Industrial Crops & Products 111 (2018) 684–693

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Influence of different thermal pretreatments and inoculum selection on the biomethanation of sugarcane bagasse by solid-state anaerobic digestion: A kinetic analysis

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ARTICLE INFO

Keywords:
Sugarcane bagasse
Methane
Solid-state anaerobic digestion
Inoculum selection
Kinetic modeling

ABSTRACT

The present study investigated the potential of UASB inoculum, by itself or mixed to nitrogenous residue (fresh bovine manure – FBM) or to adapted microorganisms (bovine rumen – BR), for biomethanation of raw and pretreated sugarcane bagasse (SB) by solid-state anaerobic digestion. The influence of autohydrolysis and organosolv pretreatment conditions on the efficiency of biomethanation process was also evaluated by means of kinetic parameters. Akaike Information Criterion was used to statistically evaluate methane production, and the multi-stage model exhibited the highest fit among the models tested. FBM addition resulted in improvements in the C/N ratio, higher yield (143.3NL_{CH4} kg_{VS}^{-1}) and higher rate constant (3.05NL_{CH4} $kg_{VS}^{-1}day^{-1}$) for CH_4 production from raw SB without any lag phase when compared to UASB inoculum itself which exhibited 5.6 days of lag phase. The FBM also led to a better inoculum adaptation to substrates which contained inhibitors such as xylooligomers and lignin fragments, and had lower C5 sugar content.

1. Introduction

The energetic exploitation of lignocellulosic wastes generated by agroindustrial activities have gained prominence in Brazil and in the world (IEA, 2007). Recently, the interest for the use of lignocellulosic wastes is mainly based on their chemical compositions, which have high carbohydrate content, in the form of cellulose and hemicelluloses (~70%), that can be used for bioenergy production (Gupta and Tuohy, 2013). According to the Brazilian National Supply Company (CONAB), the 2016/2017 sugarcane harvest season is estimated at 694 million tons (CONAB, 2016). Considering that each ton of crushed sugarcane produces about 250–280 kg of SB with ~50% moisture (Rocha et al., 2015), then from 173 to 194 million tons of bagasse could be expected in this season. Although SB is used to generate steam and electricity, it is estimated that the energetic demand in a sugarcane industry is met by burning only half of the bagasse (Costa et al., 2014). This brings the opportunity of using this lignocellulosic waste to recover more added value products, such as chemical raw materials (e.g. lignin) as well as liquid and gaseous biofuels (e.g. ethanol 2G, biogas).

The high carbohydrate content makes SB an excellent substrate for methane production through solid-state anaerobic digestion (SS-AD) (Li et al., 2011). SS-AD is also known as a dry digestion and/or dry fermentation which has the advantage of operating with a high total solid (TS) load (15–40%), ensuring greater capacity to treat wastes, thereby maintaining good degradation rates and high biogas production with low generation of effluents (Brown et al., 2012; Pandey et al., 2000).

In the context of lignocellulosic feedstock biorefinery, the SS-AD process can contribute to maximize the energy production while minimizing the generation of solid wastes. In addition, SS-AD process is based on the fact that it can be fed with residual SB from sugar and ethanol production, so that biogas produced from SS-AD using lignocellulosic wastes has a high (up to 75%) methane content (Frigon and Guiot, 2010). These characteristics contribute to the SS-AD process be widely applied at an industrial scale, converting SB or pretreated SB into biogas with high efficiency.

Some alternatives to maximize SS-AD process efficiency involve the use of inocula capable of promoting higher conversion rates of lignocellulosic wastes polysaccharides into biogas; and/or the use of a pretreatment step to decrease the recalcitrance of the lignocellulosic waste (Sawatdeenarunat et al., 2015). These alternatives increase substrate hydrolysis rate with consequent reduction of lag phase, which is considered a limiting-step in the SS-AD of lignocellulosic wastes (Adney

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https://doi.org/10.1016/j.indcrop.2017.11.048
Received 25 May 2017; Received in revised form 8 November 2017; Accepted 25 November 2017

Nomenclature

SB	Sugarcane bagasse
SB-AH	SB pretreated by autohydrolysis
SB-AH-HGOD	SB pretreated by autohydrolysis followed by glycerol organosolv at conditions for higher delignification
SB-AH-LGOD	Sugarcane bagasse pretreated by autohydrolysis followed by glycerol organosolv at conditions for lower delignification
BR	Bovine rumen
UASB-BR	UASB inoculum mixed to bovine rumen
FBM	Fresh bovine manure
UASB-FBM	UASB inoculum mixed to fresh bovine manure

et al., 1991).

In this context, the alternative of using an inoculum consisting of microorganisms from different sources seems more sustainable. Microorganisms present in sewage treatment plants are adapted to different substrates when compared to those present in the bovine rumen (BR) and in fresh bovine manure (FBM). The great advantage of using microorganisms from BR and cow manure is that they are more adapted to substrates containing lignin, cellulose and hemicelluloses (Azevedo et al., 2015; Yang et al., 2015; Yue et al., 2013; Hu and Yu 2005). Thus, an inoculum composed by a variety of microorganisms could provide higher hydrolytic and methanogenic activities (Angelidaki et al., 2009). The synergism of microorganism consortium can improve lignocellulosic wastes methanation, reduce lag phase, decrease the accumulation of volatile fatty acids and favor the nutritional balance of a culture medium (Fox et al., 2003; Lozecznik et al., 2010).

Therefore, the aim of this study was to evaluate the best inoculum and substrate processing for methane production by SS-AD of sugarcane bagasse. Mixtures of microorganisms present in upflow anaerobic sludge blanket reactor (UASB), fresh bovine manure (FBM) and bovine rumen (BR) were used to improve SS-AD of raw SB, SB pretreated by autohydrolysis (SB-AH) and SB-AH followed by glycerol organosolv delignification (SB-AH-GOD) under different process conditions. Methane production was modeled by different kinetic models, which allowed evaluating the influence of pretreatment type (substrate availability) and inoculum on biogas production rate and potential.

2. Material and methods

2.1. Chemicals

SB was provided by Bioenergética Aroeira Sugar and Ethanol Plant (Tupaciguara, MG, Brazil), and was collected in the 2014/2015 season. It was stored in a freezer at −20 °C prior to use. The kraft bleached pulp (hybrid of *Eucalyptus globulus*) was provided by Cenibra Nipo-Brasileira S/A, Belo Oriente, MG, Brazil. Grade methane (99.95%) and nitrogen (99.9999%) were purchased from White Martins Praxair Technology Inc. (Brazil). Sulfuric acid (95–98% and 99.999%) was purchased from Synth (Brazil) and Sigma-Aldrich (Brazil). Glycerol (99%), cyclohexane and ethanol (99.5%) were purchased from Synth (Brazil). Chromatography-grade standards cellobiose, D-glucose, D-xylose, L-arabinose, formic acid, acetic acid, 5-hydroximethyl-2-furfuraldehyde (HMF) and 2-furfuraldehyde (FF) were purchased from Sigma-Aldrich (Brazil).

2.2. Inocula preparation

SS-AD experiments aimed to evaluate the biochemical methane potential (BMP) were performed in batch mode using 500 mL glass bottles sealed with rubber septa and screw caps capable of allowing biogas sampling. Inoculum from three different sources were evaluated, i.e. UASB reactor fed with domestic sewage at the Centre for Research and Training on Sanitation – CePTS – UFMG/Copasa, Arrudas Wastewater Treatment Plant, Belo Horizonte, MG, Brazil, FBM and BR collected at the Department of Zootechny, Federal University of Viçosa, MG, Brazil (Ethics committee number 42/2016).

The inoculum from UASB was mixed to BR and FBM to give two new inocula, i.e. UASB plus BR (UASB-BR) and UASB plus FBM (UASB-FBM). The UASB-BR inoculum was prepared by mixing 50% volatile solids (VS) of UASB with 50% VS of BR suspension directly collected from the animal; whereas the inoculum UASB-FBM was prepared by mixing 50% VS of UASB with 50% VS of FBM.

2.3. Experiments of methane production by SS-AD

Experiments of methane production by SS-AD of sugarcane bagasse were carried out in duplicate using 500 mL glass bottles as reactor. The bottles were loaded with 15% of total solids (TS), keeping a lignocellulose substrate-to-inoculum ratio of 2.0 (on VS basis), and 10.0 g of raw SB, SB-AH or SB-AH-GOD. Kraft bleached pulp (KBP) was also used as a substrate for comparison purposes. The substrate-to-inoculum ratio used in this study was based on the literature data, which demonstrated that a substrate-to-inoculum ratio of 2.0 maximized biogas production by SS-AD (Liew et al., 2012). All glass bottles were kept under constant stirring (150 rpm) at 35.1 °C ± 0.3 °C in an orbital shaker incubator (Thoth®, model 6440) and were monitored by 100 days.

For each type of inoculum (UASB, UASB-RB and UASB-FBM) control flasks were also monitored. These control flasks were not loaded with substrates, thereby allowing the evaluation of any methanogenic activity associated to degradation of organic matter present in the inoculum or by endogenous decay. The endogenous methane production of inoculum (flask control) was subtracted from the methane production observed in the assays carried out with substrate.

2.4. Biogas analyses

Quantification of biogas produced during SS-AD was made daily by recording the accumulated pressure in each glass bottle (Manometer®, model PM-9100HA). Biogas composition, in terms of methane (%, $v\ v^{-1}$), was determined by gas chromatography (Shimadzu, model 2014/TCD). The chromatograph was equipped with a thermal conductivity detector, operating at 120 °C with a current of 85 mA and a capillary column (30m × 0.53 mm Restek®) packed with molecular sieve (5 Å) at 40 °C (Shimadzu®, model 2014). Nitrogen was used as carrier gas at a flow rate of 1.66 mL min^{-1}. An external calibration curve was built using a standard gas mixture for methane quantification (24.99 $\text{mol}_{\text{CH4}}\ \text{mol}_{\text{mixture}}{}^{-1}$).

All values of methane production were expressed in $\text{NL}_{\text{CH4}}\ \text{kg}_{\text{VS}}{}^{-1}$, considering the standard temperature and pressure conditions (273.15 K and 101,315 Pa) as recommended by International Union of Pure Applied Chemistry (IUPAC).

2.5. Kinetics of methane production by SS-AD

Different kinetic models were tried to adjust the methane