INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2015) 1–8

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Single and two-stage anaerobic digestion for hydrogen and methane production from acid and enzymatic hydrolysates of *Agave tequilana* bagasse

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ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form
9 October 2015
Accepted 2 November 2015
Available online xxx

Keywords:
Acid-enzymatic hydrolysates
Agave tequilana Weber var. Azul
Anaerobic digestion
Lignocellulosic biomass
Two-stage process

ABSTRACT

A comparison study on the energy recovery from single and two-stage anaerobic digestion of *Agave tequilana* bagasse hydrolysates is presented in this contribution. Firstly, the *A. tequilana* bagasse was acid or enzymatically hydrolyzed and then both hydrolysates were digested at different concentrations (20–100% v/v) in batch reactors. Results showed that the two-stage anaerobic digestion outperformed the single-stage process. During the acidogenesis phase of the two-stage process, high hydrogen yields were reached with the enzymatic hydrolysate at a concentration of 40% (3.4 mol H_2/mol hexose); whilst during methanogenic phase, the highest methane yield was obtained at a concentration of 20% for both hydrolysates (0.24 L CH_4/g COD). The overall energy recovery analysis demonstrated that the two-stage process outperformed 3.3 times the single-stage process when comparing the highest energy recoveries obtained in each process. This is the first study that reveals such enhancement for the anaerobic digestion of lignocellulosic hydrolysates.

Introduction

Currently the vast majority of the energy used by human beings comes from fossil fuels, which are non-renewable energy sources. When these fuels are extracted from earth and burned, carbon that was previously underground is released as carbon dioxide and accumulates in the atmosphere contributing to global warming [1]. Therefore, numerous governments around the world have encouraged the introduction of alternative energy sources such as bioenergy [2]. In this regard, lignocellulosic biomass stands out as an attractive feedstock for biofuels production because of its suitable composition, abundance and renewability [3]. Indeed, the interest on lignocellulosic biomass has also increased by the fact that large amounts of this type of biomass can be recovered as byproducts of a wide range of agro-industrial processes, such as the case of the bagasse from *Agave tequilana* (*A. tequilana* Weber var. Azul), which is the main solid waste generated by the tequila industry in Mexico [4]. According to the Tequila Regulatory Council, in 2014 the consumption of *A. tequilana* for tequila production was estimated at 788.2 × 10^3 tons, from which, approximately 40% were converted to bagasse [5].

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http://dx.doi.org/10.1016/j.ijhydene.2015.11.016

The lignocellulosic biomass such as the aforementioned bagasse is composed by lignin, extractives and ashes as the minor fractions and cellulose and hemicellulose as the major fractions [6]. Even though, the hemicellulose and cellulose polymers represent the main source of fermentable sugars, these fractions require further depolymerization for biofuel production [1]. To this end, several methods have been evaluated for sugar extraction from different types of lignocellulosic biomass [1,6,7]. Among these methods, dilute acid hydrolysis has demonstrated to be highly effective for hemicellulose depolymerization. However, this method has a drawback since toxic byproducts such as phenolic and furan compounds may also be generated depending on the severity of the hydrolysis conditions [7]. Therefore, other methods such as the enzymatic hydrolysis that is carried out at milder conditions are also attractive [8].

An emerging alternative for the valorization of lignocellulosic hydrolysates is the anaerobic digestion (AD) process, since the microbial consortiums involved in this process can metabolize the different types of sugars extracted from lignocellulosic biomass, i.e. hexoses and pentoses [9]. In general, during the AD process, bacteria break down the organic matter into hydrogen and organic acids (acidogenesis phase), while methanogenic archaea consume these compounds for methane production (methanogenesis phase). Thus, this process may be performed in either a single or two stages in order to produce methane or hydrogen and methane, respectively [10].

The single stage AD process of lignocellulosic hydrolysates has already been evaluated by using different types of lignocellulosic hydrolysates such as wheat straw hydrolysate, sugarcane bagasse hydrolysate, oat straw hydrolysate and recently *A. tequilana* bagasse hydrolysate [4,9,11,12]. Nonetheless, recent reports on the comparison of single and two-stage AD processes from different types of organic substrates (thin stillage, food waste, maize silage, among others) revealed that the two-stage process is more attractive in terms of energy recovery compared to the single-stage one [10,13,14]. Thus, even though some studies have reported the two-stage AD of lignocellulosic hydrolysates [15–17], more research is needed in order to compare the energy recovery from both AD process configurations. To the best of our knowledge, such comparison has not been reported by using lignocellulosic hydrolysates, highlighting the novelty of the present study. Thus, the main objective of this research was to compare the energy recovery that can be obtained from single and two-stage AD processes by using acid and enzymatic hydrolysates from *A. tequilana* bagasse in batch reactors. The acid and enzymatic hydrolysates were evaluated at different substrate to biomass ratios (S_0/X_0) by means of increasing the hydrolysates concentration.

Materials and methods

Substrate

The *A. tequilana* bagasse was kindly supplied by a tequila distillery located in Amatitan, Jalisco, Mexico. Prior to the hydrolysis step, the bagasse was dried at room temperature and the fibers were reduced to an average length of 1 cm. Then, enzymatic or acid hydrolyses of the bagasse were conducted at previously reported conditions [4,11]. Briefly, the enzymatic hydrolysis was carried out by dispersing 4% (w/v) of the *A. tequilana* bagasse in a 50 mM citrate buffer at pH 4.5. Then, Celluclast® 1.5 L was added at a concentration equivalent to 40 Filter Paper Units (FPU)/g of bagasse and the reaction took place in an incubator at 45 °C for 10 h. For acid hydrolysis, the *A. tequilana* bagasse was dispersed at 5% (w/v) in a 2.7% HCl solution. Then, the reaction took place in an oven at controlled temperature (124 °C) during 1.3 h. At the end of both treatments, the hydrolysates were filtered through a 0.45 μm membrane for further analyses.

Inoculum

Anaerobic granular sludge from a full-scale up-flow anaerobic sludge blanket (UASB) reactor was used as inoculum. The UASB reactor treats tequila vinasses from a local distillery. The operation parameters of the digester are pH:7, temperature: 35 °C, hydraulic retention time: 4–5 d, and organic loading rate: 4 g COD/L·d. Mean values of total suspended solids (TSS) and volatile suspended solids (VSS) of the sludge were 44 g/L and 30 g/L, respectively. For the acidogenesis phase of the two-stage AD process, the inoculum was thermally treated as reported by Buitrón and Carvajal [18] in order to eliminate hydrogen consumers and to favour hydrogen spore-formers, while for the methanogenesis phase of the two-stage process as well as for single-stage process, the inoculum was used without any treatment such as it was collected from the UASB reactor.

Automatic methane potential test system (AMPTS II)

In order to compare the energy recovery from single and two-stage AD processes by using acid and enzymatic hydrolysates from *A. tequilana* bagasse as substrate, batch experiments were carried out in an automatic methane potential test system (AMPTS II) (Bioprocess Control AB, Lund Sweden). The AMPTS II has the capacity for incubating 15 reactors of 0.5 L with individual mixing motors, CO_2 removal units and biogas (CH_4 or H_2) flowrate measuring cells (see Fig. S1). During the experimental runs, real-time temperature, pressure and accumulated gas volume were recorded automatically by the AMPTS II, and at the end of the process, a report was generated with normalized values of flowrate and accumulated gas.

Batch reactors conditions

For the single-stage AD process, the reactors were inoculated with 10 g VSS/L of non-treated sludge and fed with 400 mL of acid or enzymatic hydrolysates at concentrations of 20, 40, 60, 80 and 100 (% v/v). Prior to incubation, the initial pH was adjusted to 8 and the reactors were flushed with nitrogen gas to ensure anaerobic conditions. In contrast, for the two-stage AD process, the reactors were inoculated with 10 g VSS/L of thermally treated sludge and fed with 400 mL of acid or enzymatic hydrolysates at the same concentrations used for the single-stage process. After hydrogen production was exhausted, 10 g VSS/L of the non-treated sludge were added to

Please cite this article in press as: Arreola-Vargas J, et al., Single and two-stage anaerobic digestion for hydrogen and methane production from acid and enzymatic hydrolysates of *Agave tequilana* bagasse, International Journal of Hydrogen Energy (2015), http://dx.doi.org/10.1016/j.ijhydene.2015.11.016