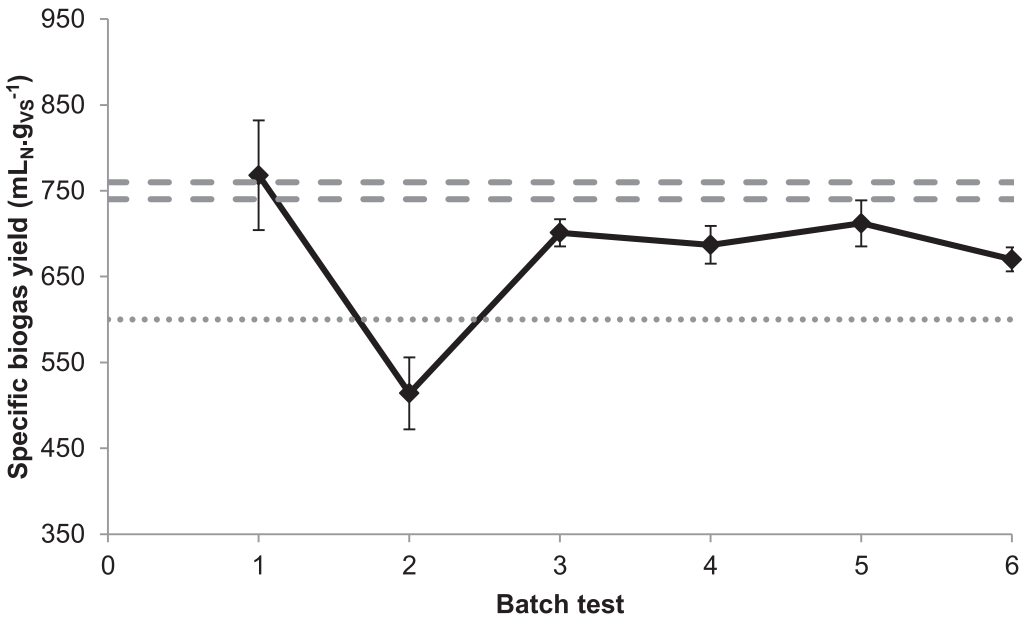

Figure 2 – Graphical control of specific biogas yield for sequential batch tests using cellulose standard. Reference range (between dashed line) = 740 to 750 $mL_N \cdot g_{VS}^{-1}$. Satisfactory level (above dotted line; ≥ 80%) ≥ 600 $mL_N \cdot g_{VS}^{-1}$.

To confirm the inoculum performance two tests with the reference samples from DBFZ (DDGS and Maize silage) were preceded. Table 3 shows the BMP for the samples and satisfactory similarity can be observed.

Table 3 – BMP results and standards for DDGS and Maize silage samples. Results from batch tests at two different periods (n=3 per test).

Sample	**LEB EMBRAPA**					**DBFZ**				
	Biogas ($mL_N.g_{VS}^{-1}$)	SD	CH_4 (%)	CH_4 ($mL_{N\ CH4}.g_{VS}^{-1}$)	SD	Biogas ($mL_N.g_{VS}^{-1}$)	SD	CH_4 (%)	CH_4 ($mL_{N\ CH4}.g_{VS}^{-1}$)	SD
DDGS	707	19	60	424	11	650	15	60	390	9
	640	11	63	403	7	643	6	63	405	4
Average* (n=6)	674^a	22	62	414^b	13	647^a	16	62	398^b	10
Maize silage	710	18	57	405	10	692	23	53	367	12
	628	12	55	345	7	732	35	51	373	18
Average* (n=6)	669^c	22	56	375^d	12	712^c	42	52	370^d	22

LEB EMBRAPA – Laboratory of Biogas Studies from Brazilian Agricultural Research Corporation.
DBFZ – Laboratory from Germany Biomass Research Center.
DDGS – Sample of dried distilled grain with soluble
*Biogas or methane average with the same letter don't show significantly difference ($\rho = 0.05$) by t-test.

By analysis of the results in the Table 3 it is possible to verify a very similar biogas and methane yield from the reference samples between the interlaboratories batch tests. Not only the amount of biogas was in the same range, but the methane content was quite similar.

The DBFZ laboratory use a inoculum source from a co-digestion biogas plant (dairy manure, grass silage and other energetic croups) and proceed a verification efficiency by a laboratory ring study (VDLUFA, 2014). The Table 3 data indicate a strong similarity performance by the inoculum used in both laboratories.

Conclusions

The proposed procedure resulted in a good strategy to obtain anaerobic mesophilic inoculum submitted to laboratory acclimatization, for use according VDI 4630, and applied to verify the BMP recovery of agriculture, livestock and food industry wastes.

The acclimation procedure can be used by laboratories in countries that don't have a large availability of co-digestion biogas plants. The acclimated anaerobic mesophilic inoculum could use for BMP batch testes to build a database of biogas and/or biomethane yield from Brazilian agricultural, agroindustrial and livestock wastes.

The use of the reference samples shows great importance to identify accordance in inoculum activity and improve the guarantee of results by comparability from BMP batch tests.

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