Sacarificación y fermentación simultánea de olote pretratado

Simultaneous Saccharification and Fermentation process of pre-treated corn cob

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Resumen

El bioetanol puede ser producido a partir de una amplia variedad de materias primas. Estas se clasifican en tres categorías de materias primas agrícolas: los azúcares simples, almidón y lignocelulosa. El olote de maíz (residuo agronómico), utilizado en este estudio, se compone de 37.85 % de celulosa, 42.3 % de hemicelulosa y 7.01 % de lignina. Después del pretratamiento alcalino (NaOH al 1 %, 120°C, 60 min), el contenido de celulosa en el residuo de olote se incrementó de 37 % a 64 % con una disminución de 30 % y 88 % de hemicelulosa y lignina respectivamente. 59g.L^{-1} de la glucosa se obtuvo a partir de material pretratado por la sacarificación enzimática (45 °C, pH 5,5, 120 h) usando Celluclast® 1.5 L. Se evaluaron los efectos de la temperatura, el pH y la concentración de la enzima. Se buscó la condición más favorable en el proceso de sacarificación y fermentación simultánea (SFS) con el uso de *Zymomonas mobilis*. Las condiciones óptimas fueron, 38 ° C; pH 4,7; concentración de la enzima 20 FPU.g-1. Se obtuvo un rendimiento de etanol del 90 %, basado en el valor de rendimiento teórico para una concentración de 27g.L^{-1} a las 96 horas.

Palabras clave: bioetanol, lignocelulosa, Sacarificación y Fermentación Simultánea.

Abstract

Bioethanol can be produced from a wide variety of raw materials. These are classified into three categories of agricultural raw materials: simple sugars, starch and lignocellulose. The cob of corn (agronomic residue), used in this study, consists of 37.85% cellulose, 42.3% of hemicellulose and 7.01% of lignin. After the pretreatment alkaline (NaOH 1%, 120 ° C, 60 min), cellulose in COB residue content increased from 37% to 64% with a decrease of 30% and 88% of hemicellulose and lignin respectively. 59g. L-1 of the glucose was obtained from material prepared by enzymatic saccharification (45 ° C, pH 5.5, 120 h) using Celluclast® 1.5 L. Assessed the effects of temperature, pH and the concentration of the enzyme. We sought more favourable condition in the process of saccharification and fermentation simultaneous (SFS) with the use of *Zymomonas mobilis*. Optimal conditions were, 38 ° C; pH 4.7; 20 FPU.g-1 enzyme concentration. A yield of 90% ethanol, based on the value of theoretical throughput for a concentration of 27g was obtained. L-1 to 96 hours.

Key words: bio-ethanol, lignocellulose, saccharification and fermentation simultaneous.

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Introduction

Many potential benefits shows the bio-ethanol production from the fermentation of lignocellulosic biomass from agricultural by-products and forest waste (second generation bioethanol), compared with the derivative of starch or sucrose (first generation bioethanol) both from the energy and environmental point of view (Farrell et al., 2006; Olofsson, 2008). In addition this biomass provides a large amount of raw material by their high availability as Gnansounou et al. (2005) mentions it. Enzymatic hydrolysis of lignocelluloses pretreatment without is often not as effective due to the high stability of the materials to the enzymatic attacks as indicated by Taherzadeh et al. (2008). The main objective of these pretreatments is solubilizing lignin fraction and change the structure of cellulose chains, so that they are easily

attacked by enzymes. A pre-treatment is to be efficient, must meet, among other features, a low energy consumption, low costs of investment and maintenance, use of reagents cheap and easily recoverable, the possibility of application on different substrates (Almenares et al., 2008).

Z mobilis, a bacterium gram-negative, anaerobic, facultative can ferment glucose in ethanol and CO_2 through the Entner-Doudoroff pathway, that generates more ethanol because of reduced bacterial biomass production compared to the Embden-Meyerhof pathway in *S. cerevisiae* (Bai et al., 2008). In addition, z. mobilis can tolerate much higher than other bacteria concentrations of ethanol, and the product of biomass is generally recognized as safe (Generally Recognized as Safe; GRAS) by the FDA (US Food and Drug Administration) for animal feed, so this species is suitable for metabolic engineering capable of fermentation of pentose referred by Zhao and col. (2012).

An attractive option for the bioconversion of pretreated materials is the Simultaneous Saccharification and Fermentation rocess (SFS), where hydrolytic enzymes and fermentation organisms are in the same reactor, which reduces investment costs (Olofsson et al., 2008). On the other hand, the SFS process has limitations, such as different optimum temperature of hydrolytic enzymes and fermentation organisms. Many reports have mentioned that the optimum temperature for enzymatic hydrolysis is 40 - 50° C, while the microorganisms with good productivity and the performance of ethanol generally do not tolerate this high temperature (Karimi et al., 2006).

This work focused on the use of the cob as raw material for the production of ethanol in a process of SFS by hydrolysis with cellulase (Celluclast® 1.5 L) and fermentation with *Z. mobilis*. The factors of temperature, pH and enzyme concentration were evaluated in order to find the most favorable condition for hydrolysis and bacteria with good yield of ethanol.

MATERIALS AND METHODS

Corncob feedstock

The cob was provided by the Department of Plant Breeding of the Universidad Autonoma Agraria Antonio Narro, Saltillo, Mexico. The residue was ground to the size of 1 mm and dried at 65 ° C for 24 h, was hermetically stored in glass containers at room temperature.

Alkaline pretreatment

The residue cob was pretreated with 1% NaOH at 121 ° C / 15 psi for 60 min. 100 ml of a 10 g sample was added. After pretreatment the residue was filtered under vacuum, the residue was neutralized to pH 7 with distilled water, dried at 65 ° C for 24 h and stored for use as a substrate in the SSF process.

Inoculum

Z. mobilis was grown in liquid medium WL, comprising (gL-1): yeast extract 4.0, tryptone 5.0 and glucose 50.0, monopotassium phosphate 0.55, potassium chloride 0.425, calcium .125 chloride, magnesium .125 sulfate, ferric chloride 0.0025 0.0025 manganese sulfate and. The strain was provided by the Department of Biotechnology of the University of Coahuila (Saltillo, Mexico). 100 ml of medium was added to 250 ml flasks and inoculated with Z. mobilis. The flasks were incubated at 37 ° C for 24 hours.

Enzyme

Cellulose shopping complex, Celluclast®1.5L, (EC 3.2.1.4, Trichoderma reesei cellulases ATCC 26921; Novozymes, Denmark) was used in the experiments. The cellulolytic activity of the complex was 71 FPU / ml.

Enzymatic hydrolysis

Hydrolysis was carried out in 250 ml flasks at 45 ° C, with a concentration of 15 FPU cellulose / (g substrate) of Celluclast® 1.5 L, stirring speed of 130 rpm, pH 5.5 and substrate concentration was 10% (w / v) for 120 h. Samples were taken at 4, 8, 12, 24, 48, 72 and 120 h the reaction mixture during incubation, and boiled for 10 min to terminate the reaction of the enzyme were stored at -20 ° C for quantification of glucose.

Simultaneous saccharification and fermentation (SFS)

The SSF process was conducted under the following conditions, the pretreated cob was added to the medium WL, sterilized by autoclaving at 121 ° C for 15 minutes, under sterile conditions the enzyme complex and inoculum Zymomonas added mobilis to concentration 10% (v / v), he stirred at 130 rpm. 1 ml samples at 120 h were taken and centrifuged at 12,000 rpm for 10 min. The ethanol concentration and the remaining monosugars were determined as described below. PH, temperature and variable concentrations of enzyme were tested according to the experimental design.

Analytical method

The contents of cellulose, hemicellulose and lignin cob were determined by the Van Soest (1963) method. The glucose content was quantified by HPLC (Agilent series 1200 Quaternary technology). All samples were filtered through a disposable filter of 0.45 mm before injecting equipment. Samples were analyzed on a monosaccharide Rezex RCM-mark Phenomenex column. The temperature was 80 ° C and Millipore water was used as mobile phase at a flow rate of 0.6 ml / min. Ethanol was analyzed by gas chromatography (GC System Agilent 7820 A), which has flame ionization detector and a column of stainless steel (3.0 m long, 0.320 mm inside diameter).

Scanning electron microscopy (SEM) analysis of residues olote

The surface morphology and characteristics of the alkali-treated and untreated cob was studied using a scanning electron microscope (Philips XL30ESEM).

Statistical design of experiments

This paper mainly studied the effects of reaction temperature, enzyme concentration and pH in SFS by a full factorial design. The factors and their levels are presented in Table I.

Table I

Factors and levels full factorial design 3^3

Nivel	Temperatura/°C	Concentración de enzima FPU.g^{-1}	pH
1	37	10	4.5
2	41	15	5.5
3	45	20	6.5

RESULTS AND DISCUSSION

OLOTE COMPOSITION AND EFFECT OF PRETREATMENT WITH ALKALI

The chemical composition of the sample of treated and untreated corncob shown in Table II. The results suggest that alkaline pretreatment under the conditions set was able to partially remove certain fractions of the composition of the corncob, produce an enriched cellulose materials, reduce the lignin content up to 88%, with a recovery of cellulose practically complete, confirming the effectiveness of pretreatment to remove lignin and consequently make it easier enzymatic hydrolysis, agreeing with cited by different authors such as Mesa et al. (2010), Chen et al. (2008), Kumar et al. (2009) and Taherzadeh et al. (2008).

Table II

Olote chemistry before and after pretreatment and product performance after pretreatment (% dry weight)

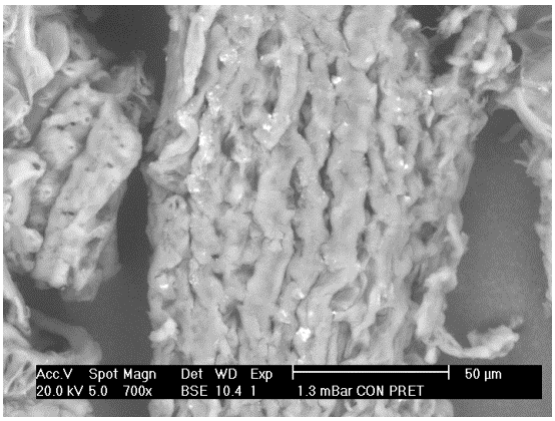
Componentes	Sin tratar	Pretratado	Rendimiento del producto[b]
Celulosa	37.85	64.20	98.8
Hemicelulosa	42.30	29.55	46.95
Lignina	7.01	0.824	7.84
Otros[a]	12.84	5.426	28.34

[a] Otros componentes incluye, cenizas, extractos y proteínas. [b] Porcentaje de cada fracción recuperada después del pretratamiento.

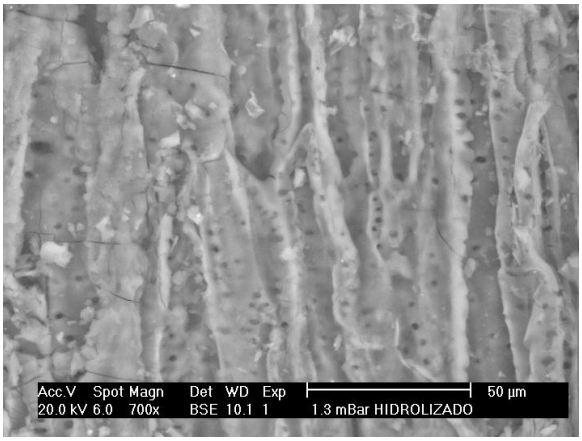
Effect of pretreatment on the surface structure of corncob

The structure of the surface without pretreatment olote was neat, smooth, entire (complete, intact), is firm, regular and homogeneous (Figure 1a). By contrast, the surface of the corncob pretreated with NaOH displayed, open, irregular (variable), comminuted and contained some micropores (Figure 1b), this might be due to the partial removal of hemicellulose and lignin, making it feasible enzymatic attack. The surface pretreated enzymatically hydrolyzed sample is perceived cracked and the more exposed and open pore (Figure 1c), similar results to those reported in working with wheat residues Han et al. (2012).

(a)



(b)



(c)

Fig.1 SEM de (a) olote untreated, (b) alkaline pretreatment corncob, (c) olote alkaline pretreatment, followed by enzymatic hydrolysis.

Enzymatic hydrolysis of pretreated olote

Table III sugar content obtained during the enzymatic hydrolysis is shown, the efficiency thereof corresponds to about 80%. Because it is difficult to know the efficiency of saccharification during SFS (being simultaneous) this amount was used to calculate the yield of ethanol. Olofsson et al. (2012) reported amounts similar to those obtained in this work in corn stover sugars, so we can consider the cob as an appropriate residue for converting sugars into ethanol production.

Table III

Results sugar content during the enzymatic hydrolysis (g.L^{-1})

	Contenido de Glucosa	Contenido de Arabinosa	Contenido de Xilosa	Contenido de Manosa
Olote pretratado	59.04	4.74	21.28	N.D*

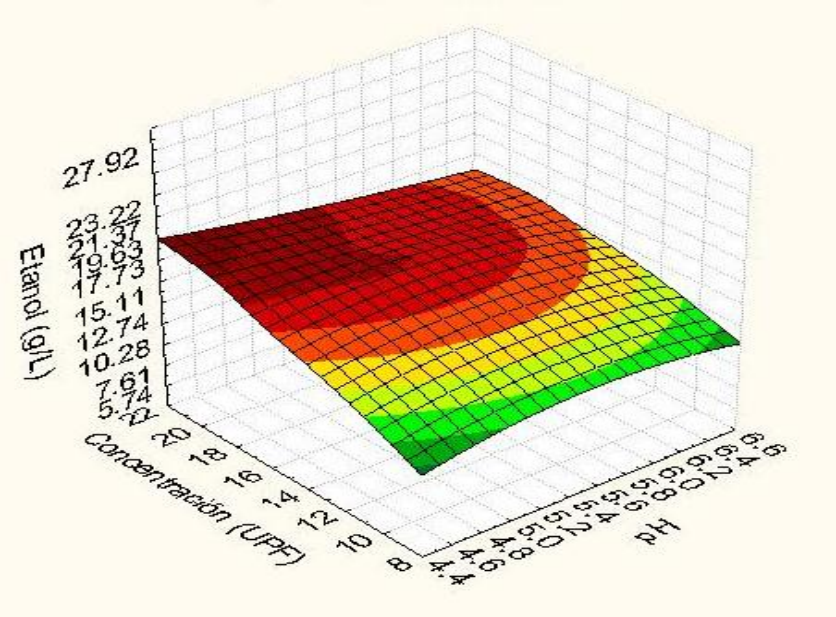
N.D.* Bajo las condiciones del análisis no fue posible detectar >10ppm

Effects of process operating conditions Simultaneous saccharification and fermentation with corncob pretreated with alkali

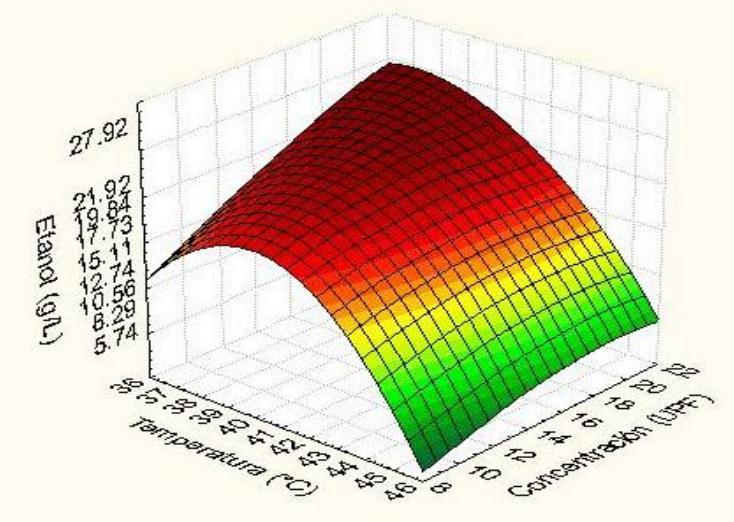
To find the effect of pH on ethanol concentration (Figure 2a), the values of 4.5, 5.5 and 6.5 in the fermentation media were used. The difference in concentration of ethanol with pH factor was not statistically significant (P> 0.5), indicating that the pH in the process has no significant effect on the generation of ethanol, however, for obtaining optimum values itself was considered. The most critical condition at pH factor is the stability of the cytoplasmic membrane in the bacteria in the enzyme is to maintain the bonds within the molecule to avoid variations in the arrangement of the

enzyme and achieve the formation of the enzyme complex / substrate and prevent denaturation as allude Madigan et al. (2004), and Hans et al. (1997), so it can be assumed that there is no involvement for ethanol production in this process for this study in this pH range.

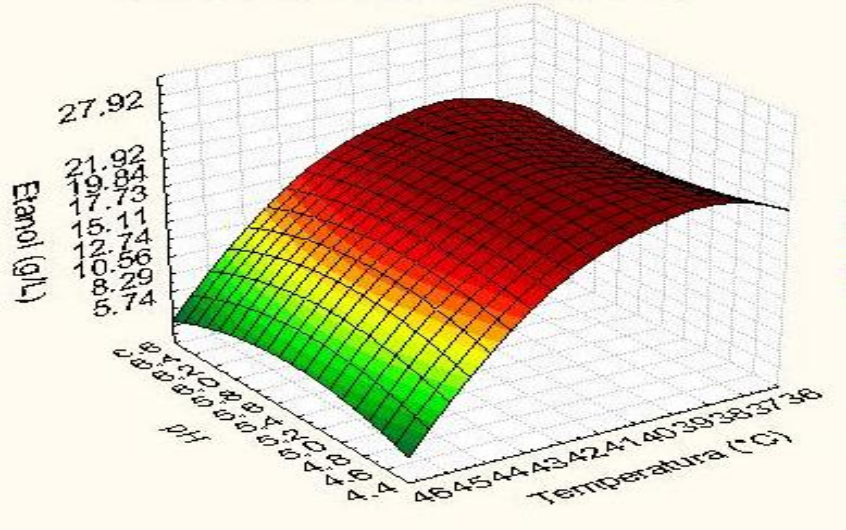
Temperature levels tested in this experiment were 37, 41 and 45 ° C. In the temperature-concentration and temperature-pH (figures 2b and 2c), interaction is achieved see a marked influence of temperature since increasing dramatically reduces ethanol production by 60%, this is due to increased temperature reaction causes a decrease in the survival of bacteria, leading to some bacteria to deactivation tendency that matches also referred to by Luo et al. (2008).



(a)



(b)



(c)

Figure 2 .graphic response surface (a) shows the effect of interaction pH- enzyme concentration in the ethanol concentration (b) effect of the concentration of enzyme-temperature ethanol concentration (c) and the effect of -temperature pH in the ethanol concentration of alkali pretreated cob.

Loads enzyme tested in this process were 10, 15 and 20 UPF finding a marked influence of this variable. Increasing concentration was directly proportional to the ethanol concentration, reaching up to 27 g / L with a load of 20 UPF. The results of the experimental design indicate that the optimal conditions for SPS are 38 ° C, enzyme loading 20UPF / g substrate and pH 4.7, obtaining a maximum concentration of 27g / L of ethanol, which is equivalent to 90% yield . This value was calculated as a percentage based on the theoretical maximum yield of ethanol is 0.51 g ethanol per gram of glucose. The results of the optimal conditions were tested in order to try to reduce processing time, which gave positive results by reducing the time of 120 h to 96 h, this being feasible in terms of productivity and process costs.

CONCLUSION

This study has shown that pretreatment favored alkali cellulose availability on the cob, which increased by 64.2%, making the residue olote favorable for ethanol production alternative. The optimum operating conditions SFS were pretreated cob 38 ° C, pH 4.7 and 20 FPU.g-1 enzyme loading, the processing time was reduced to 96h 120h under these conditions.

They were obtained 27g.L-1 ethanol, the percentage of the theoretical maximum yield was high, which makes bacteria Zymomonas mobilis a feasible in this process.

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