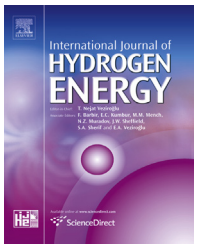
Available online at www.sciencedirect.com

ScienceDirect

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



Evaluation of hydrogen and methane production from sugarcane vinasse in an anaerobic fluidized bed reactor

Cristiane Marques dos Reis [a], *Mariana Fronja Carosia* [b], *Isabel Kimiko Sakamoto* [b], *Maria Bernadete Amâncio Varesche* [b], *Edson Luiz Silva* [a,*]

[a] *Department of Chemical Engineering, Federal University of São Carlos, Rod. Washington Luis, Km 235, 13565-905 São Carlos, SP, Brazil*

[b] *Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Av. Trabalhador São Carlense, 400, 13566-590 São Carlos, SP, Brazil*

ARTICLE INFO

Article history:
Received 18 February 2015
Received in revised form
17 April 2015
Accepted 25 April 2015
Available online 23 May 2015

Keywords:
Distillery wastewaters
Co-fermentation
Hydrogen
Hydraulic retention time
Methane

ABSTRACT

This study evaluated the hydrogen and methane production from sugarcane vinasse in an anaerobic fluidized bed reactor. Two reactors were operated with two different substrate concentrations: R5 (5 g COD L^{-1}) and R10 (10 g COD L^{-1}). During the first stage, glucose was used as the primary carbon source; vinasse was then added from 0% to 100% of the organic source in hydraulic retention time (HRT) of 6 h. Later, HRT was changed to 4, 2 and 1 h.

The best hydrogen production rate was 0.57 L h^{-1} L^{-1} (R5, HRT = 1 h, 100% vinasse). The best hydrogen yield was 3.07 mmol H_2 g^{-1} COD_{added} (R5, HRT = 6 h, vinasse:glucose = 1:3). Main metabolites were ethanol, butyric acid, propionic acid and methanol. Denaturing gradient gel electrophoresis analysis identified *Prevotella* sp. and *Megasphaera* sp. belonging to the Bacteria domain and *Methanobacterium* sp. and *Methanosphaera* sp. belonging to the Archaea domain.

Introduction

Sugar and ethanol industry is one of the main segments of the agribusiness sector in Brazil. In addition to the sugar and alcohol, the main products, nearly all of the sub-products of the industry, including bagasse, molasses and sugarcane vinasse, are processed [1]. Vinasse, one of the major byproducts of the ethanol production process with nearly 14 L of vinasse produced per liter of ethanol, can cause extensive pollution due to its high organic load (up to 40 g COD L^{-1}), and it is a potential anaerobic digestion source [2].

In general, vinasse is a low pH brown-colored residue containing particulate matter and high concentrations of organic and inorganic compounds [3]. Phenolic compounds (such as humic acid and tannic acid), the melanoidins (resulting from the reaction of sugars and proteins by the Maillard reaction), caramel and the furfural components contribute to its color [4].

* *Corresponding author*. Tel.: +55 16 33518264; fax: +55 312 16 33518266.
E-mail address: edsilva@ufscar.br (E.L. Silva).
http://dx.doi.org/10.1016/j.ijhydene.2015.04.136

The presence of these compounds makes vinasse a complex and difficult compound for degradation.

The anaerobic digestion of vinasse has the potential to reduce the organic load of this residue while generating biogases such as methane (CH_4) or hydrogen (H_2). Few studies, however, focus on the energy recovery in the form of H_2.

In general, the production of CH_4 and H_2 is a two-stage process involving separation of the acidogenic and the methanogenic stages. The combination of H_2 and CH_4 is more efficient than CH_4 alone when used as combustion engine fuel. In addition, the CH_4 and H_2 mixture reduces the emission of greenhouse gases such as CO, CO_2 and unburnt hydrocarbons [5].

Biological H_2 production can be accomplished using simple or complex substrates. Wastewater from a numerous industries including the dairy [6], brewery [7], and ethanol-sugar industries [8–10] can be used for biological hydrogen production.

The presence of inhibitory compounds in the distillery wastewaters can be a barrier to the anaerobic digestion process despite the rich amount of organic matter in the wastewater. Thus, an adjustment period is advisable to promote favorable consumption of complex substrates by the microorganisms in the environment. When added in small quantities, the microbial culture may adapt to the complex residue or to the inhibitory compounds [11]. Thus, the use of a simple co-substrate can improve the microbial degradation of refractory substances in complex residues such as stillage. Some authors [8,12,13] who employed distillery residues have also started adapting or conditioning the reactor to produce hydrogen with simple substrates such as glucose and sucrose. So far, the effect of varying proportions of vinasse-glucose in thermophilic conditions has been tested [14]. However, a study employing different vinasse-glucose ratios for hydrogen production in the mesophilic range has not been performed. The aim of this study was to evaluate the production of H_2 and CH_4 in two anaerobic fluidized reactors (AFBR) at room temperature (22 ± 3 °C) with concentrations of 5 and 10 g COD L^{-1} obtained from several ratios of diluted vinasse and glucose. Furthermore, the band profiles of the microbial communities were investigated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) in both the AFBRs.

Materials and methods

AFBR

Two acrylic fluidized bed reactors with the following dimensions were used: height 151 cm, diameter 3.1 cm, and total volume of 1452 mL (Fig. 1). The support material used for immobilizing the microorganisms was expanded clay: grain sizes between 2.8 and 3.35 mm, a real density of 1.50 g cm^{-3}, porosity of 23% and minimum fluidization velocity of 1.24 cm s^{-1}. Along the reactors, four samplers were distributed in order to collect support material for microbial characterization.

Reactor start-up procedure

The inoculum was obtained from an upflow anaerobic sludge blanket (UASB) reactor used for the treatment of swine wastewater. Swine manure is known for possessing hydrogen-producing bacteria. The sludge underwent heat treatment to activate the acidogenic cells, as described in methods proposed by Ref. [15] and adapted from Ref. [16].

The reactors R5 and R10 worked with distinct substrate concentrations of 5 g COD L^{-1} and 10 g COD L^{-1}, respectively. Some nutrients were also added according to Leite et al. [17] at the following concentrations (mg L^{-1}): $CO(NH_2)_2$ (125); $NiSO_4 \cdot 6H_2O$ (1); $FeSO_4 \cdot 7H_2O$ (5); $FeCl_3 \cdot 6H_2O$ (0.5); $CaCl_2 \cdot 6H_2O$ (47.0); $CoCl_2 \cdot 2H_2O$ (0.08); SeO (0.07); KH_2PO_4 (85.0); K_2HPO_4 (21.7); $Na_2HPO_4 \cdot 2H_2O$ (33.4). To maintain the pH of the medium between 4 and 5, hydrochloric acid (30%) and sodium bicarbonate were used as buffer solutions. Both reactors were operated in batch mode for 96 h in a closed circuit. After this activation period, the reactors were operated in continuous mode. Glucose was the only carbon source in the beginning. However, vinasse was added to the feed gradually (0, 25, 75 and 100% of g COD L^{-1}) until vinasse replaced glucose in the feed making the total concentration of 5 g COD L^{-1} or 10 g COD L^{-1} during the hydraulic retention time (HRT) of 6 h. When vinasse was the only carbon source, the HRT was varied between 4, 2 and 1 h. Table 1 illustrates the experimental stages.

To facilitate the discussion of results, each operation phase was named according to the vinasse content in the substrate mixture and the HRTs for which reactors were operated, e.g., III (75%, 6 h) indicates a phase III where the reactors were fed with a substrate mixture containing 75% vinasse and were operated for an HRT of 6 h.

The vinasse used in this experiment was collected at the Usina São Martinho distillery plant (Pradopolis, SP, Brazil), and it was stored frozen. Table 2 illustrates the main physical and chemical properties of vinasse employed. The raw vinasse had an average concentration of 40 g COD L^{-1} which was diluted to achieve the desired concentration in each reactor: R5 (5 g COD L^{-1}) and R10 (10 g COD L^{-1}). Both reactors were operated for 364 days and each stage lasted for 40 days in average.

Analytical methods

The pH, COD, nitrogen, phosphate, sulfate, magnesium, calcium, potassium and the amount of volatile suspended solids were analyzed according to the Standard Methods for the Examination of Water and Wastewater [18]. Analyses of total volatile acids were performed according to the methodology proposed by Dilalo and Albertson [19], whereas bicarbonate alkalinity was estimated as described by Ripley et al. [20]. The total reducing sugars were measured according to Dubois et al. [21]. The organic acids and alcohol concentrations were measured by liquid chromatography (HPLC Shimadzu) equipped with a pump (LC-10ADVP), an autosampler (SIL-20A HT), a column oven (CTO-20A) at 43 °C, a refractive index detector (RID-10A), a system Controller (SCL-10AVP) and column HPX-87H Aminex (300 mm, 7.8 mm, BioRad). The mobile phase consisted of H_2SO_4 (0.01 N) at 0.5 mL min^{-1}. The biogas hydrogen content was determined by gas chromatography (GC-2010, Shimadzu, Japan) using a thermal conductivity detector (TCD) with argon as the carrier gas and a Supelco Carboxen 1010 Plot packed column (30 m × 0.53 mm i.d.). Volumetric hydrogen production was measured by the Ritter