Effect of the Mixed Inocula and the pH During the Dark Fermentation Process for the Production of the Hydrogen Using Molasses as Substrate.

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ABSTRACT

In the present work, the effect of the origin of the inoculum is determined in the biological production of hydrogen through the performance of a series of experiments by batches. To do so, three different mixed inocula were tested consisting of an aerobic sludge, a methanogenic anaerobic one, and an acidogenic anaerobic one. On the other hand, the cumulative hydrogen production was quantified after selection of the best mixed inocula with a heat treatment with 70º C and 120°C per 30 min, and without treatment. As a result, it was observed that the heat treatment during the hydrogen production does not present any additional effect compared to the inoculum without heat treatment. With regard to the origin of the inoculum, the methanogenic sludge produced a higher volume of hydrogen with a performance of 225 mLH_2, unlike the acidogenic anaerobic sludge which produced 45 mLH_2. Even though the methanogenic sludge produced a higher cumulative volume of hydrogen, methane was produced during the first 15 hours, unlike the acidogenic sludge where methane was produced during the whole experiment at concentration less of 8%. It was also studied the effect of the initial pH with values of 4, 4.5, 5, 5.5 and 6.5. The results showed that at 5 pH the biogas production, and the hydrogen cumulative volume was higher with values of 815 mL and 265 mL respectively, and with a performance of 76 mLH_2/g of consumed sucrose.

1. Introduction

Hydrogen is a renewable energy source and has a high-energy content of 122 kJ/g, which is 2.75 fold greater than traditional fossil fuels. Biological systems offer a wide variety of ways to generate renewable energy. Among them, fermentative bacteria consume mainly carbohydrates in the anaerobic digestion, generating hydrogen and organic acids of small molecular weights. The theoretical maximum hydrogen production from the fermentation of pure carbohydrates is 4 mol of H_2 by hexose oxidized by and acetic acid as a byproduct of the fermentation. In practice, the production is lower due to the production of biomass and other byproducts as butyric, propionic and alcohols. Though hydrogen production via dark fermentation by pure cultures with higher hydrogen yields (2–4 mol H_2/mol

glucose) has been reported [1], mixed culture fermentation is more practical compared to pure culture process since non-sterile conditions are applied. However, one of the difficulties associated with hydrogen production using mixed communities is the coexistence of hydrogen-consuming microorganisms, such as methanogens. Various pretreatment methods including heat, acid, base, chloroform, sodium 2-bromoethanesulfonate (BESA), aeration and loading-shock have been conducted on the mixed inocula to enrich hydrogen-producing bacteria [2,3,4]. Despite the results are inconsistent from one study to another, several studies have used heat pretreatment of the inoculum used to seed the reactor as method to inactivate or eliminate these microorganisms. The pretreatment conditions reported vary in terms of temperature and time of exposure, ranging from 70 °C to 120 °C for 15 min to 2 hour. [4,5,6]. A general review shows that heat pretreatment is most widely used. Although in more recent studies it has been proposed to use the fresh inoculum without pretreatment [7]. In this study we propose the evaluation of different inocula for the production of hydrogen using the heat pretreatment to enrich the hydrogen-producing bacteria thus also determine the effect of pH on the system operation to inhibit the production of methane and its effect on yield and H_2 concentration in biogas.

2. Experimental section

Physicochemical characterization of molasses

The medium used for this experiment was molasses from a sugar factory in the center of Mexico, without any supplement. In table 1 the composition of molasses is presented. The analyses of concern were determined according to Standard Methods.[8]. These include total volatile solids VS, pH, total nitrogen and alkalinity. Sucrose was determined by the phenol sulphuric acid method [9].

Table 1. Composition of the normal Molasses

TKN	0.2-2.8 % w/w
TS	78-85% w/w
Sucre	48-58% w/w
TOC	28-34% w/w

Inocula and conditioning

A total of three sources were tested as inocula for hydrogen fermentation as it follows: activated aerobic sludge from a secondary settling tank of Municipal Wastewater Treatment Plant, anaerobic sludge from a UASB reactor of wastewater treatment plant of fruits and vegetables canning. The acidogenic sludge from a UASB pilot reactor fed with leached, operated at pH 5.5 and 35°C. The leached was obtained from a municipal landfill. The inocula were

thermally pretreatment a method commonly applied for removing hydrogen-consuming microorganisms. The inocula were incubated at 70 °C and 120°C for 30 min and also the inocula were tested without pretreatment.

Experiment in batch for hydrogen production with a variety of inocula

Batch experiments were performed in 125 mL serum bottles with 80 mL of liquid volume containing molasses and inoculum. The initial amount of biomass used in the batch experiments was approximately 2 g/L VS and the molasses concentration was 30 g/L. The initial pH of the batch experiments were adjusted to 5.5 with HCl and sprayed with nitrogen gas for 5 min to generate anaerobic conditions. The cultures were set in a controlled incubator at 33 °C.

Effect of pH on hydrogen production

To determine the effects of the initial pH values on hydrogen production, kinetic assays were performed with anaerobic sludge without heat treatment, by setting the initial pH with HCl at 4.0, 4.5, 5.0, 5.5 to perform the experiment at 6.5, the pH was controlled with NaOH concentrated solution. The pH value of fermentation solution in series changed naturally with prolonged time. The SV and molasses concentration were identical to those described in the preceding paragraph.

Biogas and liquor analyses

The amount of biogas produced in each reactor was recorded by the water displacement method. The biogas contents were analyzed by a gas chromatograph (GC) (Model 580 GOW-MAC) equipped with a thermal conductivity detector and a 2-m stainless column packed with Molecular Sieve Carbosphere (80/100 mesh). Injector, detector and column temperature were kept at 120°C the first two, and 140 °C respectively. Nitrogen gas was the carrier gas at a flow rate of 40 mL/min at a pressure of 40 psi. The liquid samples taken from the batch culture reactor were centrifuged at 5000 rpm for 15 min, and then the supernatants were filtered through 0.45 μm cellulose acetate membranes for the analysis of the volatile fatty acids (VFAs) and ethanol. The filtered samples were acidified with 50 mL of a 1:1 (V/V) HCl solution. The concentration(VFA in the liquors was determined by a gas chromatograph (HP-5890II) equipped with a flame ionization detector and a stainless AT-100 column (0.53 mmØ 1.2mm x 10m) at a temperature ramp T_{ini} 80 ºC, 25 ºC/min, T_{fin} 200°C.

3. Results and discussion

Effect of the inoculum's type in the hydrogen fermentation

Several types of inocula have been used in the studies of hydrogen production via anaerobic fermentation, such as anaerobic sludge, agricultural waste, and isolated bacteria, employing different methods of inoculum's conditioning, like acid pretreatment, alkaline, temperature, among others. These studies have been developed using ideal and

simple substrata like glucose, sucrose and synthetic culture media. Sometimes the results applied with more complex samples or actual substrata, like waste from the sugar industry, are different from the obtained with pure samples; therefore to apply the scale hydrogen production is necessary to experiment in real conditions of substrate and inoculum. In this study, experiments in batch are presented using different inocula, such as acidogenic anaerobic sludge, anaerobic sludge and activated aerobic sludge with or without thermal treatment. These were performed employing molasses as substratum at an initial concentration of 30 g/L without adding any other nutrients.

Fig. 1 shows the accumulated hydrogen yield inoculate with different inoculum without any pretreatment during the fermentation period. There was a remarkable difference in the hydrogen production yield among different inocula. The anaerobic sludge produced the highest volume with 227 mL, but surprisingly the acidogenic anaerobic sludge only produced 45 mL and the aerobic sludge produced a minimum volume of hydrogen. With concern to the biogas production, the anaerobic sludge was also the one that produced the most, and the acidogenic and activated aerobic sludge produced a biogas composed almost completely of carbon dioxide. In relation to the presence of methane in the biogas, the yield of the methane production was decreased gradually in the anaerobic sludge until it got inhibited completely. However, for the acidogenic sludge the yield remained low and steady throughout the experiment with an average value of about 5% of methane in the biogas.

The hydrogen contents in the headspace of the reactors, inoculated with anaerobic sludge, were between 0% and 55% maintaining an average of 35%. When the production of hydrogen began to be important, methane production was inhibited in the anaerobic sludge. Unlike the acidogenic sludge, the hydrogen content was less, between 0% and 20%, without inhibiting methanogenesis. And for the aerobic sludge the hydrogen content in the biogas was never significant as Fig. 1(C) shows. Unlike the results reported by Watanabe [10], which present a complete inhibition of methanogenesis with a leached as an inoculum, with no pretreatment used for the leachate in that study. It was expected that an inoculum from an acidogenic reactor, fed with leachate and operating under conditions of an acidic pH of 6, it would be prone to present a lack of methanogenic activities. But our results show the anaerobic sludge as the best producers of hydrogen above acidogenic sludge. After that, the anaerobic sludge was selected to experiment with thermally pretreated sludge at 70°C and 120 °C for 30 min.

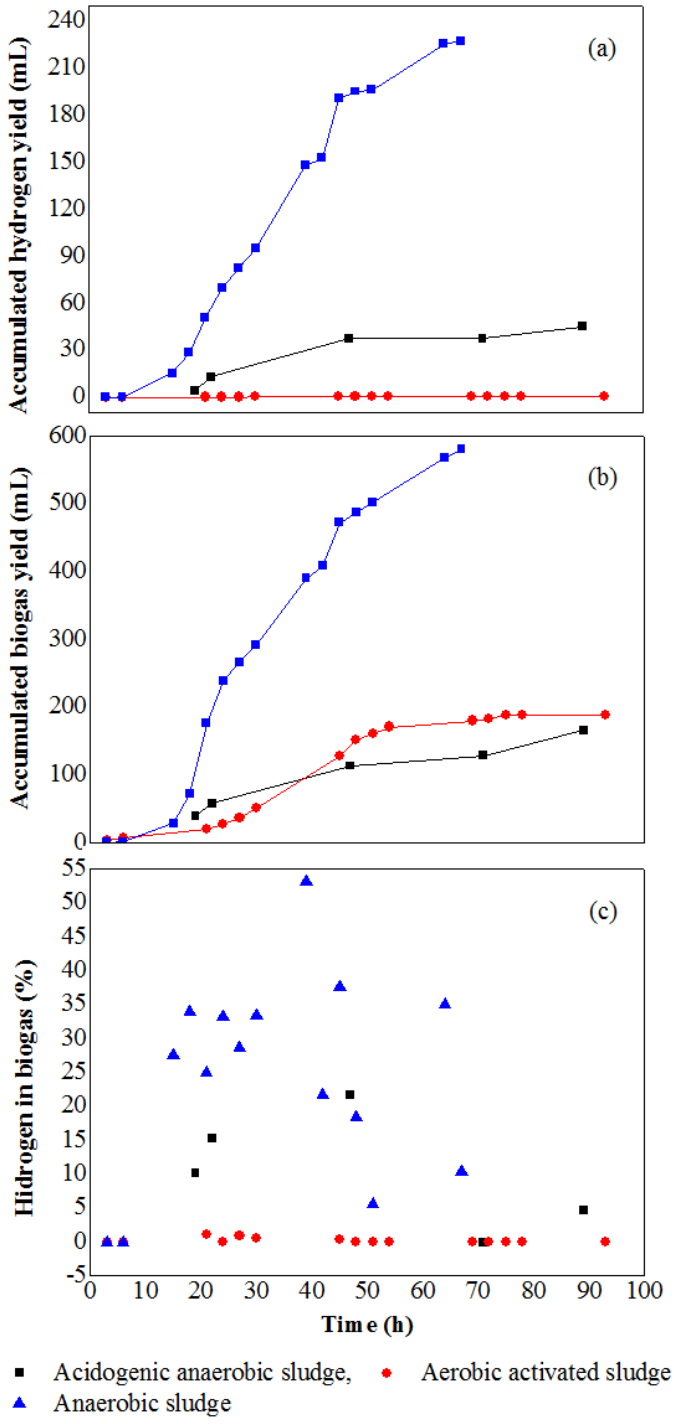

Figure1. Accumulated (a) hydrogen production and (b) biogas production without pretreatment (c) Hydrogen concentration in biogas

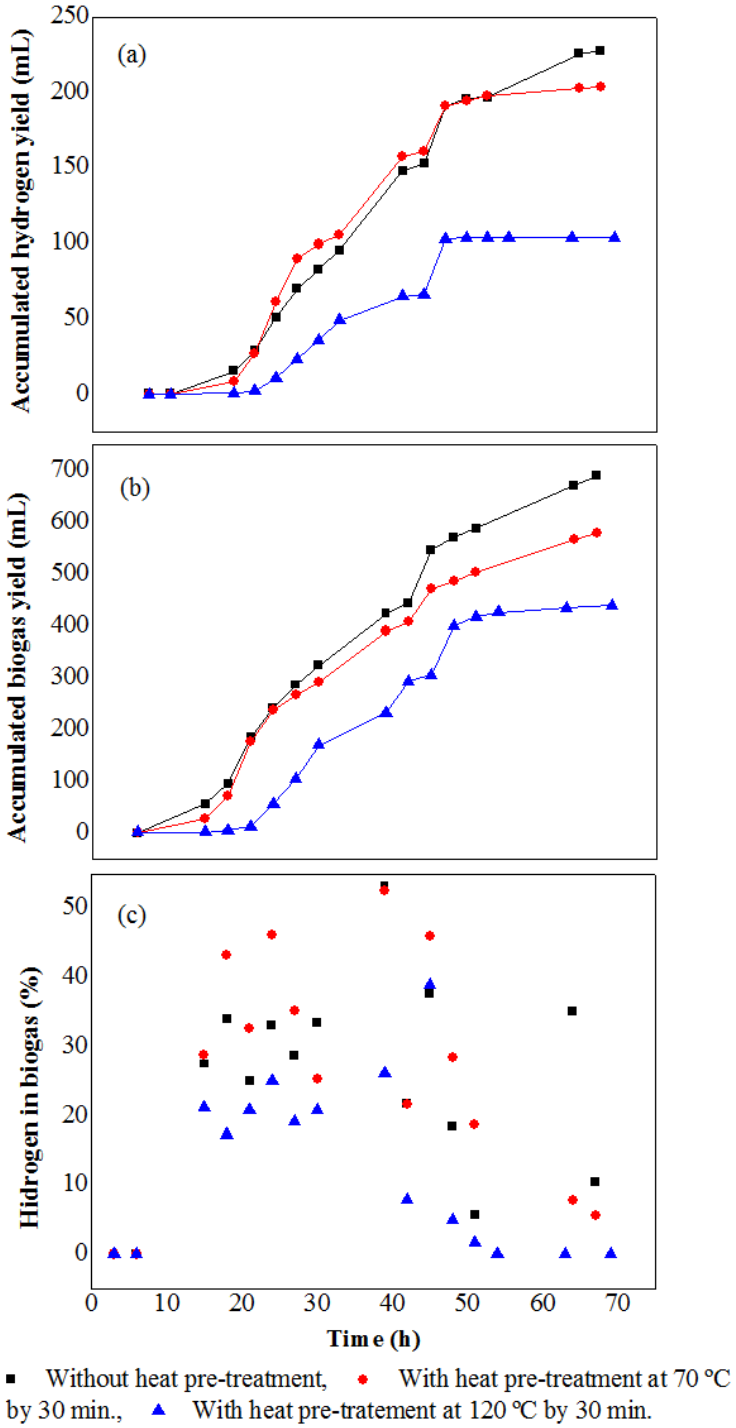

Figure2. Accumulated (a) hydrogen production and (b) biogas production by heat pretreatment (c) Hydrogen concentration in biogas

Figure 2 illustrates the cumulative hydrogen for different conditions of heat pretreatment. It is quite interesting to notice that the untreated anaerobic sludge gives the highest cumulative hydrogen production of 227 mL, while the heat treated sludge at 70°C gave relatively low hydrogen production with a volume of 203 mL, and a further heating of the inoculum at 120°C gave the lowest production of hydrogen with a volume of only 103 mL. Several authors have reported the heat treatment as the most efficient method to enrich the inoculum in hydrogen producing microorganisms [4]. However, it was reported in this study that the highest hydrogen yield was achieved with fresh inoculum and that a higher pretreatment temperature has a negative effect on the production of hydrogen and biogas. Reported conditions to perform the pretreatment vary between 70 and 120ºC for 30-60 min., and some authors when comparing different pretreatment methods like alkaline, acids, and thermal show that the thermal treatment is the most adaptable for the conditioning. However, they worked with pure substrata, and they did not compare the conditions of temperature and time to pretreat the inoculum. From our results we can conclude that it is not necessary a pretreatment to enrich the inoculum, whereas from a treatment of 70°C for 30 min, and without treatment, volumes of hydrogen production practically the same are obtained, and on the contrary, if the conditioning temperature is increased, it is detrimental to the production. Previous studies carried out with other substrata have shown that the different methods to inhibit methanogenesis can affect hydrogen production. Although the heat pretreatment has been the most widely used method to condition the hydrogen-producing inoculum, there are conflicting reports in the literature about the effects of temperature over hydrogen production. The hydrogen yields and rates increase as the temperature increases. However, the increase of increasing temperature can also have detrimental effects over hydrogen production. The results obtained in this study suggest that inoculum pretreatment is not always effective in enhancing hydrogen productivity, and it will depend the particular characteristics of the inoculum. In this case, a pretreatment was not necessary, because the inoculum was costumed to a substratum with a high concentration of carbohydrates, such as fruits and vegetables waste.

Effect of initial pH value

Accordingly, the effects of acclimation and pH on hydrogen production were investigated by subculture experiment at different pH conditions. Figure 3 shows the cumulative hydrogen amount and sucrose consumption rate for every batch culture with no pretreatment of the anaerobic sludge at each pH condition. The initial pHs of 4.0, 4.5 5.0 and 5.5, were adjusted, and 6.5 pH was constant. The corresponding average H_2 production rate and total H_2 yield are summarized in table 2. It is apparent from figure 3 that the pH values of fermentation molasses decreased with increasing time due to the production of acids derived from accelerated metabolism of carbohydrate-rich substrata. For pH 4 and 4.5, the sucrose was rapidly removed within 25 h, and then it was consumed slowly. The hydrogen production fellow the opposite tendency, due to that in the first 25 h it was produced and then this production increased slowly.

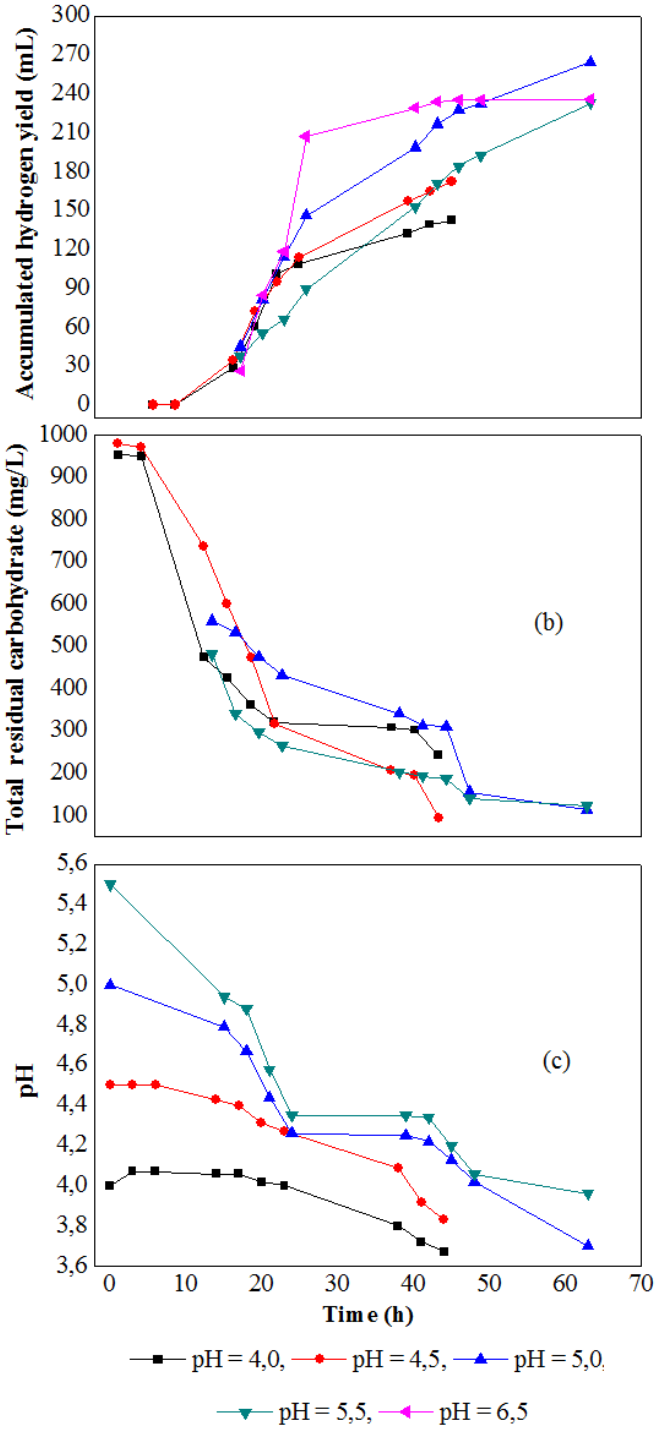

Figure 3. a) Biogas production in every bath culture with no pretreatment anaerobic sludge at different conditions. The initial pH was not controlled, except 6.5 pH was controlled through the experiment. (b) carbohydrate consumption through time. (c) Evolution of pH.

In case of pH, it decreased in a short time, and about 45 h, it reaches the value of hydrogen production inhibition, whose value is below 4 units of pH. For 5.0 and 5.5 initial pH, the carbohydrate consumption occurred in the first 25 hours after consumption was slowly but continues gradually, and at the same time the hydrogen production continuous producing a maximum volume of 265 and 232 ml, respectively, until reaching the limit value of pH after of 69 h. Curiously for adjusted 6.5 pH, the maximum value of hydrogen production (236 ml) was reached in a short time, about 25 h, after that the biogas production increased indicated biological activity, but not towards H_2 production, given that the biogas was formed almost by CO_2. This decrease in the concentration of hydrogen in the biogas with pH adjusted culture has been reported by Li.[11]. They reported inhibition in the hydrogen production with the addition the NaOH to control the pH at 7. They hey explain an inhibitory effect of NaOH in the production of hydrogen and recommend pH adjustment with bicarbonate.

In table 2 Total H_2 yield and the Average hydrogen production rate at different initial pH values were presented. Based on the best experimental result obtained of maximum H_2 production rate (76.11 ml H_2/ g sucrose) and H_2 percentage of biogas (36%), the optimal operational condition for initial pH value and complex substrate was at pH 5.0. It has both better yield and faster production rate of hydrogen. However these values obtained are lower compared with the reported studies where molasses is supplemented with nutrients.

Table2. Total H_2 yield and the Average hydrogen production rate at different initial pH values

pH	Hydrogen yield (ml H_2/g sucrose consume)	Average hydrogen production Rate (mlH_2/h)
4.0	41.26	3.6
4.5	49.49	4.4
5.0	76.11	4.4
5.5	66.94	4.2
6.5	69.02	4.0

4. Conclusions

From these results, we demonstrated that depending on the source of inoculum the pretreatment is not necessary. We also demonstrate that an excessive thermal pretreatment may generally affect the bacterial flora, including the hydrogen producing bacteria. Moreover, the optimum pH value for the hydrogen fermentation is 5.0, and even without adjusting, the system itself can regulate, and can be improved by using a buffer, $NaCO_3$ is recommended at pH 6.5.

Working at a pH near neutrality is not beneficial for the production of hydrogen, because the accelerated metabolism of carbohydrate consumption and subsequent production of acid compounds require constant adjustment of pH with NaOH, which causes negative effect to produce a biogas composed almost exclusively of CO_2. In effect at pH 6.5 the metabolism of microorganisms is not inhibited, but if the production of hydrogen.

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6. References

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