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MICROPLATE SCREENING OF ADSORBENTS TO REMOVE KEY CONTAMINANTS FROM HYDROLYSATES AND OBTAIN XYLOOLIGOSACCHARIDES AS BIOACTIVE FOOD CHEMICAL.

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ABSTRACT

Xylooligosaccharides (XOs) have been associated with several health benefits. X2 to X6 can be obtained from hydrolysates of biomass. However, it is necessary to purify the hydrolysates to obtain a product to include in food supplies. The present investigation aimed to evaluate three different adsorbents into 96-well plate assays. The percentage adsorbed of furfural and hydroxymethylfurfural (HMF) were analyzed to verify the detoxication capabilities of the adsorbents. Results to detoxication of the pretreated liquor and enzymatic hydrolysate were included, indicating that Activated Carbon was the only solid that removed both components to an undetectable level. Despite of that, Silica-C18 removed around 30% of these contaminants while kept most of the XOs into the water. Therefore, results indicated that Silica-C18 could also be used in detoxication and improve XOs recovery into the water solution.

Keywords: Xylooligosaccharides. Adsorption. Screening. furfural. hydroxymethylfurfural.

1 INTRODUCTION

Xylooligosaccharides (XOs) have been associated with several health benefits, such as prebiotic effects.[1] Other benefits already reported include anti-inflammatory, antioxidant, antitumor, and antimicrobial effects[2]. XOs can be produced by the hydrolysis of biomass material. There are reports of XOs obtained from many sources, furthermore, it is an alternative to compose the products of biorefineries.[3] The interest usually lies in relatively low Polymerization Degree (PD) for food application, mainly PD 2-6.[4] Also, glucose and xylose change the calorific value and sweetness power of XOS mixtures and are undesirable. Consequently, a crucial step towards the application into food industry is the separation steps. Moreover, the presence of contaminants brings negative effects into the bioactive functionality.[4,5] They are produced during the pretreatment, and include furfural and hydroxymethylfurfural (HMF). Many purification processes were proposed to detoxication of hydrolysates. [3] The present investigation aimed to evaluate three different adsorbents to purify hydrolysates from sugarcane bagasse. Pretreated liquor and the liquor after enzymatic hydrolysis were used into the adsorption assays. A XOs solution prepared with a commercial product (*Corncob*) was applied in order to compare results. The adsorption took place into a 96-well plate, followed by filtration and HPLC analysis.

2 MATERIAL & METHODS

Three resins were tested: Silica-C18 (Kopp technologies), Silica-C8 (Kopp technologies) and Activated Carbon (Sinth). The adsorption process took place into a 96-well plate, and each well had a total volume around 2 mL. 50mg of resin was added into the desired well in a manner that six repetitions could be obtained for each resin and solution. A multichannel pipette (Thermo Scientific) was used to add 600μL of the desired solution into the wells. Therefore, each assay happened into a different well, and consisted of maintaining contact between 600μL of a solution and 50mg of the adsorbent. The solution could be pretreated liquor, enzymatic hydrolysate and XOs *Corncob* (*Corncob xylan* da Carl Roth (> 95%, Karlsruhe, Alemanha). The plate was kept under mixing for one hour. After mixing, the contend of all wells was removed with the multichannel pipette and added into a 96-well plate with filters in each well. The solution was filtered into a vacuum system (Supelco, PlatePrep 96-well Vacuum Manifold) in a manner that the contend of each well was received by a different well of a new 96-well plate. The contend of each well was stored in closed flasks and froze to -75ºC until HPLC analysis.

The concentration of each XOs molecule into a well was determined by High Performance Liquid Chromatography (HPLC). A Sugar-Pak column (10μm, 6.5 x 300mm) was applied. The mobile phase was ultrapure water with 50 mg/L of EDTA and the temperature was kept at 80ºC. Concentrations of Furfural and HMF were determined also by HPLC, but with a Rezex column (, using water with 5mmolL^{-1} of H_2SO_4.

Data from HPLC was used to calculate the Adsorption Recovery (AD) in the solid for the molecules in each assay. In other words, AD(%) represents the percentage of the molecule that was adsorbed in the well:

$$AD_i(\%) = \frac{C_{i0} - C_{iL}}{C_{i0}} \quad (1)$$

C_{i0} is the concentration (g/L) added into the resin, in other words, the measured concentration into the solution before the adsorption process. C_{iL} is the concentration (g/L) measured in the sample after adsorption and filtration. The total area of the peaks before the retention time of X_4 were used as a rough estimation of AD(%) for molecules with PD ≥ 5. However, the species were not quantified individually because there were no standards for PD ≥ 7. Additionally, the peaks began to merge for high PDs, and individual determination got imprecise. As six samples were obtained in each solution-resin combination, the set of each combination was divided into two. Hence, three samples were used to measure XOs molecules concentrations. The other three were used to measure Furfural and HMF concentrations. As a result, there was a triplicate to define average and sample standard error for the AD(%) calculations.

Table 1 include information about each solution applied (initial solution). The concentration of X_2 was increased by 329% with the enzymatic hydrolysis, X_3 by 124%, while the concentration of X_4 decreased. The chromatogram also indicated that the concentration of fractions with a PD ≥ 5 decreased. This was evaluated by comparing the chromatograms for enzymatic hydrolysate and pretreated liquor. Table 1 presents the concentration of each fraction measured by HPLC.

Table 1 Concentration of each XOs molecule measured directly by HPLC into the water solution before the adsorption assays. C is concentration (g/L), and s the standart error of the sample (g/L).

	X_2		X_3		X_4	
	C (g/L)	s (g/L)	C (g/L)	s (g/L)	C (g/L)	s (g/L)
Pretreated liquor	0.842	0.005	1.184	0.001	1.355	0.002
After enzymatic hydrolysis	3.610	0.053	2.652	0.049	0.871	0.023
XOs (*Corncob*)	2.228	0.047	2.597	0.064	1.417	0.068

3 RESULTS & DISCUSSION

Table 2 summarizes the results for the adsorption of XOs molecules from pretreated liquor, enzymatic hydrolysate and XOs from Corncob in each adsorbent tested. It is not possible to strictly compare the values measured for hydrolysates and XOs from *Corncob*, since the initial concentration was different and may not lead to the solid saturation. In other words, the results may not represent the approximation of a favorable isotherm in the higher concentration zone. However, the results on Table 2 should suffice to analyze the overall tendency, which is the purpose of microplate assays.

Table 2 Percentage of XOs adsorbed for eah tested material in differente degrees of polymerization. AR is the Adsorption Recuperation, s is its standart error (in the sample). -C18: silica-C18; -C8: silica-C8; AC: Activated Carbon.

	X2		X3		X4		> X5	
After enzymatic hydrolysis								
	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)
-C18	2.8%	1.4%	0.1%	4.6%	13.5%	4.8%	12.7%	1.9%
-C8	0.6%	2.6%	2.8%	1.7%	0.0%	3.1%	2.1%	2.8%
AC	11.8%	2.6%	5.1%	3.4%	15.9%	3.4%	7.8%	2.8%
Pretreated liquor								
	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)
-C18	2.2%	0.6%	5.9%	1.2%	6.2%	4.3%	16.0%	1.2%
-C8	0.9%	1.0%	2.2%	1.8%	9.5%	5.5%	16.7%	4.3%
AC	6.7%	0.1%	12.0%	0.2%	36.8%	0.9%	28.4%	2.9%
XOs (*Corncob*)								
	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)	AR (%)	s (%)
-C18	0.1%	1.1%	1.1%	1.3%	4.1%	1.7%	4.1%	0.8%
-C8	7.9%	8.4%	6.8%	7.4%	2.5%	3.6%	5.1%	4.6%
AC	19.7%	4.2%	25.2%	6.5%	27.8%	9.1%	11.5%	2.6%

Activated Carbon (AC) was the adsorbent that removed most of the measured contaminants from the pretreated liquor and the enzymatic hydrolysate (Table 3). In this case, 100% means that the chromatogram did not indicate the compound in any measurable amount. Silica-C18 adsorbed more HMF and Furfural than Silica-C8.

Silica-C18 and Silica-C8 does not adsorb a high percentage of XOs, in fact, some percentages indicate that the amount adsorbed would be statistically indistinguishable from 0%, if the experimental uncertainty is taken into consideration. However, Activated Carbon (AC) adsorbed higher percentages of XOs, especially for XOs molecules with lower PD. AC could adsorb much higher percentages of measured molecules in XOs from *Corncob* medium, since hydrolysates present a much more complex system, including contaminants such as Furfural and HMF.

Table 3 Percentage of HMF and furfural adsorbed for eah tested material. AR is the Adsorption Recuperation, s is its standart error (in the sample). -C18: silica-C18; -C8: silica-C8; AC: Activated Carbon.

	Pretreated liquor					After enzymatic hydrolysis				
	HMF		Furfural			HMF		Furfural		
	AR (%)	s (%)	AR (%)	s (%)		AR (%)	s (%)	AR (%)	s (%)	
-C18	22.8%	8.1%	32.4%	2.8%	C18	27.5%	5.6%	31.4%	4.3%	
-C8	9.9%	9.2%	18.0%	1.8%	C8	6.8%	2.2%	18.7%	2.1%	
AC	100.0%	-	100.0%	-	AC	100.0%	-	100.0%	-	

Therefore, results indicate that AC is the best adsorbent to remove these contaminants from hydrolysates. Despite of that, Silica-C18 and Silica-C8 adsorb less XOs from the hydrolysates (Table 2). Consequently, increasing the amount of activated silica in the system could improve the detoxication, while increasing the recovery of XOs into the water solution. These results indicate that adsorbent selection may be a factor to balance detoxication and XOs recovery. In this case, the costs of the separation processes could increase, and the application need to be evaluated in economic aspects too. In either way, this tradeoff can represent an important step towards the economic feasibility of the XOs production as bioactive food chemical. The results can also be important in future studies applying adsorption in columns.

Batch assays will be important to validate the observations, since results from microplates have uncertainties or even bias due to the limited mixing during assays. In this case, hydrophobized adsorbents may be more affected than other solids, which may be a source for the differences observed between AC and silica-C18 or – C8. Nonetheless, future batch validation results could exclude silica-C8 from the tests because this adsorbent performed similarly to silica-C18 to adsorb XOs molecules, but much worse to adsorb Furfural and HMF.

4 CONCLUSION

Activated Carbon removed approximately 100% of Furfural and HMF from hydrolysates. However, this adsorbent also adsorbed high percentages of XOs molecules. Silica-C18 adsorbed around 30% of these contaminants. However, in general, Silica-C18 kept higher XOs concentrations into the solution (especially for lower PD), observation that was corroborated by the XOS *Corncob* assays. If these results are verified by batch assays, it could indicate that Silica-C18 could be used in order to detoxicate XOs from hydrolysates and increase the recovery of the target molecules into the water solution.

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