Vol. 17, No. 3 (2018) 1095-1104
Revista Mexicana de Ingeniería Química

OPTIMIZATION OF THE ACID HYDROLYSIS OF CLADODES OF *Opuntia ficus-indica* BY RESPONSE SURFACE METHODOLOGY

OPTIMIZACIÓN DE LA HIDRÓLISIS ÁCIDA DE CLADODIOS DE *Opuntia ficus-indica* POR LA METODOLOGÍA DE SUPERFICIE DE RESPUESTA

A. Texco-López[1], A. Cadena-Ramírez[1], J. Álvarez-Cervantes[1], X. Tovar-Jiménez[1], C.A., Gómez-Aldapa[2], J. Castro-Rosas[2], A. Téllez-Jurado[1]*
[1]*Posgrado en Biotecnología, Universidad Politécnica de Pachuca, Carretera Pachuca-Cd. Sahagún, km 20, Ex-Hacienda de Santa Bárbara, C.P. 43830, Zempoala, Hidalgo, México.*
[2]*Área Académica de Química, Instituto de Ciencias Básicas e Ingeniería, UAEH, Ciudad del Conocimiento, Carretera Pachuca-Tulancingo km. 4.5, Col. Carboneras, C.P. 42184, Mineral de la Reforma, Hidalgo, México.*

Received November 10, 2017; Accepted April 17, 2018

Abstract

The self-sustaining nature of renewable energies makes it necessary to continuously search for substrates that satisfy a growing interest in the production of biofuels, taking care of areas such as food security. In this work we optimized a hydrolysis process of *Opuntia ficus-indica* cladodes using acid diluted and surface response methodology. The nopal is a secondary crop widely distributed in arid and semi-arid zones and recently investigated for its potential in the biofuel industry given its versatility as a crop. The result of this optimization was the production of 20,825 mg mL^{-1} of total reducing sugars (TRS), at 27.25 minutes of reaction and with a solution of 3.46% sulfuric acid. The main sugars obtained in the hydrolyzed were galactose (57.14%), arabinose (20.51%), glucose (13.18%) and xylose (9.17%). Also, it emphasizes the value of humidity of this culture (90%) and the low amount of lignin present (2.70%).

Keywords: optimization, *Opuntia ficus-indica*, second-generation biofuels, solid-liquid relationship.

Resumen

La naturaleza autosustentable de las energías renovables hace necesaria una búsqueda continua de sustratos que satisfagan un creciente interés por la producción de biocombustibles cuidando áreas como lo es la seguridad alimentaria. En este trabajo se optimizó un proceso de hidrólisis cladodios de *Opuntia ficus-indica* utilizando ácido diluido mediante la metodología de superficie de respuesta. El nopal es un cultivo secundario ampliamente distribuido en zonas áridas y semi-áridas, y recientemente investigado por su potencial en la industria de los biocombustibles dado su versatilidad como cultivo. El resultado de esta optimización fue la obtención de 20.825 mg mL^{-1} de azúcares reductores totales (TRS), a los 27.25 minutos de reacción y con una solución de ácido sulfúrico al 3.46%. Los principales azúcares presentes en el hidrolizado fueron galactosa (57.14%), arabinosa (20.51%), glucosa (13.18%) y xilosa (9.17%). Así mismo, en este cultivo destaca el valor de la humedad mayor al 90% y la cantidad de lignina presente (2.70%).

Palabras clave: biocombustibles de segunda generación, *Opuntia ficus indica*, optimización, relación sólido-líquido.

1 Introduction

In Mexico, as in other countries, climate change, food security, and the need to have substrates in a constant, economic and sustainable manner have favoured the search and experimentation for new raw materials for the production of Biofuels. One of the alternatives recently proposed is the use of agaves and cacti due to their tolerance to low humidity media and climatic variations (Yang *et al*., 2015). In addition to the above, due to the type of climate and soil in our country *Opuntia ficus indica* (OFI) (nopal) are a widespread crop, with a cultivated area of about 10,000 hectares by year. The annual production of nopal is around 600000 tons, with a productivity of 40-50 tons ha^{-1} year^{-1}, which is considered as a high yield, surpassing the yield of agaves, crops such as beet, ryegrass, alfalfa, wheat, and being on par with other crops such as maize, sorghum and sugar cane (Nobel, 1995; Santos *et al*., 2016).

On the other hand, the studies of hydrolysis on OFI cladodes in order to obtain sugars are scarce, and within the realized ones there is no work that analyses the optimization of this process, that is why it becomes necessary apply and develop methods aimed at maximizing this potential resource of sugars, and be able to involve it in the investigation of so-called second generation biofuels. One of these tools is optimization using Surface Response Methodology (SRM), which helps to determine the best working conditions in order to increase the yields of various processes (Kalil *et al*., 2000; Gilmour, 2006; Fuentes-Ortega *et al*., 2017).

In addition to the above, several pre-treatment methods have been developed, including mechanical, thermal, acidic, alkaline, autohydrolysis, oxidative and all possible combinations between them (Hendriks and Zeeman, 2009; Ortíz-Mendez *et al*., 2017), each with advantages and effects on the substrates applied. Of the processes mentioned, one of the most widely studied for the hydrolysis of lignocellulosic materials, has been the use of dilute inorganic acids with sulphuric acid (H_2SO_4), being one of the main reagents due to its low price (with respect to other inorganic acids), handling and high efficiency in the hydrolysis reaction, it has been described that the use of sulfuric acid can remove up to 100% of the hemicellulose present with some residues (Taherzadeh and Karimi, 2008; Saucedo *et al*., 2010).

Finally, the pre-treatment with inorganic acids has been applied to OFI biomass, the sugars obtained (cladodes and fruits) have been used for the production of ethanol by biochemical way using mainly yeasts (Retamal *et al*., 1987; Kuloyo *et al*., 2014; Santos *et al*., 2016). However, methodologies for the optimization of hydrolysis treatments have not been used, which is the objective of this work. The use of different sulfuric acid concentrations is proposed to carry out the hydrolysis of *Opuntia* cladodes. For the optimization of the process, the Response Surface Methodology is used to find the optimal conditions for the release of sugars and to determine their potential for the production of second-generation ethanol.

2 Materials and methods

2.1 Raw Material

A lot of young *Opuntia ficus indica* cladodes (sprouts of about 18 to 25 cm length) were collected in the state of Hidalgo (19°41’50.4"N, 98°47’20.5"W), México in September 2017. The cladodes were segmented into cubes of approximately 1 cm^3, which were dehydrated at a temperature of 60 °C (AOAC 1998). The dehydrated sample was ground and sieved (250 μm particle size, No. 60 mesh), the flour obtained was stored in plastic bags in a cool dry place, and this dry weight fraction was designated as Solid Fraction (SF). The physicochemical analyses performed on this fraction were: water content (by weight difference), determination of ether extract (TAPPI T-204 om-88), ash (AOAC 1998), lignin (TAPPI T222 om-88), cellulose (Browning, 1967) and percentage of mucilage as part of the compounds that are soluble in water (Rodríguez, 2011).

2.2 Acid hydrolysis of OFI cladodes and optimization by RSM

To carry out the hydrolysis, a stainless steel autoclave operated at 1 atm and 121 °C was used, and flasks were placed inside with aluminium caps in order to avoid leakage during this process. A solid-liquid ratio of 1:15 was used (cladode flour of OFI:dilute sulphuric acid solution in g mL^{-1}), this ratio was chosen since according to several reports, is close to water content in the young OFI cladodes (Malainine *et al*., 2003; Ginestra *et al*., 2009; Kuloyo *et al*., 2014; Yang *et al*., 2015). Under these conditions, the hydrolysis was carried out (for triplicate) for 20 minutes with the following concentrations of sulphuric acid solutions: 0.5, 1, 2, 3, 4, 5 and 6%. At the end of this hydrolysis, the total reducing sugars (TRS) were measured and the treatment that released the largest amount of TRS was selected. Afterwards, three residence times were examined, that were 10, 20 and 30 minutes. At the end of each treatment (triplicate), the obtained samples were filtered with Whatman # 1 filter paper and washed with distilled water, the solid residue obtained was dried at 105 °C for 24 h in order to quantify the hydrolysed matter.

The data obtained were analysed by Design-Expert® program to make the experimental design in order to optimize the pre-treatment. The variables selected for the optimization were: acid percentage and residence time, leaving a fixed solid:liquid (S:L) ratio, as response variable was used the TRS obtained. After completing the design, we proceeded to validate the already optimized model and to determine the amount of the hydrolysed sample under this methodology.

2.3 Analytical methods and statistical analysis

The technique developed by Miller (1959), using a wavelength in the 525 nm UV-Vis spectrophotometer (Biochrom Libra S11^{TM}) was used for the determination of the TRS. For the identification of the sugars released during the hydrolysis, an HPLC (Thermo Scientific Ultimate 3000) coupled to a refractive index detector (Refractomax 520^{TM}) was used, Rezex^{TM} RPM-Monosaccharide Pb^{+2} 8% column, at a flow rate of 0.6 mL min^{-1} and water as mobile phase was used for identification of sugars. Triplicated tests were performed; the statistical analyzes of the data were in the program SigmaPlot^{TM} 11.0.

3 Results and discussion

3.1 Composition of the raw material

The results obtained from the physicochemical characterization of the OFI cladodes show a high water content (94.73%), low lignin content (2.65%) and water-soluble compounds (mucilage) (53%). The S:L ratio obtained from the raw material was 15, which can be compared with that described in other studies. The high percentage of moisture is typical of plants with CAM metabolism. These plants produced hydrocolloids in their tissues whose objective is the water storage. This feature is essential for the survival of plants that live in arid zones (Yang *et al*., 2004). Factors such as the age of the cladodes also have a strong impact on the composition of the mucilage, in plants with greater age; the mucilage promotes cell adhesion decreasing its concentration en the plant (Chang *et al*., 1994; Habibi *et al*., 2004). On the other hand, the moisture content varies depending on the season of the year, in the rainy season; the moisture content of the cladodes increases considerably, but has little effect on the carbohydrate content (Ribeiro *et al*., 2010). The results obtained indicated a high humidity (94%) and a concentration of sugars very similar to those described by other authors. The main differences found were observed in the lignin content, probably due to the age of the cladodes used in this work (two months).

Table 1 shows the results obtained in the present work with those reported by other authors, it is observed that there are differences in the values, these differences may be due to climatic conditions, plant varieties, cultural practices, etc. Stintzing and Carlem (2005) and Ribeiro *et al*. (2010) indicated that edaphic factors and site of crops among others have a strong impact on the composition of OFI.

3.2 Exploratory analysis of acid hydrolysis

The results obtained in the treatments with the different concentrations of acid solution and an S:L ratio of 15 indicated that, from the concentration of 3% sulfuric acid there were no significant differences ($p > 0.05$) about a the release of TRS (Figure 1). In addition, it can be seen that there is a relationship between the amount of acid used and the release of TRS, reaching a point where despite increasing acid concentration, no more TRS can be obtained from the samples this may be due to the acid hydrolyses of the hemicellulose, some proteins, lipids and other biomolecules such as the polyphenols, leaving finally the crystalline cellulose, difficult hydrolysis (Wyman *et al*., 2005; Börjesson and Westman, 2015).

Table 1. Physicochemical characteristics of *Opuntia ficus-indica* cladodes, results are presented on a dry basis, with the exception of water content (n.r. not reported).

Autor	Water content (%)	Ethereal extract (%)	Ash (%)	Lignin (%)	Water soluble compounds (%)	Holocellulose (%)
In this Work	94.7±0.2	3.9±0.4	15±0.1	2.7±0.1	63.1±1.6	18.9±0.6
Malainine *et al*. 2003	n.r.	7.2	19.6	3.6	48	21.6
Kuloyo *et al*. 2014	88-95	n.r	16.8	7.9	24.3	23.1
Yang *et al*. 2015	93.9	n.r	23.7	12.3	25.0	13.1
Ginestra *et al*. 2009	n.r	0.63	n.r	16	17.7	13.5

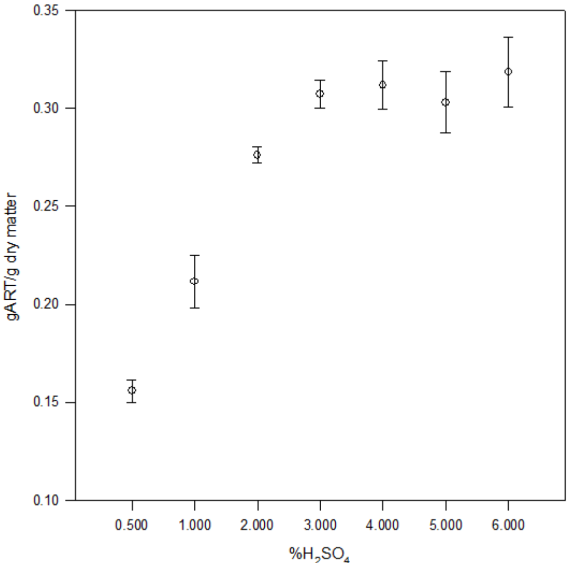

Fig. 1. Effect of sulfuric acid concentration on TRS release at 1 atm and 121 °C.

On the other hand, an excessive exposure of lignocellulosic material to acid conditions can promote the degradation of carbohydrates by generating compounds such as furfurals that are toxic for fermentative processes (Chandel *et al*., 2007; Geddes *et al*., 2010). In similar processes of chemical hydrolysis with barley straw, it has been seen that the reduction of particle size considerably improves the process. The increasing of the surface area make more available the holocellulose and therefore improves the release of TRS (Quintanar-Gómez *et al*., 2012).

Once the effect of the H_2SO_4 concentration on the hydrolysis of the raw material was determined, it was evaluated the effect of the residence time on the release of the TRS. For this, a series of hydrolysis tests were carried out using the following times: 1, 5, 10, 20 and 30 minutes, for these tests a relationship was used a S:L ratio of 15 and 3% sulfuric acid solution. The results are shown in Figure 2.

It was observed that the hydrolysis of the cladodes flour takes place in a relatively short period of time and that stabilizes between 10 and 30 minutes. Part of this phenomenon can be due to the fact that the mucilage in its hydrocolloid that is composed of neutral polysaccharides, integrated mainly of arabinose, rhamnose, galactose and xylose, which are easily hydrolysed (Wyman *et al*., 2005). The results suggest that the TRS liberated come mainly from the hydrocolloids and hemicellulose of cladodes. Some authors describe that soft conditions of acid hydrolysis affect the methylated derivatives of the *Opuntia* mucilage, yielding sugars like arabinose, xylose, galactose and a great diversity of oligosaccharides. Stronger hydrolysis conditions yield other types of sugars, such as rhamnose and glucose (McGarvie and Parolis, 1981). The type of sugars detected and the absence of oligosaccharides suggests that with the conditions used there was access to the crystalline matrix of the cellulose of the *Opuntia* cladodes.

3.3 *Hydrolysis optimization*

Based on the results obtained previously, the process was optimized to maximize the released of the TRS. The time and percentage of the hydrolysis was chosen as variables for optimization and central composite design rotatable using the response surface methodology for this process. The factors and levels of work are shown below (Table 2).

From this design, it was analysed 8 experimental units (4 for -1 and 4 for +1), with 5 central points (0), all the analyses were by triplicate, the uncoded matrix is shown following (table 3).

When the measurements indicated in Table 3 were done, the adjustment of a quadratic model was obtained, where was related the released of the TRS (mg L^{-1}) with the variables time (A) (min) and percentage of Acid (B), equation 1 shows the relation obtained.

$$TRS = 20.59 + 2.63A + 1.62B - 0.39AB - 1.42A^2 - 0.50B^2 - 0.24A^2B - 1.11AB^2 \quad (1)$$

Table 2. Factors and levels analysed by rotational central compound design.

Variables	Symbol used for the variable	Encoded Variables Low (-1)	 Central (0)	 Upper (+1)
Time	min	10	20	30
Acid percentage	%A	2	3	4

Table 3. Design obtained by the program DesignExpert®.

Treatment	Time (min)	Acid (%)
1	20	3
2	30	2
3	20	3
4	10	4
5	20	3
6	20	3
7	5.858	3
8	20	4.414
9	34.142	3
10	30	4
11	10	2
12	20	3
13	20	1.586

From the adjusted model, a response surface graph (Figure 3) was generated, where it can be seen that the central conditions for the optimization of the released of the TRS is between 3% and 5% of acid solution and, according to this graph, the optimal residence time is around 30 minutes. The statistical analysis shows a correlation coefficient (R^2) higher than 0.8. It has been described that an R^2 greater than 0.8 is a good model and can be used to explain the observed variability. Values of R^2 less than to 0.8 cannot be used to explain the relationships between the variables (Little and Hills, 1978). A $p - value$ below the level of significance are desirable for a better understanding of the processes studied (Koocheki *et al.*, 2009). The $p - value$ obtained is sufficiently precise to predict the most relevant variables in the hydrolysis process of *Opuntia* flour. The fit data of model are shown in table 4.

Finally, based on the results of the response surface, a rotatable central composite design was proposed in order to corroborate the observed curvature and determine the optimal conditions of TRS release. The five experiments carried out, representing the four axial points (2% acid with 10 min and 30 min; 4% acid with 10 min and 30 min) and a central point (3% acid, 20 min) in agreement to the model used (figure 4). The optimal conditions for the release of TRS were with acid at 3.47% and a hydrolysis time of 27.25 minutes.

In order to verify this given point and to validate the model obtained, a hydrolysis was carried out by triplicate at 1 atm and 121 °C, with the percentage of acid and time indicated by the model.

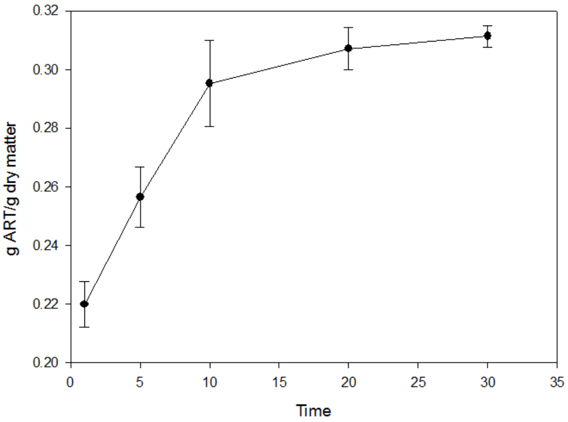

Fig. 2. Effect of time over *O. ficus indica* cladode hydrolysis at 1 atm and 121 °C.

The result obtained was 20.825 mg mL^{-1} of TRS release, with a standard deviation of 0.128 mg mL^{-1}, with this value it is verified that there is reproducibility between the theoretical and experimental data. After this step, it was noted that for the comparison of the value given in the present work with other reported, it is very important to take into account the ratio S/L used, time and percentage of acid, since when changing these values, the quantity and hence the concentration of biomass (and polysaccharides) to be hydrolysed increases or decreases, with disparate results. An example of the above is presented in the work of Kuloyo *et al.* (2014) in which pre-treatment of OFI cladodes obtained 22.6 mg mL^{-1} of TRS, with an employed S/L ratio of 3.36 (50 min, 120 °C, 1.5% H_2SO_4), which implies working with a greater amount of biomass in a lower volume of work. In other work Santos *et al.* (2016) produced with three varieties of cactus 19.18 mg mL^{-1}(O. ficus indica Var. Round palm), 22.87 mg mL^{-1} (*Nopalea cochenillifera* var. Small palm) and 26.36 mg mL^{-1} (*O. ficus-indica* var. Giant palm) of TRS with an S:L ratio of 13.33 under the following hydrolysis conditions: 60 min, 121 °C, 1% H_2SO_4, results suggesting that there is a difference between the type of varieties with respect to the sugars that could be obtained, it should be noted that this could be independent of age, soil variables and harvesting time (Stintzing and Carle, 2005).

Table 4. Values of the statistical analysis with a level of significance of 95%. R^2 (data adjustment), CV (coefficient of variability), P (significance), S.D (standard deviation).

Variable	R^2	CV (%)	P	S.D.
Sugars	0.9732	2.25	<0.0001	0.43

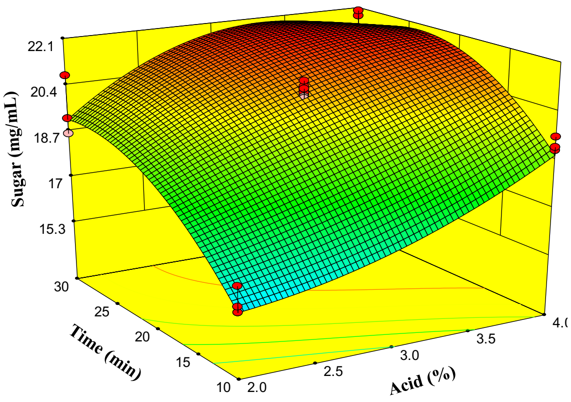

Fig. 3. Response surface of the sugar release.

Finally, Akanni *et al*. (2016), reported similar results obtained by Kuloyo *et al*., (2014). According to observed, it can be concluded that the value obtained in the present investigation is in a range with other works, and that applying some changes such as the variety of OFI, could increase the amount of TRS obtained using an optimization using the SRM.

Padrón-Pereira et al, (2009) reported 16.7% of carbohydrates released by enzymes in *Opuntia boldinghii*. On the other hand, Alencar *et al*. (2108) reached 60% hydrolysis with *Nopalea cochenillifera* but with a load of 30% solids. Souza *et al*. (2016) found a yield in the release of total sugars of 57% with *Opuntia* sp and 61% with *Nopalea* sp when used an acid-alkaline hydrolysis. Astello-García *et al*. (2015) obtained 19.1% of free sugars when OFI cladodes are hydrolysed with acid conditions.

The optimization of the chemical hydrolysis carried out in the present work, substantially improves the yields of sugars released at 75%. When comparing the results of the hydrolysis with other substrates, it is observed that Saucedo *et al*. (2010) performed an optimization of the hydrolysis of *Agave tequilana* Weber bagasse, obtaining 26.9 mg mL^{-1} (10 min, 151 °C, 2% H_2SO_4); a value that is very close to those obtained with a similar pre-treatment on OFI cladodes biomass.

Another of the substrates considered promising for the production of sugars from lignocellulosic residues is the bagasse of sugarcane, in this respect, the data obtained by Gámez *et al*. (2006) were 26.9 g L^{-1} of TRS, with an S:L ratio of 8 and under the following pre-treatment conditions: 300 min, 122 °C, 4% H_3PO_4; Geddes *et al*. (2010) obtaining 50.13 g L^{-1} of xylose from cane bagasse (27 min, 121 °C, 1.09% H_2SO_4), with an S:L ratio of 1:2.8, low ratio involving working with a reduced volume, which results in a higher concentration of TRS.

As previously mentioned, the S:L ratio used influences the amount of TRS obtained, since when using a ratio of 1:10 the value of TRS obtained, without optimization, was 30.29 g L^{-1} with the following pre-treatment conditions 30 min, 140 °C, 2.5% HCl (Chandel *et al*., 2007). Based on these and other results (Kumar *et al*., 2009; Karp *et al*., 2013; Molina *et al*. 2014) some important observations can be made, one of them being that there is a small difference between the sugars obtained with cane and agave bagasse and wheat straw and those obtained with cladodes of OFI.

Another point is that conditions such as S:L ratio, pre-treatment type and subsequent treatments can be varied to increase the concentration of sugars in the hydrolyzed (including enzymatic hydrolysis, hydrolysate concentration, purification, etc.).

Finally, under the optimization conditions obtained in this work, a hydrolysis of 74.65% of the OFI cladodes flour was obtained (Table 5). Of the hydrolysed fraction, the percentage of sugars present was 30.72% with respect to the total sample, and the remaining 43.93% probably corresponds to unhydrolysed biopolymers, lipids, proteins, phenolic compounds, pigments, etc. (Agbor *et al*., 2011). This percentage of hydrolysis is considered to be acceptable for a treatment of lignocellulosic-type substrates.

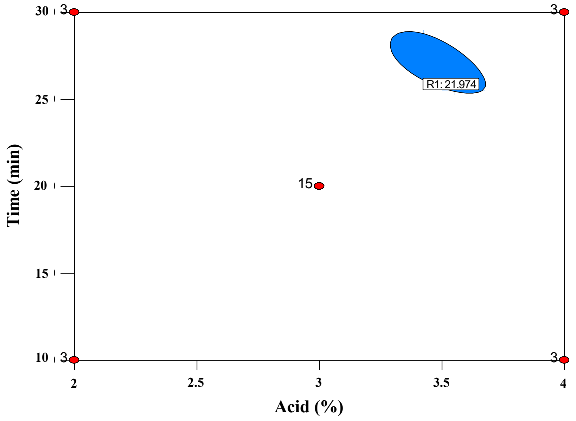

Fig. 4. Optimization of the response surface using the central composite rotatable model, for the release of TRS.

Table 5. Hydrolysis percentage of *O. ficus indica* cladodes.

	Sample weight (mg)	Solid residue weight (mg)	Hydrolysed weight (mg)	Weight of ART (mg)	Weight of other biomolecules (mg)
	2000	507	1493	614.34	878.66
Percentage	100%	25.35%	74.65%	30.72%	43.93%

With regard to the sugars profile identified, when samples were submitted to HPLC, it was determined that 57.14% corresponded to galactose, followed by arabinose with 20.51%, glucose 13.18% and xylose with 9.17%. These sugars are present in the polysaccharide chains that make up the nopal mucilages (Domínguez, 1995) which when hydrolysed are released into the medium.

Conclusions

According to the results obtained, it was observed that the process of optimization of the hydrolysis of the OFI cladodes for the release of monosaccharides was 74.56%. Factors such as *Opuntia* variety, age of the cladodes, harvest season and edaphic factors have a strong impact on the yields of the TRS. The optimization methodology used allowed to improve the release of sugars and can be a useful tool to be applied to similar crops and improve fermentative processes for the production of second-generation fuels.

Acknowledgements

This work was carried out thanks to the support of the SAGARPA-CONACYT fund with project No. 195157.

References

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B. (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances 29*, 675-685. doi: 10.1016/j.biotechadv.2011.05.005.

Akanni, G. B., du Preez, J. C., Steyn, L., Kilian, S. G. (2014). Protein enrichment of an *Opuntia ficus-indica* cladode hydrolysate by cultivation of Candida utilis and Kluyveromyces marxianus. *Journal of Science Food Agriculture 95*, 1094-1102. doi 10.1002/jsfa.6985.

Alencar, B.R.A., Dutra, E.D., Sampaio, E.V.D.S.B., Menezes, R.S.C., Morais Jr, M.A. (2018). Enzymatic hydrolysis of cactus pear varieties with high solids loading for bioethanol production. *Bioresource Technology 250*, 273-280. doi: 10.1016/j.biortech.2017.11.042.

AOAC. (1998). Official Methods of Analysis. 16th Edition. Arlington.

Börjesson, M. and Westman, G. (2015). *Crystalline Nanocellulose Preparation, Modification, and Properties. Cellulose-Fundamental Aspects and Current Trends*. Chapter 7. Ed. INTECH.

Astello-García M.G., Cervantes, I., Nair, V., Santos-Díaz M.S., Reyes-Aguero A., Guéraud F., Negre-Salvayre A., Rossignol M., Cisneros-Zevallos L., Barba de la Rosa A.P. (2015). Chemical composition and phenolic compounds profile of cladodes from *Opuntia* spp. cultivars with different domestication gradient. *Journal of Food Composition and Analysis 43*, 119-113. doi: 10.1016/j.jfca.2015.04.016.

Browning, B. L. (1967). Holocellulose preparations. In: *Methods of Wood Chemistry*, Chapter 2. Interscience Publisher. U.S.A.

Chandel, A.K., Kapoor, R.K., Singh, A., Kuhad, R.C. (2007). Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM 3501. *Bioresource Technology 98*, 1947-1950. doi: 10.1016/j.biortech.2006.07.047.

Chang, K.C., Dhurandhar, N., You, X., Miyamoto, A. (1994). Cultivar/location and processing methods affect yield and quality of sunflower pectin. *Journal of Food Science 59*, 602-605. doi 11.1111/j.1365-2621.1994.tb05572.x.

Chaturvedi, V., Verma, P. (2013). An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *Biotech 3*, 415-431. doi: 10.1007/s13205-013-9167-8.

de Souza Filho, P.F., Ribeiro, V.T., dos Santos, E.S., de Macedo, G.R. (2016). Simultaneous saccharification and fermentation of cactus pear biomass-evaluation of using different pretreatments. *Industrial Crops and Products 89*, 425-433. doi: 10.1016/j.indcrop.2016.05.028.

Fuentes-Ortega, T., Martínez-Vargas, S., Cortés-Camargo, S., Guadarrama-Lezama, A.Y., Gallardo-Rivera, R., Baeza-Jiménez, R. (2017). Optimization of microencapsulation process of sesame oil (*Sesamun indica* L.) using a surface response methodology. *Revista Mexicana de Ingeniería Química 16*, 477-490. http://rmiq.org/ojs/index.php/rmiq/article/view/19.

Gámez, S. González, J. J., Ramírez, J. A., Garrote, G., Vázquez, M. (2006). Study of the sugarcane bagasse hydrolysis by using phosphoric acid. *Journal of Food Engineering 74*, 78-88. doi: 10.1016/j.jfoodeng.2005.02.005.

Geddes, C.C., Peterson, J.J., Roslander, C., Zacchi, G., Mullinnix, M.T., Shanmugam, K.T., Ingram, L.O. (2010). Optimizing the saccharification of sugar cane bagasse using dilute phosphoric acid followed by fungal cellulases. *Bioresource Technology 101*, 1851-1857. doi: 10.1016/j.biortech.2009.09.070.

Gilmour, S.G. (2006). Response surface designs for experiments in bioprocessing. June 2006. *Biometrics 62*, 323-331. doi: 10.1111/j.1541-0420.2005.00444.x.

Ginestra, G., Parker, M.L., Bennett, R.N., Jim Robertson, J., Mandalari, G., Narbad, A., Lo-Curto, R. B., Bisignano, G., Faulds, C. B., Waldron, K. W. (2009). Anatomical, chemical, and biochemical characterization of cladodes from prickly pear [*Opuntia ficus-indica* (L.) Mill.]. *Journal of Agricultural and Food Chemistry 57*, 10323-10330. doi: 10.1021/jf9022096.

Habibi, Y., Heyraud, A., Mahrouz, M., Vignon, M.R. (2004). Structural features of pectin polysaccharides from the skin of *Opuntia ficus-indica* prickly pear fruits. *Carbohydrate Research 339*, 1119-1127. doi: 10.1016/j.carres.2004.02.005.

Hendriks, A.T.W.M., Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology 100*, 10-18. doi: 10.1016/j.biortech.2008.05.027.

Kalil, S. J., Maugeri, F., Rodrigues, M. I. (2000). Response surface analysis and simulation as a tool for bioprocess design and optimization. *Process Biochemistry 35*, 539-550. doi: 10.1016/S0032-9592(99)00101-6.

Karp, S.G., Lorenci, W.A., Soccol1, V.T., Soccol1, C.R. (2013). Pretreatment strategies for delignification of sugarcane bagasse: A review. *Brazilian Archives of Biology and Technology 56*, 679-689. doi: 10.1590/S1516-89132013000400019.

Koocheki, A., Taherian, A.R., Razavi, S.M.A., Bostan, A. (2009). Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of micilage extracted from *Lepidium perfoliatum* seeds. *Food Hydrocolloids 23*, 2369-2379. doi: 10.1016/j.foodhyd.2009.06.014.

Kuloyo, O.O., du Preez, J.C., García, A.M del P., Kilian, S.G., Steyn, L., Görgens, J. (2014). *Opuntia ficus-indica* cladodes as feedstock for ethanol production by *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. *World Journal Microbiology and Biotechnology 30*, 3173-3183. doi: 10.1007/s11274-014-1745-6.

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial Engineering Chemistry Research 48*, 3713-3729. doi: 10.1021/ie801542g.

Little, T.M., Hills, F.J. (1978). *Agricultural Experimentation Design and Analysis*. New York: John Wiley.

Malainine, M.E., Dufresne, A., Dupeyre, D., Mahrouz, M., Vuong, R., Vignon, M.R. (2003). Structure and morphology of cladodes and spines of *Opuntia ficus-indica*. Cellulose extraction and characterisation. *Carbohydrate Polymers 51*, 77-83. doi: 10.1016/S0144-8617(02)00157-1.

McGarvie, D., Parolis, H. (1981). The acid labile, peripherical chains of the mucilage of *Opuntia ficus-indica*. *Carbohydrate Research 94*, 57-65. doi: 10.1016/S0008-6215(00)85595-0.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry 31*, 426-428. doi: 10.1021/ac60147a030.

Molina, C., Sánchez, A., Sarafín-Muñoz, A., Folch-Mallol, J. (2014). Optimization of enzymatic saccharification of wheat straw in a micro-scale system by response surface methodology. *Revista Mexicana de Ingeniería Química 13*, 765-778. http://www.rmiq.org/iqfvp/Pdfs/Vol.%2013,%20No.%203/IA3/IA3.html.

Nobel, P.S. (1995). Recent ecophysiological advances for *Opuntia ficus-indica* and other cacti, in: *Annual Conference of the Professional Association for Cactus Development*. 1, 1995. San Antonio, Professional Association for Cactus Development, Dallas, 1995, 1-11.

Ortíz-Méndez, O.H., Morales-Martínez, T.K., Rios-González, L.J., Rodríguez-de la Garza, J.A., Quintero, J., Aroca, G. (2017). Bioethanol production from agave lechuguilla biomass pretreated by autohydrolysis. *Revista Mexicana de Ingeniería Química 16*, 467-476. http://www.rmiq.org/iqfvp/Pdfs/Vol.%2016,%20No.%202/Alim7/Alim7.html.

Padrón-Pereira, C.A., Moreno-Álvarez, M.J., Medina-Martínez, C.A., García-Pantaleon, D.M. (2009). Obtention of enzymatically hydrolyzed product from cactus (*Opuntia boldinghii* Briton and Rose) cladodes whole flour. *Pakistan Journal of Nutrition 8*, 459-468. doi: 10.3923/pjn.2009.459.468.

Quintanar-Gómez, S., Arana-Cuenca, A., Mercado-Flores, Y., Gracida-Rodríguez, J.N., Téllez-Jurado, A. (2012). Effect of particle size and aeration on the biological delignification or corn straw using *Trametes* sp. *Bioresources 7*, 327-344. http://stargate.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_07_1_0327_QuintanrG_CFRJ_Particle_Size_Aeration_Bio_Delignification/1309.

Retamal, N., Duran, J.M., and Fernández, J. (1987). Ethanol production by fermentation of fruits and cladodes of prickly pear cactus [*Opuntia ficus-indica* (L.) Miller]. *Journal of the Science of Food and Agriculture 40*, 213-218. doi: 10.1002/jsfe.2740400304.

Ribeiro E.M.O., Silva N.H., Lima F.J.L., Brito J.Z., Silva M.PC. (2010). Study of carbohydrates present in the cladodes of *Opuntia ficus-indica* (fodder palm), according to age and season. *Ciência e Tecnología de Alimentos 30*, 933-939. doi: 10.1590/S0101-20612010000400015.

Rodríguez, G.S. (2011). Optimización de la extracción del mucílago de nopal (*Opuntia ficus-indica*). Cartel del XIV Congreso Nacional de Biotecnología y Bioingeniería. Querétaro.

Sáenz, C., Sepúlveda, E., Matsuhiro, B. (2004). *Opuntia* spp mucilage's: A functional component with industrial perspectives. *Journal of Arid Environments 57*, 275-290. doi: 10.1016/S0140-1963(03)00106-X.

Santos, T.N., Dutra, E.M., do Prado, A.G., Leite, F.C.B., de Souza, R.F.R., dos Santos, D.C., de Abreu, C.A.M., Simoes, D.A., de Morais, M.A., Menezes, R.S.C. (2016). Potential for biofuels from the biomass of prickly pear cladodes: Challenges for bioethanol and biogas production in dry areas. *Biomass and Bioenergy 85*, 215-222. doi: 10.1016/j.biombioe.2015.12005.

Stintzing F.C., Carle R. (2005). Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. *Molecular Nutrition and Food Research 49*, 175-194. doi: 10.1002/mnfr.200400071.

Taherzadeh, M. J. and Karimi, K. (2008). Pretreatment of lignocellulosic wastes to

Improve ethanol and biogas production: A review. *International Journal of Molecular Sciences 9*, 1621-1651. doi: 10.3390/ijms90911621.

TAPPI.(1963). *Standard methods*. Solvent extractives of wood and pulp (T204). Tappi, Atlanta.

TAPPI. (1963). *Standard methods*. Acid-insoluble lignin in wood and pulp, T222.Tappi, Atlanta.

Vargas, B.G.J., Pereira Jr, N. (2010). Sugar cane bagasse as feedstock for second generation ethanol production. Part I: Diluted acid pretreatment optimization. *Electronic Journal of Biotechnology 13*. doi: 10.2225/vol13-issue3-fulltext-3.

Wyman, C.E., Decker, S.R., Brady, J.W., Viikari, L., Himmel, M.E. (2005). Hydrolysis of cellulose and hemicellulose. In: *Polysaccharides*. Ed. Dumitriu S. CRC Press. doi: 10.1201/9781420030822.ch.43.

Yang, L., Lu, Mi., Carl, S., Mayer, J. A., Cushman, J. C., Tian, E., Lin, H. (2015). Biomass characterization of Agave and *Opuntia* as potential biofuel feedstocks. *Biomass and Bioenergy 76*, 43-53. doi: 10.1016/j.biombioe.2015.03.004.