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RESEARCH ARTICLE

Simultaneous effects of pH and substrate concentration on hydrogen production by acidogenic fermentation

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Keywords: acidogenic, Gompertz modified, heat treatment, hydrogen, surface response.

Abbreviations: COD: Chemical Oxygen Demand
GHG: greenhouse gases
VFA: Volatile Fatty Acids
VSS: Volatile Suspended Solid

The present research examined the effects of initial substrate concentration and pH on the yield and productivity of hydrogen production by acidogenic fermentation. Assays were carried out at three different initial pH levels (5.5, 6.5 and 7.5) and three initial substrate concentrations (3, 5 and 10 g COD/L). Glucose was used as carbon source and the experiments were conducted at 37°C in batch tests, after a thermal pretreatment to eliminate methanogenic microorganisms. Conversions of glucose into hydrogen were between 16.75 and 27.25% of theoretical maximum, and high values of hydrogen productivity were obtained. An optimum value for the yield of glucose between initial pH of 6.3 and 3.7 g COD/L and productivity of the 5.95 H_2/gVSS h and initial pH of 6.7 and 10 g COD/L were obtained from the response surface.

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Hydrogen is an important and efficient energy carrier which can be produced by renewable processes and that presents numerous advantages: it produces minimum levels of greenhouse gases (GHG) during its combustion (water is the final product), and it has a very specific energy yield per unit of mass (3.3 times more than that of methane) (Park et al. 2005). Most hydrogen is currently produced mainly by reforming of natural gas which makes it non renewable and therefore carry greenhouse emissions in its life cycle. If hydrogen is to make an impact in global reduction of GHG emissions it must be produced from renewable resources such as bioconversion of organic residues.

Hydrogen is an intermediate compound in the anaerobic digestion process. It is a co-product during the acidogenic conversion of monosaccharides, amino acids or lipids and subsequently consumed by hydrogenotrophic methanogenic microorganisms that produce methane from H_2 and CO_2. Biological waste and wastewater treatment by anaerobic digestion is an economically and environmentally sustainable technology (Noike and Mizuno, 2000) that has grown substantially over the last two decades. With hydrogen production through anaerobic conversion not only renewable energy is produced, but it is also a system for efficiently wastewater treatment.

Most of the reactions that produce hydrogen during anaerobic digestion are not far from thermodynamic equilibriums (Valdez-Vasquez et al. 2005) and therefore in order to avoid process inhibition the concentration of the reactions products in the liquid phasemust remain low (Speece, 1996). Hydrogen concentration in the liquid is furthermore reported to much higher than the equilibrium values (Kraemer and Bagley, 2008). Indeed, a high partial pressure or hydrogen concentration has been found to suppress hydrogenase enzyme activity (Logan et al. 2003).

pH, microbial species and substrate type and substrate concentration have been reported as the most important variables affecting the productivity of hydrogen by anaerobic digestion.

The pH of the culture medium is a fundamental parameter during the acidogenesis process due to its implications on the volatile fatty acids (VFA) speciation and well known inhibitory effects. pH can decrease sharply due to VFA accumulation, so its control is of crucial importance (Oh et al. 2002). Van Ginkel et al. (2001) studied hydrogen production at different pH values. The best results were obtained between a pH of 5.5 and 6.0, using sucrose as the carbon source. Batch assays were carried out by Oh et al. (2003), and the best results were reported at a pH of 6.2. Zhao and Yu (2008), using sucrose-wastewater, observed

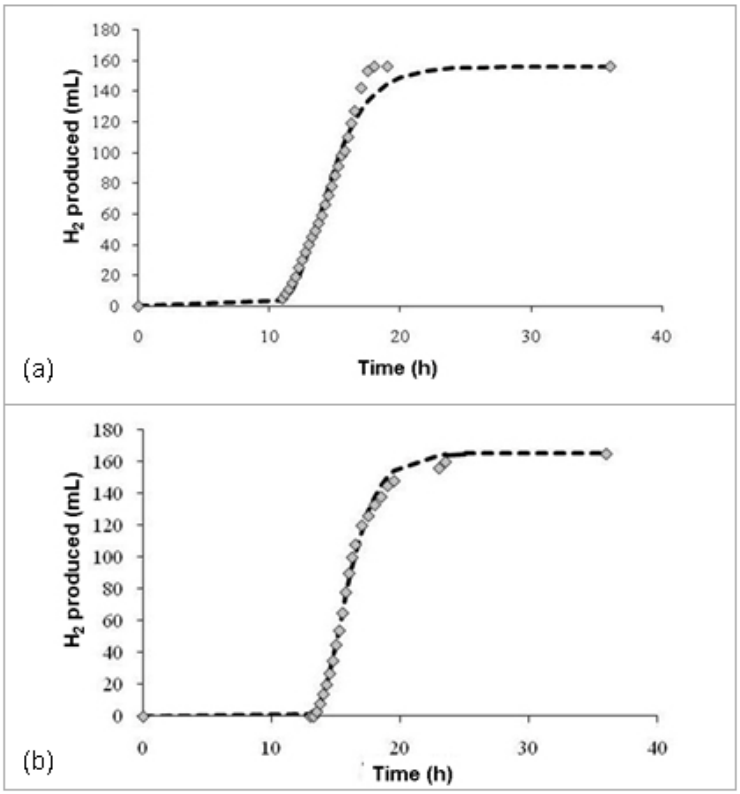

Figure 1. Profiles of hydrogen produced at two different initial pHs: (a) pH 5.5 and (b) pH 7.5. Initial glucose concentrations in both trials were 5 g COD/L. ◇ indicates experimental data while — — indicates data predictedby the Gompertz model.

Table 1. Yield and productivity obtained at different initial pH and substrate concentrations, during the acidogenic fermentation assays.

		Yield [mol H_2/mol of glucose]		Maximum specific productivity [mmol H_2/ gVSS·h]		
pH	**[g COD/L]**	**Average**	**Standard deviation**	**Average**	**Standard deviation**	**Glucose removal (%)**
5.5	**3**	0.87	0.02	2.90	0.96	97.3
	5	0.82	0.03	3.35	1.22	97.0
	10	0.76	0.03	3.27	1.03	91.0
6.5	**3**	1.07	0.04	5.01	0.72	100
	5	0.93	0.04	5.18	0.35	98.8
	10	0.72	0.05	4.64	0.78	98.5
7.5	**3**	0.70	0.04	2.02	0.52	100
	5	0.88	0.01	3.27	0.03	100
	10	0.78	0.06	6.06	0.62	99.4

the optimum hydrogen production rate between pH of 6.5 and 7.5 in a UASB reactor, however the hydrogen production in a UASB reactor is unstable because it has high solids residence time and the methanogenic microorganisms have time to grow and consume the hydrogen produced.

The aim of this study was to assess the simultaneous influence of pH and substrate concentration on the yield and productivity of hydrogen production by acidogenic fermentation using glucose as the carbon source.

MATERIALS AND METHODS

Batch experiments

The study of hydrogen production was carried out using glucose as carbon source at three concentrations: 3, 5 and 10 gCOD/L. For each concentration, three different initial pH levels were studied: 5.5, 6.5 and 7.5. Serum bottles with total volumes of 310 mL containing 250 mL of medium were used. Assays were performed at 37ºC by means of a thermostatic bath. The culture media had the following composition: NH_4HCO_3 (2.0 g/L), KH_2PO_4 (1.0 g/L), $MgSO_47H_2O$ (0.1 g/L), NaCl (0.01 g/L), $NaMoO_4{\cdot}2H_2O$ (0.01 g/L), $CaCl_2{\cdot}2H_2O$ (0.01 g/L), $MnSO_4{\cdot}7H_2O$ (0.0 15 g/L) and $FeCl_2$ (0.2 g/L). Each bottle was inoculated with sludge in order to obtain an initial biomass concentration of 1.5 g VSS/L. The biomass used as an inoculum was obtained from an anaerobic continuous stirrer tank reactor (CSTR) treating sludge from an aerobic biological wastewater plant. The sludge was washed to remove residual organic matter. To eliminate the methanogenic biomass, each seeded bottle was subjected to thermal treatment in an oven for 2 hrs at 100ºC. The volume of water lost during this pretreatment was restored to maintain the initial biomass concentration. Each assay was carried out in triplicate.

Before fermentation, bottles were flushed with pure nitrogen for 10 min to remove the oxygen from the culture medium and headspace. The pH was then adjusted to the previously defined levels with different amounts of hydrochloric acid (0.1N HCl) and sodium hydroxide (0.1N NaOH) were used, and phosphate buffer (0.1M) was used to maintain the pH during each experiment. This buffer concentration should not induce phosphate inhibition (Preliminary study, data not shown).

The volume of hydrogen produced was measured by volume displacement of a 2N sodium hydroxide solution that absorbed the carbon dioxide present in the biogas. The displaced volume was then considered to be equal to the hydrogen production. Biogas composition was verified on a daily basis by gas chromatography and negligible methane concentrations were found in all cases.

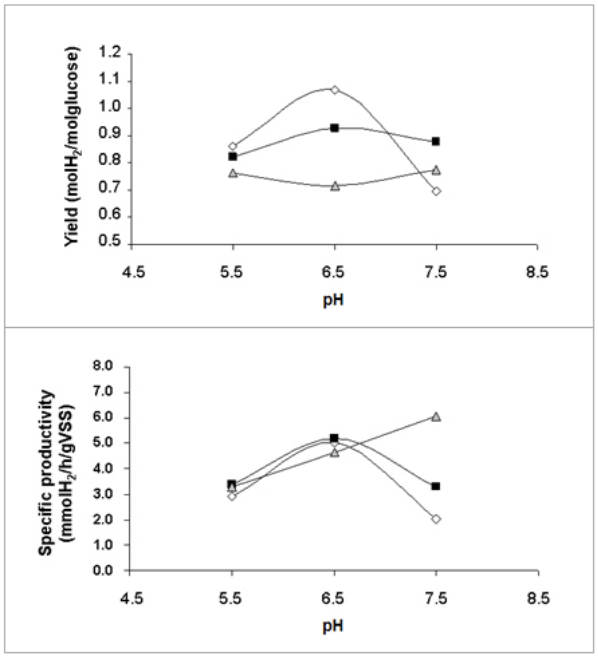

Figure 2. Effect of initial pH on the yield and maximum productivity at the following initial concentrations of glucose: (◇) 3 gCOD/L, (■) 5 gCOD/L and (△) 10 gCOD/L.

Determination of yield and productivity

Substrate (glucose) to product (hydrogen) yield was evaluated according to equation 1:

$$Y_{p/s} = \frac{\Delta P}{-\Delta S} \quad [1]$$

where $Y_{P/S}$ is the yield, ΔP is the amount of produced H_2 and ΔS is the amount of consumed substrate.

The maximum productivity was evaluated by the Gompertz modified model (Equation 2), commonly used in the modeling of hydrogen production by acidogenic fermentation (Khanal et al. 2004; Fang et al. 2006):

$$H(t) = H_m \cdot \exp\{-\exp[\frac{R_m}{H_m}(\lambda - t) + 1]\} \quad [2]$$

where H is the hydrogen production in the time (mL/h), H_m is the total final production of hydrogen (mL), R_m is the maximum hydrogen productivity (mL/h) and λ is the lag time (period between inoculation until observation of H_2 production) (Mu et al. 2007). The Gompertz model provided an adequate fit to experimental.

In order to assess the combined effect of pH and initial concentration of glucose (the factors considered in this study) on the yield and productivity (the responses), a surface response test for both factors was performed. Equation 3 was used for the surface response, which corresponds to a 2nd order polynomial equation with interactions between both independent factors.

$$Z = \beta_0 + \beta_1 \cdot pH + \beta_2 \cdot C_{Glu} + \beta_3 \cdot pH^2 + \beta_4 \cdot pH \cdot C_{Glu} + \beta_5 \cdot C_{Glu}^2 \quad [3]$$

where β_1, β_2..., β_5 are constant parameters of both responses, yield and productivity. The parameter fit was carried out in *Statgraphic Plus®*.

Analytical methods

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were analyzed according to the procedures described in APHA et al. (1995). Chemical Oxygen Demand (COD) was measured using colorimetric methods, Method 5220C, Standard Methods (APHA, 1995). Glucose concentrations were measured by the method developed by Miller (1959), which determines the reducing sugar concentration. Prior to COD and glucose determinations, samples were centrifuged at 15,000 rpm for 10 min in order to remove suspended solids.

RESULTS

Each experiment lasted for around 30 hrs initial lag periods (λ) were observed in all the experiments in the range of 11-13 hrs for the initial substrate concentrations of 3 and 5 g COD/L. At the highest substrate concentration, 10 g COD/L, larger λ of 16 hrs was observed. Hydrogen production commenced after approximately 50% of the substrate was consumed, around 7 hrs after substrate consumption started. Glucose was completely consumed in the assays at lower COD concentration after 30 hrs, under most of the conditions tested. At high COD concentrations, some glucose left was detected after this time period. As shown in Table 1, the degradation efficiency of glucose was greater than 97% at most of the pH values tested. The

Table 2. Constants obtained in the response surface.

Constants	Yield	Productivity
Intercept	-3.1500	-50.4320
β_1	1.3156	17.7336
β_2	0.0343	-1.2297
β_3	-0.1066	-1.4638
β_4	0.0096	0.2667
β_5	-0.0035	-0.0240

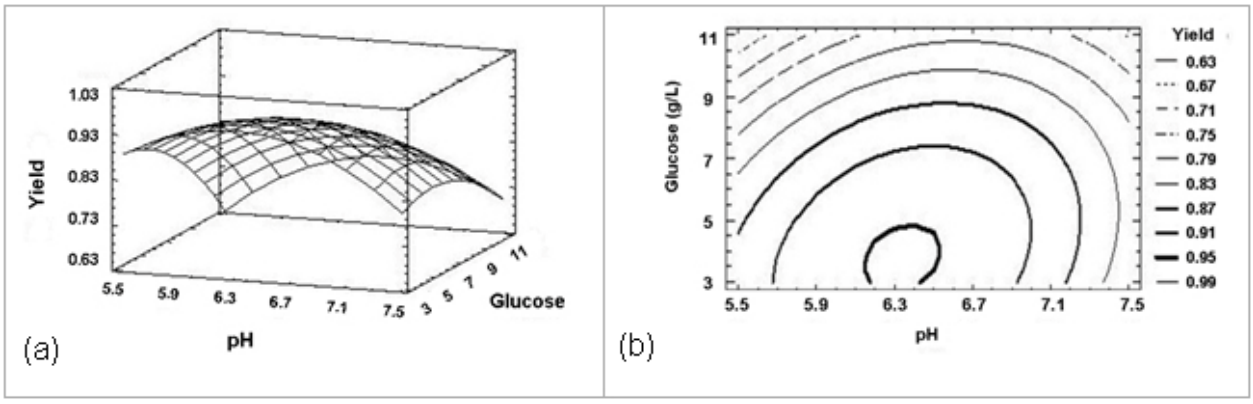

Figure 3. (a) Response surface and (b) Boundary layers for the yield of the fermentation.

maximum degradation efficiencies (100%) were observed at an initial pH of 6.5 and a glucose concentration of 3 g/L and at an initial pH of 7.5 with glucose concentrations of 3 and 5 g/L, while the minimum value (97.0%) was found at an initial pH of 5.5. This indicates initial pH does not significantly affect glucose degradation. This can be explained by the increase of VFA concentration, which caused a decrease in the pH since the buffer was not working at its most effective range (pKa = 7.21) and thus hydrogen production was somewhat inhibited (Khanal et al. 2004). For example, Duangmanee et al. (2007) observed that a low fermentation pH of approximately 4.0 inhibits hydrogen production. The initial and final pH values of fermentation were measured to determine whether the buffer used was able to maintain a constant pH. As expected, lower pH variations were obtained at the lowest substrate concentrations (0.31-0.72 units of pH), whereas the greater variations were obtained at the highest concentrations (0.78-1.78 units of pH).

Table 1 shows the results of the yield and maximum specific productivity obtained for each experiment.

Taking into account that 1 mol of glucose can theoretically produce a maximum of 4 mol of H_2 considering acetic acid as the final product of the reaction, a 26.75% conversion efficiency (related to the maximum theoretical) was achieved at pH 6.5 and initial substrate concentration of 3 g COD/L as maximum value. These results are similar than those obtained by Logan et al. (2003) and Oh et al. (2003), who respectively reported glucose conversion efficiencies into hydrogen of 23% at a pH of 6.0 and between 24.2-18.5% at a pH range of 6.2-7.5.

A maximum specific hydrogen productivity of around 6.06 mmolH_2/gVSS h (Table 1) was obtained, lower than that reported by Oh et al. (2002), within the range 16.1-18.5 mmol H_2/gVSS h at a pH between 6.0-8.0, although they used a specific strain of the bacteria *Rhodopseudomona palustres* P4, in pure culture for hydrogen production.

Figure 1 shows hydrogen production over time in two essays, which correspond to the typical profiles obtained during the studies. The Gompertz model is capable of fitting most profiles obtained; however, some disagreement between the experimental data and the model was found at a pH of 5.0 due to a loss of linearity in the last part of the Gompertz curve that was not observed at a pH of 5.0. This effect could be caused by a decrease in pH during fermentation.

Observations indicate that, for initial substrate concentrations of 5 and 10 g COD/L, initial pH values did not have a significant effect on the hydrogen yield, as it is shown in Figure 2. At an initial glucose concentration of 3 g COD/L however, a maximum was observed in the yield at initial pH of 6.5 higher that observed at pH 5.5 and 7.5. Similar results were obtained by Fang et al. (2006), who observed higher yields at pH 4.5 than those at pH 4.0 and

Table 3. Optimum initial pH and substrate concentration values derived from the response surface.

	Yield [mol H_2/mol glucose]: 0.95	**Productivity** [mmol H_2/ gVSS·h]: 5.9
pH	6.3	6.9
[g COD/L]	3.7	10

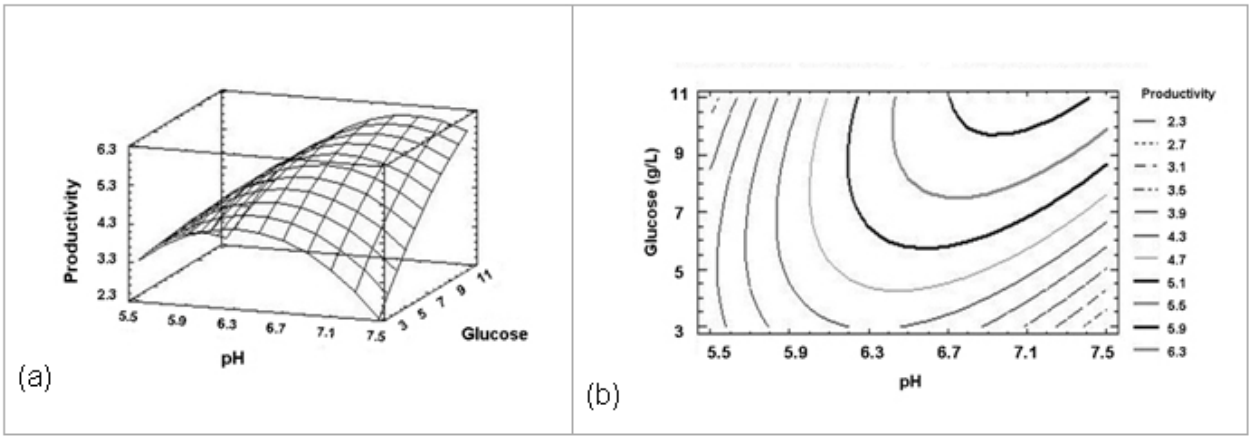

Figure 4. (a) Response surface and (b) Boundary layers for the productivity of the fermentation.

7.0 using rice starch as a carbon source. It is worth noting that starch needs to be hydrolyzed to glucose prior to fermentation although it is considered relatively easily hydrolysable.

Was observed a similar correlation between the maximum specific hydrogen productivity values at concentrations of 3 and 5 g COD/L. These results clearly indicate that initial substrate concentration and pH affect both hydrogen yield and productivity like reported Fang et al. (2006) the productivity increased from 0.3 L H_2/gVSS d to 2.1 L H_2/gVSS d with an increase in concentration from 2.7 to 5.5 g/L, respectively. Afterwards, this parameter dropped to 0.4 L/gVSS d at a concentration of 22.1 g/L. Similar results were obtained by Duangmanee et al. (2007) using sucrose as the substrate at five different initial pHs ranging from 4.5 to 6.5. The highest specific productivity was 0.54 mmolH_2/gVSS h, recorded at a pH of 5.5.

Table 2 presents the parameter values, and Table 3 presents the values of the different factors that produced the maximum response for both the yield and the productivity.

Figure 3 shows the yield surface versus initial pH and glucose concentration. The boundary conditions of the response surface are presented, which show the area where the optimum values of the variables are found. An optimum value for yield was encountered closes the experimental region (the area defined as the experimental range of each variable) at initial pH values of 6.3 and 3.7 g COD/L. At these conditions, the hydrogen yield was 0.95 mol H_2/mol glucose, which also corresponds to the optimum value of the operability region (the set of values where the process might be operated).

Figure 4 shows the response surface for the productivity of hydrogen and its boundary layer. An optimal productivity value around 5.95 mmol H_2/gVSS h was obtained at a initial pH of 6.7 and a glucose concentration of 10 g COD/L. Higher values of the concentration, which were studied, can be found within the optimum region. Thus, more research is required. It should be noted that these productivity values are the optimal values within the range of the experimental data, but they do not necessarily represent the absolute optimum values.

A similar multiple regression analysis was carried out by Pan et al. (2008) who studied the effect of glucose concentration, buffer concentrations and vitamin solution on hydrogen production by *Clostridium* sp. Fanp2. A considerable correlation was observed between glucose and buffer concentration but negligible between buffer concentration and vitamin solution.

The conditions that maximized yield differ from those maximizing productivity such that a compromise exists between these two parameters that must be considered for scap-up purposes.

CONCLUDING REMARKS

pH and glucose concentration exert a simultaneous influence on the yield and maximum specific productivity of hydrogen production using glucose as the carbon source.

Conversion values of glucose into hydrogen were found to be 17.5 and 26.75% of the maximum theoretical yield (assuming acetic acid as the final product of the fermentation). The greatest yield attained was 1.07 mol H_2/mol glucose, recorded at an initial pH of 6.5 and an initial concentration of 3 g COD/L. In terms of the maximum specific productivity, a value of 6.06 mmol H_2/gVSV h was observed at an initial pH of 7.5 and an initial glucose concentration of 10 g COD/L.

From the response surface, the optimum yield was estimated to be around 0.954 mol H_2/mol glucose at an initial pH of 6.34 and an initial glucose concentration of 3.78 g COD/L. An optimum area for productivity was found in the response surface, and, according to the

experimental conditions, the optimum value was close to the value obtained in this study.

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