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BIOHYDROGEN PRODUCTION BY MICROBIAL MIXED CULTURE FIXED ON Opuntia imbricata NATIVE AND MODIFICATED

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Resumen

Opuntia imbricata como soporte natural fue utilizado para inmovilizar un cultivo mixto microbiano para la producción de hidrógeno bajo condiciones anaerobias. Piezas secas de *Opuntia imbricata* fueron expuestas a diferentes tratamientos. El objetivo de este trabajo fue estudiar el efecto de diferentes tratamientos del soporte sobre el proceso de producción de H2, a través de las variables de respuesta: consumo de la demanda química de oxígeno (DQO) y producción de H2. Las piezas fueron expuestas a tratamientos con KIO3 y O3 de acuerdo a un diseño factorial 23 y posteriormente transferidas a reactores tipo batch. Los ensayos en batch fueron llevados a cabo a pH 5.5, 25 °C, 20 g de glucosa L-1 y una velocidad de agitación de 150 rpm. El análisis termogravimétrico y el análisis espectroscópico por transformadas de Fourier (FTIR) fueron usados para el estudio del efecto de los tratamientos sobre el soporte. Los reactores con las piezas tratadas alcanzaron una mayor producción de H2 que los reactores con piezas sin tratar, dando como resultado que el tratamiento soporte desempeña un papel crucial en la adhesión celular y la producción de H2.El aumento en los niveles de producción de H2 fue atribuido a la transformación de los grupos alcohólicos de la celulosa a aldehídos, los cuales están unidos a enlaces –CH=N producidos por microorganismos. Los soportes tratados con KIO3 (0.02M), a 90°C, así como pH 2 fue encontrado como el tratamiento más efectivo con una máxima producción de H2 de 13.42 mmol y 1.86 mmol H2 L-1 h-1.

Palabras clave: células inmovilizadas, lodo anaerobio, Opuntia imbricata, piezas secas pretratadas producción de H2

Abstract

A natural substratum (*Opuntia imbricata*) was used to immobilize a microbial mixed culture for H2 production under anaerobic conditions. *Opuntia imbricata* dried stems were subjected to different treatments. The objective of this work was to study the effect of the support treatments on H2 production process through the following response variables: soluble chemical oxygen demand (CODs) and H2 production. The steams were subject to treatments with KIO3 and O3 according to a 23 factorial design, afterward they were transferred to batch reactors. Batch tests were conducted at pH 5.5, 25°C, 20 g glucose L-1 and an agitation rate of 150 rpm. Thermogravimetric analysis and Fourier transform infrared spectroscopy (FTIR) were used to study the effect of the treatments on the support. Reactors with treated stems achieved a higher H2 production than reactors with non-pretreated stems, resulting that the substratum treatment plays a crucial role in cell adhesion and H2 production. The enhancement of the H2 production was attributed to the transformation of cellulose alcohol groups to aldehydes, which are bonded to links –CH=N produced by microorganisms. The 0.02M KIO3 treated support at 90°C and pH 2 was found to be the most effective treatment with a maximum H2 production of 13.42 mmol and 1.86 mmol H2 L-1 h-1.

Keywords: Anaerobic sludge, H2 production, Immobilized cell, Opuntia imbricata, pretreated dried stems.

Introduction

H2 has been recognized as the ideal energy carrier of the future, because it is clean, recyclable, and efficient. Direct and highly efficient conversion of H2 into electricity by fuel cells makes the application of H2 energy even more attractive (Das & Veziroglu, 2001; Levin, Pitt & Love, 2004). Fermentative H2 production can be achieved by dark fermentation or by photo fermentation. Dark fermentation normally achieves a much higher H2 production rate and is considered more applicable for simultaneous waste reduction and H2 generation (Chang, Lee & Lin, 2002; Hawkes, Dinsdale, Hawkes & Hussy, 2002). Suspended-cell culture has been the most frequently used system for H2 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 H2 production activity. Effective retention of biomass can also be achieved by utilizing immobilized cells or membrane reactors (Kumar & Das, 2001; Kim, Kim, Ryu, Song, Kim & Yeom, 2005). The immobilized-cell system is also gifted with a feature of creating a local anaerobic environment, which is well suited to fermentative H2 production (Wu, Lin, Chang & Chang, 2005; Wu, Lin, Chang, Lee & Lin, 2002; Wu, Lin & Chang, 2003). However, the technology has not been widely adopted to H2 production through dark fermentation, whereas there have been some examples describing the use of immobilized cells for phototrophic H2 production (Najafpour, Younesi & Mohamed, 2004).

In the present study a natural substratum *Opuntia imbricata* (coyonostle) was used to immobilize a microbial mixed culture for H2 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 (Ilyina, Huerta, Martinez, Rodriguez & Gorokhovsky, 2008; Prieto, Filardo, Pérez, Beltrán, Román & Méndez, 2006).

The present work describes the effect on two response variables, soluble chemical oxygen demand (CODs) and H2 production of various support treatments; performed either in KIO3 solution at different pH and temperature or in O3 solution at different pH and exposure time. Heat treatment is a wood modification method which increases its dimensional stability, permeability and performance (Korkut, 2008). There are a number of ways to modify solid support *Opuntia imbricata* for covalent cell immobilization. This article focuses on preactivation of support by a strong oxidant to produce aldehyde groups. The mechanism of periodate activation suggests the transformation of cellulose alcohol groups to aldehydes, which are bonded to links –CH=N produced by microorganisms. (Becerra, Martínez, Rodríguez & Ilyiná, 2006). The periodate substratum activation increases the oxidation level and the immobilization percentages (Bilbao, Valdés & Blanco, 2000).

Materials and methods

H2-producing inoculum

Anaerobic microbial mixed culture obtained from a beer plant in Zacatecas was used as inoculum. It was heat-treated at 90◦C for 30 min to inactivate H2 consumers and to harvest spore forming anaerobic bacteria before being subjected to cell

immobilization (Wu & Chang, 2007; Wang & Wan, 2008a)

Medium composition

The medium used for H2 fermentation was as followed (g L-1) NH4HCO3 5.24; NaHCO3 6.72; K2HPO4 0.125; MgCl2•6H2O 0.1; MnSO4•6H2O 0.015; FeSO4•7H2O 0.025; CuSO4•5H2O 0.005; and CoCl2•5H2O 1.25 x 10-4 (Ogino, Miura, Ishimi, Seki & Yoshida, 2005). Glucose concentration in the medium was set at 20 g L-1.

Substratum (Opuntia imbricata) and pretreatment

Small stem pieces of Opuntia imbricata measuring 1.8 (R) X 7 (L) cm were cut, cleaned, numbered and weighed at 25°C.

Stem pieces were treated with either KIO3 or O3 aqueous solutions as oxidant agent. Experimental design (23) was applied for pretreatment analysis (Montgomery, 2005; Wang & Wan, 2008b). Three independent factors were selected: concentration, pH and temperature for KIO3 solutions and concentration, pH and time for O3 solutions. Factors were considered at a high and a low level of a multifactor design (see Table 1). Pretreated stems were dried at 25°C for 96 hours.

Table 1. Parameters of the 2^3 factorial design for the substratum pretreatments.

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

Multifactor variance analysis (ANOVA) and linear regression models were analyzed using a statistical software program in Excel.

The optimum pretreatment condition was estimated considering the effect of pretreatments on each response variable.

The ozone treatments were performed using an ozone generator (Pacific Ozone Technology L22 model), the pressure and temperature were kept at 0.859 bar and 27°C respectively. The KIO3 treatments were performed using 500 ml of periodate solution that was kept under agitation at 250 rpm for 1 hr (Bilbao, Valdés & Blanco, 2000). All experiments were conducted by triplicate.

H2 production in Batch

20 ml of acclimated H2-producing microbial mixed culture was placed in a 250-ml serum vial containing 180 ml of medium and the pretreated substratum for biofilm formation. Silicone rubber stoppers were used to avoid gas leakage from the bottles. All experiments were carried out at constant

temperature of 25°C, a pH of 5.5 and an agitation rate of 150 rpm (Wang & Wan, 2008c; Van Ginkel, Sung & Lay, 2001; Mu, Zheng, Yu & Zhu, 2006; Fang & Liu, 2002; Lay, Fan, Chang & Ku, 2003; Okamoto, Miyahara, Mizuno & Noike, 2000; Gomez, Moran, Cuetos & Sanchez, 2006). pH was adjusted by 0.1 N NaOH and 0.1 N HCl.

Analytical Methods

During fermentation process, pH, soluble COD and H2 production were monitored. H2 was sampled from the head space of the bottles by using gas-tight glass syringes. The H2 production in batch reactors was determined by gas chromatography of headspace taking into account the volume of the chromatographic syringe and the volume of reactor headspace. H2 was calculated by comparing the sample biogas with a standard of pure H2 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. COD and pH were monitored and measured every 12 hours. (Greenberg, Clesceri & Eaton, 2005).

Thermogravimetric analysis

A Thermogravimetric analyzer Shimadzu TGA-50 was used to determine the thermal effect on dried stems. 5.705 mg of dried stems was used for the analysis. Samples were heated from 25°C to 500°C at a heating rate of 10°C/min.

Fourier transform infrared spectroscopy (FT-IR)

Perkin-Elmer Spectrum GX FT-IR instrument with a Universal ATR Diamond/ZnSe crystal with one reflection was used for this study. FT-IR spectra for native and each treated sample that was obteined. The FT-IR spectra for each treatment were transformed into absorbance spectra and normalization to 1.5 absorbance units for the highest peak.

Results and discussion

The H2 production for the different pretreatments is summarized in Table 2. Results in table show that 0.02 M, pH 2, 90°C periodate treatment had the highest H2 production. The maximum H2 production was 1.86 mmol H2 L-1 mineral medium h-1 and in all cases the H2 production of pretreated dried stems was higher than that observed in non-pretreated dried stems.

H2 production related to COD removal is also shown in Table 2. The optimal relation was 3.35 mmol H2 g COD removed-1. In almost all cases treated specimens showed higher levels than that observed in untreated specimens. Results show that modification to dried stems due to pretreatments remarkably improved H2 production and COD removal compared with results of previous studies (Mohan, Bhaskar & Sarma, 2007; Yang, Zhangb, McGarveyc & Benemann, 2007; Kim, Han & Shin, 2004; Lin & Chang, 1999; Chen, Lin & Chang, 2001; Mizuno, Dinsdale, Hawkes, Hawkes & Noike, 2000; Wu & Chang, 2007).

The preactivation of support by oxidation suggested the transformation of cellulose alcohol groups to aldehydes. The aldehyde groups of the activated support are able to react with amino groups of microorganism to form covalent bonds and result in the immobilization of the microbial mixed culture. The decrease in the oxidant concentration after support activation was demonstrated by means of common analytical technique and it could be considered as an indirect evidence for aldehyde group formation (Becerra,

Martínez, Rodríguez & Ilyiná, 2006; Bilbao, Valdés & Blanco, 2000).

Table 2. Hydrogen production yield in reactors with a microbial mixed culture fixed on *Opuntia imbricata* native and modificated.

Treatment	mmol H_2 L^{-1} h^{-1}	mmol H_2 g COD removed^{-1}
30 g/m^3, pH 2, 1 min	0.58921418	1.834526318
30 g/m^3, pH 2, 5 min	1.06056168	1.339656864
30 g/m^3, pH 4, 1 min	0.63183104	1.229509059
30 g/m^3 pH 4 5 min	0.52124263	1.825757952
80 g/m^3 pH 2 1 min	0.60955017	0.997445732
80 g/m^3 pH 2 5 min	0.67713966	1.250103984
80 g/m^3 pH 4 1 min	0.46616971	1.307696845
80 g/m^3 pH 4 5 min	1.15819094	1.969836568
0.01 M pH 2 25°C	0.72219932	1.316413945
0.01 M pH 2 90°C	0.50872605	1.762858195
0.01 M pH 4 25°C	0.84166687	3.030000721
0.01 M pH 4 90°C	0.80043133	2.376538393
0.02 M pH 2 25°C	0.83009354	1.757845152
0.02 M pH 2 90°C	1.8641256	3.355426084
0.02 M pH 4 25°C	0.54877908	1.922209979
0.02 M pH 4 90°C	0.46948273	1.94675562
No treated support	0.37799703	1.122300462

Thermal analyses

Figure 1 shows the effect of temperature in the sample weight loss. As can be seen from Figure 1 the specimens weight decreases with the increase of temperature. Based on these results it can be supposed that the thermal treatments could modify the support properties. The aim of thermal analyses was only to validate the use of thermal treatments for the support modification therefore the analyses was only carried out on non-pretreated dried stems.

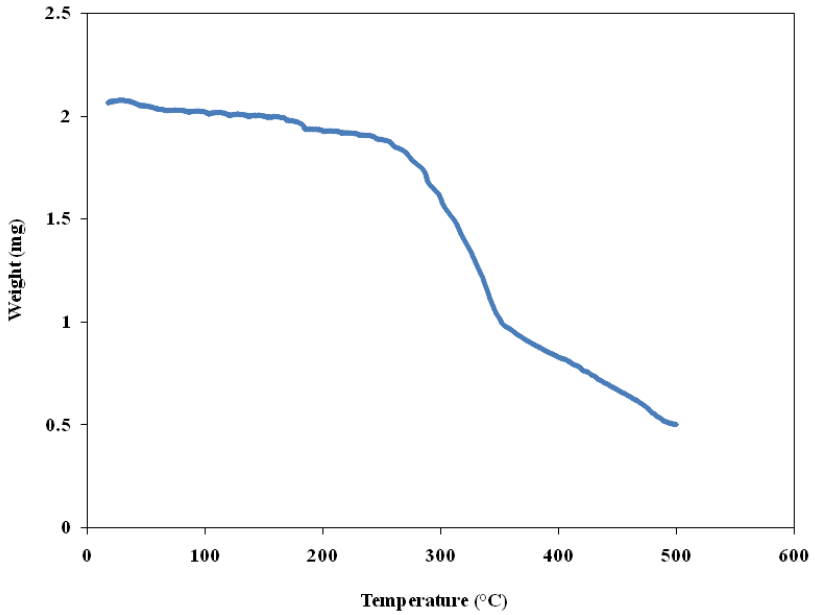

Figure 1. Native *Opuntia imbricata* TGA analysis.

FT-IR spectra

Figures 2 and 3 shows the FT-IR for the pretreated specimens associated to the highest H2 production levels. The 1660-1760 cm-1 band is characteristic for the ester, acid and aldehyde groups, however the preactivation of support by oxidation suggested the transformation of cellulose alcohol groups to aldehydes. Within that range aldehyde C=O stretch tend to absorb in the wavelength 1660-1740 cm-1, normal aliphatic aldehyde tend to absorb in the wavelength 1740-1725 cm-1, conjugation with double bond tend to absorb in the wavelength 1700-1680 cm-1 and conjugation with phenyl group tend to absorb in the wavelength 1700-1660 cm-1 (Socrates, 2004). Figures 2 and 3 show the absorption band characteristic for aldehyde groups and it can be observe a significant change for the treatments associated to the highest H2 production levels. The results implied that the support activation transforms the cellulose alcohol groups to aldehyde and this change could facilitate cell adhesion to substratum. The FTIR spectra curves for the support after the treatments were similar although the intensities of characteristic absorption peaks changed. This indicated that the functional groups of the support changed due to treatments. As shown in figure 2, the peak 1 values are ranked in the order of T1>T3>T4>ST>T2 and peak 2 are ranked in the order T3>T4>T2>ST>T1. As describe above these changes in dried stems due to pretreatments could facilitate cell adhesion and therefore improve H2 production.

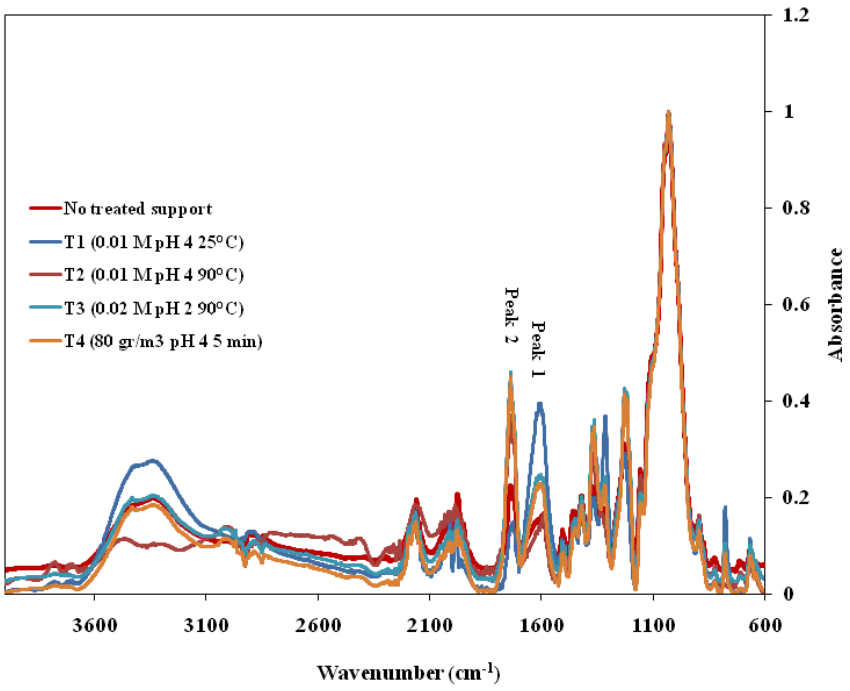

Figure 2. FT-IR spectra of treated samples associated to the highest H_2 yields.

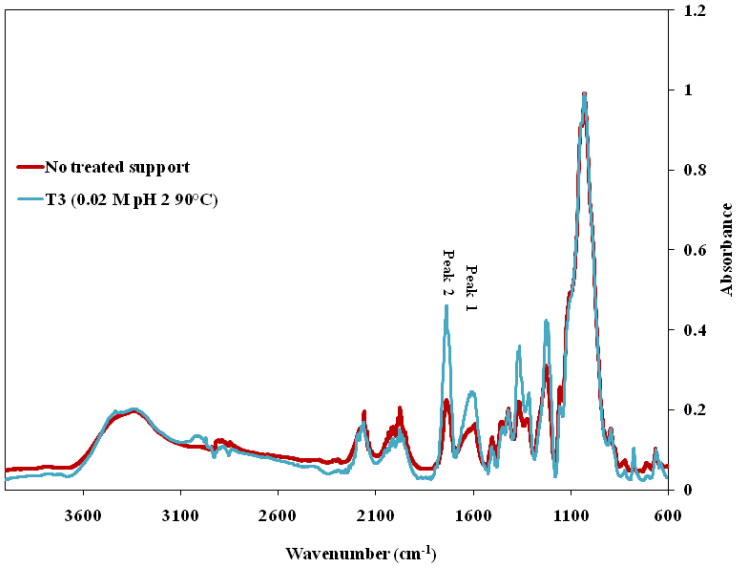

Figure 3. FT-IR spectra of treated sample associated to the maximum hydrogen production yield.

Conclusion

The pretreated substratum has proved to enhance H2 production by microbial mixed culture and this can be attributed to changes produced by pretreatments on dried stems structure making suitable for cell adhesion than not treated dried stems. The maximum

H2 production of 1.86 mmol H2 L-1 mineral medium * h-1 and the optimal relation between H2 produced and COD removal of 3.35 mmol H2 g COD removed-1 were observed with 0.02 M periodate treated support at pH 2 and 90°C. The FT-IR indicated that the functional groups of the support have changed due to treatments, resulting in the improve of the H2 yields.

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