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HYDROGEN PRODUCTION IN UASB REACTOR USING ENZYMATIC HYDROLYSATES FROM PAPER INDUSTRY WASTES BY ANAEROBIC BIOFILMS: INFLUENCE OF HRT

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ABSTRACT

The present study examines the effect of hydraulic retention time (HRT) in a 4-L upflow anaerobic sludge blanket (UASB) reactor using enzymatic hydrolysates obtained of paper industry wastes by anaerobic biofilms for hydrogen production. Paper industry wastes contain: lignin (22.6%), hemicellulose (42%) and cellulose (35.06%). The milled and mashed paper wastes had an average diameter corresponding to 710 µ. Three hydraulic retention times were tested in a reactor UASB: 1, 3, 6 h using mineral medium with glucose concentration of 20 g L^{-1}, by anaerobic biofilms developed in spheres of ixtle fiber to pH 5.0. Enzymatic hydrolysis of 50 g L^{-1} of paper industry wastes with a particle size of 710 µ to 125 rpm in 4 L of citrates buffer pH 4.8, temperature 45 ºC and enzyme concentration of 5 mg mL^{-1} was performed. Enzymatic hydrolysates (3.1 L, pH 7) were initially tested at HRT 1, there was no hydrogen production. Subsequently the HRT was decreased to 0.5 h, reaching a cumulative hydrogen production of 1.15 mol at 101 h of reaction.

Key words: Hydrogen, hydraulic retention time (HRT), paper industry wastes, enzymatic hydrolysates.

1. INTRODUCTION

Degradation of the natural environment and the energy crisis are two vital issues for sustainable development worldwide. Hydrogen is considered as one of the most promising candidates as a substitute for fossil fuels. In this context, biological processes are considered as the most environmentally friendly alternatives for satisfying future hydrogen demands [1]. In particular, biohydrogen production from waste materials is very advantageous since minimizes waste accumulation and maintain a sustainable ecosystem and hence, it has been considered as a conservative approach [2]. Considering that such wastes are complex substrates and can be degraded biologically by complex microbial ecosystems, the present paper focuses on dark fermentation as a key technology for producing hydrogen from crop residues, livestock waste and food waste [1].

It is often reported that the rate of hydrogen evolution from an anaerobic fermentation was dependent on the pH, loading rate, biogas recirculation and hydraulic retention time (HRT) for the acidogenic phase [3].Therefore, in this study, the effect of HRT on the anaerobic bio-hydrogen production process on hydrolysates from paper industry wastes was investigated through the operation of three different HRTs.

2. MATERIALS AND METHODS

2.1 Anaerobic microbial mixed culture pretreatment.

Anaerobic microbial mixed culture (500 ml) was obtained from a upflow anaerobic sludge blanket (UASB) reactor that treated wastewater from brewery Modelo (Zacatecas, Mexico). Pretreatment was carried out as describe by Chen and Hu [4]. Sludge was macerated and was subject to heat pretreatment; it was heated in boiling water bath for a short period of time (30 min) first and then, cooled down. Heat pretreatment was followed by acidic pretreatment that involved decreasing the pH of the sludge or granule solution to 3.0 using 0.1 N HCl solution for 24 h and a readjustment of pH 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 [5] (Table 1).

2.3 Support pretreatment and preparation

The fixation of microorganisms achieved through a natural material [6]. Two meters of ixtle fiber with an approximated diameter of 2.24 mm were used to wrap a plastic ball of 25.54 mm of external diameter. Sixty spheres of ixtle fiber were hydrated with distilled water by a period of 18 hours.

2.4 Enzyme activity

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) [7]. Endoglucanase activity was determined using carboxymethyl cellulose as a substrate. Reducing sugar content was estimated using 3,5 -dinitrosalicylic acid colorimetric method (DNS method) . [7].

2.5 Enzymatic saccharification

The reactor was seeded with 50 gL^{-1} of paper industry wastes, 5 L of 0.05 M citrate buffer at pH 4.8 and Enzyme powder at 5 mg per mL. The reactor was incubated at 45±1°C for 24h at 125 rpm in an orbital shaker. The residual sugars analysis was carried out at regular time intervals. Reducing sugar content was estimated using 3, 5 -dinitrosalicylic acid colorimetric method (DNS method) [7].

2.6 Hydrogen production

2.6.1 Biofilm formation and hydrogen production

A 5 L (working volume 4 L) UASB reactor was set up with 400 mL of pretreated anaerobic sludge (0.2408 gL^{-1} VSS), sixty pretreated spheres. Reactor was fed by peristaltic pump (Manostat – division of Barnant Company, Simon varistaltic pump, USA. The medium used for cell growth and H_2 production (mgL^{1}) contained the following: glucose 20,000 (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 [8]. The medium was adjusted to pH 5.0 using 3M HCl. The reactor was operated at three HRTs: 6, 3 and 1 h, was seeded with fresh medium for each HRT. At regular time intervals, the amount of hydrogen and methane were measured by gas chromatography (GC-TCD). Reducing sugar content was estimated using 3,5-dinitrosalicylic acid colorimetric method (DNS method) [7].

2.6.2 Hydrogen production using hydrolysates enzymatic

The UASB reactor was seeded with 3.1 L of hydrolysates enzymatic pH 7 and was operated at HRT of 1 and 0.5 h; it was seeded with fresh hydrolysates for each HRT.

At regular time intervals, the amount of hydrogen and methane were measured by gas chromatography (GC-TCD). Reducing sugar content was estimated using 3, 5-dinitrosalicylic acid colorimetric method (DNS method) [7].

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 10 mL $min^{-1.}$ The pH was determined by pH meter (HI 2550, HANNA instruments). Standards Methods [9] were used to determined biomass concentration (in terms of volatile suspended solid; VSS). Samples were first centrifuged at 10000 rpm for 10 min. Reducing sugar content was estimated using 3,5-dinitrosalicylic acid colorimetric method (DNS method) [7], and the total sugar content was determined using phenol sulphuric acid method [10].

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 0.40 FPU mL^{-1} . Endoglucanase activity was determined using carboxymethyl cellulose as a substrate, and was 1.52 U $mL^{-1.}$

3.3 Hydrogen production

3.3.1 Biofilm formation and hydrogen production

Figure 1 shows hydrogen production at three hydraulic retention times (HRT: 6, 3 and 1 h) in a UASB reactor during the growth and maturing of anaerobic biofilms in spheres wrapped with ixtle fiber.

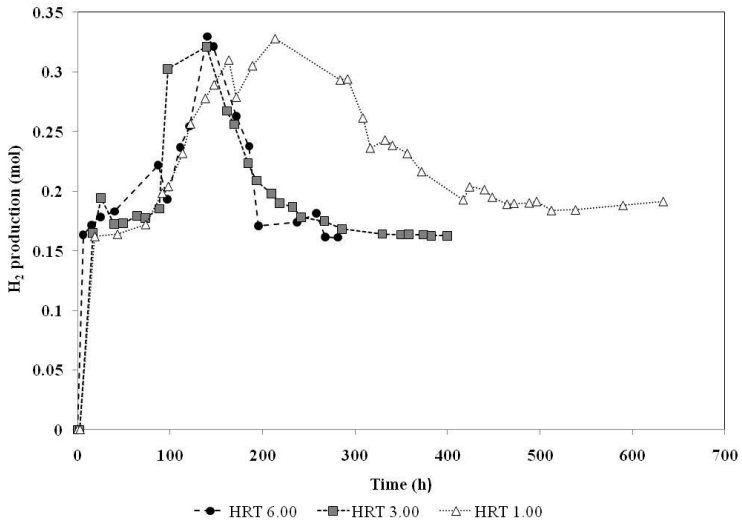

Figure 1. Hydrogen production in UASB reactor at 1, 3, 6 h HRT

The maximum production of H_2 in anaerobic reactor UASB operating at HRT of 6 h occurred to 140 h of reaction, producing a maximum of 0.33 mol H_2, with an accumulated of 4.1 mol at 292 h (Fig 2). A similar behavior was observed when the reactor was operated at 3 h HRT, a maximum hydrogen production (0.32 mol) is observed at the same time of reaction, with an accumulated of 1 mol at 400 hours.

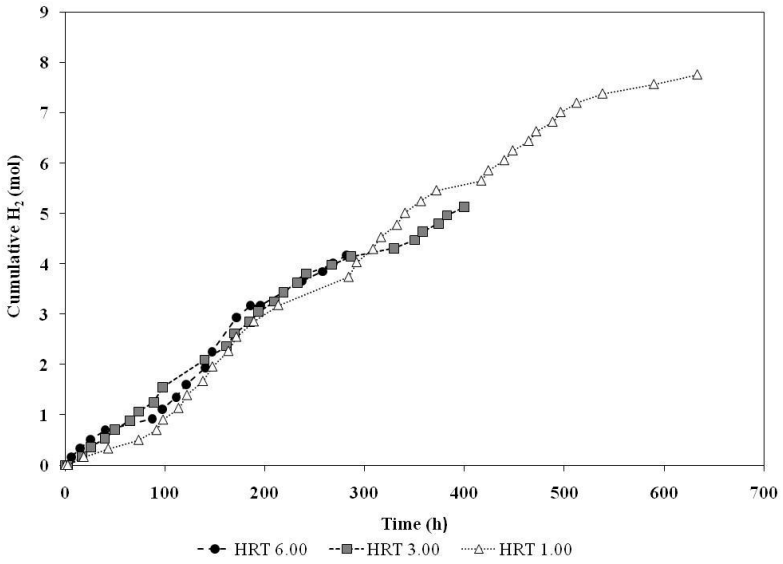

Figure 2. Cumulative hydrogen production in UASB reactor at 1, 3, 6 h HRT.

The effect of low HRT (1 h) had a negative impact on hydrogen production, being reflected in a displacement of the time in which the maximum production is reached (213 h, 0.33 mol), and an accumulated production of 7.7 mol at 633 hours of reaction (Figure 2). Zhu et al [11] reported that short retention times (<4 h) were advantageous to produce hydrogen because that biological process cut short the metabolic pathway as opposed to methane fermentation. Yu et al. [12] employed a range of HRT (from 2 to 24 h) to examine its effect on HRT on H_2 production. Their results indicated that the influence of HRT on H_2 yield was limited bus was significant on the specific H_2 production rate, with shorter HRTs corresponding to higher production rates. Chen et al. [13] using sucrose as substrate for H_2 production by anaerobic cultures also revealed that the H_2 production rate decreased from 0.094 mol h^{-1} to 0.032 mol/h when the HRT increased from 6 to 13.3h.

However the use of HRTs too short could be unfavorable bringing an excessive washing of biomass. Wu et al., (2007), evaluated the effects of different HRTs on the hydrogen production efficiency, using a reactor with cells immobilized on Poly (methyl methacrylate) (PMMA), observing a similar behavior at HRTs below 4 h. On the other hand, the use of long HRT (6 and 3 h) is a high metabolic speed due to the prolonged contact between the substrates and the microorganisms; however a decrement in the rate of hydrogen production can be reduced by the action of homoacetogenic microorganisms and metanogenic hydrogen consumers (MCH) [15]. Table 2 shows pHs results in UASB reactor after each HRT. Final pH varied in a range from 4.0 to 4.5. The selection of initial pH 5.0 was based on previous work by other researchers who observed good hydrogen production at this pH value [16].

Table 2. Influence of pH on 1, 3 and 6 h HRT.

HRT (h)	Initial pH	Final pH	CH_4 (g/l)
1	5.0	4.0	0
3	5.0	4.5	0
6	5.0	4.5	0

Hydrogen production by dark fermentation occurs in a wide range of pHs, from 4.0 to 12.0. It has been reported that can be carried out in optimal conditions in a range of pHs from 3.3 to 5.0 [17], contributing to the inhibition of metanogenic bacteria, which are reported like intolerant to pHs below 5.0 [18].

3.4. Effect of the HRT on sugar degradation

Table 3 shows the speed of consumption of reducing sugars of three HRTs. Sugar consumption-velocity data at 3 h HRT ($12.2 \cdot 10^{-2}$ mg L^{-1} h^{-1}; see Table 3) being consumed to the 100% to the 160 hours of reaction, whereas at 6 h HRT showed to a sugar consumption-velocity of $5.910 \cdot 10^{-2}$ mg L^{-1} h^{-1} and being consumed at 185 h.

Table 3. Initial velocities of the consumption of reducing sugars on hydrogen production at 1, 3, 6 h HRT

TRH (h)	V_0 de Consumo de A.R. (mg/l h^{-1})
1	2.7×10^{-2}
3	12.2×10^{-2}
6	5.9×10^{-2}

This fast decrement in concentration of reducing sugars for these HRTs influenced in a fast acceleration on hydrogen production (Figure 3). On the other hand it was possible to observe at 1 h HRT, the sugar consumption velocity was strongly affected (1.1 mg L^{-1} h^{-1}), showing an efficiency of 50% the 630 h of reaction, this behavior impact negatively on hydrogen production (displacement on the time).

3.5 Hydrogen production from hydrolysates

3.5.1 Hydrogen production from hydrolysates at 1.0 h HRT

The effect of 1.0 HRT was assayed using enzymatic hydrolysates of paper industry wastes at enzyme concentration of 0.40 mL^{-1} FPUasa and 1.52 U mL^{-1} CMCasa, pH 7. There was no hydrogen production for 1h HRT.

3.5.1 Hydrogen production from hydrolysates at 0.5 h HRT

Figure 3 shows the effect of hydrogen production using enzymatic hydrolysates of paper industry wastes at enzyme concentration of 0.40 FPU mL^{-1} pH 7. Endoglucanase activity was determined using carboxymethyl cellulose as a substrate, and was 1.52 U mL^{-1}. The best hydrogen-producing performance from hydrolysates of paper industry wastes occurred during HRT decrease (at 0.5 h HRT), giving the highest hydrogen production of 0.21 mol H at 61 h of reaction, and a cumulative hydrogen production of 1.15 mol (Fig. 3).

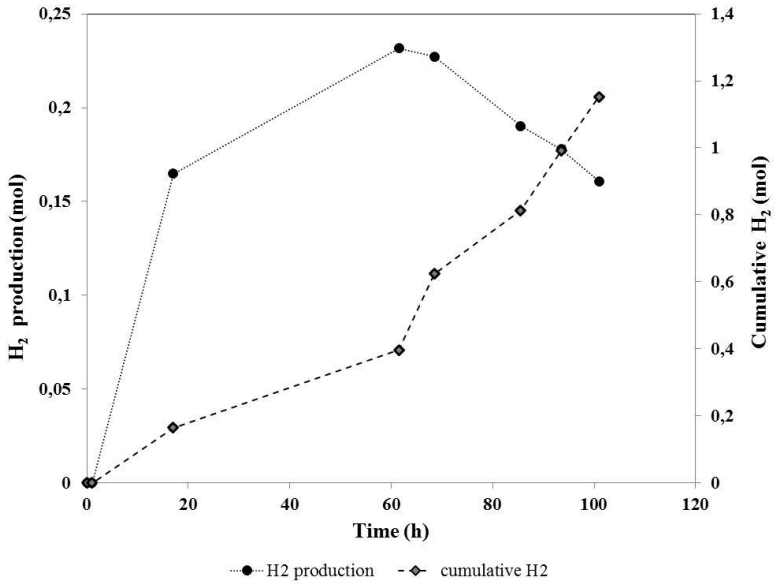

Figure 3. Hydrogen production from hydrolysates of paper wastes industry

The hydrolysis reaction was found to be fast initially and then slowed down gradually with the reaction time. Figure 4 shows the effect of fermentation time on sugar degradation. Reducing sugar content was estimated using 3,5-dinitrosalicylic acid colorimetric method (DNS method) [7].

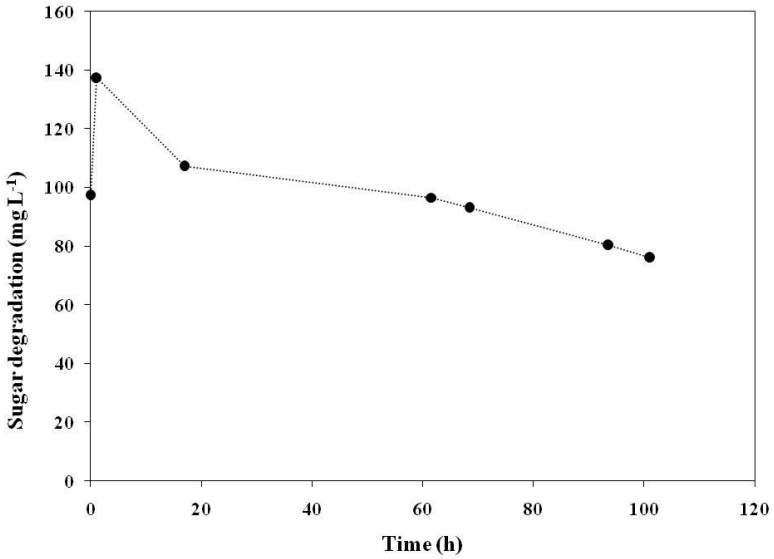

Figure 4. Effect of fermentation time in the sugar degradation for hydrogen production at 0.5 h HRT.

The experimental results show that during HRT-decreasing operation, using hydrolysates of paper industry wastes as carbon substrate resulted in increasing H_2 production with a decrease in HRT.

4. CONCLUSION

This study demonstrates a feasible bioreactor operation for hydrogen production, using a UASB reactor, hydrogen production was dependent on the sugar substrate used and also varied with the HRT shifting operation. It is of great interest to observe that sequential HRT decreasing and increasing had significant impact on hydrogen production. Environmental factor such pH and hydraulic retention time have been claimed to be able to govern the process efficiency of continuous hydrogen production.

Indeeded, we found that H_2 production for hydrolysates would be favorable when a lower HRT (0.5 h) operation was employed.

5. ACKNOWLEDGEMENTS

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