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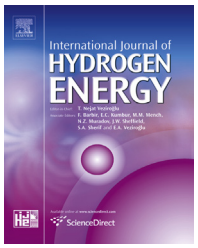

Hydrogen production from acid and enzymatic oat straw hydrolysates in an anaerobic sequencing batch reactor: Performance and microbial population analysis

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ABSTRACT

Feasibility of hydrogen production from acid and enzymatic oat straw hydrolysates was evaluated in an anaerobic sequencing batch reactor at 35 °C and constant substrate concentration (5 g chemical oxygen demand/L). In a first experiment, hydrogen production was replaced by methane production. Selective pressures applied in a second experiment successfully prevented methane production. During this experiment, initial feeding with glucose/xylose, as model substrates, promoted biomass granulation. Also, the highest hydrogen molar yield (HMY, 2 mol H_2/mol sugar consumed) and hydrogen production rate (HPR, 278 mL H_2/L-h) were obtained with these model substrates. Gradual substitution of glucose/xylose by acid hydrolysate led to disaggregation of granules and lower HPR and HMY. When the model substrates were completely substituted by enzymatic hydrolysate, the HMY and HPR were 0.81 mol H_2/mol sugar consumed and 29.6 mL H_2/L-h, respectively. Molecular analysis revealed a low bacterial diversity in the stages with high hydrogen production and vice versa. Furthermore, *Clostridium pasteurianum* was identified as the most abundant species in stages with a high hydrogen production. Despite that feasibility of hydrogen production from hydrolysates was demonstrated, lower performance from hydrolysates than from model substrates was obtained.

1. Introduction

Fermentative hydrogen production is recognized as a cost effective and environmentally friendly process. The type of substrate and type of reactor are factors that substantially affect fermentative hydrogen production parameters, i.e. the hydrogen production rate (HPR) and the hydrogen molar yield (HMY) [1,2]. Thus, evaluation of different organic wastes as

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substrates for hydrogen production has become relevant [1–5]. Agricultural by-products may be a potential substrate for hydrogen production at commercial scale, given that they are abundant, easily available and inexpensive [3–5]. However, the direct conversion of this biomass to hydrogen is limited by the low biodegradability of the lignocellulosic matrix. Due to this reason, pretreatment of the agricultural by-products is needed in order to release the biodegradable sugars contained in the hemicellulose and cellulose fractions of this biomass [6,7]. Common treatments applied before the production of biofuels from lignocellulosic biomass are acid, alkaline, enzymatic and hydrothermal hydrolysis. Sole or in combination, these types of hydrolysis have been used prior to the fermentative production of hydrogen from wheat straw [8,9], sugarcane bagasse [10,11], cornstalk and corn stover [12–15], rice straw [16] and oat straw [17].

Regarding the type of reactor, fermentative hydrogen production has been conducted in a variety of reactors operated under continuous feeding mode [1,2]. However, it has been reported that hydrogen production in anaerobic sequencing batch reactors (ASBR) has some advantages over continuous feeding mode [18]. These advantages include high degree of process flexibility, better control of the microbial population due to the cyclic operation and the decoupling of the solids retention time (SRT) from the hydraulic retention time (HRT). Some studies on ASBR have reported the effect of different operational parameters (pH, HRT, substrate concentration, etc.) over the hydrogen production [18–22]. However, there is no report on the use of an ASBR for the production of hydrogen from lignocellulosic hydrolysates.

Therefore, the aim of this research was to study the feasibility of fermentative hydrogen production in an ASBR from oat straw hydrolysates. Oat straw was used as an agricultural by-product model. In order to solubilize the hemicellulose and cellulose fractions of the oat straw, it was sequentially hydrolyzed by means of a dilute acid hydrolysis followed by an enzymatic hydrolysis. The effect of both hydrolysates (acid and enzymatic) on the hydrogen production performance was evaluated. Performance of the processes was also correlated with changes in the microbial community.

2. Materials and methods

2.1. Experimental strategy

ASBR was initially fed with a mixture of glucose/xylose 1:1 on COD basis (5 g/L total COD). Then, the mixture was substituted in a step-wise mode with increasing amounts of acid and enzymatic oat straw hydrolysates.

In a first experiment (Experiment A), hydrogen production was initially observed, but complete suppression of hydrogen and an increase on methane production was observed. This result led to a second experiment (Experiment B) where several selective pressures against methanogens were applied. Table 1 summarizes the operational periods for both experiments; each condition was maintained for at least 20 cycles. Steady state was assumed after three similar values of hydrogen production and sugar removal were achieved; once steady state was reached a new condition was evaluated. Hydrogen produced throughout this study is reported at standard temperature and pressure conditions (0 °C and 1 atm).

2.2. Inoculum and mineral medium

Experiment A: anaerobic granular sludge from a full-scale upflow anaerobic sludge blanket (UASB) reactor was used as inoculum for hydrogen production. The UASB reactor treats wastewater from a confectionery factory in San Luis Potosí, México. Prior to inoculation, the granular sludge was thermally treated, powdered and stored as previously described [19]. The powder was used as inoculum in the bioreactor at a concentration of 5.5 g/L (4.5 g VSS/L). The mineral medium

Table 1 – Operational stages of the ASBR during experiments A and B.

Stage	Purpose	Operation period (d)	Influent substrate concentration (g COD/L)	Equivalent HRT (h)	pH	Bioreactor operation mode
Experiment A						
I	Start-up	1–13	5[a]	24	5.5	ASBR
II	Acid hydrolysate effect	14–32	3.75[a] + 1.25[b]	24	5.5	ASBR
Experiment B						
I	Start-up	1–7	5[a]	6	4.5	CSTR
II		7–12	5[a]	8	4.5	ASBR
III	Acid hydrolysate effect	12–14	4.5[a] + 0.5[b]	8	4.5	ASBR
IV		15–18	4[a] + 1[b]	8	4.5	ASBR
V		19–22	3.5[a] + 1.5[b]	8	4.5	ASBR
VI		23–27	3[a] + 2[b]	8	4.5	ASBR
VII		28–34	2.5[a] + 2.5[b]	8	4.5	ASBR
VIII	Effect of the acid and enzymatic hydrolysates mixture	34–37	2.5[b] + 2.5[c]	8	4.5	ASBR
IX	Enzymatic hydrolysate effect	38–42	5[c]	8	4.5	ASBR

a Model substrate: mixture of glucose-xylose (1:1).
b Acid hydrolysate.
c Enzymatic hydrolysate.