academicJournals

Vol. 16(41), pp. 1997-2008, 11 October, 2017
DOI: 10.5897/AJB2017.16214
Article Number: 48C7FC366259
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Xylose recovery from dilute-acid hydrolysis of oil palm (*Elaeis guineensis*) empty fruit bunches for xylitol production

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Received 18 August, 2017; Accepted 28 September, 2017

The aim of this study was to evaluate the effect of different process conditions, such as the sulfuric acid concentration, contact time, solid:liquid ratio and particle size, on the xylose recovery and the formation of by-products from oil palm empty fruit bunch (OPEFB) with dilute-acid hydrolysis. Moreover, an adapted and non-adapted strain of the yeast *Candida guilliermondii* was used to obtain xylitol from the optimal hydrolysate. Xylose, glucose, hydroxymethylfurfural (HMF) and acetic acid in the acid hydrolysate were analyzed with high performance liquid chromatography (HPLC). A Box-Behnken-based design was used to find the combination of factors that maximized the formation of xylose in the hydrolysate optimization process. The fermentation to obtain xylitol from the optimized hydrolysate of OPEFB with adapted and non-adapted *C. guilliermondii* strains was made in 100 ml Erlenmeyer flasks at 30°C for 96 h at 200 rpm in an incubator shaker. The maximum xylose concentration (32.59 g L^{-1}) was obtained at 121°C, for 30 min, with a 1:8 solid:liquid ratio, 2% acid concentration and particle size of around 4 cm. With the same conditions, the inhibitor concentrations, such as HMF, glucose and acetic acid, were 0.023, 1.033 and 11.078 g L^{-1}, respectively. The optimized conditions were the same as previously described. The higher xylitol productivity (10.3 g L^{-1}) and yield (0.43 g g^{-1}) were obtained by fermentation with non-adapted *C. guilliermondii* strains from the optimized hydrolysate of OPEFB without the need for detoxification. It is not necessary to make an adaptation of *C. guilliermondii* in the optimized hydrolysate of OPEFB to produce xylitol.

Key words: Oil palm, empty fruit bunches, xylose, dilute-acid hydrolysis, xylitol.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.*)* is the highest yielding edible oil crop in the world. It is cultivated in 42 countries, with 11 million ha worldwide. Colombia is the largest producer of oil palm in Latin America and the fourth largest producer in the world (Fedepalma, 2014). Oil palm empty fruit bunches (OPEFB) constitute 21% of the total weight of harvested fruits. OPEFB fiber is one of the most promising significant resources of biomass waste

and it is produced from the oil palm extraction of fresh fruit bunches. This biomass is a rigid cellulose structure combined with an amorphous hemicellulose and lignin cross-linked structure and is usually burned in incinerators by oil palm mills, which creates environmental pollution problems in the neighboring areas (Kim and Kim, 2013). The OPEFB biomass contains 42.7% cellulose, 17.1% hemicellulose and 13.2% lignin (Hassan et al., 2013).

Moreover, OPEFB contain 24% xylan, a sugar polymer made up of pentose sugar xylose, which can be used as a substrate for the production of different compounds with chemical and biochemical processes (Rahman et al., 2007). Xylan is the most abundant polysaccharide in hemicelluloses and is more susceptible to hydrolysis by mild acid treatments due to its amorphous structure, as compared to cellulose, which needs severe treatment conditions because of its crystalline nature (Shatalov and Pereira, 2012). Although xylose was the main sugar obtained from the hemicellulose, other by-products, such as glucose, acetic acid and furfural, were also produced in low amounts during the hydrolysis process (Dominguez et al., 1997; Silva et al., 1998; Rahman et al., 2007).

Acetic acid and furfural are considered potential inhibitors to yeast metabolism by causing cell morphological change or ultimate death of the organism. Further, high glucose concentration can repress the xylose reductase activity, which is involved in the conversion of xylose to xylitol (Mardawati et al., 2015). Thus, the concentration of these by-products should be kept at a low level to run the hydrolysis reaction at less severe conditions. The pretreatment of lignocellulose to obtain fermentable sugars is an essential step for lignocellulose conversion with microbial fermentation. A variety of pretreatment methods using mechanical, chemical and biological processing have been developed to change the structural and chemical composition of lignocellulose in order to improve sugar yields (Carvalho et al., 2013; Duangwang and Sangwichien, 2013; Liu and Wang, 2016). Among these methods, an acid hydrolysis pretreatment can be done with concentrated or diluted-acid, but the use of concentrated acids is less desirable because inhibitory compounds are formed. Furthermore, concentrated acid pretreatment methods generate more problems with equipment corrosion and acid recovery (Rocha et al., 2014). It has been recommended to use an acid concentration of about 1% (w/v) for optimal xylose yields (Zhang et al., 2012). Dilute-acid hydrolysis is probably the most commonly applied and frequently investigated method among the chemical pretreatment methods that are used. The main objectives of the acid hydrolysis pretreatment stage are to solubilize the hemicellulosic fraction of the biomass and to increase the accessibility of the cellulose to the enzymes (Zhang et al., 2012; Rocha et al., 2014).

The dilute-acid hydrolysis process usually uses sulphuric acid and hydrochloric acid at concentrations of 1 to 10% at a moderate temperature (in the range of 100 to 150°C) and produces an aqueous fraction containing mainly hemicellulosic sugars (Lenihan et al., 2010). These sugars consist of monosaccharides (xylose, glucose, arabinose) and xylo-oligosacharides (Alfaro et al., 2009; Yañez et al., 2009), which can be further hydrolyzed and fermented to obtain several products like xylitol (Ferrer et al., 2013).

Xylitol, a naturally occurring five-carbon sugar alcohol, has applications in the pharmaceutical, food, and odontological industries owing to its similar high sweetening power, but fewer calories, relative to sucrose. However, current commercial xylitol production processes require high-pressure conditions (up to 50 atm), as well as the toxic nickel catalyst, rendering the chemical process expensive and environmentally unfriendly (Hong et al., 2016). The alternative biotechnological production of xylitol from hemicellulosic hydrolysates and industrial by-products has been proposed, since the process is relatively easy and the cost of production is cheaper than that with chemical methods. The yields of xylitol correspond to only 50 to 60% of xylan present in the raw materials. Among the microorganisms that can assimilate xylose, yeasts belonging to the genus *Candida* sp. are the best xylitol producers (Miura et al., 2015).

In general to produce value-added compounds such as ethanol, xylitol, etc., cells must cope with different conditions in industrial processes such as high concentration of inhibitory products, osmotic problems, high process temperatures, and growth conditions that are not well controlled (Tomás-Pejó et al., 2010). Evolutionary engineering or adaptive strategies are promising alternatives to develop more tolerant yeasts. Directed adaptation of yeast to inhibitory prehydrolysate is one interesting strategy to enhance the process efficiency (Tomás-Pejó et al., 2010). Several studies reported that adapted yeast strains, such as *Pichia kudriavzevii* and *Saccharomyces cerevisiae*, had higher ethanol production from sugarcane juice, wheat straw or sugarcane bagasse hydrolysates compared to the non-adapted strains (Martín et al., 2007; Tomás-Pejó et al., 2010; Dhaliwal et al., 2011). With regards to *C. guilliermondii*, the xylitol production from concentrated sugarcane bagasse and rice straw hemicellulosic hydrolysates was performed using adapted and non-adapted strains. The xylitol yield increased with the first strain, showing that the more adapted strains can

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efficiently reduce xylose to xylitol in hemicellulose hydrolysates (Sene et al., 2001; Silva and Roberto, 2001).

This study aimed to evaluate the effect of the sulfuric acid concentration (2, 4 and 6% (w/v)); contact time (20, 30 and 40 min), solid:liquid ratio (1:8; 1:10 and 1:12) and particle size (0.5, 3 and 4 cm) on the release of xylose and the formation of by-products [glucose, acetic acid and hydroxymethylfurfural (HMF)] from the dilute-acid hydrolysis of OPEFB biomass. A Box-Behnken-based design was used to find the combination of factors that maximized the formation of xylose in the hydrolysate optimization process and to assess the influence of these treatments on the morphology and microstructure of OPEFB. Moreover, bioconversion of the optimal hydrolysate to xylitol by the yeast *C. guilliermondii* was investigated using an adapted and non-adapted strain.

MATERIALS AND METHODS

Raw material

The OPEFB (20 kg) were collected in August, 2015 from Palmar del Oriente S.A.S., a local oil palm mill located at 4.40073 latitude and 75.40819 longitude, in Villanueva, Casanare, Colombia. They were disinfected, oven-dried at 60°C for 24 h and ground using a blade mill. The milled OPEFB was sieved using a Ro-Tap (Model B, W.S. Tyler Inc., Gastonia, NC, USA), resulting in the separation of different particle sizes, between 0.05 and 4 cm. The milled and sieved OPEFB were packed in polyethylene bags and stored at 30°C and 60% relative humidity (RH). The moisture, ash, and cellulose, hemicellulose and lignin contents of the dried OPEFB were determined according to National Renewable Energy Laboratory protocols, NREL/TP 510-42621, NREL/TP 510-42622 and NREL/TP 510-42618, respectively (Sluiter et al., 2008, 2012).

OPEFB dilute-acid hydrolysis

The dilute-acid hydrolysis of the OPEFB biomass was carried out in 125 ml Erlenmeyer flasks at 121°C for 20, 30 and 40 min, with a solid/liquid ratio of 1:8; 1:10 and 1:12, an aqueous H_2SO_4 solution of 2, 4 and 6% (w/v) and particle size of 0.05, 3 and 4 cm. After the hydrolysis process, the Erlenmeyer flasks were quickly immersed in an ice water bath to stop the reaction. The solids were separated from the aqueous solution with filtration. The hydrolysate was analyzed for xylose, glucose, acetic acid and HMF (Roberto et al., 2003).

Xylitol production

The most effective treatment was chosen from the OPEFB dilute-acid hydrolysis. Then, two treatments with *C. guilliermondii* (ATCC 6260, Microbiologics®, St. Cloud, MN) inoculation were performed: adapted and non-adapted strains to the hydrolysate. The adapted strains were obtained by mean of successive inoculations in mediums with optimized hydrolysate component, as follows: a loop full of yeast was transferred to sterilized 100-ml Erlenmeyer flasks containing 20 ml of yeast extract, peptone and glucose (YPG) medium as the primary inoculation, which was incubated at 30°C for 48 h at 108 rpm in an incubator shaker. An aliquot of 2 ml was then inoculated into 100 ml Erlenmeyer flasks containing 18 ml of medium, YPG and the optimized hydrolysate of OPEFB, at the same incubation conditions. Sequential transferring of the inoculum (2 ml) was done as described previously for increasing concentrations of the optimized hydrolysate of OPEFB (20, 50, 70, and 100%), so that strains could adapt to the physiological conditions of the medium. Cell culture growth at potato/dextrose/agar (PDA) was monitored at each step during successive inoculation of strains until a significant increase in cell concentration was observed (Aguilera and Benitez, 1989; Oberoi et al., 2010).

The batch fermentation was performed in 100 ml Erlenmeyer flasks with 47 ml of the optimized hydrolysate of OPEFB at 30°C for 96 h at 200 rpm in an incubator shaker. The system pH of the medium was maintained at 5.5. The inoculum concentration (3 g L^{-1}) with yeast viability of 98% determined by Neubauer chamber (Saeed et al., 2010) and xylose concentration (40 g L^{-1}) were also kept constant in each fermentation batch. These flasks were inoculated separately with adapted and non-adapted *C. guilliermondii* strains, under sterile conditions. The parameters evaluated were xylitol concentration and product yield coefficient (Yp/s), which is the xylitol yield based on the xylose consumption (g of xylitol per g of xylose) (Manjarres-Pinzón et al., 2016). At least four repetitions for this process were carried out.

Analytical methods

Sugars and inhibitor concentrations

The xylose, glucose and HMF in the acid hydrolysate, and the xylitol in the fermentation process were analyzed with HPLC (Shimadzu Prominence 20A, Kyoto, Japan), using an Aminex HPX-87H (Biorad) column and RI detector. Aqueous H_2SO_4 (0.005 M) was used as the mobile phase with a flow rate of 0.6 ml/min. The oven temperature was maintained at 65°C. The injection volume was 20 µl, with an isocratic method (Piñeros-Castro et al., 2011). Moreover, the acetic acid was determined using the same HPLC and column, but with a UV detector and at room temperature. The detection wavelength of this acid was set at 203 nm. D(+)-xylose (142080.1208, Pancreac, Química SAU, Spain), D(+)-glucose anhydrous (141341.1211, Pancreac, USA), 5-hydroxymethyl-2-furaldehyde (CAS 67.47.0, Sigma-Aldrich, USA), acetic acid glacial (CAS 64.19.7, Scharlab S.L., Spain) and xylitol (CAS 87.99.0, Sigma-Aldrich, USA) were used as the internal standards (IS). The quantification was done by measuring the ratio of the peak area of the sample to that of the IS. Primary standard stock solutions of xylose, glucose, acetic acid, HMF and xylitol were prepared in aqueous H_2SO_4 at a concentration of 0.005 M.

Morphological changes of OPEFB

Scanning electron microscopy (SEM)

The morphology and changes in the physical structure of the untreated and treated OPEFB, with dilute-acid hydrolysis in sulfuric acid at concentrations of 2, 4 and 6% for 30 min at 121°C, were examined with a scanning electron microscope (SEM) (Jeol JSM 5910LV XL,Jeol Ltd., Tokyo, Japan), operating at 15 kV and 29 Pa. The samples were dried at 60°C for 24 h and coated with 20 nm of gold and palladium (Hassan et al., 2013).

Experimental design

The response surface methodology (RSM) was used to optimize the hydrolysis process. The basic theoretical aspects, the fundamental assumptions and the experiment implications of RSM have been discussed elsewhere (Myers et al., 2009). A Box-

Behnken-based design was used with 30 runs and six replicates of the central point. The independent variables were sulfuric acid concentration [2, 4 and 6% (w/v)], contact time (20, 30 and 40 min), solid:liquid ratio (1:8; 1:10 and 1:12), and particle size (0.05, 3, and 4 cm). The xylose, glucose, acetic acid and HMF contents were taken as dependent variables in the production of fermentable sugars and inhibitors for the acid hydrolysis of OPEFB. For each of the dependent variables, a complete second order model of the equation was fitted (Equation 1).

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_0 \left(X_i\right) + \sum_{i=1}^{4} \beta_{4+i} \left(X_i^2\right) + \sum_{i=i}^{4} \sum_{i'>i}^{4} \beta_{6+i+i'} \left(X_i X_{i'}\right), \qquad i = 1,2,3,4 \tag{1}$$

Where, Y represents the expected value of the response variable (xylose, glucose, acetic acid and HMF contents), β_i represent the estimated coefficients of the model, and X_1, X_2, X_3 and X_4 are the independent variables (acid concentration, contact time, solid:liquid ratio and particle size). The regression analysis was carried out using R software (Lenth, 2009). Fischer-test was used for determination of type of model equation, while the Student t-test was performed for determination of statistical significance of regression coefficients. For the xylitol concentration and Yp/s, a one-way analysis of variance (ANOVA) was used at a 5% significance level, considering the factor as type of strain: adapted and non-adapted. The least significant difference (LSD) was used to compare the treatments when significant differences were found. All analyses were performed using R software.

RESULTS AND DISCUSSION

Composition of empty fruit bunches

The cellulose and hemicellulose contents of the OPEFB were 45.36 and 28.83%, respectively, which makes this OPEFB a suitable raw material for the production of fermentable sugars. Other compounds, such as total lignin (21.87%), ash (1.90%), extractives in water (3.80%), extractives in ethanol (10.01%) and moisture (6.46%), were similar to those reported in the literature (Hamzah et al., 2011; Ferrer et al., 2013; Palamae et al., 2014). However, the differences seen in the OPEFB compositions as compared with other studies could be due to the maturity degree of the fresh fruit bunches, the geographic regions, and the soil conditions (Hazir et al., 2012).

OPEFB dilute-acid hydrolysis

Sugars, as xylose and glucose, were released during the dilute-acid hydrolysis, according to the experiment operating conditions, which are shown in Table 1. The maximum xylose concentration (32.59 g L^{-1}) was released when the reaction was carried out at 121°C for 30 min with an acid concentration maintained at 2%, solid:liquid ratio of 1:8 and 4 cm particle size. Under the same conditions, the inhibitor concentrations, such as HMF, glucose and acetic acid, were 0.023, 1.033 and 11.078 g L^{-1}, respectively. Hydrolysis procedures at high temperatures under acid conditions lead to the formation and release of acetic acid. The maximum acetic acid concentration (11.078 g L^{-1}) was seen with these conditions. On the other hand, the highest release of glucose (1.80 g L^{-1}) and the maximum concentration of HMF (0.038 g L^{-1}) were obtained at 121°C for 30 min, with the acid concentration maintained at 4%, a solid:liquid ratio of 1:8 and a 0.05 cm particle size.

OPEFB fibers have been used to produce xylose using dilute-acid hydrolysis, with the following optimum conditions: 1:10 solid:liquid ratio and sulfuric acid concentration between 0.25 to 0.5% (w/v). As the solid:liquid ratio increased, the xylose recovery was maximum at temperatures between 140 to 160°C (Zhang et al., 2012). Rahman et al. (2007) reported that the maximum xylose concentration (30.81 g L^{-1}) was seen when the reaction was carried out at 115°C for 60 min with an acid concentration of 4% and particle size <1 mm. This xylose concentration was similar to the data of this study, but with a different contact time (30 min) and particle size (3 cm). More importantly, similar xylose yields were obtained with a particle size of 3 cm, which is much higher than that used in the literature (less than 1 mm). Furthermore, Zhang et al. (2012) reported that, when the particle size was further reduced to 1 mm, the xylose yield dropped and the highest xylose production was obtained with a particle size of 2 mm. In this study, a high concentration of xylose was produced in the hydrolysate of the OPEFB, with large particle sizes and a low concentration of glucose. A large particle size and dilute-acid hydrolysis were probably not able to reduce the crystallinity of the cellulose, and the glucose concentration in the medium was less than that with smaller sizes of fiber (Goh et al., 2016).

Furthermore, the HMF, as an inhibitory compound exclusively produced with acetic acid by dehydrating hexoses, would hinder the microorganism growth in fermentation. Some studies have shown that lignocellulosic hydrolysates containing greater than 1 g L^{-1} of furfural or HMF had a significant inhibitory effect on the xylose fermentation using *Candida tropicalis* (Huang et al., 2011). Under this criterion, the formation of HMF during the dilute-acid hydrolysis of OPEFB could be ignored because it had relatively low concentrations, according to the maximum data of this study (0.038 g L^{-1}). Chiesa and Gnansounou (2014) reported an acetic acid concentration (1.53 g L^{-1}) when the hydrolysis was carried out at 161.5°C, 9.44 min and 1.51% acid sulfuric concentration. However, the severity of this compound was not particularly evident. This acid is probably of minor relevance with respect to the possible inhibition of microorganisms. In fact, concentrations up to 10 g L^{-1} have been found not to affect fermentation process (Mussatto and Roberto, 2004). Taking into account these results, detoxification procedures should be avoided

Table 1. Box-Behnken design (BBD) applied for the OPEFB dilute-acid hydrolysis and **t**he corresponding experiment responses used for the RSM modeling.

RUN	Coded variable*				Responses**			
	X1	X2	X3	X4	Y1	Y2	Y3	Y4
1	0	0	1	1	32.59	1.03	11.08	0.023
2	0	1	-1	0.5	19.83	0.55	6.18	0.011
3	-1	0	0	1	27.81	0.67	9.79	0.014
4	-1	0	1	0.5	30.16	0.97	9.68	0.020
5	1	0	0	1	23.78	0.97	9.80	0.026
6	1	1	0	0.5	24.88	1.04	10.36	0.023
7	0	0	0	0.5	26.43	1.03	8.68	0.031
8	0	0	0	0.5	27.38	1.03	8.27	0.031
9	1	-1	0	0.5	24.45	0.95	6.73	0.013
10	0	1	1	0.5	27.98	0.75	9.23	0.013
11	1	0	0	-1	23.32	1.67	8.23	0.035
12	-1	0	-1	0.5	22.65	0.53	7.68	0.024
13	-1	-1	0	0.5	24.17	0.53	6.89	0.004
14	1	0	1	0.5	29.09	1.47	10.48	0.033
15	1	0	-1	0.5	26.26	1.36	9.94	0.028
16	0	-1	0	-1	19.81	0.79	6.10	0.013
17	0	-1	-1	0.5	20.03	0.55	5.92	0.008
18	0	0	0	0.5	27.95	1.03	7.92	0.019
19	0	0	0	0.5	24.81	1.28	8.17	0.025
20	-1	1	0	0.5	25.75	0.51	8.47	0.003
21	0	0	1	-1	31.60	1.80	9.09	0.038
22	0	0	-1	1	22.76	0.69	7.86	0.021
23	0	0	-1	-1	22.67	0.91	7.01	0.015
24	0	-1	0	1	21.81	0.43	7.10	0.006
25	0	-1	1	0.5	29.40	0.79	8.60	0.015
26	-1	0	0	-1	22.03	1.19	7.16	0.022
27	0	0	0	0.5	25.27	0.90	8.10	0.015
28	0	1	0	1	23.25	0.53	6.81	0.010
29	0	0	0	0.5	25.86	0.93	8.78	0.023
30	0	1	0	-1	20.84	0.98	6.65	0.020

*X1, Acid concentration; X2, Contact time; X3, Solid:liquid ratio; X4, Particle size; **Y1, Xylose (g L^{-1}); Y2, Glucose (g L^{-1}); Y3, Acetic acid (g L^{-1}); Y4, Hydroxymethylfurfural (g L^{-1}).

under the conditions of this study. The estimation of the xylose yield over the independent variables X1, X2, X3 and X4 in terms of the response surfaces is shown in Figure 1. The optimum condition for the release of xylose from the OPEFB was set with the second-order polynomial model (Table 2), which was fitted to the experiment data and the regression coefficients were calculated with multiple regression analysis ($p<0.05$).

The model showed the relationship of the different parameters on the xylose recovery of the OPEFB where, the particle size of the samples (4 cm) was higher than that reported in similar studies with this material (Duangwang and Sangwichien, 2013; Hassan et al., 2013). The maximum xylose concentration of 32.59 g L^{-1} can be obtained by conducting a hydrolysis experiment with a 30 min reaction time, 2% acid concentration, solid:liquid ratio of 1:8, and 4 cm particle size, at a 121°C reaction temperature (Figure 1).

The regression analysis showed that the xylose, acetic acid, glucose and HMF production were significantly affected by the linear effect of solid:liquid ratio, negatively for the first two compounds, and positively for the latter two compounds, respectively, and by the quadratic negative effect of contact time (Table 2). Moreover, the glucose, acetic acid and HMF were linearly affected by variations in acid concentration and particle size. The glucose and HMF production were negatively affected by the interaction between solid:liquid ratio and particle size. In the case of correlation coefficients of the polynomial equation, the R^2 values were higher than 85%, which

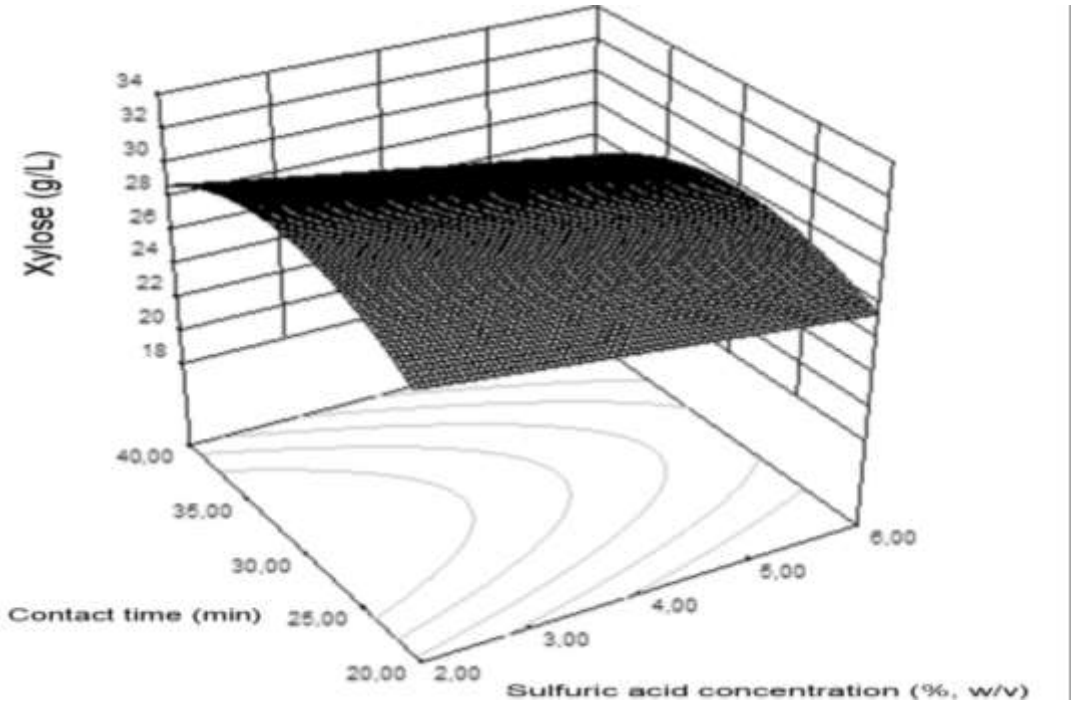

Figure 1. Production of xylose as a function of the acid concentration (%) and contact time when the solid:liquid ratio was maintained at 1:8 and the particle size was 4 cm.

Table 2. Regression equation coefficients and correlation coefficient (R^2) of the response surface models for xylose, glucose, acetic acid and HMF production from the OPEFB dilute-acid hydrolysis.

Variable and interaction*	Estimated regression coefficients			
	Xylose	Glucose	Acetic acid	HMF
β_0	-8.385	-5.224	4.886	-0.134
X_1	3.857	0.170†	-1.099†	-0.006†
X_2	1.886	0.227	0.640	0.008
X_3	-301.881†	26.198†	-165.953†	0.203†
X_4	3.235	0.491†	-0.039†	0.018†
X_1X_1	0.007	0.015	0.197†	0.000
X_1X_2	-0.014	0.001	0.002	0.000
X_1X_3	-28.052	-1.922	-8.789	0.050
X_1X_4	-0.222	-0.003	-0.033	-0.000
X_2X_2	-0.027†	-0.003†	-0.012†	-0.000†
X_2X_3	-1.45919	-0.045	0.452	0.006
X_2X_4	-0.003	-0.001	0.000	-0.000
X_3X_3	3138.79	14.156	1109.69	1.488
X_3X_4	-3.580	-4.156†	3.975	-0.144†
X_4X_4	-0.337	-0.028	0.020	-0.000
R-square (R^2) (%)	85.5	93.8	84.9	88.7

*X_1, Acid concentration; X_2, Contact time; X_3 , Solid:liquid ratio; X_4 , Particle size; β_0 is the intercept of quadratic model. †Significant at P<0.05.

indicated good agreement between experimental data and the model. Thus, the response could be sufficiently explained by the model. Figure 2 shows the 3D response surface plots constructed on the basis of Table 2. With regards to the OPEFB hydrolysis process, glucose, acetic acid and HMF were produced, but the low concentration

of these compounds would not affect the possible production of metabolites by fermentation with microorganisms. The 3D response surface plots represent a quadratic model, explaining the behavior of inhibitors, such as glucose, acetic acid and HMF, with determination coefficients (R^2) near to 0.93, 0.85 and 0.88, respectively.

The xylose production, taking into account variables such as particle size, solid:liquid ratio, sulphuric acid concentration and contact time during the hydrolysis process, is shown in Figure 1. The effects of the solid:liquid ratio and contact time were the more important factors for the released xylose. Furthermore, the maximum xylose concentration was reached with contact times close to 30 min and 2% sulphuric acid concentrations according to the model, where R^2 was 0.85, which explained 85% of the variability in the xylose response. These values are similar to other studies, where the acid hydrolysis of OPEFB fiber for the production of xylose was evaluated using RSM and a R^2 of about 0.83 (Rahman et al., 2007).

By applying the desirability function method, 29 solutions were obtained for the optimum criteria, with a desirability value in the range of 0.8 to 0.9 (Results not shown). The criteria for this optimization were the maximum xylose concentration, the minimum glucose concentration, and HMF and acetic acid in the test range. All of the conditions of the desirability functions were close to each other, for example, the sulphuric acid concentration varied between 2 to 2.2%, the contact time was in the range of 30 to 37.5 min, the solid:liquid ratio was 1:8 and the particle size was around 4 cm. Under these circumstances, the solution that had the highest xylose concentration values was selected. Therefore, the optimum process conditions with a desirability value of 0.9 were 1:8 solid:liquid ratio, 2% acid concentration and 4 cm particle size, at 121°C for 30 min. At this point, the xylose, glucose, HMF and acetic acid concentrations were calculated as 32.597, 0.769, 0.012 and 11.065 g L^{-1}, respectively.

Morphological changes of OPEFB

The micrographs of the OPEFB surfaces examined under SEM are shown in Figure 3. The G and H images showed the presence of silica bodies, which are formed by soil minerals moving into the sedimentary cavities between and within cell walls during plant growth. These structures have been found in great numbers in OPEFB (Hassan et al., 2013). Circular craters are completely filled with a solid, transparent silica body, which is silicon dioxide (SiO_2) (Ilvessalo-Pfaffli, 1995). A component, such as lignin, is a physical barrier for any lignocelluloses biomass. The presence of silica bodies on the OPEFB fiber surface is an additional resistance and increases the protective layer of the fiber, which obstructs the penetration of chemicals into the hemicellulose matrix and cellulose (Yunus et al., 2010). The dilute-acid hydrolysis could penetrate the lignin without other pretreatments and was able to remove the silica bodies. The presence of empty sedimentary cavities revealed perforations, which is shown in images E and F, when the fibers were treated with 4% sulfuric acid. However, the same effect was achieved with acid concentrations of 2 and 6%, where the silica bodies were removed, a breakdown of the OPEFB fibers was observed and a lot of the fiber surfaces were damaged. The acid hydrolysis with 2% sulfuric acid and a particle size of 4 cm were selected as the best optimization condition for xylose production from OPEFB (images A, B, C and D). This treatment had fibers with a homogenous surface and no silica bodies.

Xylitol production

The bioconversion of xylose to xylitol was carried out with adapted and non-adapted *C. guilliermondii* strains. The non-adapted strains could convert xylose to 10.3 ± 0.6 g L^{-1} xylitol with a yield of 0.43 g g^{-1}; whereas the adapted strains produced 6.8 ± 0.4 g L^{-1} xylitol with a yield of 0.31 g g^{-1}. The xylitol production and the yield were significantly ($p < 0.05$) higher for non-adapted strains, indicating that it is not necessary to make an adaptation of *C. guilliermondii* in the optimized hydrolysate of OPEFB. In addition, this study focused on maximizing the recovery of xylose contained in the hemicellulosic fraction of the OPEFB with the lower amount of inhibitors to increase the bioconversion to xylitol using *C. guilliermondii* without the need for detoxification.

Previous studies conducted with *C. guilliermondii* on hydrolysates of agroindustrial residues had different xylitol productions and yields. Arruda et al. (2011) performed a fermentation in Erlenmeyer flasks (125 ml) containing 50 ml of the medium (pH 5.5), on a rotary shaker at 200 rpm, 30°C for 120 h and used *C. guilliermondii* yeast in sugarcane bagasse hemicellulosic hydrolysate with 70 g L^{-1} xylose. They reported that the xylitol production and Yp/s were 40 g L^{-1} and 0.6 g g^{-1}, respectively. In addition, the xylitol production from sorghum forage hydrolysate using *C. guilliermondii* were carried out in 125 ml Erlenmeyer flasks containing 50 ml medium, under 200 rpm, at 30°C for 96 h, and the highest xylitol production and Yp/s were 15.45 g L^{-1} and 0.35 g g^{-1}, respectively (Camargo et al., 2015). The xylitol production was also performed with the same yeast in 250 ml Erlenmeyer flasks, containing 100 ml of brewer's spent grain hydrolysate; which were agitated in a rotatory shaker at 200 rpm, at 30°C for 96 h. The optimized conditions resulted in 0.78 g g^{-1} of Yp/s (Mussatto and Roberto, 2008). Therefore, the fermentation for xylitol production using different types of hydrolysate as carbon source can be affected by several factors such as, the

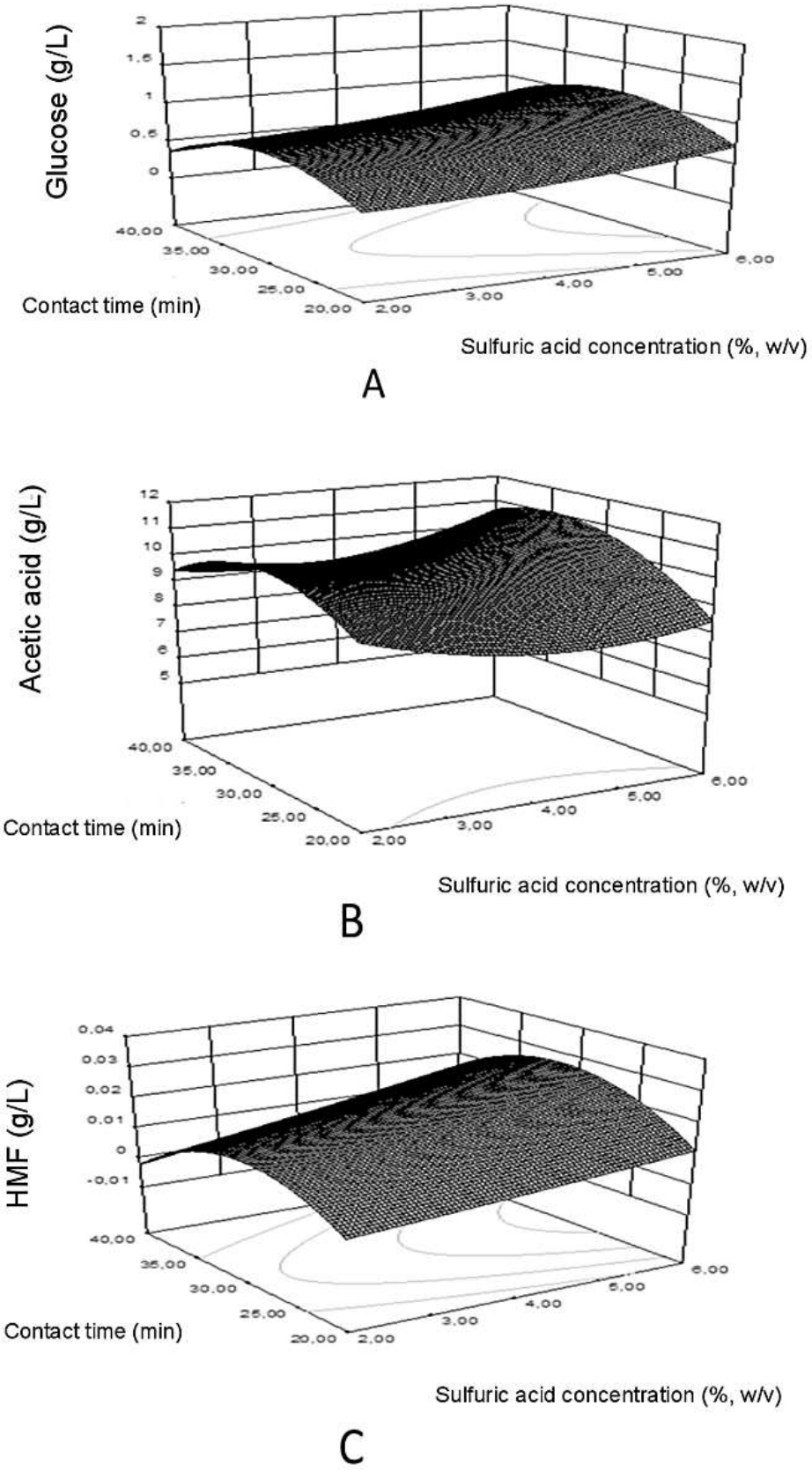

Figure 2. Production of glucose (A), acetic acid (B) and HMF (C) as a function of the acid concentration (%) and contact time (min) when the solid:liquid ratio was maintained at 1:8 and the particle size was 4 cm.

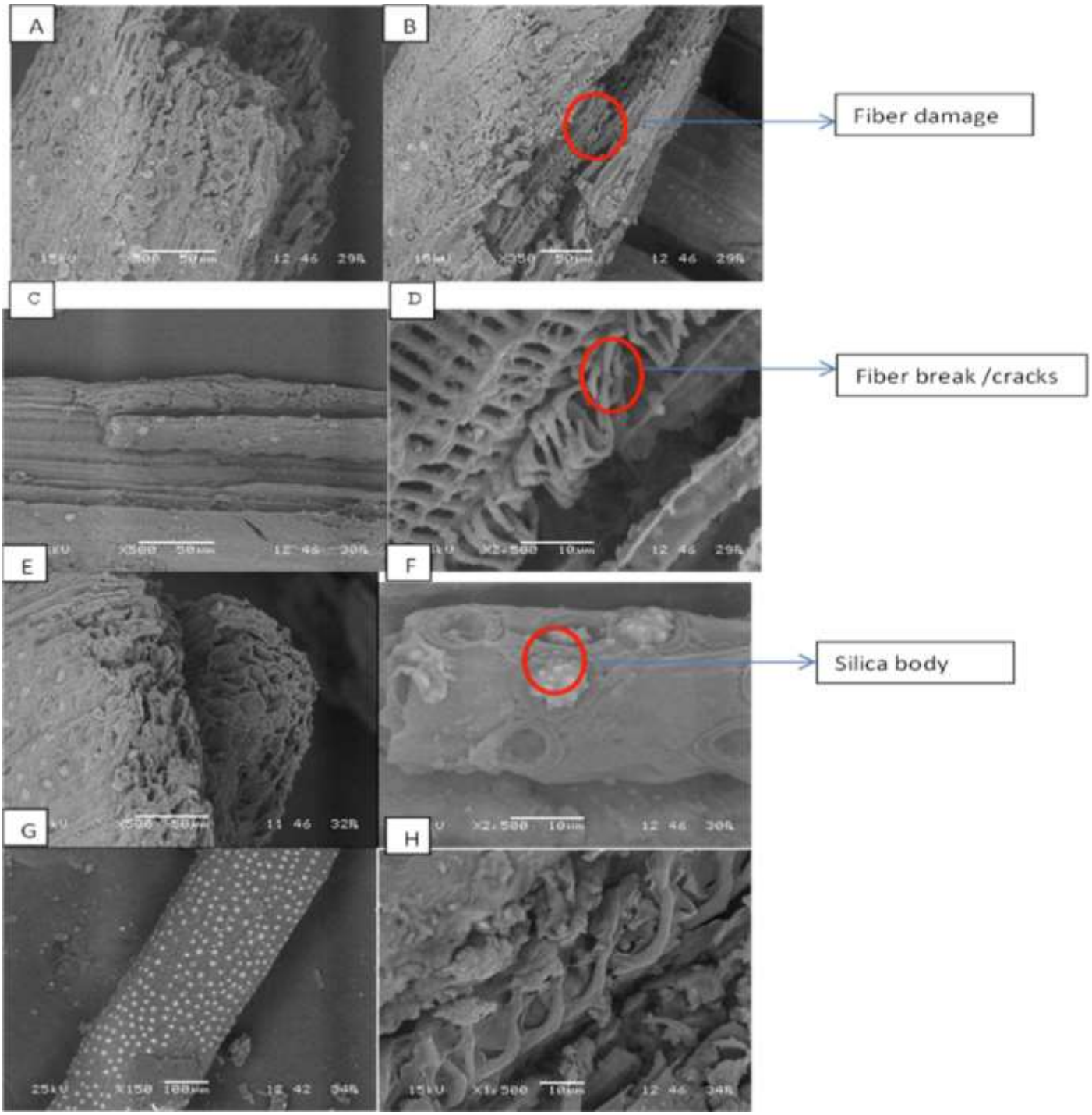

Figure 3. SEM images of the OPEFB pretreated with 2% sulfuric acid + 3 cm particle size (A and B), OPEFB pretreated with 6% sulfuric acid + 3 cm particle size (C and D), OPEFB pretreated with 4% sulfuric acid + 3 cm particle size (E and F) and the untreated OPEFB (G and H).

initial cell concentration, pH, temperature, type and concentration of nutrients in the culture medium, initial xylose concentration, presence of carbon sources other than xylose, the presence of inhibitor compounds in the hemicellulosic hydrolysates and the dissolved oxygen level (Silva et al., 1998; El-Baz et al., 2011; Salgado et al., 2012; Morales-Rodriguez et al., 2016).

The operational aspects of xylitol production and the cell metabolism are affected by the dissolved oxygen concentration, which is one of the most important

parameters to be considered in the production of xylitol (Salgado et al., 2012). An increase in xylitol productivity occurs when the oxygen supply is limited or the maximum oxygen supply is lower than the cell demand (Aguiar et al., 2002). Under anaerobic conditions, yeasts are unable to metabolize D-xylose. At a low oxygen level, the electron transport system is unable to oxidize intracellular nicotinamide adenine dinucleotide (NADH) completely, increasing the NADH concentrations and permitting xylitol excretion. At a high oxygen level, the oxidation favors xylitol oxidation to xylulose (Mohamad et al., 2015).

Walther et al. (2001) simulated different aeration conditions according to the levels of medium volume in 250 ml Erlenmeyer flasks. The medium volume at 26, 40 and 54% with respect to the Erlenmeyer flask volume was established for aerobic, semi-aerobic and microaerobic aeration, respectively. The medium volume used in the fermentation was 47%, which could be considered aeration with microaerobic tendency. Optimal values of oxygen concentration for xylitol production usually correspond to microaerobic conditions (Aguiar et al., 2002; Salgado et al., 2012). However, the strains grow vigorously at the beginning of fermentation at high initial xylose concentrations and high aeration. This leads to high cell densities and low oxygen levels in the later stages of the fermentation and results in high xylitol production rates. At lower initial xylose concentrations, cell densities are low and the level of dissolved oxygen remains high, therefore, less xylitol accumulates (Walther et al., 2001). Furthermore, extremely high initial xylose concentrations are detrimental to xylitol yields due to osmotic stress, which could be induced in the microorganism by the excess amount of sugar in the medium. Thus, with a careful manipulation of the both, the aeration and initial xylose concentration could result in high xylitol yields. In both the productivity and the yield of xylitol, aeration appeared to be a very important factor (Nolleau et al., 1993). If the fermentation medium contained glucose, then higher yields and productivities are obtained under aerobic conditions, while in the absence of glucose or with low initial glucose concentrations, microaerobic conditions improve yields. This behavior can be attributed to increased oxygen demand by the high cell densities achieved in the presence of glucose (Walther et al., 2001). The xylitol productivity from the optimized hydrolysate of OPEFB could be improved by controlling the dissolved oxygen in the flask. But, several factors should be taken into account, such as the initial concentration of xylose, glucose and strains.

Conclusion

The hydrolysis of the OPEFB fibers catalyzed by dilute sulfuric acid was optimized using a Box-Behnken-based design. The combination of operating at 121°C, for 30 min, with a 1:8 solid:liquid ratio, 2% acid concentration and 4 cm particle size was effective in terms of maximum xylose (32.597 g L^{-1}) and minimum glucose (0.769 g L^{-1}) concentrations, according to the prediction model. At these optimized conditions, the HMF and acid acetic concentrations were 0.012 and 11.065 g L^{-1}, respectively. However, when hydrolysis takes place at higher temperatures and higher acid concentrations, the presence of inhibitors can affect the rate of reaction during the fermentation process. Non-adapted *C. guilliermondii* strains were able to produce xylitol (10.3 ± 0.6 g L^{-1}) with a yield of 0.43 g g^{-1} from the optimized hydrolysate of OPEFB without detoxification. The xylitol productivity could be improved by controlling the dissolved oxygen in the flask and taking care of the initial concentration of xylose, glucose and strains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Colciencias is acknowledged for providing a grant to Katherine Manjarres-Pinzón.

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