BIOHYDROGEN PRODUCTION BY BIOFILMS DEVELOPED ON *Opuntia imbricata* NATIVE AND MODIFICATED

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ABSTRACT

Biohydrogen production by anaerobic biofilms using dried stems of *Opuntia imbricata* as substratum or carrier media has proved successful, leading to a considerable increase in hydrogen production rates. *Opuntia imbricata is a* cactus found in arid parts of North America. Dry stems are highly rough and porous, offering a strong, steady and intricate structure, facilitating adhesion of microorganisms to its surface.

This research focused on determine the effect of thermochemical treatments on dried stems of *Opuntia imbricata* over the biohydrogen production, COD removal and biomass fixation. Experimental series were carried out in batch, using 180 ml of mineral medium (20 g L^{-1} $C_6H_{12}O_6$, pH 5.5) adding 20 ml of macerate anaerobic sludge and small pieces of dried stems of Opuntia imbricata previously treated.

Results showed that biohydrogen production rate was in the range of 0.09-0.37 mmol H_2 h^{-1} with a biohydrogen production in the range of 1-3.5 mmol H_2/g COD. Maximum biohydrogen production was obtained for the case were treatment with sodium periodate was applied, with 13.42 mmol H_2/g COD and a production rate of 0.37 mmol H_2 h^{-1}.

Oral Presentation

1.- Introduction

Hydrogen has been recognized as the ideal energy carrier of the future, because it is clean, recyclable, and efficient. Direct and highly efficient conversion of H_2 into electricity by fuel cells makes the application of H_2 energy even more attractive [1,2]. Fermentative H_2 production can be achieved by dark fermentation or by photo fermentation. Among them, dark fermentation normally achieves a much higher H_2 production rate and is considered more applicable for simultaneous waste reduction and H_2 generation [3,4]. Suspended-cell culture has been the most frequently used system for H_2 production via dark fermentation. However, continuous operation of suspended-cell systems often encounters problems with washout of biomass at high dilution rates and would require the recycling of biomass from the effluent to maintain sufficient cell density for high H_2 production activity. Effective retention of biomass can also be achieved by utilizing immobilized cells or membrane reactors. The immobilized-cell system is also gifted with a feature of creating a local anaerobic environment, which is well suited to fermentative H_2 production [5,6]. However, the technology has not been widely adopted to H_2 production through dark fermentation, whereas there have been some examples describing the use of immobilized cells for phototrophic H_2 production [7].

In the present study a natural support called *Opuntia imbricata* (coyonostle) was applied to immobilize acclimated sludge for H_2 production under anaerobic conditions. *Opuntia imbricata* is an abundant cactus in the northern region of Mexico which is composed of 28.68+/–6.27% hemicellulose, 34.02+/–5.04% cellulose and 37.64+/– 6.31% lignin [8].

The present work describes the effect on three response variables of various support treatments; performed either in KIO_3 solution at different pH and temperature or in O_3 solution with different pH and time. Heat treatment is a wood modification method which increases its dimensional stability, permeability and performance [9]. There are a number of ways to modify solid support *Opuntia imbricata* for the covalent cell immobilization. This article focuses on preactivation of support by a strong oxidant to produce aldehyde groups. The aldehyde groups of the activated carrier were able to react with amino groups of microorganisms to form covalent bonds and result in the cell immobilization [10].

2.- Materials and methods

2.1.- H_2-producing sludge

Anaerobic sludge originating from a beer plant in Zacatecas was used as the seed sludge. It was heat-treated at 90◦C for 30 min to inactivate hydrogen consumers and to harvest spore forming anaerobic bacteria before being subjected to cell immobilization [11].

2.2.- Medium composition

The medium used for H_2 fermentation consisted of glucose as the sole carbon substrate and of sufficient inorganic supplement including [12] (mg/l) NH_4HCO_3, 5240; $NaHCO_3$, 6720; 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; and $CoCl_2 \cdot 5H_2O$, 0.125. The glucose concentration in the medium was set at 20 g / l.

2.3.- Coyonostle samples

Wood specimens measuring 1.8 (R) X 7 (L) cm were cut from coyonostle (*Opuntia imbricata*). All wood specimens were cleaned, numbered and weighed at T_{amb}.

Coyonostle specimens were treated with either KIO_3 or O_3 aqueous solutions as oxidant agent. Wood specimens were pretreated according to 2^3 experimental designs [13]. Three independent factors were selected: concentration, pH and temperature for KIO_3 solutions and concentration, pH and time for O_3 solutions. All these factors were considered at a high and a low level of a multifactor design (see Table 1).

Multifactor variance analysis (ANOVA) and linear regression models were computed using a statistical software program in Excel. The optimum pretreatment condition was estimated considering the effect of pretreatments on each response variable.

Table 1. Multifactor Design

Oxidant	**Concentration**		**pH**		**Temperature**		**Time**	
Agent	High Level	Low Level	High Level	Low Level	High Level	Low Level	High Level	Low Level
KIO_3	0.02 M	0.01 M	4	2	90°C	25°C		
O_3	80 gr/m^3	30 gr/m^3	4	2			5 min	1 min

The ozone treatments were performed using an ozone generator (Pacific Ozone Technology L22), the pressure and temperature were kept at 0.859 bar and 27°C. The KIO_3 treatments were performed using 500 ml of periodate solution under agitation at 250 rpm for 1 hr [14]. Each experimental condition was carried out in triplicate.

2.4.- Batch fermentation for H_2 production

20 ml of acclimated H_2-producing sludge was placed in a 250-ml serum vial containing 180 ml of the aforementioned medium and the pretreated support for the immobilization of anaerobic sludge. Silicone rubber stoppers were used to avoid gas leakage from the bottles. In general, the batch reactor was performed at a constant temperature of 25°C, a pH of 5.5 and an agitation rate of 150 rpm [15]. The desired pH was adjusted by 0.1 N NaOH and 0.1 N HCl. Each experimental condition was carried out in triplicate.

2.5.- Monitoring and analysis

During the hydrogen fermentation, the monitoring parameters were protein concentration, pH, soluble COD, and hydrogen production.

The hydrogen gas production was calculated by comparing the sample biogas with a standard of pure hydrogen using a gas chromatograph (VARIAN 3400) equipped with a thermal conductivity detector (TCD). The temperatures of injector, detector and column were kept at 200°C, 200°C and 50°C. Helium was used as a carrier gas.

The protein concentration was measured according to the Peterson method. Measurements of soluble CDO and pH were performed according to the Standard Methods [16].

Samples removed from the liquid and gas phases were analyzed every 12 hours.

3.- Results and discussion

Using the favorable conditions for H_2 fermentation (i.e., 25°C, pH 5.5, 20 $g*l^{-1}$ of glucose in feed), batch cultures were carried out for H_2 production with immobilized mixed consortium on modified *Opuntia imbricata*.

3.1.- H_2-production with ozone treated support

Figure 1 shows that after a lag time of 12–16 h, H_2 rapidly evolved. The comparison between ozone treatments indicates that mainly the H_2 evolution rate in treated support was greater than that observed in no treated support.

The 30 gr/m^3, pH 2, 1 min, treatment reaches the maximum H_2 production of 11.31 mmol at 96 hours.

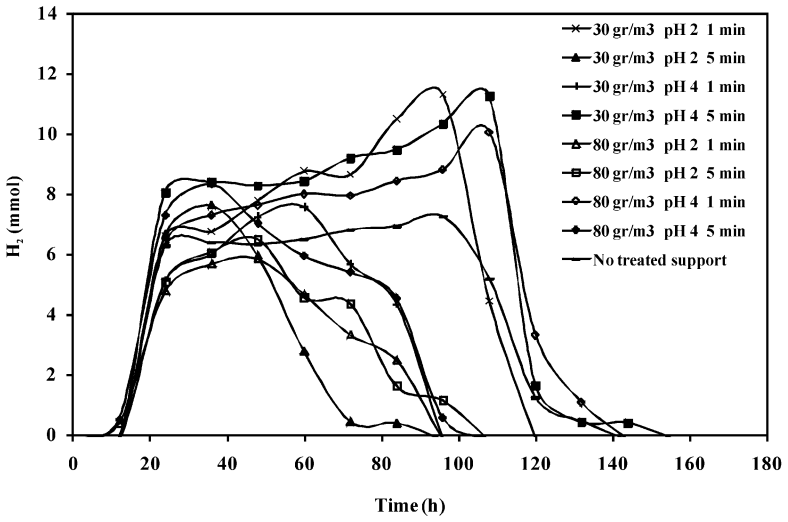

Figure 1. H_2 production with ozone modified Opuntia imbricata.

3.2.- H_2-production with periodate treated support

Figure 2 depicts the effects of periodate pretreated support versus hydrogen production. As can be seen from Figure 2, the H_2 yield increases with periodate treated support. The maximum H_2 production of 18.18 mmol occurred at 132 hours with 0.01 M periodate pretreated support at pH 4 and 25 °C.

The lag period varies between 12-25 hours and the depletion of the medium was observed after a process time in the range of 50-200 hours.

Our test results showed that the modification of the support remarkably affected the hydrogen yield.

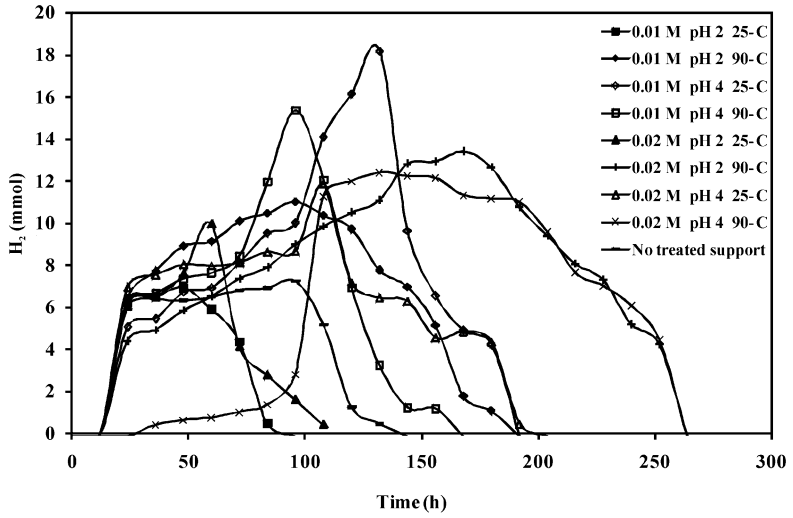

Figure 2. H_2 production with periodate modified Opuntia imbricata.

3.3.- Protein concentration with ozone treated support

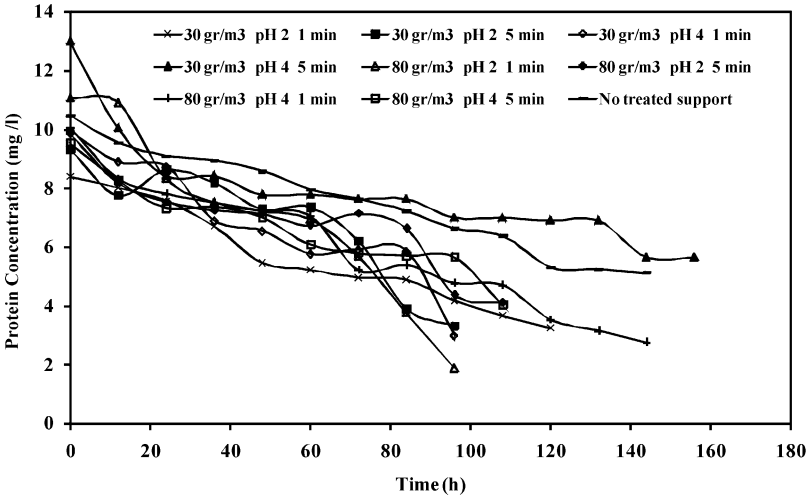

Figure 3. Protein concentration with ozone modified Opuntia imbricata.

Figure 3 depicts the effects of ozone pretreated support versus protein concentration of liquid phase. As can be seen from Fig.3 the protein concentration decreases with the increase of the hydrogen production due to the biofilm formation.

Our experiments results showed that the modification of the support also affected the biofilm formation.

3.4.- Protein concentration with periodate treated support

Fig.4 depicts the effects of periodate pretreated support in the biofilm formation. The rates of biofilm formation varied depending on the type of treatment used.

Utilization of periodate treated support for bio-hydrogen production by dark fermentation of glucose was proven to be more advantageous as compared to no treated support due the higher rate of biofilm formation.

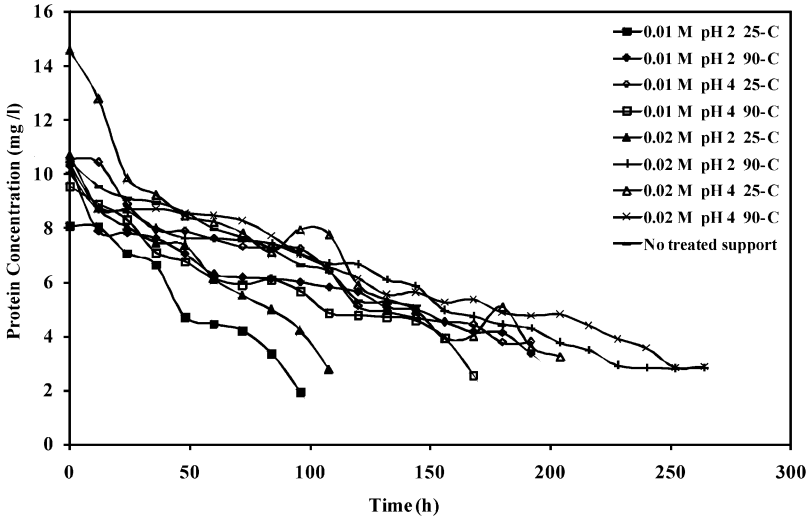

Figure 4. Protein concentration with periodate modified Opuntia imbricata.

3.5.- COD consumption with ozone treated support

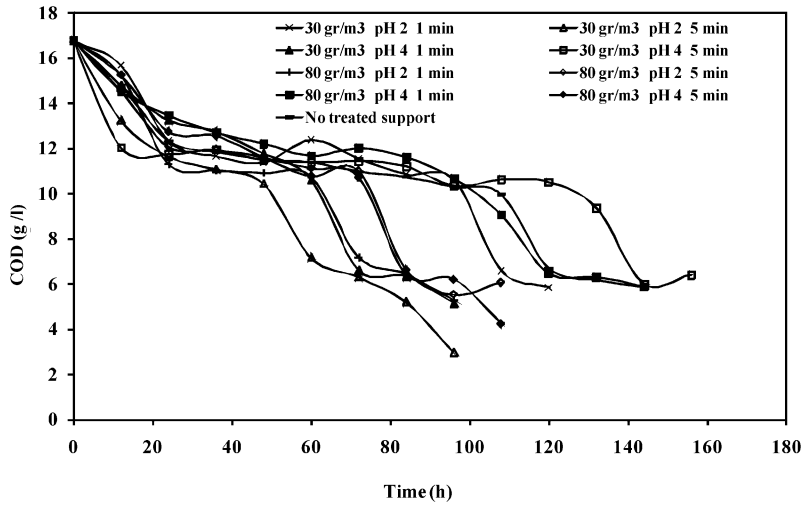

Figure 5. COD consumption with ozone modified Opuntia imbricata.

Figure 5 depicts the effects of ozone pretreated support in the COD consumption. The comparison between ozone treatments indicates that mainly the COD consumption rate in modified coyonostle was greater than that observed in no treated support. As can be seen from Figure 5, the COD consumption rate increased sharply with the increase of the H_2 production rate. Our tests results showed that the modification of the support also affected positively the COD consumption.

3.6.- COD consumption with periodate treated support

Figure 6 depicts the effects of periodate pretreated support in the COD consumption.
Utilization of periodate treated support for hydrogen production by dark fermentation of glucose was proven to be more advantageous as compared to no treated support due the higher rate of COD consumption. The results implied that the support conditions control could stimulate the microorganisms to form the biofilm and increase the H_2 production yields.

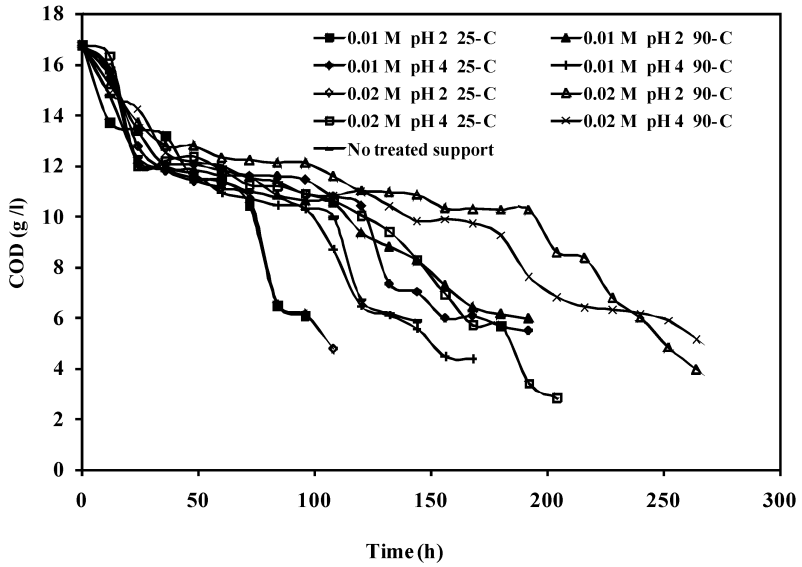

Figure 6. COD consumption with periodate modified Opuntia imbricata

3.7.- Effect of support pretreatment on hydrogen yield

The hydrogen yield for the different pretreatments is summarized in Table 2. As can be seen from Fig.7 the comparison between all support pretreatments indicates that 0.02 M, pH 2, 90°C periodate treatment had the highest hydrogen production yield.

The maximum hydrogen production yield was 1.86 mmol H_2 * lt^{-1} mineral medium * hr^{-1} and in all cases the hydrogen production yield of treated specimens was higher than that observed in no treated specimens.

Table 2. Hydrogen yield

Treatment	#	mmol H_2/ lt / hr
30 g/m^3 pH 2 1 min	1	0.58921418
30 g/m^3 pH 2 5 min	2	1.06056168
30 g/m^3 pH 4 1 min	3	0.63183104
30 g/m^3 pH 4 5 min	4	0.52124263
80 g/m^3 pH 2 1 min	5	0.60955017
80 g/m^3 pH 2 5 min	6	0.67713966
80 g/m^3 pH 4 1 min	7	0.46616971
80 g/m^3 pH 4 5 min	8	1.15819094
0.01 M pH 2 25°C	9	0.72219932
0.01 M pH 2 90°C	10	0.50872605
0.01 M pH 4 25°C	11	0.84166687
0.01 M pH 4 90°C	12	0.80043133
0.02 M pH 2 25°C	13	0.83009354
0.02 M pH 2 90°C	14	1.8641256
0.02 M pH 4 25°C	15	0.54877908
0.02 M pH 4 90°C	16	0.46948273
No treated support	17	0.37799703

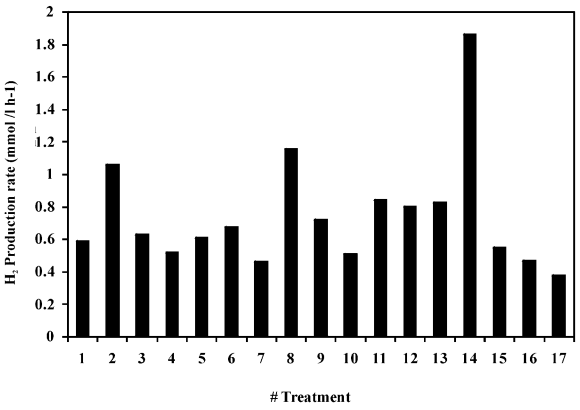

Figure 7. Hydrogen yield for pretreated support

Table 3. Relation between H_2 produced and COD removed

Treatment	#	mmol H_2/g COD
30 g/m^3 pH 2 1 min	1	1.834526318
30 g/m^3 pH 2 5 min	2	1.339656864
30 g/m^3 pH 4 1 min	3	1.229509059
30 g/m^3 pH 4 5 min	4	1.825757952
80 g/m^3 pH 2 1 min	5	0.997445732
80 g/m^3 pH 2 5 min	6	1.250103984
80 g/m^3 pH 4 1 min	7	1.307696845
80 g/m^3 pH 4 5 min	8	1.969836568
0.01 M pH 2 25°C	9	1.316413945
0.01 M pH 2 90°C	10	1.762858195
0.01 M pH 4 25°C	11	3.030000721
0.01 M pH 4 90°C	12	2.376538393
0.02 M pH 2 25°C	13	1.757845152
0.02 M pH 2 90°C	14	3.355426084
0.02 M pH 4 25°C	15	1.922209979
0.02 M pH 4 90°C	16	1.94675562
No treated support	17	1.122300462

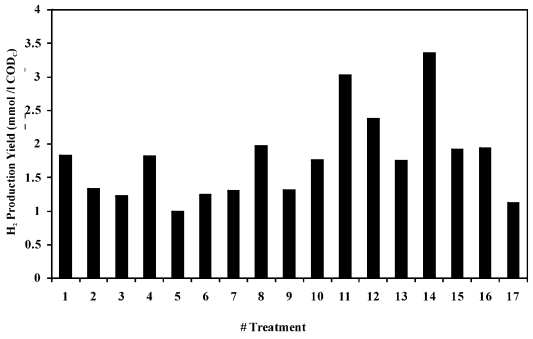

Figure 8. Relation between H_2 produced and COD removed for pretreated support

The relation between H_2 produced in mmol and COD removed in grams for the different pretreatments is summarized in Table 3. Fig.8 depicts the comparison between all support pretreatments and indicates that 0.02 M, pH 2, 90°C periodate treatment had the optimal relation.

The optimal relation was 3.35 mmol H_2 * gr^{-1} COD and almost in all cases treated specimens showed higher levels than that observed in no treated specimens.

4.- Conclusion

The pretreatment of the support proved to be more advantageous as compared to no treated support due the higher rate of biofilm formation and played a key role in conversion of glucose into biohydrogen gas by the consortia. The maximum hydrogen yield of 1.86 mmol H_2 * lt^{-1} mineral medium * hr^{-1} was observed with 0.02 M periodate treated support at pH 2 and 90°C.

Support treated under those conditions also resulted in the highest relation between H_2 produced and COD removed among the other treatments tested.

The aim of the statistical tools, in this case multifactor variance analysis (ANOVA), was useful in helping to observe these results globally at same time that facilitates the decision taking; the optimum pretreatment condition was 0.02 M KIO_3 at pH 2 and 90°C and it was estimated considering the effect of pretreatments on each response variable.

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