INFLUENCE OF CLARIFICATION STAGE ON THE FRACTIONATION OF XYLOOLIGOSACCHARIDES BY NANOFILTRATION

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ABSTRACT

Xylooligosaccharides (XOS) are oligosaccharides derived from xylan, a component of hemicellulose, with potential health benefits, including prebiotic effects and antioxidant properties. Membrane separation processes, particularly ultrafiltration and nanofiltration, are effective for fractionating XOS with different degrees of polymerization. In this study, a clarification step using the UP010 ultrafiltration membrane was introduced before the nanofiltration process to remove larger compounds that could interfere with nanofiltration performance. The clarification step reduced fouling and improved nanofiltration efficiency but provided an increase of approximately 10 hours in total processing time. However, implementing this step using the UP010 ultrafiltration membrane did not significantly change the composition of the XOS. Thus, despite the effectiveness of the clarification step in improving nanofiltration performance, the significant increase in processing time suggests the need to explore alternatives to optimize the process without compromising process efficiency and benefiting XOS fractionation.

Keywords: Nanofiltration Membranes. Utrafiltration Membranes. Dietary Fiber. Oligosaccharides.

1 INTRODUCTION

Xylooligosaccharides (XOS) are oligosaccharides composed of xylose monomers linked by β-(1,4) glycosidic bonds, derived from the hydrolysis of xylan, a polysaccharide present in hemicellulose. XOS are prebiotic dietary fibers with potential applications in preventing diabetes and colon cancer, as well as possessing antioxidant and antimicrobial activities, which can be influenced by the degree of polymerization of the oligosaccharides.[1,2] Therefore, it is believed that fractionation of these molecules can enhance their biological activities, thereby improving the industrial application of the different fractions.

Membrane separation processes have shown promising results for the fractionation of oligosaccharides with various degrees of polymerization. These processes are easily scalable, achieve high recovery rates without the need for solvents, and produce concentrated products.[3,4,5,6] Considering that the product of xylan hydrolysis comprises XOS with varying degrees of polymerization and compounds with higher molar masses, this study aimed to evaluate the implementation of a clarification step using the UP010 ultrafiltration membrane prior to the fractionation of XOS by nanofiltration.

2 MATERIAL & METHODS

For the fractionation of XOS, a beechwood xylan hydrolysate[7] previously produced was utilized. The filtration process was conducted using a conventional laboratory-scale filtration system (Trevisan Tec. LTDA, Campinas, Brazil)[8]. This system comprised a stainless-steel filtration cell equipped with a feed batch, a magnetic stirrer, and a support structure holding a membrane disc with an effective permeation area of 14.52 cm². The system was pressurized with nitrogen gas, while the temperature was controlled by circulating water in the equipment jacket through a thermostatic bath.

Initially, the beech hydrolysates produced through enzymatic hydrolysis were diluted to a concentration of 30 $g.L^{-1}$. To evaluate the clarification stage, the UP010 ultrafiltration membrane with 10 kDa MWCO and the NP030 nanofiltration membrane with 500-600 Da MWCO were used, both produced by Microdyn-Nadir® (Wiesbaden, Germany).

During the clarification stage, the filtration system was fed with 125 mL of previously diluted hydrolysate and completed upon collecting 100 mL of permeate. Subsequently, the 100 mL of permeate obtained from the ultrafiltration stage was used as feed for the nanofiltration stage. Nanofiltration was conducted with a volume reduction factor of 2, where the system was fed with 100 mL of previously clarified hydrolysate and finished after collecting 50 mL of permeate.

In parallel, a test was carried out without applying the clarification step, where the nanofiltration system was fed with 100 mL of non-clarified hydrolyzate and completed after collecting 50 mL of permeate. All tests were carried out at 30 °C, with the ultrafiltration process carried out using 1 MPa and the nanofiltration processes at 3 MPa. To evaluate the membranes, the permeate flux, retention coefficient and membrane selectivity were estimated.

The permeate flux (J_p) was calculated according to Equation 1, where V_p represents the accumulated volume of permeate, A_m the membrane permeation area and t the time for the accumulation of V_p.[9]

$$J_p = \frac{V_p}{A_m . t} \quad (1)$$

The retention coefficient (R_i) of each solute *i* was calculated according to Equation 2, with $m_{p,i}$ being the mass of species *i* in the permeate and $m_{f,i}$ being the mass of species *i* in the feed. While the selectivity of the membranes ($S_{i,j}$) was estimated by the ratio between the observed retention coefficients of two species (*i* and *j*) present in the solution, according to Equation 3.[10,11,12]

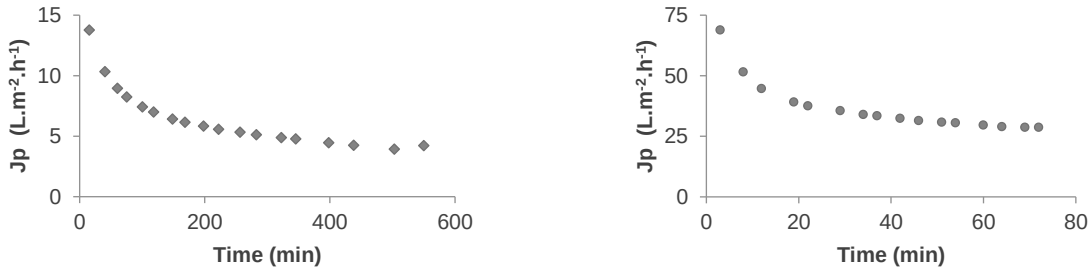
$$R_i = 1 - \frac{m_{P,i}}{m_{f,i}} \quad (2)$$

$$S_{i,j} = \frac{R_{obs,i}}{R_{obs,j}} \quad (3)$$

For the quantification of XOS and xylose, the fractions obtained were filtered through a 0.22 µm polyvinylidene fluoride membrane (Milipore, Burlington, United States) and subsequently analyzed on a high-performance liquid chromatograph (Prominence®, Shimadzu, Japan). The equipment was equipped with a refractive index detector (RID-10A), column oven (CTO-20A), and auto injector (SIL-20AHT). Samples of 20 µL were automatically injected and eluted at 0.4 mL.min^{-1} with ultrapure water (Milli-Q®, Permution). Quantification was performed using an Aminex HPX-42A column (Bio-Rad Laboratories, United States) at 50 ºC and a running time of 40 min.[13] Control of chromatographic conditions and data acquisition was carried out using LC Solution® software. Xylose and XOS concentrations were determined using a previously constructed standard curve. The xylobiose, xylotriose, xylotetraose and xylontose standards were purchased from Megazyme (Bray, Ireland), and the xylose standard from Sigma-Aldrich (San Luis, USA).

3 RESULTS & DISCUSSION

The clarification stage was implemented to remove compounds with higher molar masses, such as non-hydrolyzed xylan and residues from the xylan extraction process from lignocellulosic material. These compounds can cause fouling in nanofiltration membranes, interfering with their selectivity and limiting process performance. Figure 1 shows the flux (Jp) curves for the nanofiltration process of both non-clarified and previously clarified hydrolysate, while Table 2 presents the average permeate fluxes and the durations of the nanofiltration processes. The implementation of the clarification step reduced scaling and the formation of the polarized layer on the surface of the nanofiltration membrane, resulting in improved performance of the nanofiltration process. Additionally, the clarification step reduced the time required to collect 50 mL of permeate, demonstrating a significant improvement in the overall efficiency of the process.



(a) (b)

Figure 1 Permeate flux curves of (a) non-clarified hydrolyzate and (b) previously clarified hydrolyzate, both using the NP030 membrane at 30 ºC and 3 Mpa.

Table 1 Permeate flow of XOS hydrolyzate with and without clarification using the NP030 membrane at 30º C and 3 MPa.

Clarification	J_p (L.m^{-2}.h^{-1})	Time (h)*
Yes	34.04±6,61^a	1.06±0.20^b
No	4.27±0,04^b	8.98±0.26^a

Mean ± standard deviation (n=2). Different lowercase letters between the lines indicate that there were significant differences between the means at the 90% confidence level using the t test (p<0.10). *Time required to collect 50 mL of permeate fraction (FRV = 2).

However, it is important to consider the total process time for the fractionation of the clarified hydrolysate, including the time required for the clarification stage, which lasted approximately 18 hours to collect 100 mL of permeate. Consequently, when considering the total fractionation time for the clarified hydrolysate (clarification plus nanofiltration), an increase of approximately 10 hours was observed compared to the fractionation of the non-clarified hydrolysate. This underscores the need to balance efficiency and process time in the optimization of this procedure.

As shown in Table 2, no significant differences (p<0.10) were observed between the processes when the clarification step was implemented using the UP010 membrane. Therefore, from the perspective of XOS fractionation, under the conditions studied, the clarification of the XOS hydrolysate did not have a significant impact.

Table 2 Influence of clarification on retention coefficients and selectivities using the NP030 membrane at 3 MPa and 30°C.

Clarification	Retention Coefficient			
	R_{X1}	R_{XOSb}	R_{XOSa}	$R_{XOStotal}$
Yes	0.49±0.02[a]	0.68±0.04[a]	0.69±0.13[a]	0.69±0.05[a]
No	0.48±0.03[a]	0.58±0.11[a]	0.62±0.09[a]	0.59±0.11[a]
	Selectivity			
	$S_{XOS/X1}$	$S_{XOSb/X1}$	$S_{XOSa/X1}$	$S_{XOSa/XOSb}$
Yes	1.41±0.06[a]	1.42±0.21[a]	1.42±0.21[a]	1.01±0.14[a]
No	1.22±0.16[a]	1.21±0.17[a]	1.29±0.10[a]	1.05±0.07[a]

Mean±standard deviation (n=2). Captions: X_1=xylose; X_2=xylobiose; X_3=xylotriose; X_4=xylotetraose; X_5=xylopentose;XOS_b= sum of xylobiose and xylotriose; XOS_a= sum of xylotetraose and xylontose; XOS_{total}= sum of xylobiose, xylotriose, xylotetraose and xylontose. Different lowercase letters between the lines indicate that there were significant differences between the means at the 90% confidence level using the t-test ($p<0.10$).

4 CONCLUSION

Although the clarification step contributed to optimizing the performance of the nanofiltration process, it was not effective in terms of XOS fractionation. Furthermore, it is important to note that the implementation of the clarification stage significantly increased the total process time by approximately 10 hours. Therefore, these results underscore the importance of a judicious approach when selecting process steps, reinforcing the need for strategic adjustments to achieve the desired efficiency in XOS fractionation without compromising the total process time.

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