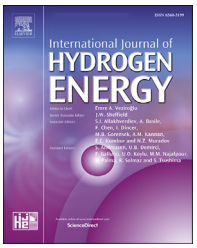
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2018) 1–9

Available online at www.sciencedirect.com

ScienceDirect

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



Evaluation of semi-continuous hydrogen production from enzymatic hydrolysates of *Agave tequilana* bagasse: Insight into the enzymatic cocktail effect over the co-production of methane

Alma Toledo-Cervantes [a], *Jorge Arreola-Vargas* [b], *Silvia V. Elias-Palacios* [a], *Erika N. Marino-Marmolejo* [c], *Gustavo Davila-Vazquez* [c], *Víctor González-Álvarez* [a], *Hugo O. Méndez-Acosta* [a,*]

[a] *Departamento de Ingeniería Química, CUCEI-Universidad de Guadalajara, Blvd. M. García Barragán 1451, C.P. 44430, Guadalajara, Jalisco, Mexico*

[b] *División de Procesos Industriales, Universidad Tecnológica de Jalisco, Luis J. Jiménez 577-1 de Mayo, C.P. 44979, Guadalajara, Jalisco, Mexico*

[c] *Tecnología Ambiental, Centro de Investigación y Asistencia en Tecnología y Diseño Del Estado de Jalisco, Av. Normalistas 800, C.P. 44270, Guadalajara, Jalisco, Mexico*

ARTICLE INFO

Article history:
Received 10 March 2018
Received in revised form
22 May 2018
Accepted 24 May 2018
Available online xxx

Keywords:
ASBR
Anaerobic digestion
Biohydrogen
Granular biomass disaggregation
Lignocellulosic biomass

ABSTRACT

This work addresses the hydrogen production from enzymatic hydrolysates of *Agave tequilana* bagasse and the valorization of the acidogenic effluent for methane production in anaerobic sequencing batch reactors (ASBRs). Regarding hydrogen production, the ASBR was operated at four organic loading rates (OLRs), which were modified by decreasing the cycle time (from 24 to 12 h) and increasing the COD concentration (from 8 to 12 and 16 g L^{-1}). Results showed that the highest OLR promoted the highest hydrogen production rate of 25.2 ± 2.1 NmL L^{-1} h^{-1}. Conversely, the hydrogen molar yield remained constant, obtaining similar values to the highest reported for lignocellulosic hydrolysates in continuous reactors (1.6H_2-mol $mol^{-1}_{consumed\ sugar}$). Regarding methane production from the acidogenic effluent, an unexpected methane suppression was observed during the first 5 cycles of the ASBR operation. Such event was attributed to the disaggregation of the granular sludge due to the remaining hydrolytic activity of the enzymatic cocktail used for the hydrolysates production. This was corroborated by feeding acetate to an ASBR (positive control) and supplying the enzymatic cocktail. Overall, even though the ASBR configuration demonstrated its suitability for hydrogen production, further studies are needed to coupling the methanogenic phase in different reactor configurations.

* *Corresponding author.*
E-mail address: hugo.mendez@cucei.udg.mx (H.O. Méndez-Acosta).

https://doi.org/10.1016/j.ijhydene.2018.05.134

Introduction

Current concerns about centralizing energy production and the environmental issues caused by fossil fuel burning have driven attention to the wide spectrum of alternative energy sources. In this context, hydrogen (H_2) and methane (CH_4) have become promising alternatives not only because of their high calorific values but due to the versatility of their production. These gases may be produced through non-energy intensive processes such as anaerobic digestion, and can be converted to heat and electricity in industries and/or used as the so-called biohythane gas [1,2].

Anaerobic digestion is a cost-effective and mature technology, which provides dual benefits as waste treatment and simultaneous energy production [3]. Among the wide organic feedstock used for hydrogen and methane production, lignocellulosic wastes have been recognized as one of the promising sources of energy due to their abundance, renewability, and non-competition with human food demand [4]. However, pretreatment is essential to achieve the efficient release of the carbohydrate content, enhancing sugars bioavailability [5]. A variety of physical, chemical and biological pretreatment methods have been evaluated, being dilute acid hydrolysis the most commonly applied method due to its high sugar extraction efficiency [6]. Nonetheless, toxic byproducts such as furans, organic acids, and phenolic compounds can be produced due to the high temperatures and low pH of the pretreatment [4]. In contrast, milder hydrolytic procedures such as the enzymatic deconstruction of lignocellulose by the synergistic action of cellulases and hemicellulases release no toxic byproducts; however, enzymatic deconstruction of lignocellulose is complex due to its recalcitrant structural features [7].

In this context, *Agave tequilana* (*A. tequilana* Weber var. Azul) bagasse, the main agro-industrial waste generated by the tequila industry in Mexico, is considered an excellent candidate for enzymatic deconstruction, since it is generated after cooking the heads of *A. tequilana* at 100 °C for 24 h, which may be considered as an in-situ thermal pretreatment [8]. Thus, the *A. tequilana* bagasse contains high amounts of readily available carbohydrates (≈70% of holocellulose). According to the Tequila Regulatory Council, in 2016 the consumption of *A. tequilana* for tequila production was 941 800 tons, being 40% converted to bagasse which represents an environmental problem in the tequila producing regions of Mexico [9].

Because of the aforementioned characteristics of the *A. tequilana* bagasse, this lignocellulosic biomass has recently emerged as an attractive feedstock for hydrogen and methane production via two-stage anaerobic digestion [10,11].

To the best of our knowledge, only two studies have been reported on the co-generation of hydrogen and methane from enzymatic hydrolysates of *A. tequilana* bagasse, one in batch mode and one in continuous systems [10,11]. Nonetheless, several works have been reported on the use of different lignocellulosic hydrolysates aiming hydrogen and/or methane production in different reactor configurations [2,12–14]. In general, continuous stirred tank reactor (CSTR) [2,10], anaerobic sequencing batch reactor (ASBR) [13], upflow anaerobic sludge blanket reactor (UASB), anaerobic filter (AF) [14], trickling bed reactor (TBR) [8], and their combinations have been evaluated. Among these configurations, the semi-continuous operation of the ASBR has proved to provide several advantages, including low operation costs, easy operation, process flexibility, and decoupling of the solids retention time (SRT) from the hydraulic retention time (HRT), allowing to treat high organic loading rates (OLR) and better control of the microbial population [13,15]. In spite of the several reported advantages of the ASBR [16], there are no studies in the current literature that evaluate the co-production of hydrogen and methane from enzymatic hydrolysates of *A. tequilana* bagasse in this reactor configuration. Therefore, the present work aims to contribute to the first study on the semi-continuous production of hydrogen and methane from this type of lignocellulosic hydrolysates.

Materials and methods

Enzymatic hydrolysates and inocula

The *Agave tequilana* bagasse was kindly supplied by Casa Herradura distillery (Amatitan, Jalisco, Mexico), dried at room temperature and milled to an average length size of 1–1.5 cm. The enzymatic hydrolysis was carried out by mixing 4% (w/v) of the *A. tequilana* bagasse in a 50 mM citrate buffer solution at pH 4.5. Celluclast ® 1.5 L (Novozymes, Bagsværd Denmark) with an activity of 67 Filter Paper Units (FPU)/mL was added at a concentration equivalent to 40 FPU/g of bagasse and the reaction took place in an incubator at 45 °C for 12 h [17]. At the end of the hydrolysis, the hydrolysate was filtered through gauze cloth and characterized in terms of total sugars and chemical oxygen demand (COD) according to the analytical methods section.

An anaerobic granular sludge from a full-scale UASB reactor treating tequila vinasses in Casa Herradura distillery was used as inoculum. Such digester operates at pH 7, 35 °C and treats an OLR of 4 g COD L^{-1} d^{-1}. Mean values of total suspended solids (TSS) and volatile suspended solids (VSS) of the granular sludge were 12 and 9%, respectively. Finally, for the acidogenesis phase of the two-stage anaerobic digestion process, where hydrogen is produced, the inoculum was thermally treated as previously reported [11].

Reactors set up

Both ASBRs, acidogenic and methanogenic, were made of polyvinyl chloride with a working volume of 1.25 L. The reactors were completely instrumented to online determine variables such as temperature, pH, oxidation-reduction potential, pressure, H_2 and CH_4 gas percentage, and gas flow rates (Fig. 1). The acquisition and storage of data were carried out through a National Instruments cRIO-9004 device equipped with analogical and digital cards, and a graphical interface using the LabVIEW 8.2® software. The temperature of the reactors was controlled at 37 °C by means of a water jacket, while the pH was regulated by adding 2 N NaOH solution as required.

Please cite this article in press as: Toledo-Cervantes A, et al., Evaluation of semi-continuous hydrogen production from enzymatic hydrolysates of *Agave tequilana* bagasse: Insight into the enzymatic cocktail effect over the co-production of methane, International Journal of Hydrogen Energy (2018), https://doi.org/10.1016/j.ijhydene.2018.05.134