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High organic loading rate on thermophilic hydrogen production and metagenomic study at an anaerobic packed-bed reactor treating a residual liquid stream of a Brazilian biorefinery

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HIGHLIGHTS

- An optimum OLR in a packed-bed reactor producing hydrogen from vinasse was reached.
- A decrease in the specific OLR negatively affected hydrogen production.
- An equation to estimate the biomass growth in packed-bed reactors is presented.
- *Megasphaera* were the microorganisms that most contributed to hydrogen production.
- Thermophilic fermentation decreased contamination by autochthonous microorganisms.

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ABSTRACT

This study evaluated the influence of a high organic loading rate (OLR) on thermophilic hydrogen production at an up-flow anaerobic packed-bed reactor (APBR) treating a residual liquid stream of a Brazilian biorefinery. The APBR, filled with low-density polyethylene, was operated at an OLR of 84.2 kg-COD m^{-3} d^{-1}. This value was determined in a previous study. The maximum values of hydrogen production and yield were 5,252.6 mL-H_2 d^{-1} and 3.7 mol-H_2 $mol^{-1}_{total\ carbohydrates}$, respectively. However, whereas the OLR remained constant, the specific organic load rate (sOLR) decreased throughout operation from 1.38 to 0.72 g-Total carbohydrates g-VS^{-1} h^{-1}, this decrease negatively affected hydrogen production. A sOLR of 0.98 g-Total carbohydrates g-VS^{-1} h^{-1} was optimal for hydrogen production. The microbial community was studied using 454-pyrosequencing analysis. Organisms belonging to the genera *Caloramator*, *Clostridium*, *Megasphaera*, *Oxobacter*, *Thermoanaerobacterium*, and *Thermohydrogenium* were detected in samples taken from the reactor at operation days 30 and 60, suggesting that these organisms contribute to hydrogen production.

1. Introduction

Sugarcane vinasse (the traditional liquid stream generated from first-generation of ethanol production) has the potential to produce hydrogen due to its high COD content (35.2 ± 2.6 g-O_2 L^{-1}), amounts of nutrients, low pH (4.6 ± 0.2) and large generation volume; 312 billion liters were generated (10–14 L of vinasse L^{-1} ethanol produced) during the 2012–2013 sugarcane harvest in Brazil (UNICA, 2014).

A previous study has demonstrated that up-flow anaerobic packed-bed reactors (APBR) can be successfully used for continuous thermophilic hydrogen production using sugarcane vinasse as a substrate (Ferraz Júnior et al., 2014a). In the cited study, an optimal organic loading rate (OLR) of 84.2 kg-COD m^{-3} d^{-1} was determined, predicting a maximum volumetric hydrogen production (VHP) and yield of 1117.2 mL-H_2 d^{-1} $L^{-1}_{reactor}$ and 2.4 mol-H_2 $mol^{-1}_{total\ carbohydrates}$, respectively. These values were determined using an experimental setup that was monitored for 30 days. However, in long-term operation, natural biomass growth in the APBR might

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reduce the specific organic loading rate (sOLR), thereby negatively affecting hydrogen production; that is, assuming a constant initial substrate concentration, the substrate availability can decrease due to the natural biomass growth in the APBR, as presented by Fontes Lima and Zaiat (2012) and Penteado et al. (2013). In these studies, homoacetogenic activity (the Wood–Ljungdahl pathway) increased throughout the operation, resulting in a decrease in the amount of hydrogen released into the gas phase. Thus, a longer period of monitoring is necessary to evaluate the stability of hydrogen production.

In a later study, Fontes Lima et al. (2013) compared the use of sucrose and glucose as substrates for hydrogen production in an APBR. Sucrose and glucose differed most, regarding the stability of the process. Homoacetogenic microorganisms might have caused the hydrogen production instability when the reactor was fed with sucrose, and the inability of such microorganisms to grow on glucose might explain the stabilization of hydrogen production that was observed in an APBR fed with the monosaccharide. In contrast, Yu and Mu (2006), Dong et al. (2011) and Braga et al. (2015) increased the organic loading rate (OLR) in up-flow anaerobic sludge blanket reactors (UASB) and observed continuous and stable hydrogen production during long-term operation (3 years, 310 days and 366 years, respectively) using sucrose-based laboratory-made wastewater.

"Long-term" operation appears to be a subjective term in discussions of biological hydrogen production. Nevertheless, a lack of knowledge about long-term hydrogen production exists, particularly in relation to packed-bed reactors. Several previous studies have indicated that hydrogen production is ephemeral in this type of reactor as homoacetogenic activity becomes established due to the accumulation of biomass in the bed. For this type of reactor, operations of 60 days can be considered long-term because within this period is sufficient to evaluate the biochemical pathways that are primarily responsible for operating instabilities and to evaluate possible hydrodynamic sickness resulting from the accumulation of biomass in the packed bed. Moreover, in most operations using this type of reactor configuration, decreases in hydrogen production have been observed at an early stage (i.e., from the 25th day of operation).

Thus, the aim of the current study was to evaluate hydrogen production during a Brazilian sugarcane harvest period, that corresponds to approximately 210 days, in a thermophilic APBR under a high OLR of 84.2 kg-COD m^{-3} d^{-1} and using raw sugarcane vinasse as a substrate. The biomass that developed in the APBR was characterized using next-generation sequencing (454-pyrosequencing).

2. Methods

2.1. Reactor and support materials

An up-flow APBR was constructed of acrylic tube and filled with low-density polyethylene (LDP) as the support material. The total volume and liquid volume were 3.5 and 2.3 L, respectively. Details regarding the dimensions of the reactor and the characteristics of the LDP used have been previously presented in Ferraz Júnior et al. (2014b).

2.2. Sugarcane vinasse

Wastewater (raw sugarcane vinasse) from the sugarcane industry (São Martinho, Pradópolis, São Paulo, Brazil) was used as a substrate. Before being fed into the reactors, the raw wastewater was filtered through a paper filter (Nalgon, density 80 g m^2, porosity 3 μm) to reduce the concentration of suspended solids. The COD value was used as a parameter for the OLR calculation. The chemical composition of the wastewater had been determined previously as follows (in g L^{-1}): total carbohydrates (4.1 ± 0.9); $COD_{filtered\ (3\ \mu m)}$ (35.2 ± 2.6); soluble $COD_{(0.45\ \mu m)}$ (25.8 ± 4.5); BOD (16.7 ± 1.1); total nitrogen (0.7 ± 0.02); phosphorus (0.16 ± 0.05); and sulfate (1.4 ± 0.3).

2.3. Operation of the reactor for hydrogen production

The inoculum was obtained through the natural wastewater fermentation process, in which the raw filtered vinasse had its pH set to 6.5 using 50% (w/v) NaOH. Then, the vinasse was maintained for three days in a dark chamber to allow the autochthonous microorganism growth. After this period, the fermented effluent with the cultured biomass was pumped into the reactor and recirculated for 5 days to enhance the biomass attachment to the LDP particles (Leite et al., 2008). The APBR was operated continuously at an OLR of 84.2 kg-COD m^3 d^{-1} and a hydraulic retention time (HRT) of 10.2 h at 55 °C (Ferraz Júnior et al., 2014a). The initial pH of vinasse was 6.5, however, no pH control was performed along the packed-bed reactor.

2.4. Analytical methods

To evaluate the performance of the reactors, influent and effluent liquids were sampled periodically to determine total carbohydrate concentration using sucrose (1%, w/v) as the standard curve (Dubois et al., 1956), pH, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) and volatile suspended solids (VSS) (APHA et al., 2005).

The concentrations of organic acids (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acids) and solvents (methanol, ethanol and *n*-butanol) were analyzed using a gas chromatograph equipped with a FID detector (Perna, 2011).

The volume of gas produced was measured using a Ritter MilligasCounter. The analytical composition of the biogas, which comprised hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4), was determined using a Shimadzu gas chromatograph-2010® GC equipped with a capillary column Carboxen 1010 PLOT (30 m × 0.32 mm) and a thermal conductivity detector (Perna et al., 2013).

2.5. Determination of the specific organic loading rate (sOLR)

sOLR, which was measured in g-Total Carbohydrates g-VSS^{-1} h^{-1}, was calculated based on the ratio of organic loading rate (OLR) to the estimated concentration of biomass (Cx_n/t) for a given period (Eq. (1)):

$$sOLR = \frac{OLR}{Cx_n/t} \tag{1}$$

OLR was calculated based on the total concentration of carbohydrates (C_{tc}) using Eq. (2):

$$OLR = \frac{C_{tc} \cdot Q}{V_w} \tag{2}$$

In Eq. (2), Q is the vinasse flow rate, and V_w is the working volume of the reactor.

The concentration of biomass throughout the experimental period (Cx_n/t) was estimated using the biomass yield ($Y_{x/s}$) and the carbohydrate concentration converted throughout the experimental time (C_{tc}/t), as show in Eq. (3):

$$Cx_n/t = Y_{x/s} \cdot C_{tc}/t \tag{3}$$

The biomass yield ($Y_{x/s}$) was calculated with data obtained for biomass concentration in respect to the total carbohydrate