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Continuous hydrogen production from enzymatic hydrolysate of *Agave tequilana* bagasse: Effect of the organic loading rate and reactor configuration

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HIGHLIGHTS

- Enzymatic hydrolysates of agave bagasse were suitable for H_2 production.
- An inverse correlation between VHPR and HMY was observed in CSTR.
- VHPR and HMY were simultaneously enhanced through increasing OLR in TBR.
- CSTR may be more susceptible to inhibition by hydrogen accumulation than TBR.
- Apparent H_2 consumption and high solids loading limited further OLR increments.

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ABSTRACT

Lignocellulosic biomass is a promising alternative energy source, which after pretreatment can be efficiently used as feedstock for biological production of second generation biofuels. Hydrogen is considered an ideal biofuel and its production through dark fermentation has been recognized as a sustainable process. However, more studies with continuous systems are needed for its application at industrial scale. In this study, enzymatic hydrolysate of *Agave tequilana* bagasse was used for long-term continuous hydrogen production in both, a continuous stirred tank reactor (CSTR) and a trickling bed reactor (TBR), which were operated up to 87 days under different organic loading rates (OLR) ranging from 17 to 60 g COD/L-d. Volumetric hydrogen production rate (VHPR) and hydrogen molar yield (HMY) in CSTR displayed an inverse correlation with maximum values of 2.53 L H_2/L-d and 1.35 mol H_2/mol substrate, attained at OLR 52.2 and 40.2 g COD/L-d, respectively. In contrast, increasing OLR up to 52.9 g COD/L-d simultaneously enhanced VHPR and HMY in TBR, attaining values of 3.45 L H_2/L-d and 1.53 mol H_2/mol substrate, respectively. Acetate and butyrate were the main metabolites in both reactors, while lactate and propionate were detected in minor concentrations. Metabolites distribution, electron balances and hydrogen production trends obtained from both reactors suggest that CSTR may be more susceptible to inhibition by hydrogen accumulation than TBR. Apparent hydrogen consumption and susceptibility to high solids load were found to limit further OLR increments in CSTR and TBR, respectively.

1. Introduction

Lignocellulosic biomass possess a high potential as a renewable energy source for biofuel production due to its high abundance, high carbohydrate content and no competition with food feedstock [1]. Hydrogen, a dense energy carrier is considered an ideal biofuel because its combustion only generates water vapor. It can be produced biologically by dark fermentation of sugars such as glucose, sucrose or fructose, which generally exhibit higher productivities as compared to complex substrates such as effluents from food and beverage industries [2]. Nonetheless, hydrogen production

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through dark fermentation of organic wastes, including residual lignocellulosic biomass, is recognized as an environmental friendly, cost effective sustainable process for energy production along with waste and biomass valorization [3]. *Agave tequilana* bagasse is an interesting source of fermentable sugars for hydrogen production [4] because its high carbohydrate and low lignin content (46% cellulose, 23% hemicellulose, 19% lignin), with the advantage of being produced as a specific concentrated waste in tequila distilleries. In this regard, there are 3.6×10^5 tons of agave bagasse that might be used each year for energy production in Mexico [5].

In order to release sugars, lignocellulosic materials typically require pretreatment to hydrolyze linkages between lignin, hemicellulose and cellulose [6]. However, the agave bagasse used in this study may be considered as a pretreated feedstock since it is generated after cooking (100 °C for 24 h) and grinding agave heads for syrup extraction during the tequila production process. Thus, the application of direct enzymatic hydrolysis over agave bagasse, without any further pretreatment, to produce a suitable substrate for hydrogen production is highly attractive from both the economic and energetic point of view. In fact, batch experiments showed greater hydrogen production and energy recovery by applying enzymatic rather than acid hydrolysis over the *Agave tequilana* bagasse fibers [7].

Research on hydrogen production from lignocellulosic biomass has been developed since the past decade mainly in batch experiments [1,6]. However, in order to evaluate the viability of the process scale-up, the study of continuous systems is required. Only few studies dealing with the use of lignocellulosic hydrolysates for continuous hydrogen production are available in the current literature, most of which have been carried out in continuous stirred tank reactors (CSTR). The highest volumetric hydrogen production rate (VHPR) of 16 L H_2/L-d has been reported in a CSTR fed with detoxified rice straw acid hydrolysates [8]. Typically, higher VHPR are registered when the organic loading rate (OLR) is increased; however, reduced hydrogen molar yields (HMY) and risk of biomass wash-out imply a limit for OLR increments in CSTR [2,9,10]. Immobilizing biomass in CSTR have led to much higher VHPR [11], due to the high biomass content in the reactor that allows treating higher OLR without biomass washout.

On the other hand, hydrogen concentration in the liquid phase has been recognized as a key factor for performance during hydrogen production in CSTR, since supersaturation may lead to reduced hydrogen production [12,13]. With regard to trickling bed reactors (TBR) they provide two advantages i.e. a higher gas-liquid interphase favoring hydrogen degasification and immobilized biomass giving flexibility to deal with OLR variations [2]. Previously, hydrogen production in TBR was evaluated by using simple sugars and lignocellulosic hydrolysates [14,15]. Such studies were focused in attaining a stable operation by avoiding clogging problems due to excessive biomass attachment as well as to evaluate the effect of simple and complex substrates utilization. Although HMY obtained from the enzymatic hydrolysate showed similar values to those observed when glucose was used as substrate (1.7 mol H_2/mol glucose) [14], the enzymatic hydrolysate was tested for only 16 days in continuous mode.

Therefore, the aim of this study was to evaluate enzymatic hydrolysates of *Agave tequilana* bagasse as substrate for long-term continuous hydrogen production using both reactors configurations CSTR and TBR. Sugars consumption efficiency, VHPR, HMY, biomass and soluble metabolites concentrations were regularly monitored, while the fermentation performance was systematically assessed through increases in OLR. Hydrogen production rates and yields related to specific operating conditions and reactors configurations are compared and discussed.

2. Materials and methods

2.1. Inoculum and medium composition

Anaerobic sludge obtained from a pilot-scale fixed-bed reactor treating tequila vinasses (CUCEI-Universidad de Guadalajara, Mexico) was heat treated at 105 °C for 24 h to eliminate hydrogen-consuming microorganisms according to Arreola-Vargas et al. [14] and used as inoculum. The total suspended solids (TSS) and volatile suspended solids (VSS) concentration of the seed sludge were 27 g/L and 20 g/L, respectively. The dry sludge was manually grinded and mixed with mineral medium and hydrolysate to attain a final concentration of 4.5 and 2.3 g VSS/L for the CSTR and TBR, respectively. The used mineral medium was modified from Arreola-Vargas et al. [16] to contain (g/L): $NH_4H_2PO_4$, 4.5; Na_2HPO_4, 0.635; K_2HPO_4, 0.125; $MgCl_2{\cdot}6H_2O$. 0.1; $ZnCl_2$, 0.075; $FeSO_4{\cdot}7H_2O$, 0.025; $MnSO_4{\cdot}H_2O$, 0.009; $CuSO_4{\cdot}5H_2O$, 0.005.

2.2. Agave bagasse hydrolysis

Agave bagasse was collected four times from a single tequila distillery (Casa Herradura, Amatitán, Jalisco, Mexico). The bagasse was sun dried at ambient temperature and then grinded to obtain fibers with lengths between 2 and 4 cm. The enzymatic hydrolysis of bagasse was performed following previously reported conditions [7]. Briefly, dry agave bagasse and the enzymatic preparation (Celluclast 1.5 L®, Novozymes, Denmark) were suspended at 4% (w/v) in 50 mM citrate buffer at pH 4.5, and the mixture was shaken at 100 rpm and 45 °C for 10 h. Total sugars and chemical oxygen demand (COD) were determined after cooling and before reactors feeding.

2.3. Reactors set up and experimental procedure

Fig. 1 shows the schematic diagram of the reactors used in this study. The 3 L CSTR (Fig. 1a) was made from PVC with a liquid volume (V_R) of 2.8 L and a liquid recycle rate of 8 L/min. The CSTR was equipped with a controller (cRIO 9074, National Instruments, USA) with signal processing and data acquisition capabilities that allowed the regulation of pH at 4.6 or 5.5 by the automatic addition of NaOH 2 N. Readings of pH, conductivity, temperature, pressure, gaseous hydrogen concentration (BCP-H2, BlueSens, Germany) and gas flow rate (μFlow, Bioprocess Control, Sweden) were acquired online with a data logging software (LabVIEW 2009, National Instruments, USA).

The TBR (Fig. 1b) was made of methacrylate, which was composed by three cylindrical modules of 6.5 cm inner diameter and 30 cm length each and a 0.5 L liquid vessel at the bottom. Vertically arranged polyvinyl ethylene (PE) tubing (inner diameter 1 cm) was used as packing material since it has been shown to be effective for preventing clogging when used in the continuous hydrogen production by dark fermentation [14]. The TBR was packed with 18 PE tubes per module with length 10.7 cm (module I) and 27.5 cm (modules II and III) with a void fraction (ε) of 0.7 and void volume of 1.5 L (V_{R*}). The total liquid volume in the TBR was 670 ml (bottom vessel plus packing hold-up). A conical nozzle with adjustable spray angle, typically used for irrigation, was placed at the top of the column for feeding and liquid recirculation. Recirculation flow rate was set at 180 ml/min while feeding flow rate was set at 1.8, 2.8 and 5.5 ml/min to obtain HRT of 6, 4 and 2 h, respectively. TBR was equipped with a controller (HI 8710E, HANNA Instruments, USA) to adjust pH at 5.5 through the automatic dosing of NaOH 2 N.

For the start-up phase, reactors were operated in batch mode with initial hydrolysate concentration of 10 g COD/L and initial