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## Hydrogen and methane production via a two-stage processes (H<sub>2</sub>-SBR + CH<sub>4</sub>-UASB) using tequila vinasses

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### ABSTRACT

#### Keywords:

Anaerobic digestion  
Biogas  
Hydrogen methane organic matter removal  
Tequila vinasse

The feasibility of producing hydrogen and methane via a two-stage fermentation of tequila vinasses was evaluated in sequencing batch (SBR) and up-flow anaerobic sludge blanket (UASB) reactors. Different vinasses concentrations ranging from 500 mg COD/L to 16 g COD/L were studied in SBR by using thermally pre-treated anaerobic sludge as inoculum for hydrogen production. Peak volumetric hydrogen production rate and specific hydrogen production were attained as  $57.4 \pm 4.0$  mL H<sub>2</sub>/L-h and  $918 \pm 63$  mL H<sub>2</sub>/gVSS-d, at the substrate concentration of 16 g COD/L and 6 h of hydraulic retention time (HRT). Increasing substrate concentration has no effect on the specific hydrogen production rate. The fermentation effluent was used for methane production in an UASB reactor. The higher methane composition in the biogas was achieved as 68% at an influent concentration of 1636 mg COD/L. Peak methane volumetric, specific production rates and yield were attained as  $11.7 \pm 0.7$  mL CH<sub>4</sub>/L-h,  $7.2 \pm 0.4$  mL CH<sub>4</sub>/g COD-h and  $257.9 \pm 13.8$  mL CH<sub>4</sub>/g COD at 24 h-HRT and a substrate concentration of 1636 mg COD/L. An overall organic matter removal (SBR + UASB) in this two-stage process of 73–75% was achieved.

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### Introduction

Recent technological developments have driven the human society towards unavoidable dependence on the fossil fuel energy resources, which are drastically being depleted due to over consumption. This has led the researchers in energy sector to find alternative energy sources or renewable energy sources as the main task. Currently, hydrogen gas has gained the credit of being a solution to the future energy demands and also bearing the possibilities of socio economic,

technological and environmental benefits, because of its unique features, such as high energy content, no green house gases emission and also demonstrated applications in the fuel cells to produce electricity [1–5].

Tequila industry in Mexico represents one of the largest economic sectors of the nation. The Tequila Regulatory Commission reported that a production of 200 million liters of tequila in Mexico so far in 2010 [6]. For every liter of tequila production, generation of 10 L of wastewater has been recorded. This wastewater causes various environmental problems due to high organic content, acidity (pH < 3.9) and high salt content [7].

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<http://dx.doi.org/10.1016/j.ijhydene.2014.04.139>

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Anaerobic digestion is an effective method of treating different organic pollutants. This process offers several advantages over aerobic systems like economic and energy saving, less biological sludge production, fewer nutrients requirements and smaller reactor volumes [8]. Biohydrogen production from various wastewater and solid organic wastes have been reported previously, such as from condensed molasses soluble [9], food industry wastewater [10], rice straw [10], mushroom waste [11], de-oiled jatropha waste [12] and water hyacinth [13]. In addition, it has been shown as an alternative method of treating the wastes meanwhile generating the energy. In this spotlight tequila vinasses could act as a feasible feed stock for the fermentative energy production. Buitrón and Carvajal [6] studied hydrogen production from tequila vinasses using initial concentrations up to 5 g chemical oxygen demand (COD)/L. In that study, it was observed that the amount of biogas and hydrogen production was affected by the initial concentration fed to the reactor, and the intensity of this effect was dependent on the HRT and the temperature utilized.

Generating methane from the effluent of  $H_2$  fermentation which is rich in organic acids could significantly enhance the total energy value of the process. Besides, it could also reduce the COD of the effluent to a certain extent. Two-stage fermentation system ( $H_2 + CH_4$ ) has been recently gained more attention due to the reduction of toxicity of the waste materials to very lower extent and also generation of more amount of energy compared to single stage fermentation [14–19]. Thus, in this work the co-generation of bio-hydrogen and methane was evaluated using the wastewater of tequila industry as carbon source. Additionally, the effect of high substrate concentrations on organic matter removal was also studied.

## Materials and methods

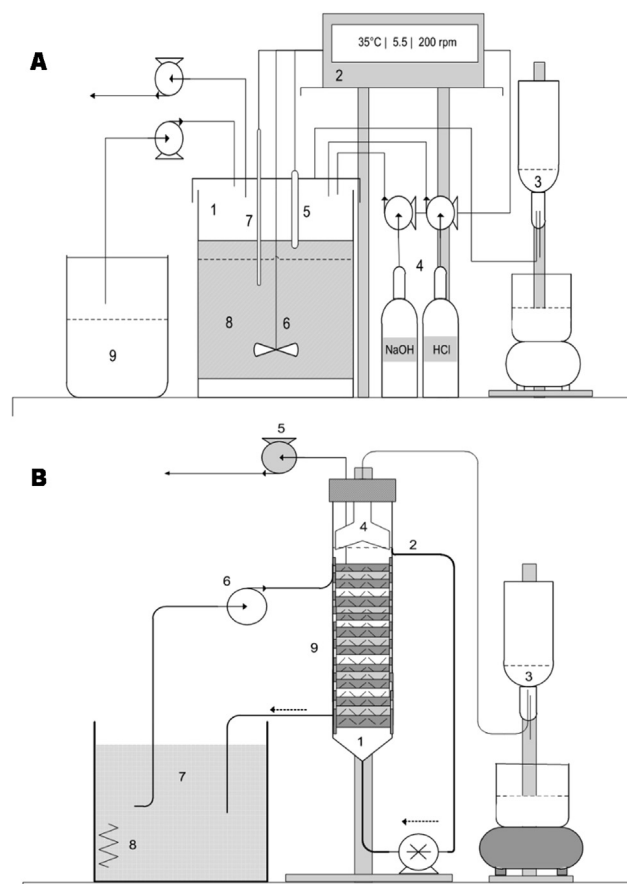
### Selection of hydrogen-producing bacteria and tequila vinasses

Anaerobic sludge from a brewery wastewater treatment plant was used as seed inoculum in this study. Heat treatment at 100 °C for 24 h was employed to eliminate the hydrogen consuming methanogens and to enrich the hydrogen-producing microorganisms. After thermal treatment, the sludge was activated with glucose until a stable hydrogen production was observed as mentioned by Ref. [6]. The vinasses used in this study contains the following characteristics: organic matter concentration between 19.8 and 20.9 as  $gBOD_5/L$  and between 29.9 and 30.5 as  $gCOD/L$ ; glucose 4.6  $g/L$ ; phenols from 44 to 81  $mg/L$ ; sulphates 915  $mg/L$ ;  $N-NH_3$  110  $mg/L$ ; and pH from 3.2 to 4.0. The  $BOD_5/COD$  ratio was 0.67 indicating that organic matter could easily be biodegraded.

### Hydrogen fermentation of tequila vinasses

It has been reported that the hydraulic retention time (HRT) influenced hydrogen production from tequila vinasses [6]. Thus, two sets of experiments, at two different HRT's, were performed each set at different the tequila vinasses concentrations. The first experiment was conducted with 18 h-HRT

using a sequencing batch reactor (SBR-1). Three different substrate concentrations (soluble COD) were employed: 0.5, 1.0 and 5.0  $mgCOD/L$ . As no inhibition was observed, a second set of experiments (SBR-2) was carried out using a lower HRT (6 h) and six concentrations of vinasses (soluble COD), covering a higher range than the previous set: 2, 6, 8, 12, 14 and 16  $gCOD/L$ . The schematic diagram of the reactor set-up is shown in Fig. 1A. The reactor operation and methodologies were previously reported [6]. In first set of experiments, the reactor had a reaction volume of 4 L, whereas for the second one, the volume was 600 mL. The SBR-1 was inoculated with heat pre-treated inoculum (3.7  $gVSS/L$ ). SBR-2 was inoculated with a glucose-adapted biomass (1.5  $gVSS/L$ ) which was harvested at the end of the experiments SBR-1. An automatic control system maintained the temperature at 35 °C and the pH of 5.5. The nutrient medium supplied was prepared as described by Mizuno et al. [20]. SBRs were run under each condition for several days and the reported data represent the average of at least three representative cycles when the hydrogen production had been stabilized.



**Fig. 1 – (A) Reactor set-up for hydrogen production (1- SBR reactor, 2- Bio controller, 3- biogas measuring device, 4- Acid and base for pH control, 5- pH electrode, 6- agitator, 7- thermometer, 8- thermal jacket for temperature control, 9- feedstock tank). (B) Reactor set-up for methane production (1- UASB reactor, 2- recirculation, 3- biogas measuring device, 4- Phase separator, 5- influent, 6- water jacket, 7- water tank at 35 °C, 8- thermostat for temperature control, 9- thermal jacket).**

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