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Research article

A need for a standardization in anaerobic digestion experiments? Let's get some insight from meta-analysis and multivariate analysis

Céline Lavergne[a,*], David Jeison[a], Valentina Ortega[a], Rolando Chamy[a], Andrés Donoso-Bravo[b]

[a] Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica Valparaíso, Avenida Brasil 2085, 234000, Valparaíso, Chile
[b] Cetaqua, Centro Tecnológico del Agua, Los Pozos 7340, Santiago, Chile

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ABSTRACT

An important variability in the experimental results in anaerobic digestion lab test has been reported. This study presents a meta-analysis coupled with multivariate analysis aiming to assess the impact of this experimental variability in batch and continuous operation at mesophilic and thermophilic anaerobic digestion of waste activated sludge. An analysis of variance showed that there was no significant difference between mesophilic and thermophilic conditions in both continuous and batch conditions. Concerning the operation mode, the values of methane yield were significantly higher in batch experiment than in continuous reactors. According to the PCA, for both cases, the methane yield is positive correlated to the temperature rises. Interestingly, in the batch experiments, the higher the volatile solids in the substrate was, the lowest was the methane production, which is correlated to experimental flaws when setting up those tests. In continuous mode, unlike the batch test, the methane yield is strongly (positively) correlated to the organic content of the substrate. Experimental standardization, above all, in batch conditions are urgently necessary or move to continuous experiments for reporting results. The modeling can also be a source of disturbance in batch test.

1. Introduction

Anaerobic Digestion (AD) is one the most sustainable technologies for waste and wastewater treatment. Little energy is required for the process, and biogas, a renewable biofuel, is obtained as a by-product. Nowadays, AD is a consolidated technology with thousands of bioreactors already working worldwide (Vasco-Correa et al., 2017). To study the anaerobic biodegradability of a certain substrate, batch tests in lab-scale are usually carried out in the so-called biochemical potential test (BMP). Both operation modes are normally carried out at mesophilic (33–37 °C) or thermophilic conditions (50–57 °C). The BMP assay provides an easy platform to test the anaerobic degradability of different organic waste as well as a proper comparison criterion to assess the degradability properties of different substrates. Continuous reactors, with a semi-continuous injection of substrate and media withdrawn, are also employed. Semi-continuous operations take considerably more time than BMP test. However, that hurdle is partially offsetted by the fact that full-scale anaerobic digesters operate mostly in this mode, such that the extrapolability of the results is improved. In addition, the influence of the inoculum quality is also lower than in batch conditions.

During BMP tests, the main quantifiable manipulated variables (which also represent, overall the most reported variables) are basically the initial conditions of the test, namely: pH, substrate/inoculum ratio and the total solid content of the assay. On the other hand, the outputs are the biogas or methane production, mostly the accumulated volume, although the discrete production rate can also be reported (Batstone et al., 2004). From this data, it is nowadays very common to draw some parameters such as the maximum production (*P*) or methane yield (cm^3 CH_4 g_{VS}^{-1}), maximum production rate (cm^3 CH_4 g_{VS}^{-1} d^{-1}) and the lag-phase (d) by using the Gompertz function. Likewise, the same *P* and the hydrolysis coefficient (d^{-1}), k_h, can be estimated by using the first order equation (Donoso-Bravo et al., 2010). In (semi)continuous operation, unlike BMP test, the initial conditions' influence phases out as the experiment goes on. Therefore, the main manipulated variables are the inlet conditions related to the substrate fed, such as, the substrate concentration, the inlet flow (*i.e.*, the organic load rate - OLR and the hydraulic retention time - HRT, temperature, pH, etc.). The outputs of this operation are the biogas or methane yield, productivity and the organic matter removal attained at steady state conditions.

There is a significant variability in the results that are reported, even for the same substrates. This is the result of the big quantity of variables that can affect the results which cannot be easily standardized. For instance, the substrate heterogeneity given by its size, composition or

* Corresponding author.
E-mail address: celine.lavergne@pucv.cl (C. Lavergne).

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bioavailability, inoculum origin and quality, biogas measurement method, etc. (Raposo et al., 2011; Strömberg et al., 2014). This has led to a several efforts to standardize the implementation of the test at an international level among several institutions or laboratories in what is known as ring test (Angelidaki et al., 2009; Eadsforth et al., 2013; Holliger et al., 2016; Steinmetz et al., 2016). Nowadays, there is significant amount of data about AD of different substrates in the literature and, to our knowledge, no studies where the results of BMP and continuous operation in AD have been analyzed in a global fashion have been published. In this regard, meta-analysis is a tool that can be used to get valuable information from large quantities of data since it is defined as the statistical procedure for combining data from multiple studies. These types of analysis are usually coupled with multivariate statistical analysis due to the large quantities of variable involved. In AD, meta-analysis has started to be employed, for instance, to analyze microbial populations in the reactors (Nelson et al., 2011), assess the methane yield of manure (Miranda et al., 2016), estimate the methane potential (Appels et al., 2011) or the anaerobic biodegradability (Mottet et al., 2010) in waste activated sludge, as well as the GHG emission from dairy farms (Miranda et al., 2015). Therefore, the aim of this study is to carry out an objective rationale, based on meta-analysis and multivariate analysis, of how the variability of the data from BMP test and continuous operation at mesophilic and thermophilic temperature for waste activated sludge as a substrate may impact the global understanding of the AD process. Sewage sludge or waste activated sludge was chosen since is one of the most relevant substrates that has been subjected to AD and, compared to other substrates such as food waste, or animal manure, present a less degree of variability.

2. Methodology

2.1. Data collection and working database generation

The data collection was carried out from existing literature found in Scopus data base, where waste activated sludge (WAS - known also as secondary sludge or biological sludge) was used as substrate in both lab- and pilot-scale experiments. The data was separated in four categories; BM: batch in mesophilic (35–37 °C), BT: thermophilic condition (55 °C), CM: continuous in mesophilic, CT: thermophilic. For each category, two types of quantitative data were considered: 1) input or independent variables and 2) outputs or dependent variables. A database was created with the results collected from the literature, which can be consulted in the Mendeley data (http://dx.doi.org/10.17632/8795jgp8cv.1). A breakdown of the specific data collected is described in Table 1. As it can be seen, only TS and VS (of substrate and inoculum) were included as input parameters, despite it is known that there are a number of other parameters that have been proven to be essential for the AD processes. This decision is explained based on screening the reported data from which we realized these two parameters are reported the most in the literature, which means that we have enough data of these parameters so that the statistical analyses will be meaningful. Qualitative data or data, such as mixing, origin of the inoculum and volatile fatty acid concentration are scarcely reported in the studies and could not be included in order to ensure a significant statistical impact of the results. Inoculum properties (VS and S/X ratio) were only considered in the batch test since they clearly exert a direct effect on the BMP results. During continuous operations the initial effect of the inoculum quality phases out as the operation proceeds.

Due to the variety of ways of presenting and reporting data, some key assumptions were established:

1) The density of WAS is assumed to be 1000 kg m^{-3}. This assumption allows us to consider 1 g L^{-1} equal to 1 g kg^{-1}, since both units are reported for substrate and inoculum concentration
2) Because of the nature of the substrate when units were given in suspended solid instead of total solid, we assume both to be the same. In other words, the soluble content was neglected
3) The methane production rate and the lag phase were taken when the Gompertz equation was used. Likewise, the hydrolysis constant was taken from articles where the first order equation was used. The total methane production or methane yield was taken from either the Gompertz or the first order equations. When no equations were employed only the methane yield was considered from the reported results by the authors
4) The results of biogas production were assumed to be reported in normal conditions
5) Whenever possible, data that were not explicitly provided in the paper were estimated or evaluated based on provided information. This was done when 1) it was possible to estimate it through a simple extra calculation, for instance, if the biogas yield and the methane content are given the methane yield can be estimated as well or (2) it was possible to use a conventional criterion to get the estimation, for instance if the biogas was given in cm^3 CH_4 g_{COD}^{-1} the conversion of 1.5 g_{COD} g_{VS}^{-1} was employed to make the transformation. The assumptions and the calculation are clearly indicated and highlighted in the whole dataset (http://dx.doi.org/10.17632/8795jgp8cv.1).

Table 1
Description of the collected data.

Inputs		Unit	Outputs		Unit
Batch (BMP)					
Substrate	TS and VS	kg m^{-3}	Biogas	Biogas yield (Y_{BIOGAS})	cm^3 g_{VS}^{-1}
Inoculum	TS and VS	kg m^{-3}		Methane yield ($Y_{METHANE}$)	cm^3 g_{VS}^{-1}
Initial ratio	VS_s/VS_i	kg kg^{-1}		Methane production rate	cm^3 g_{VS}^{-1} d^{-1}
				Lag-phase (λ)	d
				Hydrolysis constant	d^{-1}
Continuous					
Substrate	TS and VS	kg m^{-3}	Biogas	Biogas yield (Y_{BIOGAS})	cm^3 g_{VS}^{-1}
Operation	HRT	d		Methane yield ($Y_{METHANE}$)	cm^3 g_{VS}^{-1}
	OLR	kg_{VS} m^{-3} d^{-1}		Biogas Productivity ($Prod_{BIOGAS}$)	cm^3 m^{-3} d^{-1}
				Methane productivity ($Prod_{METHANE}$)	cm^3 m^{-3} d^{-1}
			Digestate	VS removal	%
				COD removal	%

2.2. Statistical analysis

All statistical analyses were performed with R software (R Core Team, 2013). Pearson tests were used to determine the correlation between the different variables. In order to identify significant influence of both temperature and operation method on methane yield, two-way ANOVA was performed using the values of methane yield (cm^3 g_{VS}^{-1}) with two levels for each factor: 1) Temperature: Mesophilic (n = 106) and Thermophilic (n = 37), and 2) Method: Batch (n = 71) and Continuous (n = 72). The values of methane yield were square-rooted transformed to fit the ANOVA assumptions. The assumptions of the ANOVA were validated by testing the normality of the residuals using a Shapiro-Wilk test and the homogeneity of variances looking at the residuals vs fitted plot. Then, post-hoc Tukey test was performed with the aim of identifying specific influence within each factor. The representation of the data was done by using boxplot representation.

2.3. Principal component analysis

Two multivariate principal component analysis (PCA) were