Article
ECORFAN Journal
June 2016 Vol.2 No. 2 33-39

Optimization of the enzymatic hydrolysis process using bagasse of delimited sweet sorghum

GUARNEROS-FLORES, Javier†*, LÓPEZ-ZAMORA, Leticia and AGUILAR-USCANGA, María Guadalupe

División de estudios de posgrado e investigación, Instituto Tecnológico de Orizaba, Oriente 9 No.582 Col. Emiliano Zapata C.P. 94320 Orizaba Veracruz.

Received January 7, 2016; Accepted June 15, 2016

Abstract

The aim of this research was to optimize the process of enzymatic hydrolysis from sweet sorghum bagasse, maximizing the production of fermentable sugars present (glucose and xylose). The bagasse was ground, crushed and dried, once free of moisture was subjected to an alkaline treatment using 4% H2O2 on a liquid-solid ratio of 16:1. To optimize the enzymatic process a Box Behnken experimental design were used, using two enzymes (hemicellulase and cellulase) and considering three independent variables (time, enzyme % w/w and liquid-solid ratio), being the g/L of xylose and glucose the response variable. A statistical analysis allowed us to determine that the best conditions are: 5 % w/w cellulase, 3 % w/w hemicellulase, reaction time of 48 hours and a liquid-solid ratio of 5:1 generating 121.12 g/L of glucose and 76.38 g/L of xylose. In this investigation is observed an excellent performance of both enzymes working simultaneously, assuring high levels of sugars from sweet sorghum bagasse and discarding the prolonged reaction times because the increase of sugars (glucose and xylose) between the 52 and the 92 hours of reaction is inferior to 14 %

Hydrolysis, sweet sorghum bagasse, hemicellulase, cellulas, optimization

Citation: GUARNEROS-FLORES, Javier, LÓPEZ-ZAMORA, Leticia and AGUILAR-USCANGA, María Guadalupe. Optimization of the enzymatic hydrolysis process using bagasse of delimited sweet sorghum. ECORFAN Journal-Democratic Republic of Congo 2016, 2-2: 33-39

* Correspondence to Author (email: letylopezito@gmail.com)
† Researcher contributing first author.

Introduction

The prospects of depletion of fossil fuels coupled with the growing demand for energy, place liquid biofuels as a renewable energy alternative, within the framework of the growing valuation of fuels that have low impact on the emission of greenhouse gases. Today, however, the damage caused to the planet by emissions from the mass consumption of petroleum-derived fuels, such as gasoline and diesel, in transportation vehicles is increasingly evident (Cardona, 2009).

Bioethanol accounts for about 90% of biofuel produced worldwide. The production of ethanol from renewable sources of environmentally friendly raw materials, such as agricultural crops, forest crop residues, and / or industrial and municipal waste, is currently of great interest. Emphasis is also placed on those that do not compete with their use as a food source, in addition to focusing on the waste of the crop not on the plant as such, that is, on the use of lignocellulosic biomass capable of To generate sugars that can be obtained by the appropriate physical and chemical treatments (Bueno et al., 2009).

Currently, one of the raw materials that is taking a major boom in the production of ethanol is sweet sorghum, defined as a promising crop because of its large biomass production and minimum general requirements (Medina et al., 2011). Sweet sorghum (Sorghum bicolor L. Moench) ertenece to the family of grasses, crop is well suited to growing in warm arid or semi - arid areas. Tolerates heat, salt, and floods. It is treated as an annual plant, although it is perennial herb and in the tropics can be harvested several times a year; has an approximate height of 1 to 3 m depending on the crop (Chuck et al., 2011).

The lignocellulosic biomass of sweet sorghum (bagasse) presents a complex structure, composed of several fractions that must be processed separately to ensure efficient conversion of these materials to ethanol (Montes et al., 2010). The major fraction of the biomass is the cellulose whose glucose chains are grouped in higher structures of great crystallinity, their intimate association with lignin makes their hydrolysis difficult to obtain fermentable sugars (Viñals et al., 2012).

The effect of pretreatment on lignocellulosic materials has been recognized over time. The purpose of pretreatments is to remove lignin and hemicellulose, reduce the crystallinity of the cellulose and increase the porosity of the material. However, an alternative option is only to use a treatment capable of removing the largest quantity of lignin and subsequently providing an enzymatic treatment to the raw material using two enzymes simultaneously, a cellulase and a hemicellulase which must be able to produce two types of sugars Different being glucose and xylose respectively (Agbor et al., 2011).

There are a number of key features for the effective pretreatment of lignocellulosic biomass. The pretreatment process should be of low capital and operational cost and should result in the recovery of most lignocellulosic components in the form of separate fractions, suitable pretreatment will provide favorable conditions for subsequent successful hydrolysis. Hydrolysis literally means decomposition or destruction of a substance, in the case of enzymatic hydrolysis the decomposition is carried out by the use of enzymes. The correct use of the type of enzymes depends on the pretreatments applied to sweet sorghum bagasse (Cuervo et al., 2009).

ISSN-On line: 2414-4924

The objective of the study was to optimize the process of enzymatic hydrolysis using sweetened bagasse of sweet sorghum, maximizing the yields of two fermentable sugars present, using a hemicellulase to unfold the hemicellulose in xylose and a cellulase to obtain glucose from the cellulose.

Methodology

10 kg of sweet sorghum were ground, producing 5.2 kg of bagasse (BSD), which was sun-dried for 48 h in order to remove most of the water present. After solar drying, a reduction was carried out by means of a cutter composed of 6 blades, in order to reduce the length of the BSD, since a smaller size of the raw material increases the efficiency of the pretreatments applied because there is a Greater contact area (Guarnizo et al., 2009). Finally the moisture of the BSD obtained was determined, before proceeding with the delignification.

BSD delignification was performed by the action of an alkaline pretreatment using H 2 O 2 to 4% in a liquid - solid ratio (RLS) 16: 1 and a pH 11.5 adjusted with NaOH 10 M. The reaction was out for 45 h to finally separate the liquid fraction from the solid, by manual pressing. To the solid sample, 2 washes were run under running water in a 5: 1 RLS in order to remove remaining trace amounts of lignin. We proceeded to a solar drying of 48 h to continue with the next stage of the process.

The delignified BSD must be treated enzymatically, for which it was necessary to determine the enzymatic charges to be used, since hemicellulose and cellulose are still present in different amounts in the BSD. For this, three different tests were performed considering different combinations of cellulase and hemicellulase in% w / w, the concentration of cellulase used being greater because bagasse contains mostly cellulose in its composition.

The enzymes used were Cellic CTec3 and Cellic HTec3 from Novozymes. The preparation of such tests is shown in Table 1, all tests were performed in duplicate.

Proof	Bagasse delignified (g)	Hemicellulose HTec3 (%)	Cellulase CTec3 (%)
1	twenty	3	5
2	twenty	4	5
3	twenty	5	6

Table 1 Preparation of samples with different enzyme concentrations relative% w / w

From the best enzymatic combination found, a Box Behnken experimental design was proposed for the enzymatic step (Table 2). The enzymatic loading in p / p ratio (4, 5 and 6% cellulase and 2, 3 and 4% hemicellulase), RLS (5: 1, 7: 1 and 9: 1) and reaction time (24, 48, and 72 h) and the concentration in g / L of Glucose and xylose quantified by HPLC using a constant stirring of 250 rpm and a temperature of 50 ° C (optimum temperature of both enzymes).

To prepare the samples, was used 0.05 M COONa CH3 initial liquid phase as varying amounts of pretreated bagasse according to different RLS, also the pH was adjusted to 5.0, the optimum working Cellic CTec3 the cellulase enzyme.

ISSN-On line: 2414-4924

Sample	CTec3: HTec3 (% w / w)	Time (h)	RLS
1	4: 2	24	7: 1
2	6: 4	24	7: 1
3	4: 2	72	7: 1
4	6: 4	72	7: 1
5	4: 2	48	5: 1
6	6: 4	48	5: 1
7	4: 2	48	9: 1
Referring to Fig.	6: 4	48	9: 1
Referring to Fig.	5: 3	24	5: 1
10	5: 3	72	5: 1
eleven	5: 3	24	9: 1
12	5: 3	72	9: 1
13	5: 3	48	7: 1
14	5: 3	48	7: 1
fifteen	5: 3	48	7: 1

Table 2 Box-Behnken design for enzymatic hydrolysis of sorghum bagasse delignification

Inactivation of the enzyme was performed by thermal shock by exposing the samples to 10 min. Baths in boiling water and 10 min in cold water, finally the liquid phase was separated from the solid by centrifugation, the first one was analyzed in the HPLC by introducing each One of the results to the NCSS 2007 software to complete the experimental design and obtain the optimum working conditions of the enzymatic process.

The results provided by the NCSS 2007 Software were validated through experimentation to corroborate the optimal working conditions found, as well as the mathematical model generated.

Results

The results of the percentage composition of the bagasse are shown in Table 3.

Bagazo	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Others (%)
Raw	39.54	29.83	22.21	8.42
Alkaline	50.07	34.56	5.33	10.04

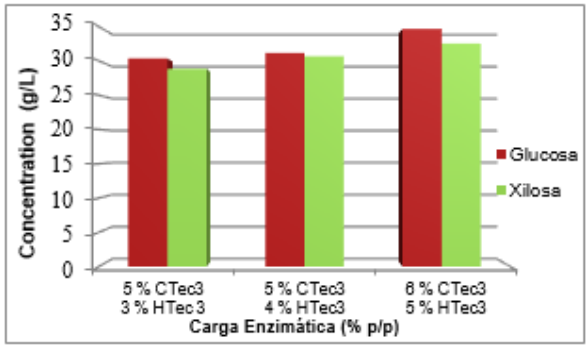
Table 3 Fiber content of sweet sorghum bagasse at different stages

The percentage of lignin removal was calculated after the alkaline pretreatment by equation 1, obtaining a total of 76% of removal, a result superior in 8% compared to that obtained by Viveros et al. (2014).

$$\%\ \mathrm{Rem}_{\mathrm{Lignina}} = \left[\frac{\%\ \mathrm{Lig_i} - \%\ \mathrm{Lig_f}}{\%\ \mathrm{Lig_i}}\right] * 100 \quad (1)$$

Graph 1 shows the results obtained from the 3 different enzymatic combinations of CTec3 and HTec3, where it is possible to observe that in the first (3% HTec3 - 5% w / w CTec3) and the second (4% HTec3 - 5% w / w CTec3) combination, there is only a 6% increase of xylose, while comparing the first with respect to the third combination (5% HTec3 - 6% w / w CTec3) the increase presented is 10.19% of Glucose and 8.75% xylose, however, the latter represents the use of a greater amount of both enzymes, especially in the hemicellulase. Considering that there must be a balance between the economy (cost of reagents) and production (generation of glucose and xylose), it was decided to use the first combination of enzymes, as it was not affected in a drastic production in the hydrolyzation process.

ISSN-On line: 2414-4924



Graphic 1 concentrations of sugars to different enzymatic loads

The glucose concentrations obtained from the experimental design are presented in Table 4, ranging from 81.00 and 135.84 g / L for glucose and 32.17 and 80.49 g / L for xylose, generating the best results in tests 5 and 6, both with a reaction time of 48 h, a RLS of 5: 1, but different enzymatic charges (4-2% and 6-4% of CTec3-HTec3 respectively), which will be adjusted by statistical analysis.

Sample	Glucose (g / L)	Xylose (g / L)
1	98.23	44,463
2	105,324	52,037
3	119,933	65,751
4	104,803	52,484
5	130,259	75.101
6	135,841	80.49
7	95,435	41,088
Referring to Fig.	95,717	43,601
Referring to Fig.	121,557	67,682
10	123,658	68,222
eleven	81.006	36,246
12	92,179	32,171
13	109.856	56,969
14	110,278	57,879
fifteen	108,138	56,912

Table 4 Results obtained from experimental design for obtaining glucose and xylose

The statistical analysis performed using the NCSS 2007 software generated the mathematical model presented in equation 2, representing an adjustment of 93.21% and a theoretical response of 134 g / L of glucose, complying with the optimal conditions of enzymatic loading (CE) Of 5% w / w CTec3 and 3% HTec3, reaction time (t) of 48 hrs and RLS of 5: 1.

$$Glucosa_{(g/L)} = 15.117 - 31.83 * CE + 2.68 * t + 10.92 * RLS + 3.68 * CE^2 - 0.010 * t^2 - 0.25 * RLS^2 - 0.23 * CE * t + 0.42 * CE * RLS + 0.002 * t * RLS \quad (2)$$

The optimum working conditions (time, RLS and enzyme loading) were tested experimentally. The average concentrations of glucose and xylose obtained are presented in Table 5.

Testing Experimental	Glucose (g / L)	Xylosa (G / L)
General test	121.12	76.38

Table 5 Concentrations of glucose and xylose (g / L) obtained by experimental verification of the optimum conditions optenidas

The response variable statistically analyzed was the concentration of glucose, however a direct relationship was observed with respect to the concentration of xylose generated. Figure 1 shows the response surface obtained from the results generated by the experimental design, combining the effects of time (h) vs. amount of delignified bagasse (g) vs. glucose obtained (g / L), showing the highest Concentration of glucose in the area of intense red color.

ISSN-On line: 2414-4924

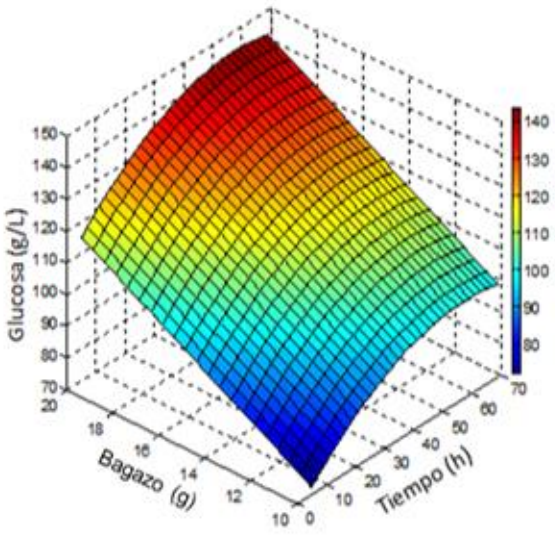


Figure 1 Response Surface glucose content enzymatic hydrolysis Effect Time (h) vs Bagasse (g)

It is observed that the highest glucose generation occurs with an amount of bagasse of 20 g (RLS 5: 1), equivalent to 5 mL / g, besides that after 48 h the glucose increase is minimal, checking the Optimal conditions provided by statistical analysis.

The glucose concentration generated by checking the optimal conditions obtained for the process (121.12 g / L), was 9.63% lower than the theoretical predicted by the model (134 g / L), in addition to generating 76.38 g / L xylose, improving the results obtained by authors such as Chen et al. (2010) and Nochebuena et al. (2012) with 29.84 and 21.01 g / L of xylose respectively by acid prehydrolysis, it is important to note that the adjustment presented by the mathematical model was higher than 90%, which guarantees the reproducibility of the process. The results generated in the design of experiments have a direct relationship between the concentration of glucose and xylose generated so it is not necessary to experiment different ranges in time and RLS for each of the enzymes making the process less complex to take finished.

Thanks

To the Technological Institute of Veracruz for making use of the facilities of the Bioengineering laboratory. To the National Institute of Agricultural and Livestock Forestry Research (INIFAP) where the lignocellulosic analyzes of the raw material were carried out. To the TecNM, for the other financing through project 5831.16-P

Conclusions

Sweet sorghum bagasse can be considered as a bioenergetic crop, when the high levels of sugars contained in it are present, showing concentrations even higher than those of sugarcane and with lower overall requirements.

The application of pretreatment with H 2 O 2 favoreción removing lignin reaching values of up to 76% removal leaving susceptible to bagasse for further enzymatic attack.

The enzymatic hydrolysis was carried out using two different enzymes (Cellic Ctec3 and Cellic HTec3) simultaneously favoring the production of Glucose and Xylose in high concentrations (121.12 and 76.38 g / L respectively), proving that it is not necessary to apply a pretreatment to eliminate the Hemicellulose and demonstrating the good performance of the two enzymes used.

References

Agbor, B. V., Cicek, N., Sparling, R., Berlin, A., & Levin D. (2011). Biotechnology Advances. Biomass pretreatment: Fundamentals toward application. 29, 675:685.

ISSN-On line: 2414-4924

Bueno, G., Cordovezm M., & Delgado, G. (2009). Sorgo dulce: sus potencialidades productivas. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar 18 (3) 2-15.

Cardona, A. C. A. (2009). Perspectives of biofuels production in Colombia: Latinamerican and world contexts 29 (1): 109-120.

Chen, Y., Sharma, R., & Chen, C. (2010). Potential of agricultural residues and hayfor bioethanol production. Applied Biochemistry and Biotechnology Part A: Enzyme engieneering and biotechnology. 142:276-290.

Chuck, H. C., Carrillo, E. P., & Serna, H. S. (2011). Sorgo como un cultivo multifacético para la producción de bioetanol en México: tecnologías, avances y áreas de oportunidad. Revista Mexicana de Ingeniería Química. 10 (3):529-549.

Cuervo, L., Folch, L. J., & Quiroz, E. R. (2009). Lignocelulosa como fuente de azúcares para la producción de etanol. Biotechnology and Bioengineering. (13)3.

Guarnizo, F. A., Yépez, M. P., & Sánchez V. H. (2009). Pretratamiento de la celulosa y biomasa para la sacarificación. Scientia et Technica (42)15.

Medina, M. M. A., Fernández, L. L., Aguilar, C. L., & De la Garza, H. (2011). Aprovechamiento de materiales lignocelulósicos para la producción de etanol como carburante. Revista Científica de la Universidad Autónoma de Coahuila. 3(6), 35-41.

Montes, G. N., Quintero, P. V., López, C. M. E., & García, A. M. (2010). RB. Cañero: sorgo dulce [sorghum bicolor (L.) Moench] para la producción de etanol. Folleto técnico 43. ISBN: 978-607-425-355-9.

Nochebuena, M. L. E., López, Z. L., Aguilar, U. M. G., & Dominguez, G. C. X. (2012). Optimización del tratamiento oxidativo sobre la hidrólisis enzimática de bagazo de caña de azúcar empleando superficie de respuesta. Revista Latinoamericana de Química ISSN 0370-5943.

Viñals, V. M., García, B. A., Álvarez, M. G., & Ramil, M. (2012). Obtención de etanol a partir de biomasa lignocelulósica. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA). 7-16.

Viveros, R. I. P., López, Z. L., & Partida, S. G. (2014). Maximization of glucose production using hydrogen peroxide as an alkaline pretreatment of sweet sorghum bagasse. Journal of chemical, Biological and Physical Sciences. ISSN 2249-1929 (4) 3-2

ISSN-On line: 2414-4924