JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

A comparison of process performance during the anaerobic mono- and co-digestion of slaughterhouse waste through different operational modes

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ARTICLE INFO

Article history:
Received 26 December 2016
Revised 6 February 2017
Accepted 5 June 2017
Available online xxxx

Keywords:
Co-digestion
Biomethane potential test
Second feeding
Specific methanogenic activity
Slaughterhouse waste

ABSTRACT

The use of consecutive feeding was applied to investigate the response of the microbial biomass to a second addition of substrates in terms of biodegradation using batch tests as a promising alternative to predict the behavior of the process. Anaerobic digestion (AD) of the slaughterhouse waste (SB) and its co-digestion with manure (M), various crops (VC), and municipal solid waste were evaluated. The results were then correlated to previous findings obtained by the authors for similar mixtures in batch and semi-continuous operation modes. AD of the SB failed showing total inhibition after a second feeding. Co-digestion of the SB + M showed a significant improvement for all of the response variables investigated after the second feeding, while co-digestion of the SB + VC resulted in a decline in all of these response variables. Similar patterns were previously detected, during both the batch and the semi-continuous modes.

Introduction

Anaerobic digestion (AD) technology has gained an increasing attention because of its environmental and economical benefits. The worldwide interest for this technology has led to continuous testing and evaluations of different kinds of materials, which are suitable for AD. The first stage in evaluations of the feasibility of any substrates to be used for AD is the determination of its biomethane potential (BMP). This parameter gives valuable information regarding the capacity of the substrates to be converted into methane, hence, for designing the operational details, as well as for the economical evaluation when establishing the new biogas plants (Angelidaki et al., 2009). A simple and reliable method to determine the methane potential of a substrate is a batch anaerobic fermentation assay, so called BMP test. Such method provides useful information regarding the biodegradability of the substrates, the possible biogas yield, and makes it possible to estimate the kinetic parameters concerning the degradation rate, as well as the possible toxicity levels (VDI-4630, 2006). Nevertheless, this test provides no information about the stability of the process, which would be important to know for future continuous operation. Further studies, running time-consuming continuous AD assays, are therefore usually required to ensure the long-term effects. Depending on the complexity of the materials treated, the

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http://dx.doi.org/10.1016/j.jes.2017.06.004
1001-0742/© 2017 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

degradation rate, the interactions between the mixture of substrates and the possible inhibitions of the microbial community; the reactor must be operated using different retention times. It leads to long experimental running (*i.e.*, running at the same conditions for a period of at least 3 retention times) to evaluate the stability and the performance of the process (Bayr et al., 2012; Zhang et al., 2012). Therefore, decision makers are facing long time delays to get final and reliable results applicable for full-scale implementations.

Using models describing the AD process is also a valuable and cost-effective tool, which is widely applied to evaluate the system and to predict the biogas production. With the development of the AD models, like ADM1, a joint effort has been dedicated to increase the understanding of this complex biodegradation process (Batstone et al., 2002). However, despite the general acceptance of these models, there are still some emerging areas regarding the characterization of the substrates as well as the resulting interaction of the mixtures when the co-digestion process is applied (Batstone, 2013).

The use of consecutive feeding (*e.g.*, second feedings) in batch experiments, is an intermediary step between the batch and semi-continuous operation, and it can be a promising alternative to predict the behavior of the process, *i.e.*, the response of the microbial biomass or the stability and performance of the reactors in a long-term operation, since this test requires a shorter time compared to that needed for running the continuous experiments. Hence, the hypothesis is that consecutive feeding in batch assays would give a fast forecast of the process in order to select the substrate combinations and operational parameters, which would likely be appreciated by both the industrial and research world.

The stability of the process is highly dependent on the symbiotic growth and activity of the principal groups of microorganisms involved in the anaerobic degradation chain (Angelidaki et al., 2009). Thus, for monitoring the reactor behavior, it is important to determine the specific methanogenic activity (SMA). SMA is able to measure the direct enzymatic activity of the microorganisms involved in the last step of the anaerobic degradation, *i.e.*, in the methanogenesis (Sorensen and Ahring, 1993). In fact, evaluating the activity of the biomass is a key parameter when degrading complex organic matter, reflecting the stability of the reactor during a period of time (Ahring, 1995).

In the literature, most of the applications of the SMA test have been focused on measuring the activity of the specific groups of methanogens (*i.e.*, hydrogenotrophic or acetoclastic methanogens) in granular and non-granular sludges by adding specific substrates, such as hydrogen and carbon dioxide, acetate, and methanol (Ahring, 1995; Dolfing and Bloemen, 1985; Sorensen and Ahring, 1993).

The objective of this work was to investigate the response of the microbial biomass to a second feeding addition in terms of methane yield, degradation kinetics and SMA. The process was evaluated under the batch operation mode when mono-digesting slaughterhouse wastes or during its co-digestion with other waste fractions from the agro-industrial activities. To the best of our knowledge, the utilization of consecutive feeding to predict the performance of the co-digestion of different agricultural waste streams under practical conditions has not yet been reported. The methane yield, degradation kinetics, and SMA were determined and then compared with the results obtained previously by the authors in both the batch assays and in the semi-continuous operation modes for similar mixtures (Pagés-Díaz et al., 2014, 2015) in order to establish a correlation between process performance and operation modes. The goal was to examine whether there is a possibility for the use of consecutive feeding in batch assays as a quick prediction method for determining the expected process performance under a semi-continuous operation.

1. Materials and methods

1.1. Substrates and inoculum

Solid cattle slaughterhouse waste (SB) and its mixtures with animal manure (M), various crops (VC), and the organic fractions of the municipal solid waste (MSW) were prepared. According to the previous results obtained from the batch and semi-continuous operations (Pagés-Díaz et al., 2014, 2015), mixtures of SB + M (50%: 50%), SB + VC + MSW (33%: 33%: 33%), and SB + VC (50%: 50%), each based on the wet weight (ww), were investigated. Detailed information of the composition, and the preparation of the materials are presented in Pagés-Díaz et al. (2014). The characteristics of the SB and its different mixture combinations are summarized in Table 1. The inoculum (*i.e.*, biomass) with 3.3% total solid (TS) and 1.9% volatile solid (VS) used in the assays, was obtained from a full-scale thermophilic (55 ± 1°C) co-digestion biogas plant (Borås Energy and Environment AB, Borås, Sweden) treating MSW. The inoculum was first filtered with a 2-mm sieve to remove the indigested particles and then stored for stabilization at (55 ± 1)°C during three days, before starting up the assays.

1.2. Batch experimental set-up

The experimental set-up was performed in accordance with a method previously described by Hansen et al. (2004). Four set-ups, one for the mono-digestion of the SB and three for the co-digestion assays (*i.e.*, SB + M, SB + VC + MSW, and SB + VC) were performed, all in triplicates. Controls, which included either only the inoculum (blank reactor) to determine the gas production from the inoculum itself, or the cellulose (Cellulose Fibrous Long, Sigma Aldrich, Germany) as a substrate (positive control) to ensure the quality of the inoculum, were also run in parallel. Glass bottles of 2 L were used to carry out the tests. Each bottle was fed with 400 mL of inoculum and fresh substrate to keep the ratio of inoculum to substrate equal to 2 (g VS/g VS). The reactors were then sealed with tick rubber septa and aluminum caps (Apodan Nordic, Copenhagen, Denmark). Finally, the headspace of each reactor was flushed with a mixture of 80% N_2 and 20% CO_2 (V/V) during 3 min to attain anaerobic conditions. The reactors were then incubated at thermophilic conditions (55 ± 1°C) during the experimental period (incubator MMM-group, Einrichtungen GmbH, Venticell) and manually shaken every day.

Gas samples were regularly taken from the headspace and analyzed by gas chromatography (GC). To avoid an overpressure inside the bottles, the gas was released after

Please cite this article as: Pagés-Díaz, J., et al., A comparison of process performance during the anaerobic mono- and co-digestion of slaughterhouse waste through different operational modes, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.06.004