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SIMULTANEOUS PRODUCTION OF CELLO- AND XYLO-OLIGOSACCHARIDES FROM BIOMASS PRETREATED WITH IONIC LIQUID MIXTURE

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ABSTRACT

Lignocellulosic biomass is used for obtaining various bioproducts, but pretreatments need to be applied to reduce the recalcitrance of the matrix and facilitate enzymatic access. Among the pretreatments, ionic liquids are capable of deconstructing lignocellulosic biomass and facilitating enzymatic hydrolysis, resulting in the production of biomolecules. Oligosaccharides are found in biomass, and specific enzymes are used to release these bioproducts. Thus, the aim of our study was to explore the simultaneous release of COS and XOS from pretreated sugarcane straw with ionic liquids. The total release of COS (77.66 mg $g_{biomass}^{-1}$) was achieved after 3 hours of processing at 40 °C, using 50 FPU $g_{biomass}^{-1}$ of Novozym® and 30 U $g_{biomass}^{-1}$ of Shearzyme®. Therefore, oligosaccharide release was achieved in a shorter enzymatic hydrolysis time, reducing costs and processing time.

Keywords: Enzymatic hydrolysis. Experimental design. Kinetics. Oligosaccharides. Sugarcane Straw

1 INTRODUCTION

Lignocellulosic residues are widely available and low-cost, making them attractive sources for reuse and the production of various products. Among these sources, sugarcane residues such as bagasse and straw are utilized for energy generation through burning, biofuel production, or the creation of bioproducts. However, the lignocellulosic matrix is resistant to enzymatic attack due to its complex cellulose-hemicellulose-lignin structure and requires pretreatment to convert it into biomolecules[1].

Pretreatments are applied to residual lignocellulosic biomass and involve breaking down the rigid biomass matrix to facilitate enzymatic hydrolysis and obtain bioproducts, such as oligosaccharides. Among the pretreatments applied, ionic liquids stand out, which are salts and remain liquid at temperatures below 100°C, formed exclusively by cations and anions. Their properties include low volatility, high thermal and chemical stability, and structural adjustability. Additionally, they are seen as an environmentally friendly approach, as they can be recycled and reused[1,2].

Oligosaccharides are naturally found in lignocellulosic biomass and consist of a small number of sugar molecules linked together, typically composed of 2 to 10 monosaccharides joined by glycosidic bonds. These carbohydrates have attracted interest in the food industry for incorporating these biocomponents into food products for the development of functional foods. Among these compounds, cellulose-derived oligosaccharides, known as cellooligosaccharides (COS), and those derived from xylan, known as xylooligosaccharides (XOS), are particularly prominent[3].

Enzymes are utilized in biomass after pretreatment to obtain products. Enzymes, biological sources, are widely used for obtaining specific products due to their high specificity and selectivity in breaking bonds and minimal production of undesirable byproducts compared to chemical processes or autohydrolysis, as they typically exhibit a high degree of polymerization. Moreover, enzymes are non-toxic, which contributes to their use and future application of the biocomponent in product development[3,4]. Thus, we explored the synergistic action of two enzymatic cocktails for the simultaneous release of COS and XOS over time, aiming for time and process cost savings.

2 MATERIAL & METHODS

The biomass used in the enzymatic assays was sugarcane straw biomass resulting from pretreatment using a mixture of protic ionic liquids (91% (w w^{-1}) of 2-Hydroxyethylammonium acetate ([Mea][Ac]) and 9% (w w^{-1}) of 2-hydroxyethylammonium hexanoate ([Mea][Hex])), with a water content of 20% (w/w) and a temperature of 89 °C for 3 hours of processing. The biomass after the pretreatment step was washed and dried. The enzymes used in the enzymatic assays were the Novozym® 50013 cellulase enzyme cocktail (250 FPU mL^{-1}) and the Shearzyme® 500 L xylanase enzyme (135 U mL^{-1}).

A Central Composite Rotatable Design (CCRD 2^3)[5] with 4 replications at the central condition, totaling 18 experiments, was employed as a strategy to assess the simultaneous release of COS and XOS based on the variables temperature (°C) and enzyme mixture. Cellulase loads ranged from 0 to 250 FPU g_{biom}^{-1} and xylanase from 0 to 135 U g_{biom}^{-1}, including axial points (±1.68) (Table 1). The effect of each variable was determined using the Protimiza Experimental Design online software (http://experimental-design.protimiza.com.br/)[5].

The assays were conducted in 2 mL plastic tubes in sodium citrate buffer pH 5.0, with 5% (w v^{-1}) of pretreated biomass and agitation at 1500 rpm in a thermoblock for 12, 24, and 48 h. Following the experimental design, enzymatic assays for validation were performed at 40 °C, with 50 FPU g_{biom}^{-1} of cellulase and 30 U g_{biom}^{-1} of xylanase, performed in triplicate. Subsequently, the tubes were centrifuged at 9000 × *g*, and the supernatant was collected and filtered for subsequent chromatographic analysis. COS concentration was determined by HPAEC-PAD using a Dionex DX-500 system. The concentrations of COS and XOS (2 ao 6) were determined by calibration curves of standard compounds purchased from Megazyme®.

3 RESULTS & DISCUSSION

The mixture of enzymatic cocktails has the potential to promote the simultaneous release of COS and XOS. Enzyme load is a key variable for oligosaccharide release, and the low loads of cellulase and xylanase applied in the process resulted in the highest release of COS (88.85 mg $g_{biomass}^{-1}$), along with low temperature (41 °C) over 12 h of processing (Run 1 - Table 1). We found that the release of XOS was consistent over time (~19 mg $g_{biomass}^{-1}$), but the presence of xylanase enzyme was crucial for enzymatic synergy to increase COS release. We observed that time is crucial for oligosaccharide release, as evidenced in the kinetics of the experimental design and process validation; the shorter the enzymatic hydrolysis time, the greater the release of COS, avoiding conversion into monomers.

From the analysis of the effects on COS and XOS responses over time, no variable was statistically significant, resulting in no model and response surface in relation to the studied variables. However, the low variation in the results of the center points indicates good repeatability of the process. Thus, the experimental design was used as a strategy to reduce the number of assays, enabling simultaneous analysis and improvement of variables, and process optimization[5]. In this study, we reduced the temperature by 10 °C, reduced cellulase by 80%, and xylanase by 78%, resulting in the release of 77.66 mg $g_{biomass}^{-1}$ of COS and 19.27 mg $g_{biomass}^{-1}$ of XOS in a 3 h process (Figure 1). According to the results of this study, the simultaneous release of COS and XOS occurred, leading to cost and time reduction in the process of obtaining oligosaccharides.

Table1. Experimental design conditions, coded and real values, and total release of COS and XOS at different times of enzymatic hydrolysis using sugarcane straw pretreated with an ionic liquid mixture.

Run	T (°C)	Novozym	Shearzyme	COS release (mg $g_{biomass}^{-1}$)			XOS release (mg $g_{biomass}^{-1}$)		
				12 h	24 h	48 h	12 h	24 h	48 h
1	-1 (41)	-1 (50.6)	-1 (27.3)	88.85	76.06	56.67	19.80	18.36	17.29
2	1 (59)	-1 (50.6)	-1 (27.3)	80.70	66.59	62.23	15.12	14.55	15.33
3	-1 (41)	1 (199.4)	-1 (27.3)	37.22	20.27	10.89	19.88	19.36	16.67
4	1 (59)	1 (199.4)	-1 (27.3)	54.09	58.17	53.24	13.02	13.20	14.23
5	-1 (41)	-1 (50.6)	1 (107.7)	85.42	74.86	48.15	18.43	19.81	19.35
6	1 (59)	-1 (50.6)	1 (107.7)	87.13	65.78	61.87	16.19	13.11	13.95
7	-1 (41)	1 (199.4)	1 (107.7)	33.47	20.57	11.84	18.32	19.98	15.80
8	1 (59)	1 (199.4)	1 (107.7)	57.74	51.32	53.67	12.75	10.51	12.77
9	-1.68 (35)	1 (125)	0 (67.5)	21.62	13.90	5.06	21.40	18.53	12.53
10	1.68 (65)	1 (125)	0 (67.5)	53.95	52.68	68.49	13.25	10.51	13.89
11	0 (50)	-1.68 (0)	0 (67.5)	44.63	45.57	55.79	9.04	9.59	10.50
12	0 (50)	1.68 (250)	0 (67.5)	66.72	46.72	36.46	19.70	12.29	12.63
13	0 (50)	0 (125)	-1.68 (0)	61.50	47.10	52.04	5.49	5.17	5.56
14	0 (50)	0 (125)	1.68 (135)	73.57	50.16	78.49	17.36	14.82	9.09
CP	0 (50)	0 (125)	0 (67.5)	70.92	61.26	55.97	17.00	19.02	14.71
CP	0 (50)	0 (125)	0 (67.5)	70.86	56.41	53.77	17.95	13.02	14.08
CP	0 (50)	0 (125)	0 (67.5)	74.20	55.33	49.41	18.23	15.05	12.55
CP	0 (50)	0 (125)	0 (67.5)	71.79	58.69	44.04	18.34	13.33	11.98
CP_m	0 (50)	0 (125)	0 (67.5)	71.94±1.56	57.92±2.63	50.80±5.27	17.88±0.61	15.11±2.76	13.33±1.28

CP, center point; CPm, mean os center point runs.

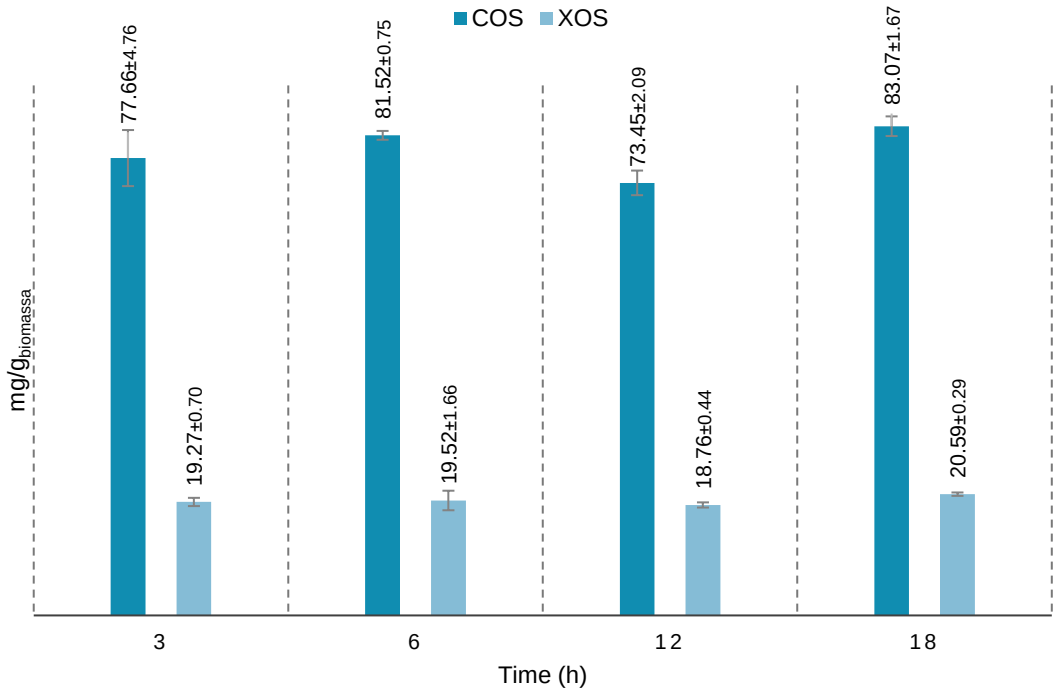

Figure 1 Total cellooligosaccharides (COS) and xillooligosaccharides (XOS) released from sugarcane straw using mixture of enzyme as assessed over time using 50 FPU g_{biom}^{-1} of cellulase and 30 U g_{biom}^{-1} of xylanase at 40 °C.

4 CONCLUSION

The use of enzymatic cocktails resulted in the simultaneous release of COS and XOS from sugarcane straw pre-treated with the mixture of ionic liquids. Through experimental design analyzing different enzyme loads and temperatures, it was possible to evaluate the optimal condition for oligosaccharide release, contributing to reducing enzyme load, temperature, and processing time observed from enzymatic kinetics. COS and XOS have been widely employed in the pharmaceutical, nutraceutical, and food industries for the development of various products with prebiotic capacity. Thus, the study enabled the extraction of oligosaccharides from residual biomass of the sugarcane industry.

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