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INFLUENCE OF THE pH ON HYDROGEN PRODUCTION BY SSF OF PAPER INDUSTRY WASTES USING ANAEROBIC BIOFILMS

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ABSTRACT

The present research studies the influence of pH in the fermentative hydrogen production in batch reactors, by simultaneous saccharification and fermentation (SSF) from paper industry wastes using anaerobic biofilms. Paper industry wastes contain: lignin (22.6%), hemicellulose (42%) and cellulose (35.06%). The milled and mashed paper waste that was used had an average particle diameter of 710 μ. The hydrolysis conditions of the commercial enzyme were verified (temperature, pH and speed of agitation). Results of optimal conditions were as follows: temperature 45 ºC, pH of buffer 5.5 and 100 rpm. Studies of the influence of the pH were investigated by experimental design in fermentative production of the hydrogen with pH values of: 4, 5 and 6, and taking optimal conditions of hydrolysis process: 100 rpm, temperature 45ºC and concentration of enzyme 10 FPU (filter paper assay) U mL^{-1}, and using anaerobic biofilms developed in spheres of ixtle fiber, obtaining a production of hydrogen of : 1.88, 1.63, 1.78 mol of H_2 for 6, 5 and 4 at 288 h of reaction.

Key words: Hydrogen, saccharification, fermentation, pH, anaerobic biofilms.

1. INTRODUCTION

Fossil fuels (specially petroleum) are currently the dominant energy resource in the planet. As the depletion of limited fossil fuels is inevitable, there is an urgency to search for replacement source or energy [1]. The extensive use of fossil fuel has also created an environmental issue where emission of carbon dioxide during combustion of fossil fuels has caused a global warming effect. [2]. As a consequence, most developed countries have devoted to the development of alternative energy carriers.

Hydrogen is a clean fuel with a high energy content of 122 kJ g^{-1} and no CO_2 emissions. Hydrogen is considered as the major energy carrier of the future and can be used in fuel cells for electricity generation [3. Anaerobic hydrogen fermentation seems to be favorable, since hydrogen can be yielded at higher rates and at lower cost in degrading various organic wastes enriched with carbohydrates so maintains a sustainable ecosystem [4].

Paper and pulp industry, one of the prime industrial sectors, depends majorly on surplus quantity of lignocellulosic components of plants. This industry is considered to be a main consumer of natural resources and chemicals for paper manufacturing [5]. The pulp and paper industry generates about 80 million tones of solid waste every year, and only about 42% of the waste is recycled [6]. Indeed wastewater treatment units of paper mills produce a large quantity of sludge, which represents currently a major pollution problem, urgently requiring an alternative disposal solution [7]. Paper industry wastes has a high content of polysaccharides, it can be further processed to obtain products with high added value produced from sugar, such as hydrogen. This conversion requires the polysaccharides on waste to be broken down into the constitutive monomers and the released sugars to be fermented by anaerobic mixed culture [3]. Enzymatic hydrolysis is often preferable method for conversion of a polysaccharide into monomers since it can not only economize energy on account of the relatively mild reaction conditions, but also avoid using toxic and corrosive chemicals [8].

The enzymatic hydrolysis and fermentation steps for hydrogen production can be performed as simultaneous saccharification and fermentation (SSF) process, wich offers various

advantages such as the use of a single-reaction vessel for both steps (allowing process integration with the consequent reduction on capital cost), rapid processing time, reduced end-product inhibition of hydrolysis and increased productivity [9].

The use of biofilms system in which regularly the specific activity is increased and also this type of systems offer a higher operational stability [10]. Also this is mentioned by Wu and Chang (2007), in which the microbial community is protected by the polymeric matrix and allows tolerating extreme environmental conditions [11].

The objective of this work was to determine the effect of initial pH on the hydrogen production by SSF process from paper industry wastes using low loading of commercial enzyme and anaerobic biofilms supported in natural fiber.

2. MATERIALS AND METHODS

2.1 Anaerobic microbial mixed culture pretreatment

An anaerobic microbial mixed culture (500 ml) was obtained from an Upflow Anaerobic Sludge Blanket (UASB) reactor used for treating wastewater from brewery Modelo (Zacatecas, Mexico). Sludge obtained from UASB reactor was macerated and pretreated following the method described by Chen (2007) [12]. In the heat pretreatment, sludge was heated in a boiling water bath for a short period of time (30 min) and then, it was cooled down. Heat pretreatment was followed by acidic pretreatment that involved decreasing the pH of the sludge or granule solution to a value of 3.0 using 0.1 N HCl solution during 24 h and a readjustment of pH value back to 7.0 by 0.1N NaOH solution.

2.2 Characterization and pretreatment of paper industry wastes

The paper industry wastes were collected from paper industry located in Ramos Arizpe, Coahuila. They were cut, dried, milled and sieved until a size of particle of 710µ. The partial characterization of the residues was realized according to methodologies described by Charkov [13] (Table 1).

2.3 Support pretreatment and preparation

The fixation of microorganisms was achieved through a natural material. Two meters of ixtle fiber (natural material), with an approximated diameter of 2.24 mm were used for covering a plastic ball of 25.54 mm of external diameter. Sixty spheres covered with ixtle were hydrated with distilled water by a period of 18 hours.

2.4 Enzyme activity

FPU activity was determined to the enzyme Celluclast® 1.5L (EC 3.2.1.4 cellulase from *Trhichoderma reesei* ATCC 26921; Novozymes, Denmark). Total cellulase assay method accounts the combined activity of complex cellulolytic enzymes and the total cellulase activity in the enzyme extract was measured in terms of filter paper units (FPU) [14]. Reducing sugar content was estimated using 3,5-dinitrosalicylic acid colorimetric method (DNS method) [14], and the total sugar content was determined using phenol sulphuric acid method [15].

2.5 Biofilm formation

Sixty pretreated spheres were placed inside of 5L UASB reactor (working volume 4L) which contained 400 mL of pretreated anaerobic sludge (0.2408 gL^{-1} volatile solid suspended, VSS) and mineral media with following composition (mg L^{-1}): glucose 20gL^{-1} as the sole carbon source, NH_4Cl, 2,000; $NaHCO_3$, 6,720; K_2HPO_4, 125; $MgCl_2{\cdot}6H_2O$, 100; $MnSO_4{\cdot}6H_2O$, 15; $FeSO_4{\cdot}7H_2O$, 25; $CuSO_4{\cdot}5H_2O$, 5; $CoCl_2{\cdot}5H_2O$, 0.125. The initial pH was adjusted to 5.0 with 3M HCl [16]**.** The reactor was fed by a peristaltic pump (Manostat – division of Barnant Company, Simon varistaltic pump, USA) at 1 h hydraulic retention time (HRT) during 633 h.
Hydrogen and methane were determinate by gas chromatography (GC-TCD). Reducing sugar content was estimated using DNS method [14], and the total sugar content was determined using phenol sulphuric acid method [15].

2.6 Simultaneous saccharification and fermentation

SSF experiments were carried out in the 250 mL glass flask with 7 g of paper industry wastes, two spheres of immobilized biofilm (4.4 g biomass) and an enzyme loading of 10 FPU mL^{-1} dissolved in 50 mM sodium citrate buffer in order to complete a final volume of 140 mL. Experiments were performed at pH values of: 4, 5 and 6 ±0.02 adjusting with NaOH 3N or HCl 3N. The fermentation reactors were fitted with rubber stoppers and purged with argon for 20 min to ensure an anaerobic environment. All the experiments were carried out in duplicate. The fermentation temperature was controlled at 45 ºC ±0.1 and the solution was stirred to 100 rpm in an orbital shaker (Lumistell IRO 70). Hydrogen and methane were determinate by gas chromatography (GC-TCD). The pH was determined by pH meter (HI 2550, HANNA instruments). Reducing sugar content was estimated DNS method [14], and the total sugar content was determined using phenol sulphuric acid method [15].

2.7 Analytical methods

Hydrogen and methane was measured by gas chromatography equipped with a thermal conductivity detector (VARIAN 3400) and a CP Molecular Sieve 5A Capillary Column (VARIAN). GC conditions were as followed: injector and detector temperatures. 200ºC and column temperature: 50°C, using Argon as carrier gas with flow rate 10mLmin^{-1}. Standards Methods [17] were used to determined pH (HI 2550, HANNA instruments). Samples were first centrifuged at 10000 rpm for 10 min. Reducing sugar content was estimated using DNS method [14], and the total sugar content was determined using phenol sulphuric acid method [15].

3. RESULTS AND DISCUSSION

3.1 Characterization and pretreatment of paper industry wastes

Partial characterization of paper industry wastes contains: cellulose, hemicellulose and lignin as noted in Table 1.

Table 1. Partial characterization of paper industry wastes.

Substrate	Hemicellulose	Cellulose	Lignin
Paper industry wastes	**42%**	**32.06%**	**22.6%**

3.2 Enzyme activity

The total cellulase activity in the enzyme extract was measured in terms of filter paper units (FPU) was 74 FPU mL^{-1} [14].

3.3 Hydrogen production

3.3.1 Biofilm formation and hydrogen production

Figure 1 shows hydrogen production in a UASB reactor during biofilm formation. In order to eliminate methanogenic bacteria and make fermentation condition suitable for hydrogen production, initially pH value was controlled at 5.0, but during fermentation pH value was no adjusted, with a final pH of 3.0

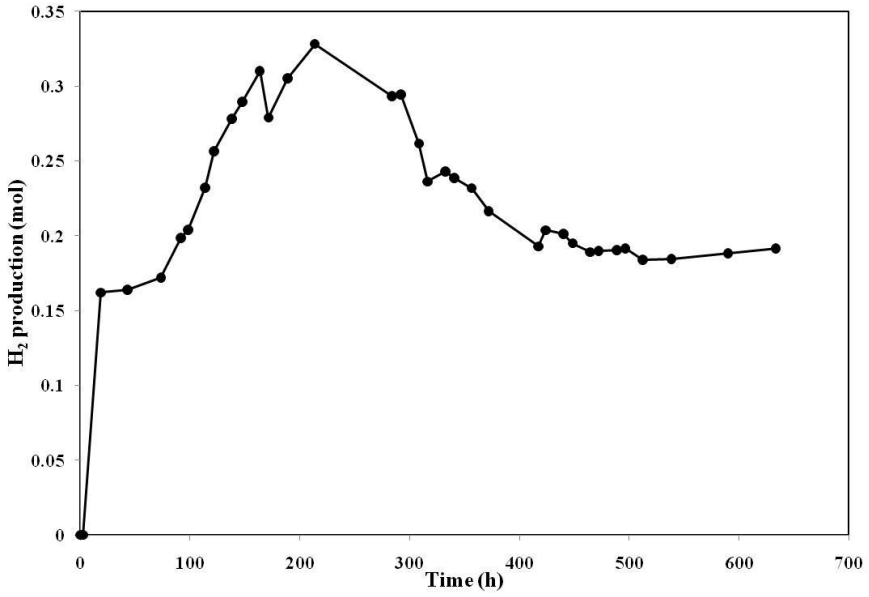

Figure 1. Hydrogen production for anaerobic microbial mixed culture adaptation.

Hydrogen production begins at 18 hours, obtaining a maximum at 213 hours, and during the period of fermentation methane was no detected. The activity of the methanogens has generally been reported to be inhibited by weakly acidic conditions below pH 5.0 [18] and inhibition methanogenic bacteria was observed during period biofilm formation and fermentation (633 h). The immobilized anaerobic biofilm displayed good H_2-producing activity.

3.3.2 Simultaneous saccharification and fermentation

Experiments were carried out simultaneously performing the enzymatic hydrolysis and the microbial fermentation steps. The hydrogen production efficiency was evaluated by the cumulative hydrogen production, as well as the hydrogen content in the biogas.

Figure 2 shows hydrogen production at three pH values. Hydrogen production velocity were $1.16 \cdot 10^{-2}$ mol h^{-1}, $9.8 \cdot 10^{-3}$ mol h^{-1}, $1.05 \cdot 10^{-2}$ mol h^{-1} at pH value of 6, 5 and 4, respectively. Maximum hydrogen production were achieved at 81.5 h, 61.5 h, and 63.9 h at pH value of 6, 5 and 4, respectively, for 24 h of start up.

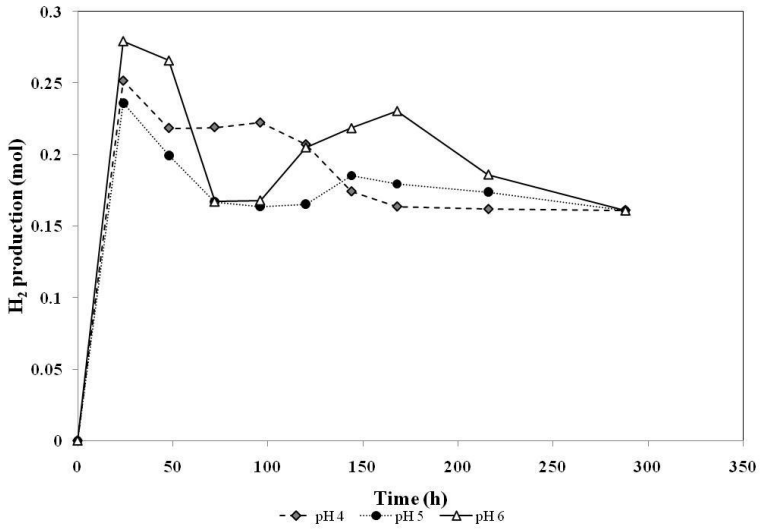

Figure 2. Effect of initial pH on hydrogen production at various initial cultivation pH (4, 5, 6) at enzyme loading 10 FPU and 45°C.

The speed of hydrogen generation was strongly affected by pH value. Initial pH may influence the lag phase in batch hydrogen production, since alkaline pH decrease lag time, however it make cause low yield of hydrogen. The reported optimal pH range for hydrogen production ranged 5.0-7.5 stated that the optimal pH for hydrogen production can be less than 5.0 or around 4.5 for the thermophilic culture. Decline in pH value is noted in hydrogen production process owing to the production of organic acids that depletes the buffering capacity of the medium [19]. Moreover pH

affects the activity of iron containing hydrogenase and cellulolase enzymes, decreases in pH will inhibit hydrogen production [20].

Fig. 3 shows a cumulative hydrogen production was obtaining at 288 h: 1.88 mol, 1.63 mol and 1.78 mol for pH values of 6, 5 and 4 pH respectively.

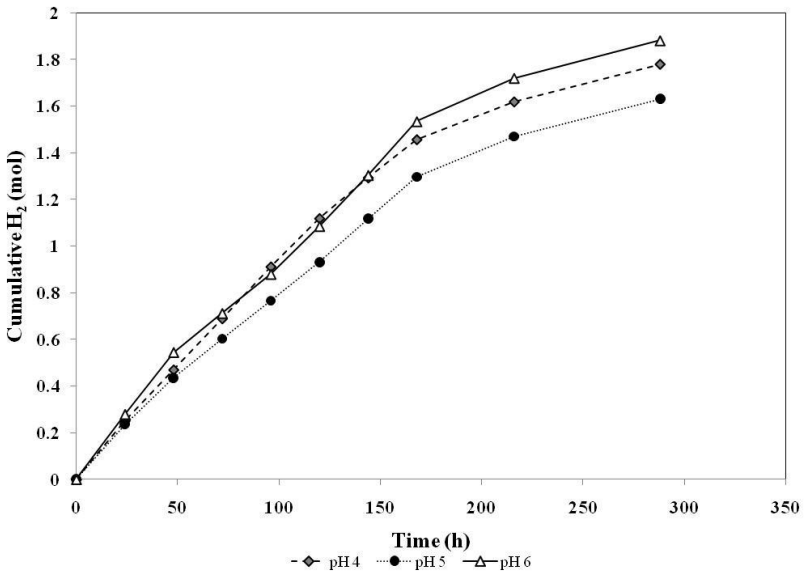

Figure 3. Cumulative hydrogen production (mol) at various initial cultivation pH (4, 5, 6), at enzyme loading 10 FPU and 45°C.

The reactor at pH 6.0 provided the highest hydrogen production as the hydrogen production at pH 4.0 was slightly lower than at pH 6.0. The initial pH 6.0 and 4.0 were consequently deemed the optimal initial pH for hydrogen production, indicating that anaerobic biofilm was not inhibited at too low pH . These results implied the initial pH could stimulate the microorganisms to produce hydrogen and would achieve the system having maximum hydrogen production. Reactors at three pH showed an slight increasing on pH during the first hours fermentation, this is due to the components of paper industry wastes. Then tend to fall with respect to the initial pH by accumulation of volatile grasses acids, showing the tendency of pH reported in literature [21].

The maximum production of reducing sugars was observed during the first 24 h, and afterwards it was kept nearly constant. Even though the reducing sugars concentration has never decrease throughout the all process.

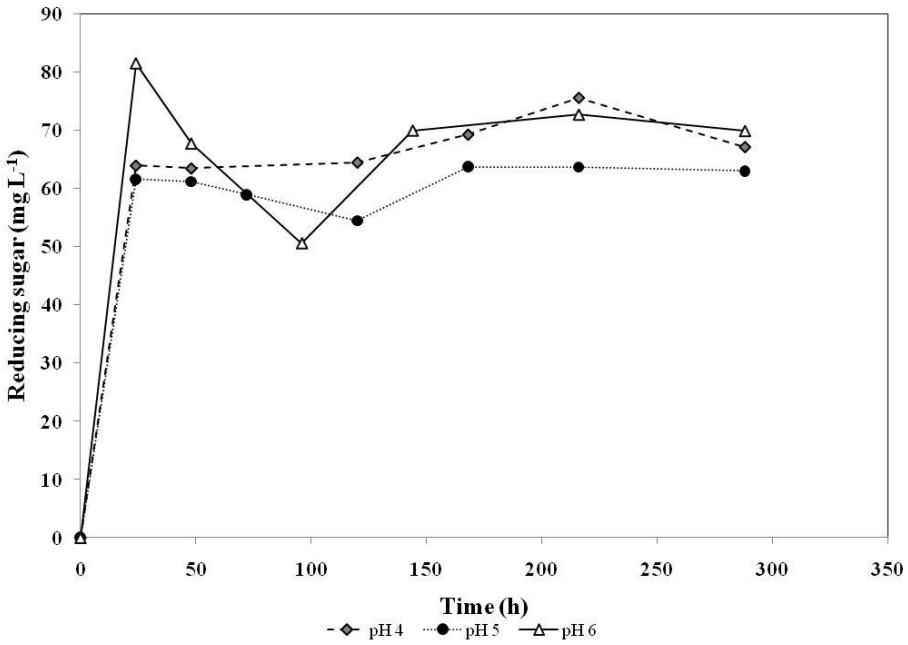

Figure 4. Concentration profile of sugars released with respect to time at different pH´s.

4. CONCLUSIONS

The fermentative hydrogen production was carried out using paper industry wastes from anaerobic biofilms with enzyme Celluclast® at low loading. Effect of the pH on hydrogen production by SSF of paper industry wastes showed higher hydrogen production for initial pH 6.0. This indicates the feasibility of hydrogen production by SSF from paper industry wastes without prehydrolysis, which is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzyme that convert the carbohydrate polymer to fermentable sugars. Moreover, the utilization of anaerobic biofilm on SSF process is feasible, because a biofilm system increases biomass level, leading to elevation of the production rate of hydrogen gas.

5. ACKNOWLEDGEMENTS

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