Universidade Estadual Paulista
"Júlio de Mesquita Filho"

Faculdade de Ciências Farmacêuticas

Avaliação de pré-tratamento hidrotérmico de palha e bagaço de cana-de-açúcar para produção de xilo-oligossacarídeos e hidrolisado enzimático de glicose

Lídia Manfrin Dias

Tese apresentada ao Programa de Pós-graduação em Alimentos e Nutrição para obtenção do título de Doutor em Alimentos e Nutrição.

Área de Concentração: Ciência dos Alimentos.
Orientador: Prof. Dr. Fernando Masarin
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TÍTULO DA TESE: Avaliação de pré-tratamento hidrotérmico da palha e bagaço de cana-de-açúcar visando à produção de xilo-oligossacarídeos e etanol de segunda geração

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Dedico este trabalho ao meu esposo Thales e
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RESUMO

A busca por fontes de energias renováveis e ambientalmente corretas vem aumentando nos últimos anos culminando no conceito de biorrefinaria, o qual refere-se ao aproveitamento integral da biomassa lignocelulósica através da produção de múltiplos bioprodutos de interesse comercial. Uma forma de aproveitamento dos materiais lignocelulósicos durante o processo de produção de etanol de segunda geração (etanol celulósico) é a produção de xilo-oligossacarídeos (XOS) durante o pré-tratamento da biomassa. **Objetivo:** O objetivo deste trabalho foi otimizar a produção de XOS através de pré-tratamento hidrotérmico (PTH) em bagaço (BCA) e palha (PCA) de cana-de-açúcar e hidrolisar enzimaticamente a fração celulósica obtida após o PTH para produção de etanol (etanol 2G). **Metodologia:** O BCA e a PCA foram pré-tratadas hidrotermicamente sob diferentes condições de temperatura, tempo e consistência (carga mássica). Essas variáveis foram incorporadas ao um delineamento composto central rotacional em resposta a produção de XOS. As frações sólidas (resíduo rico em celulose e lignina) e líquidas (hidrolisado hemicelulósico) obtidas após os diferentes PTHs foram caracterizadas quimicamente. As frações sólidas obtidas na condição otimizada (BCA e PCA) foram hidrolisadas com preparado enzimático Cellic CTec 2 com diferentes cargas mássicas e enzimáticas. **Resultados:** As condições de PTH para máxima produção de XOS foram de 161,9 °C por 75,3 min e 10% de consistência para o BCA e 177,2 °C por 64,8 min e 10% de consistência para a PCA. Os PTHs do BCA e da PCA nessas condições resultaram na produção de 53,3 e 96,3 mg de XOS por grama de subproduto (base seca), respectivamente. As frações sólidas do BCA e da PCA obtidas após os PTHs nas condições previamente otimizadas apresentaram conversões máximas de celulose em glicose de 70 e 94%, respectivamente. O aumento da consistência não resultou em diminuição expressiva das conversões de celulose em glicose. As concentrações de glicose nos hidrolisados oriundos do BCA e da PCA com 20% de consistência foram de 64 e 112 $g.L^{-1}$, respectivamente. Desta forma, a condição de 20% de consistência foi selecionada para verificar o efeito do aumento da carga enzimática, a qual não resultou em aumento significativo das conversões de celulose em glicose, ambos BCA e PCA, com excessão a carga enzimática de 30 FPU por grama de substrato (base seca). **Conclusão:** Os resultados indicam que os PTHs nas condições otimizadas (BCA e PCA) foram excelentes para produção de XOS. Além disso, as frações sólidas ricas em celulose obtidas após os PTHs em condição previamente otimizada apresentaram potencial na hidrólise enzimática com preparado enzimático Cellic CTec 2, portanto, podendo ser uma estratégica para a produção de etanol de segunda geração (etanol celulósico).

PALAVRAS-CHAVE: Subprodutos de cana-de-açúcar; Pré-tratamento hidrotérmico; Delineamento Composto Central Rotacional; Metodologia de Superfície de Resposta; Xilooligossacarídeos; Hidrólise enzimática.

ABSTRACT

The search for renewable and environmentally correct energy sources has increased in recent years, culminating in the concept of biorefinery, which refers to the full use of lignocellulosic biomass through the production of multiple bioproducts of commercial interest. One way of using lignocellulosic materials during the production process of second-generation ethanol (cellulosic ethanol) is the production of xylo-oligosaccharides (XOS) during the pre-treatment of biomass. **Objective:** The objective of this work was to optimize the production of XOS through hydrothermal pretreatment (PTH) in sugarcane bagasse (BCA) and straw (PCA) and to enzymatically hydrolyze the cellulosic fraction obtained after PTH to produce ethanol (2G ethanol). **Methodology:** BCA and PCA were hydrothermally pretreated under different conditions of temperature, time and consistency (mass load). These variables were incorporated into a central rotational composite design in response to XOS production. The solid (cellulose and lignin-rich residue) and liquid (hemicellulose hydrolyzate) fractions obtained after the different PTHs were chemically characterized. The solid fractions obtained in the optimized condition (BCA and PCA) were hydrolyzed with Cellic CTec 2 enzymatic preparation with different mass and enzymatic loads. **Results:** The PTH conditions for maximum XOS production were 161.9 °C for 75.3 min and 10% consistency for BCA and 177.2 °C for 64.8 min and 10% consistency for PCA. BCA and PCA PTHs under these conditions resulted in the production of 53.3 and 96.3 mg of XOS per gram of by-product (dry basis), respectively. The solid fractions of BCA and PCA obtained after PTHs under the previously optimized conditions showed maximum cellulose conversions to glucose of 70 and 94%, respectively. The increase in consistency did not result in a significant decrease in cellulose to glucose conversions. Glucose concentrations in hydrolysates from BCA and PCA with 20% consistency were 64 and 112 $g.L^{-1}$, respectively. Thus, the condition of 20% consistency was selected to verify the effect of increasing the enzymatic load, which did not result in a significant increase in cellulose to glucose conversions, both BCA and PCA, with the exception of an enzyme load of 30 FPU per gram of substrate (dry weight). **Conclusion:** The results indicate that PTHs under optimized conditions (BCA and PCA) were excellent for XOS production. In addition, the cellulose-rich solid fractions obtained after PTHs in a previously optimized condition showed potential in enzymatic hydrolysis with the Cellic CTec 2 enzyme preparation, therefore, it could be a strategy for the production of second-generation ethanol (cellulosic ethanol).

KEYWORDS: Sugarcane by-products; Hydrothermal pre-treatment; Central Composite Rotational Design; Response Surface Methodology; Xylooligosaccharides; Enzymatic hydrolysis.

LISTA DE ABREVIATURAS

BCA	Bagaço de cana-de-açúcar
PCA	Palha de cana-de-açúcar
PTH	Pré-tratamento Hidrotérmico
XOS	Xilooligossacarídeos
GP	Grau de polimerização
X_2	Xilobiose
X_3	Xilotriose
X_4	Xilotetraose
X_5	Xilopentaose
X_6	Xilohexaose
X_7	Xiloheptaose
DCCR	Delineamento Composto Central Rotacional

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INTRODUÇÃO

CAPÍTULO 1

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1. INTRODUÇÃO

O aumento substancial da população e urbanização têm aumentado cada vez mais a demanda e o consumo de energia em todo o mundo. O uso de combustíveis fósseis tem atendido essa necessidade, porém, nas últimas décadas ficou evidente que este é um recurso limitado e as reservas não suprirão a demanda em um futuro próximo. Além disso, questões relacionadas ao impacto ambiental causado pela queima dos combustíveis fósseis têm gerado preocupações e esforços na busca por novas fontes de origem renovável e ecologicamente corretas (1). Um dos principais objetivos do uso dos biocombustíveis é a substituição de combustíveis fósseis, permitindo a diminuição da dependência por recursos não renováveis e a redução das emissões de gases de efeito estufa (2).

Para se alcançar transformações sociais sustentáveis é necessário adotar tecnologias ambientalmente corretas e formas de reaproveitamento de todos os subprodutos da cadeia produtiva (biorrefinaria). Este conceito integra tecnologias unitárias de conversão de biomassa para produzir biocombustíveis e produtos bioquímicos de valor agregado a partir de subprodutos agroindustriais (3).

O Brasil, um dos maiores produtores agrícolas do mundo, gera quantidades significativas de subprodutos de biomassa em atividades decorrentes da colheita e transformação de produtos. De acordo com a Companhia Nacional de Abastecimento (4), na safra 2021/22, foram produzidos 585,1 milhões de toneladas de cana-de-açúcar. A produção brasileira de etanol, para esta safra, será de cerca de 26,7 bilhões de litros.

Dois principais subprodutos são produzidos no processamento da cana-de-açúcar: palha de cana-de-açúcar (PCA, durante a colheita) e bagaço de cana-de-açúcar (BCA, na etapa de extração de sacarose, no processo industrial). No Brasil, cerca de 40% da oferta interna de energia é baseada em matéria-prima do tipo renovável (bioenergia); sendo 15,7% de energia oriunda de biomassa vegetal, que está diretamente associada com o cultivo de cana-de-açúcar e seus derivados (5). A utilização do BCA tem a vantagem de já se encontrar disponível na indústria, pois é gerado após a etapa de extração do caldo de cana-de-açúcar (6). Quanto ao destino da PCA, o governo do estado de São Paulo criou uma lei estadual que proíbe de forma gradativa à queima da palha de cana-de-açúcar no campo resultando no aumento da mecanização da colheita da cana-de-açúcar por parte das indústrias e consequente acúmulo da PCA no campo (7). Atualmente em média 80% do BCA são queimados nas plantas termoelétricas das Usinas do setor sucroenergético para cogeração de energia o que é fundamental para tornar o processo viável economicamente. Todavia, a PCA não é utilizada para cogeração de energia, pois essa biomassa contem altos teores de matéria inorgânica (principalmente sílica) (8). Desta forma, parte da PCA deve ser removida do campo para sua transformação em bioprodutos e/ou bioenergia, todavia, parte da PCA deve permanecer no campo para a manutenção dos níveis adequados de microrganismos no solo, que são fundamentais para sua fertilidade, além do crescimento das plantas, de forma a sustentar o cultivo de cana-de-açúcar (9).

Uma alternativa de melhor aproveitamento dos subprodutos de cana-de-açúcar (BCA e PCA) é a produção de xilo-oligossacarídeos (XOS, fração hemicelulósica), etanol 2G (etanol de segunda geração ou celulósico, fração celulósica) e lignina. Para este fim, o pré-tratamento hidrotérmico (PTH) é uma alternativa para fracionar o material em duas frações: fração líquida (remoção da fração hemicelulósica por hidrólise química resultando na formação de XOS) e fração sólida (rica em celulose e lignina) (10–13). A fração sólida pode ainda ser fracionada em mais duas frações: fração líquida (extração de lignina por uso de solventes orgânicos convencionais e líquidos iônicos) e fração sólida (rica em celulose) (14–17). Por fim a fração celulósica pode ser hidrolisada enzimaticamente por celulases obtendo-se hidrolisado de glicose que pode ser fermentado a etanol (etanol celulósico). Desta forma, os subprodutos de cana-de-açúcar podem ser utilizados para à produção de bioprodutos de alto valor agregado (XOS e lignina de alta pureza), além de combustível de fonte renovável (etanol celulósico e lignina de baixa pureza).

Os XOS, os quais são constituídos principalmente por unidades de xilose, são responsáveis por diversos efeitos benéficos como a prevenção de cáries, a diminuição de níveis séricos de colesterol e o estímulo do crescimento de bifidobactérias no trato-gastrointestinal. Por serem moderadamente doces, estáveis em uma ampla faixa de pH e temperatura, também conferem características organolépticas aos alimentos. A utilização de XOS já é uma realidade em alguns países. No Japão, por exemplo, essas substâncias já são comumente empregadas em vários produtos alimentícios para humanos e animais (18–24).

Os subprodutos de cana-de-açúcar podem ser mais bem aproveitados como, por exemplo, na produção de XOS (bioproduto de alto valor agregado), pois a hemicelulose e celulose têm baixo aproveitamento de energia em sua oxidação (queima). Desta forma, a proposta é produzir XOS a partir da fração hemicelulósica e etanol celulósico a partir da fração celulósica culminando em um “resíduo rico em lignina” que pode ser de baixa ou alta pureza. A lignina de baixa pureza pode ser aplicado na queima para cogeração de energia (produção de vapor d´água/energia elétrica), pois a lignina tem um alto aproveitamento de energia em sua queima e a lignina de alta pureza tem aplicação nos setores de química e de construção civil a partir da formulação de aglutinantes naturais, adesivos, compósitos, fibra de carbono, dentre outros bioprodutos.

Cana-de-açúcar

A cana-de-açúcar é uma gramínea da classe das monocotiledôneas, pertencente à família *Poaceae* e gênero *Saccharum*, seu crescimento em altura ocorre até a limitação no abastecimento de água, baixas temperaturas, ou floração. Devido à falta de resistência a baixas temperaturas, é uma planta amplamente cultivada em regiões tropicais e subtropicais e foi a primeira cultura introduzida no Brasil, há quatro séculos, no litoral do nordeste (25,26).

A cana-de-açúcar é uma importante fonte de renda e desenvolvimento, sendo a principal matéria-prima para a produção de açúcar e uma das mais importantes fontes para a produção de etanol e bebidas alcoólicas (26). No Brasil, a área total de mudas, plantio e colheita de cana-de-açúcar na safra de

2021/22 atingiu 8.317,3 mil hectares e a produtividade deve atingir 70.357 Kg/ha (4).

Atualmente o Brasil ocupa o primeiro lugar como maior produtor mundial de açúcar e o segundo lugar no *ranking* dos países maiores produtores de etanol, ficando atrás dos Estados Unidos, que produzem etanol a partir do milho, porém é o maior exportador mundial de etanol (27).

Provavelmente o desenvolvimento mais significativo na produção de co-produtos da cana-de-açúcar no Brasil tenha ocorrido com o estabelecimento do Programa Nacional do Álcool (Programa ProÁlcool), criado em novembro de 1975 em resposta aos altos preços do petróleo e ao aumento dos custos de importação dessa matéria-prima. O ProÁlcool aumentou a demanda interna por etanol, resultando na rápida expansão da indústria da cana-de-açúcar, aumentando a escala das fábricas, reduzindo custos de produção de açúcar e etanol e aumentando a capacidade técnica. O ProÁlcool representou e ainda representa impacto no mercado mundial de açúcar e etanol, tornando o Brasil uma importante potência global na produção de cana-de-açúcar além de apresentar essa cultura como uma importante matéria-prima para produção de produtos energéticos e não apenas alimentícios (28).

Espera-se que o Brasil seja o país que contribua com a maior parte do aumento no consumo e produção de etanol segundo as projeções da FAO (27) até 2030. Esse aumento é devido, principalmente, ao programa RenovaBio, assinado em janeiro de 2018 e que entrou em vigor a partir de 2020. RenovaBio é a Política Nacional de Biocombustíveis, instituída pela Lei n°13.576 de 2017 que possui como objetivos a contribuição com a relação de

eficiência energética e redução na emissão de gases causadores do efeito estufa na produção, comercialização e uso de biocombustíveis; contribuir para o cumprimento aos compromissos do Brasil no contexto do Acordo de Paris; promover a expansão na produção e uso dos biocombustíveis na matriz energética nacional, destacando a regularidade do abastecimento de biocombustíveis além da promoção da participação competitiva dos diversos biocombustíveis no mercado nacional.

O principal mecanismo do RenovaBio é a instituição de metas anuais para a redução de emissões na matriz de combustíveis no país. Essas metas foram definidas pela Resolução CNPE n°5, de 5 de julho de 2018 para um período de 10 anos e serão desdobradas em metas individuais aplicadas a todos os distribuidores de biocombustíveis, sendo proporcionais à sua respectiva participação no mercado na comercialização de combustíveis fósseis em relação ao ano anterior.

Dessa forma, fica claro o crescente aumento para os próximos anos na demanda e consequente produção de biocombustíveis no Brasil, sendo o etanol de cana-de-açúcar o mais importante deles.

A biorrefinaria de cana-de-açúcar

Biorrefinarias são instalações de processamento integrado que produzem múltiplos coprodutos a partir da biomassa, por exemplo a cana-de-açúcar, a partir de um processamento sustentável da biomassa a fim de produzir uma variedade de bioprodutos com alto valor agregado, além de biocombustíveis (29).

A cana-de-açúcar é considerada uma das melhores fontes de matéria-prima para uma biorrefinaria, por apresentar vantagens como ser uma planta C_4, ou seja, possui alta eficiência na capacidade de processamento de energia solar em biomassa, sem muita exigência de água e nutrientes; é uma cultura amplamente distribuída no mundo, com uma prática agrícola, controle de pragas e doenças e desenvolvimento de variedades bem compreendida e estabelecida além do fato de tanto o BCA quanto a PCA já estarem presentes em uma instalação de processamento, como as fábricas do setor sucroenergético (28).

Em uma biorrefinaria de cana-de-açúcar, a indústria não apenas processa a cana produzindo açúcar e etanol como também produz energia renovável e produtos biotecnológicos a partir da biomassa lignocelulósica. A biomassa lignocelulósica refere-se à matéria vegetal composta principalmente por celulose, hemicelulose e lignina (28).

O processamento da cana-de-açúcar na indústria sucroenergética gera subprodutos em quantidades significativas, que devem aumentar nos próximos anos em decorrência do aumento da produção agroindustrial para a obtenção de etanol, principalmente. O uso desses subprodutos como matéria-prima para produção de novos produtos torna-se imprescindível tanto do ponto de vista ambiental como uma forma de evitar o desperdício de material rentável (30).

Durante a colheita da cana-de-açúcar, as folhas e ponteiras são deixadas no campo, enquanto os colmos (caules) são transportados para a fábrica, onde são triturados e moídos para a extração do caldo rico em açúcar

para a produção de açúcar. Dessa forma, os principais subprodutos gerados no processamento de cana-de-açúcar são o BCA e a PCA. O BCA é a fração resultante da limpeza, preparação e extração do caldo de cana e é heterogêneo no tamanho e formato de partículas. A PCA compreende as folhas verdes e secas, ponteiras e suas frações, frações de raízes e partículas de terra aderidas a elas (8,31).

O BCA atualmente é queimado/oxidado nas fábricas para cogeração de energia, no entanto, há mais energia no BCA em relação a necessidade que é requerido para o processo da fábrica resultando em acúmulo de BCA. Cada tonelada de cana-de-açúcar colhida e processada pelas usinas gera cerca de 270-280 kg de BCA e 140 kg de PCA (ambas com aproximadamente 50% de umidade) (28,32).

No entanto, estudos sobre o comportamento da celulose, hemicelulose e lignina, principais componentes do BCA e PCA, na pirólise e combustão para a produção de energia através do calor gerado, indicam que biomassas com maior teor de carbono e alto teor de lignina, resultam em uma maior conversão em gramas de material para *kilojoule* (kJ) de energia térmica produzida, além de apresentarem maior rendimento em carvão, que pode estar relacionada à maior conversão termoquímica. Desta forma, biomassas com maior teor de lignina apresentam melhor eficiência na combustão, podendo produzir mais calor por quilograma de material (33,34).

O BCA apresenta teores de celulose, hemicelulose e lignina que variam de 34,1 a 45,5%; 16,8 a 32,2% e 17,1 a 32,4% (base seca), respectivamente. A PCA apresenta teores de celulose, hemicelulose e lignina que variam de

33,6 a 40,8%; 26,2 a 30,8% e 19,6 a 31,8% (base seca), respectivamente (8,24,35). O alto teor de polissacarídeos (celulose e hemicelulose) presentes nessas biomassas em comparação ao baixo conteúdo em lignina, torna-as promissoras para a utilização na produção de produtos de maior valor agregado em uma biorrefinaria.

A celulose e hemicelulose são polissacarídeos constituintes da parede celular da cana-de-açúcar que podem ser hidrolisados e convertidos em produtos, como por exemplo: glicose, que pode ser fermentada a bioetanol; ácido glucônico; ácido aspártico, ácido glicólico, ácido levulínico; XOS e álcoois como glicerol, sorbitol, xilitol. A lignina pode dar origem a resinas, fenol, benzeno, tolueno, vanilina, ácido vanílico, e muitos outros produtos promissores que tornam os subprodutos de cana-de-açúcar uma matéria-prima ideal para transformar o processamento da cana em uma biorrefinaria ambientalmente correta e lucrativa (28,36).

Estrutura da parede celular da cana-de-açúcar

A estrutura dos tecidos de uma planta são classificadas como dérmicos, tecidos fundamentais e tecidos vasculares. O tecido vascular é envolvido pelo sistema fundamental e o sistema dérmico reveste a planta. Este é constituído pela epiderme, o revestimento mais externo dos órgãos vegetais em estrutura primária e pode ser substituído pela periderme em órgãos com crescimento secundário. O sistema fundamental é constituído por células de colênquima e esclerênquima, que são células vivas com paredes celulares não lignificadas (floema) e o tecido vascular (xilema) constituído por vasos condutores, fibras,

traqueídeos, células epiteliais (células mortas e lignificadas após sua maturação) e células de parênquima (células vivas e não lignificadas) (37).

As células eucarióticas vegetais são semelhantes às células animais, porém possuem características típicas como parede celular, vacúolos e plastídios. A parede celular envolve externamente a membrana plasmática da célula. A primeira camada de parede celular formada constitui a parede primária, esta é formada por deposição de microfibrilas de celulose em um arranjo entrelaçado. Paredes primárias são formadas enquanto as células ainda estão em crescimento e desenvolvimento, assim, não são lignificadas e podem representar a única parede de certos tipos de células, como algumas células do parênquima (37,38).

Em outras células, entretanto, ocorrem deposições de camadas adicionais internamente à camada primária, por aposição, ou seja, ocorre deposição de microfibrilas de celulose em um arranjo ordenado. A primeira, segunda e terceira camadas da parede secundária são denominadas de S_1, S_2 e S_3, respectivamente (Figura 1.1).

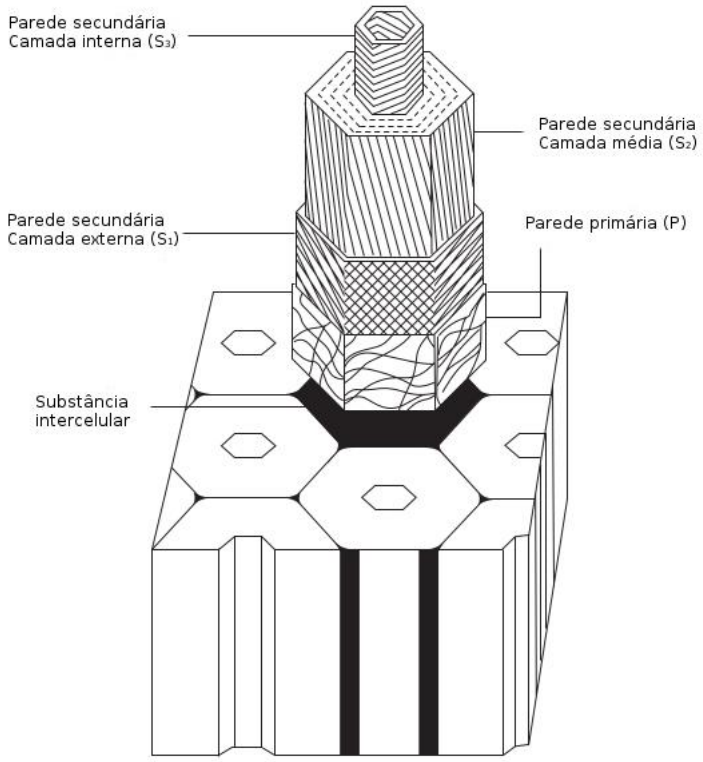

Figura 1.1. Esquema da orientação das microfibrilas nas camadas das paredes primária e secundária de uma célula vegetal. Na parede primária (P) as microfibrilas de celulose são depositadas em um arranjo entrelaçado. Na parede secundária (S_1, S_2 e S_3) a deposição ocorre de forma ordenada, variando a orientação nas diferentes camadas (adaptado de HARRIS e STONE, 2008).

Essas camadas são caracterizadas pela mudança de orientação da deposição. As paredes secundárias são depositadas internamente à parede primária após o completo desenvolvimento da célula e são caracteristicamente lignificadas após atingir a maturidade. A lamela média é a camada interfacial entre as células adjacentes e também é tipicamente lignificada (37,38).

As paredes celulares primária e secundária geralmente são compostas por longas microfibrilas de celulose e por polissacarídeos não celulósicos que

a interconectam, como (1→3,1→4)-β-D-glucanas, heteroxilanas, heteroglucanas, heteromananas e pectinas (39).

Levando-se em consideração a composição polissacarídica, três tipos de paredes celulares não celulósica foram propostas por Carpita e Gibeaut (40). A do Tipo I é rica em xiloglucana, a do Tipo II é rica em arabinoxilana e a do Tipo III é rica em manana. As paredes celulares tipicamente encontradas em cana-de-açúcar são do Tipo II (41).

Os colmos e folhas da cana-de-açúcar *in natura* são compostos principalmente por celulose na forma de microbibrilas de glucanas; arabinoxilana, β-glucana, xiloglucana e traços de manana; pectinas, incluindo homogalacturonana, arabinogalactana e arabinana e lignina. Polissacarídeos, pectinas e lignina podem ser encontrados em diferentes proporções de acordo com a idade da planta, espécie e linhagem utilizada para a análise (42).

Além das macromoléculas citadas, as paredes celulares vegetais contêm ainda pequenas quantidades de substâncias químicas, não estruturais denominadas de extrativos. São caracterizados por serem materiais cerosos e aromáticos de baixa massa molar, incluindo flavonóides, estilbenos, taninos, sais inorgânicos, ceras, alcalóides, proteínas, fenólicos simples e complexos, açúcares simples, pectinas, mucilagens, terpenos, amido, glicosídeos, óleos essenciais (43,44). Esses compostos podem ser extraídos com água, solventes orgânicos ou uma mistura destes (8,45).

As cinzas são materiais inorgânicos que estão ligados à estrutura física da biomassa e podem ser removidos em parte lavando-se ou extraindo o material (46). O BCA e a PCA de cana-de-açúcar apresentam teores variáveis

de extrativos, que podem estar de 1,6 a 9,2% (base seca). Os teores de cinzas encontrados para o BCA variam de 1,0 a 7,9% (base seca) e da PCA variam de 2,4 a 5,7% (base seca) (8).

Celulose

A celulose é o biopolímero natural mais abundante na Terra e é considerado o composto orgânico produzido mais importante na biosfera. É sintetizada por um amplo número de organismos, que varia de plantas e animais, bactérias e fungos, sendo que mais de 99% desses organismos são plantas (12,47,48).

A celulose é um homopolímero linear com fórmula molecular $(C_6H_{10}O_5)_n$ composto por unidades de D-anidroglucopiranose, também conhecidas como unidades de glicose. Estas estão unidas por ligações do tipo β-(1-4)-glicosídicas, formando um dímero conhecido como celobiose, a unidade repetitiva da molécula de celulose (Figura 1.2).

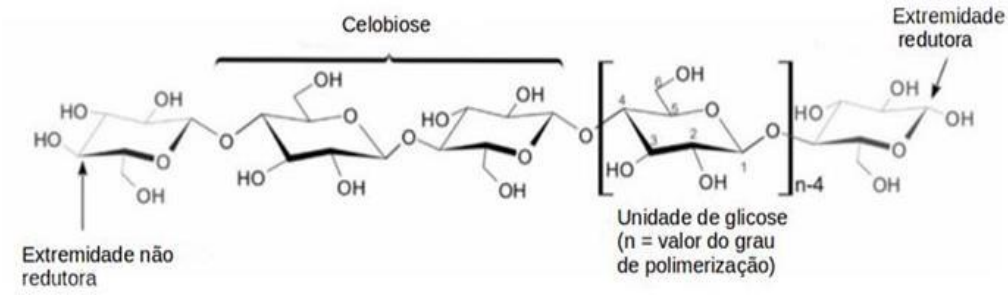

Figura 1.2. Estrutura molecular da celulose. Adaptado de Trache et al. (47).

O tamanho das moléculas de celulose é expresso como grau de polimerização (GP), que indica o tamanho em termos de número de unidades de monômero por cadeia de celulose. O grau de polimerização pode variar de 100, em celulose microcristalina a 15.000 em fibras de algodão (12,47–49).

As ligações de hidrogênio (intramoleculares e intermoleculares) e as interações de *van der waals* presentes na cadeia de celulose impedem a rotação das moléculas de glicose, enrijecendo a cadeia resultando na formação de regiões ordenadas da molécula (regiões cristalinas) e regiões desordenadas (amorfas). As cadeias de celulose se agrupam formando microfibrilas, que juntas, formam as fibras de celulose (Figura 1.3). As microfibrilas de celulose são formadas por 36 cadeias de celulose (12,47,49).

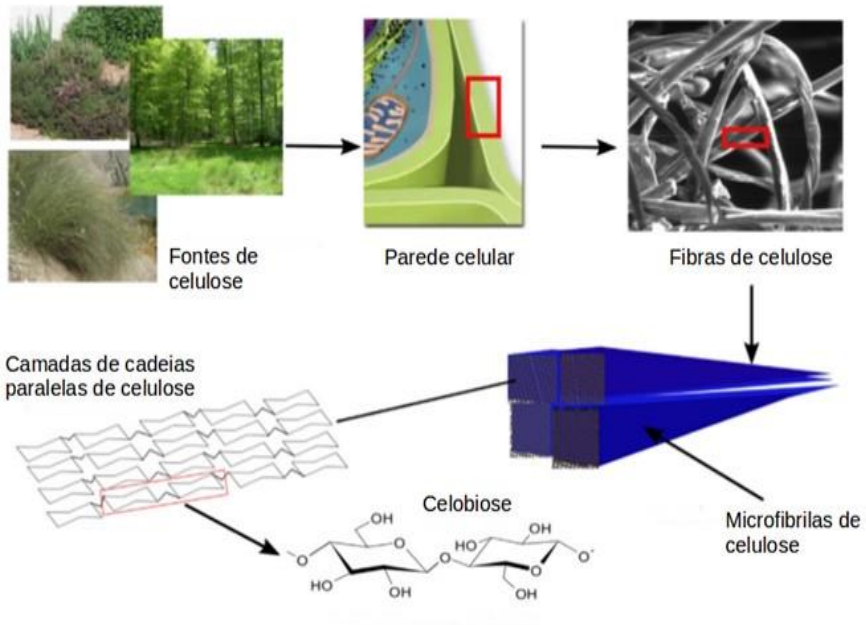

Figura 1.3. Organização estrutural da celulose. Adaptado de Trache et al. (47).

Hemicelulose

As hemiceluloses são heteropolímeros de cadeias curtas contendo grupos laterais compostos de unidades de pentoses (xilose e arabinose), hexoses (glicose, manose, ramnose e galactose) e ácidos urônicos (galacturônico e metil-glucurônico). Esses heteropolímeros entremeiam as microfibrilas de celulose conferindo resistência e flexibilidade à parede celular da planta. A hemicelulose representa o terceiro polímero mais abundante na biosfera, ficando atrás da celulose e quitina (12,36,48,50).

As hemiceluloses dos subprodutos de cana-de-açúcar são compostas principalmente por arabinoxilana (3). As arabinoxilanas possuem um esqueleto que consiste em resíduos β-(1→4)-D-xilopiranosil, sendo que alguns pontos há cadeias laterais, como por exemplo, grupos arabinosil (α-L-arabinoxilana), grupos acetil e ácido-metil-glucurônico (4-O-metil) (Figura 1.4) (51–53).

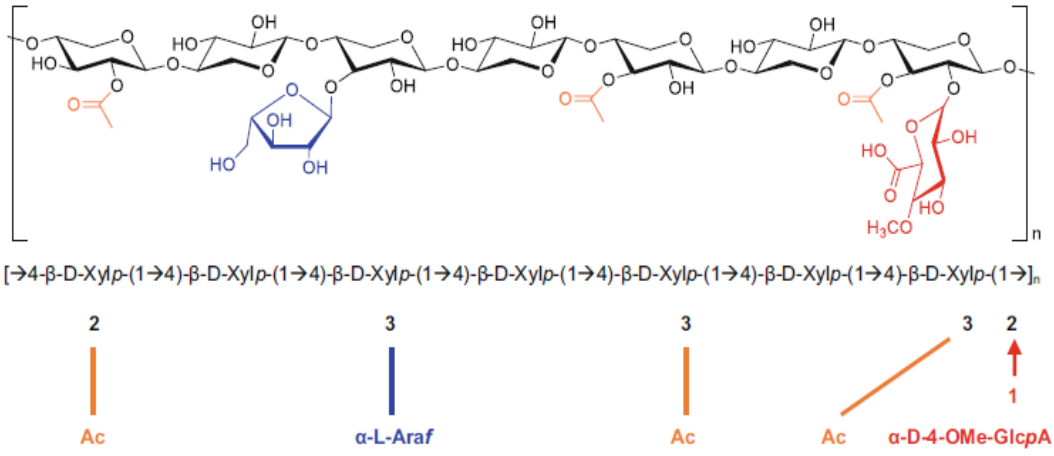

Figura 1.4. Representação esquemática da estrutura geral da molécula de glucuronoarabinoxilana (PAZ-CEDENO et al. (53)).

A arabinoxilana apresenta grande potencial para produção de bioprodutos de interesse comercial e alto valor agregado, por exemplo, os XOS, que possuem propriedades prebióticas proporcionando vários benefícios à saúde. Além de poder ser convertida em etanol, xilitol, ácido láctico, oligossacarídeos funcionais, dentre outros (36,54).

Lignina

A lignina é uma macromolécula composta por três principais tipos de álcoois hidroxicinamil: álcool *p*-cumárico, álcool coniferílico e álcool sinapílico. Quando polimerizados, esses monolignóis são denominados como unidades monoméricas da lignina, como se segue: *p*-hidroxifenil (unidade H), guaiacil (unidade G) e siringil (unidade S). Essas unidades monoméricas encontram-se irregularmente conectadas quimicamente a uma variedade de ligações do tipo éter, onde a mais comum é a arilglicerol-β-aril (β-O-4') (42).

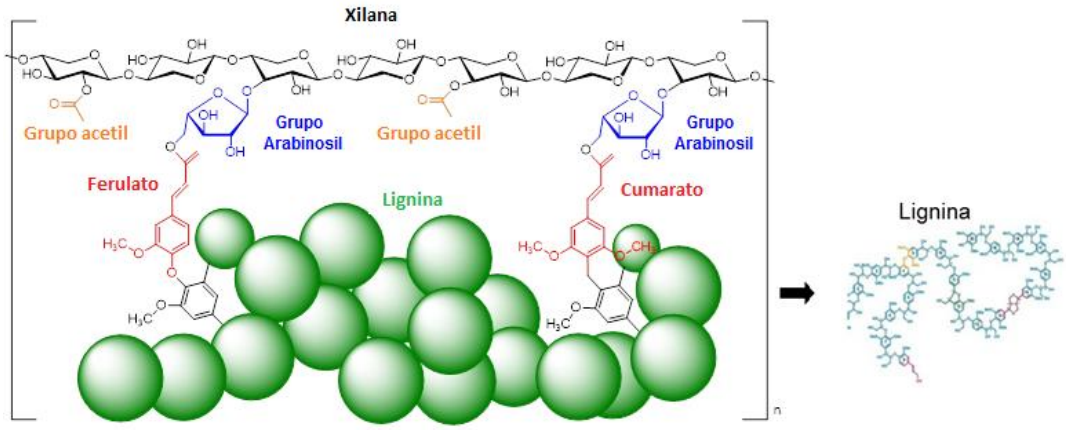

Figura 1.5. Esquema mostrando resíduos de ferulato acoplados à lignina, ambos resíduos de alcoóis coniferílico e sinapílico são ligados com ferulatos (53,55,56).

A proporção entre os constituintes da lignina varia de acordo com o tipo de célula, tecidos vegetais e a espécie da planta. A lignina de madeiras de folhosas, é composta por unidades S e G em proporções semelhantes e pequenas quantidades de unidades H. Nas madeiras de coníferas, encontra-se principalmente ligninas compostas por unidades G e apenas pequenas quantidades de unidades H. As gramíneas, por sua vez, apresentam ligninas compostas pelas três unidades, com unidades H comparativamente menores (31,42).

A lignina dos subprodutos de cana-de-açúcar está localizada principalmente nos feixes vasculares (parede celular de células de vasos e fibras) e em menor quantidade localizada nas paredes das células de células de parênquima, que armazenam sacarose (57). A arabinoxilana é a principal hemicelulose ligada quimicamente à lignina, através de seus grupos laterais (grupo arabinosil) conectados nas unidades de xilose da cadeia principal de xilana (Figura 1.5) (57).

A Figura 1.6 apresenta a estrutura geral das fibras dos subprodutos de cana-de-açúcar que é formada por longas cadeias de celulose interligadas por ligações de hidrogênio com moléculas de hemicelulose entrelaçadas, o que resulta em uma estrutura altamente complexa que é encapsulada pela lignina. Assim, as microfibrilas de celulose estão organizadas de forma paralela e encaixadas em uma matriz de hemicelulose e lignina (12,48,58).

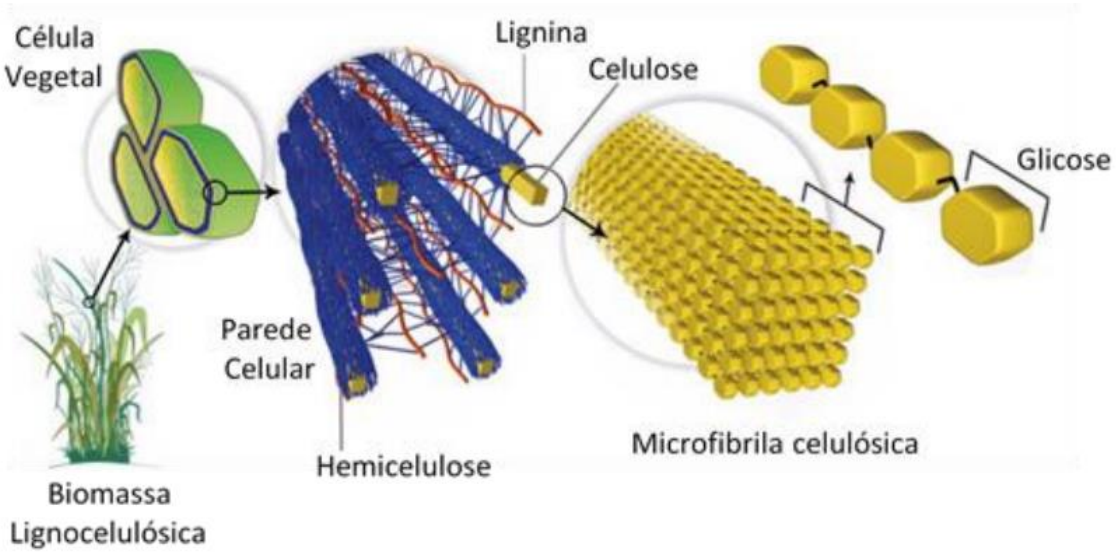

Figura 1.6. Modelo proposto para representar os componentes macromoleculares na estrutura dos subprodutos de cana-de-açúcar. Adaptado de Sjöström e Westermark (59).

Pré-tratamento hidrotérmico (PTH)

O pré-tratamento, desestruturação ou fracionamento da biomassa lignocelulósica é uma importante técnica, pois objetiva a desconstrução da organização e associações entre lignina, hemicelulose e celulose da parede celular, permitindo o fracionamento do subproduto e a recuperação de seus componentes (Figura 1.7).

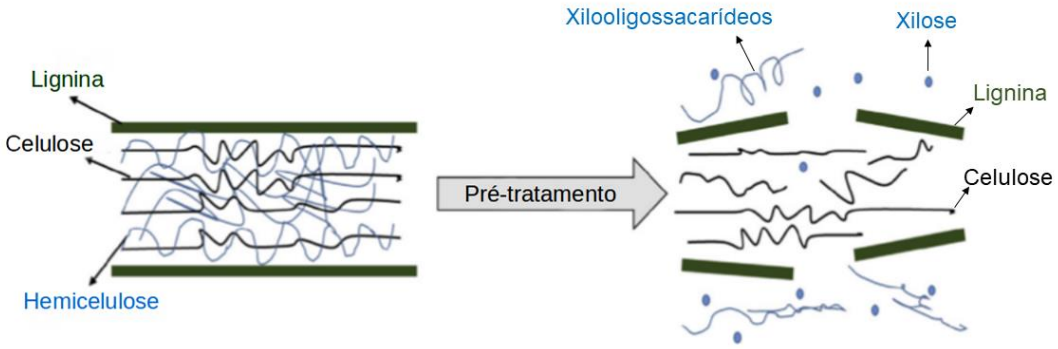

Figura 1.7. Disposição entre celulose, hemiceluloses e lignina na parede celular antes e após o pré-tratamento. Adaptado de Kumar et al. (1).

Os métodos de pré-tratamento tradicionais alteram a composição química e a ultraestrutura do material, diminuindo ou aumento o índice de cristalinidade do material e aumentando a acessibilidade da parede celular a microrganismos ou produtos químicos. Os pré-tratamentos promovem a clivagem das ligações do tipo éter e éster das macromoléculas da parede celular, podendo remover as frações de hemicelulose e lignina, além de aumentar o volume dos poros e a área de superfície do material facilitando a acessibilidade de enzimas hidrolíticas (60,61).

Vários métodos de pré-tratamentos foram desenvolvidos e podem ser classificados da seguinte forma: biológicos; físicos (trituração, extrusão mecânica, dentre outros); químicos (ácidos, bases, ozônio, clorito, solventes orgânicos, líquidos iônicos, dentre outros) e físico-químicos, ou seja, pré-tratamento químico combinado com físico (explosão a vapor, micro-ondas, ultrassom, hidrotérmico, dentre outros). Normalmente, os pré-tratamentos químicos em pH alcalino levam a dissolução/degradação da lignina em

fragmentos solúveis, enquanto os pré-tratamentos em pH ácido resultam em uma fração líquida contendo fragmentos de açúcares monoméricos e oligoméricos, produtos de degradação de açúcares e de lignina (60,62,63).

O PTH utiliza apenas água como meio reacional, que pode estar em sua forma de vapor ou líquida. O PTH consiste em uma tecnologia em que os materiais lignocelulósicos são tratados com água a quente comprimida a altas pressões (entre 5-20 $Kgf.cm^{-2}$), e temperaturas (entre 150-220°C), e a uma razão líquido/sólido (proporção de volume de água para massa de biomassa lignocelulósica) pré-determinada. O PTH apresenta vantagens por ser um processo ecológico e ambientalmente correto, ou seja, não necessita de produtos químicos e utiliza apenas água como meio reacional, não apresentando problemas de corrosão em equipamentos (10,12,13,29,60,64).

O PTH promove o decaimento do pH da solução do meio reacional devido à liberação de ácido acético e ácido metil-glucurônico presentes nas cadeias laterais da arabinoxilana. Além disso, a altas temperaturas (150-230°C), as ligações de hidrogênio da água se enfraquecem, resultando na sua autoionização em íons hidrônio (H_3O^+), que atuam como catalisadores e contribuem para a diminuição do pH do meio (11). A principal fração dos subprodutos de cana-de-açúcar a ser hidrolisada no PTH é a arabinoxilana por ser a mais instável dos três principais constituintes da parede celular (10–13,29,64).

Desta forma, no PTH a degradação da arabinoxilana ocorre em três etapas: inicialmente ocorrem reações na superfície do material que resultam na formação dos produtos primários (XOS), xilose, ácido metil-glucurônico e

ácido acético, que são produzidos pela clivagem das ligações do tipo éter e éster. Os XOS em condições mais severas de temperatura e tempo são hidrolisados a xilose. A xilose é instável em meio ácido e em altas temperaturas, sendo desidratada em furfural que por sua vez pode ser oxidado a ácido fórmico (Figura 1.8) (10,12,13,60,63,65).

Na celulose, ocorre hidrólise aleatória das ligações glicosídicas, diminuindo o grau de polimerização. Devido à natureza cristalina da celulose, as regiões amorfas, que são mais acessíveis, são removidas inicialmente. Assim como as hemiceluloses, a glicose liberada da celulose pode sofrer degradação formando produtos como hidroximetilfurfural (HMF) e ácido levulínico em meio ácido e altas temperaturas (Figura 1.8) (60,65). Segundo Sakaki et al. (66) essa degradação se inicia a temperaturas acima de 230°C e quase toda a celulose é decomposta a 295°C, todavia, o tempo de reação é outra variável importante na degradação dos açúcares a furfural e HMF.

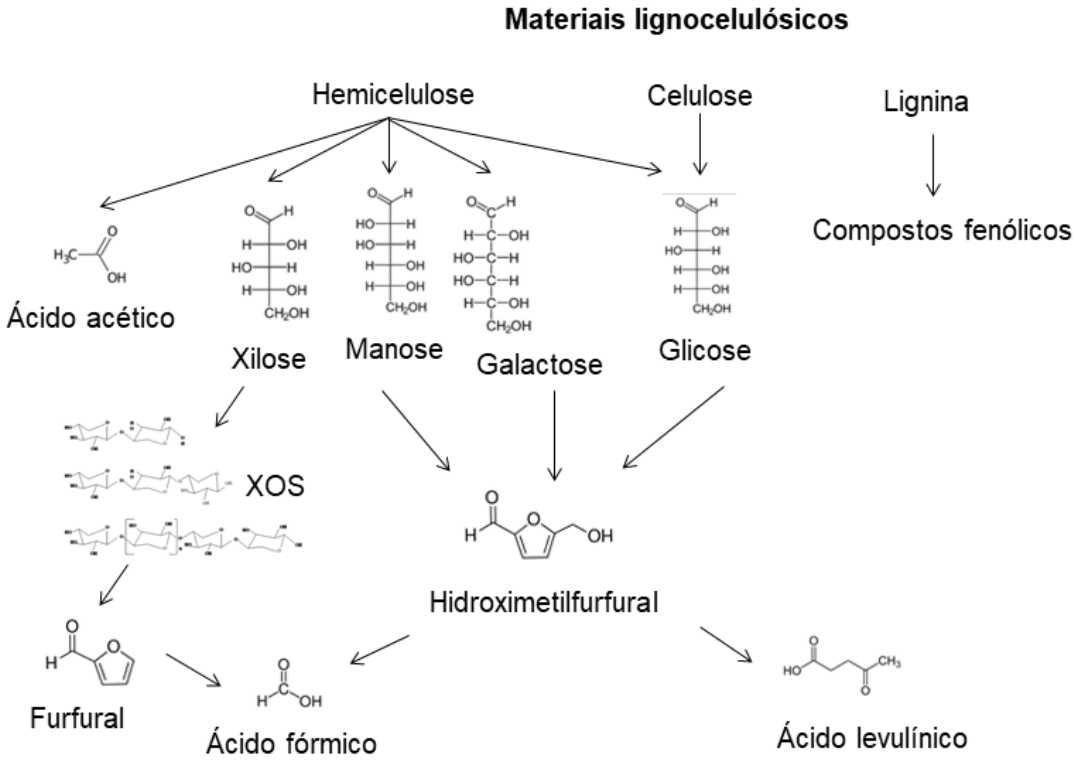

Figura 1.8. Principais produtos formados na hidrólise por pré-tratamento hidrotérmico (PTH) de arabinoxilana dos subprodutos de cana-de-açúcar. Adaptado de Xiao et al. (62).

Comparado com a hemicelulose e a celulose, a remoção de lignina durante o PTH é limitada podendo ocorrer despolimerização e repolimerização simultaneamente. Sugere-se que a lignina sofra uma reação em duas etapas: na primeira ocorre a clivagem das ligações entre lignina e carboidratos, ocorrendo hidrólise principalmente das ligações do tipo éter β-O-4 da lignina liberando fragmentos baixa massa molar e solúveis em água; e em uma segunda etapa ocorre a recondensação e repolimerização dos fragmentos solúveis na presença dos ácidos orgânicos liberados no processamento hidrotérmico (60,65).

No hidrolisado hemicelulósico são encontrados monômeros aromáticos, sendo que o tipo e quantidade destes compostos variam de acordo com as condições de hidrólise e a composição da biomassa pré-tratada. Os principais componentes derivados da fração de lignina identificados no hidrolisado hemicelulósico de BCA após PTH são ácido p-cumárico, ácido ferúlico, catecol, ácido cafeico, guaiacol, ácido hidroxibenzoico, ácido vanílico, vanilina, siringaldeído, dentre outros (67).

A severidade do PTH determina o efeito do mesmo sob a biomassa e os produtos que serão formados, além das características da biomassa em termos de composição química.

Produção de xilo-oligossacarídeos (XOS)

XOS são oligômeros constituídos por unidades de xilose unidas por ligações glicosídicas do tipo β-1,4, formando oligossacarídeos entre 2-12 unidades de xilose, como por exemplo, xilobiose (X_2), xilotriose (X_3), xilotetraose (X_4), xilopentose (X_5), xilohexaose (X_6), xiloheptaose (X_7), dentre outros (Figura 1.9) (68).

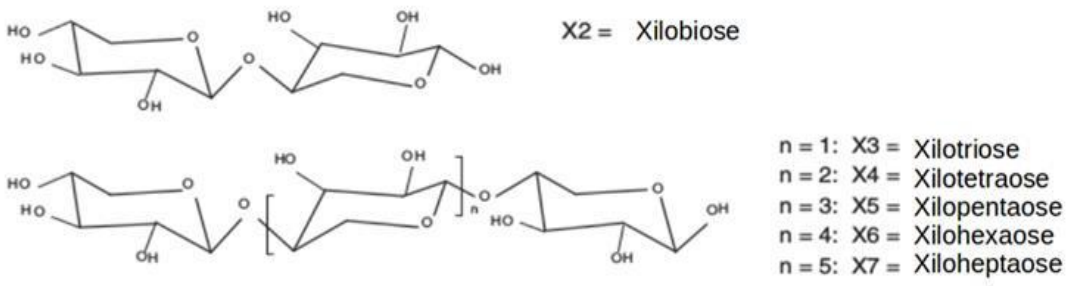

Figura 1.9. Estrutura dos xilooligossacarídeos. Adaptado de Carvalho et al. (69).

Os XOS possuem diversas propriedades biológicas, entre elas, a atividade prebiótica, ou seja, são oligossacarídeos não digeríveis, mas fermentescíveis, que possuem a capacidade de estimular seletivamente o crescimento e manutenção de bactérias benéficas no trato gastrointestinal conhecidas como probióticos. Os prebióticos aumentam o crescimento e número das bactérias benéficas, que além de excluírem várias bactérias patogênicas também conferem uma variedade de benefícios à saúde (70).

A atividade prebiótica dos XOS, no entanto, depende de sua massa molar. XOS≤ 4 unidades de monômeros promovem a proliferação de bifidobactérias benéficas no intestino humano que inibe o crescimento de bactérias patogênicas. Os XOS não são digeríveis, sendo considerados como fibras alimentares solúveis, pois o corpo humano não produz enzimas específicas para hidrólise de ligações do tipo β e, dessa forma, não possuem calorias, podendo ser consumidos em dietas de baixo valor calórico e por portadores de diabetes (19,21,24,69).

Além disso, propriedades antialérgicas e antioxidantes são conferidas pelos substituintes fenólicos (ácido metil-glucurônico) encontrados lateralmente à cadeia principal de alguns XOS. Desta forma, esses XOS podem apresentar atividades, como por exemplo, anticariogênica, imunomodulatória, citotóxica seletiva, protetora contra doenças cardiovasculares e risco de câncer de cólon devido à produção de ácidos graxos de cadeia curta pelos microrganismos da microbiota intestinal, capacidade de redução nos níveis de triglicerídeos séricos e colesterol, assim

como lipoproteína de baixa densidade e aumento nos níveis de lipoproteína de alta densidade também descritos na literatura (18,23,68,69).

Os XOS são produzidos pela hidrólise da molécula de arabinoxilana e podem diferir quanto ao grau de polimerização, ou seja, quantidade de unidades monoméricas; grau de substituição, razão entre arabinose e xilose e ligações entre eles. Podem ser produzidos por meio de duas estratégias principais, hidrólise enzimática, pela ação de endoxilanases que hidrolisam a xilana a XOS, ou pelo PTH (71). Durante o PTH há a liberação de ácido acético da cadeia de arabinoxilana, o que diminiu o pH e determina o grau de polimerização dos XOS produzidos. O teor de XOS produzidos dependerá de um equilíbrio entre a decomposição da fração de arabinoxilana em XOS e em xilose. Em pH muito ácidos, a arabinoxilana é hidrolisada em monômeros de xilose, que podem ainda ser degradados, formando produtos como furfural e ácido levulínico (68).

Hidrólise enzimática da celulose

A celulose presente na fração sólida resultante do PTH pode ser utilizada para produção de bioetanol e o mais importante passo nesse processo é a hidrólise enzimática, que agrega alto custo ao processo de sacarificação devido ao custo das enzimas utilizadas.

As enzimas responsáveis pela clivagem da celulose são as celulases, um grupo das hidrolases que clivam as ligações do tipo β-1,4-glicosídicas e são classificadas em três grupos: as endoglucanases, que clivam as ligações internas das fibras da molécula de celulose presentes na parte amorfa da

celulose; as exoglucanases ou celobiohidrolases, que atuam na região externa da molécula de celulose (redutora ou não redutora) e as β-glicosidases ou celobiases que hidrolisam oligossacarídeos solúveis e celobiose em glicose. Essas enzimas são adquiridas na forma de coquetéis comerciais com complexos de celulases e hemicelulases (Figura 1.10) (51).

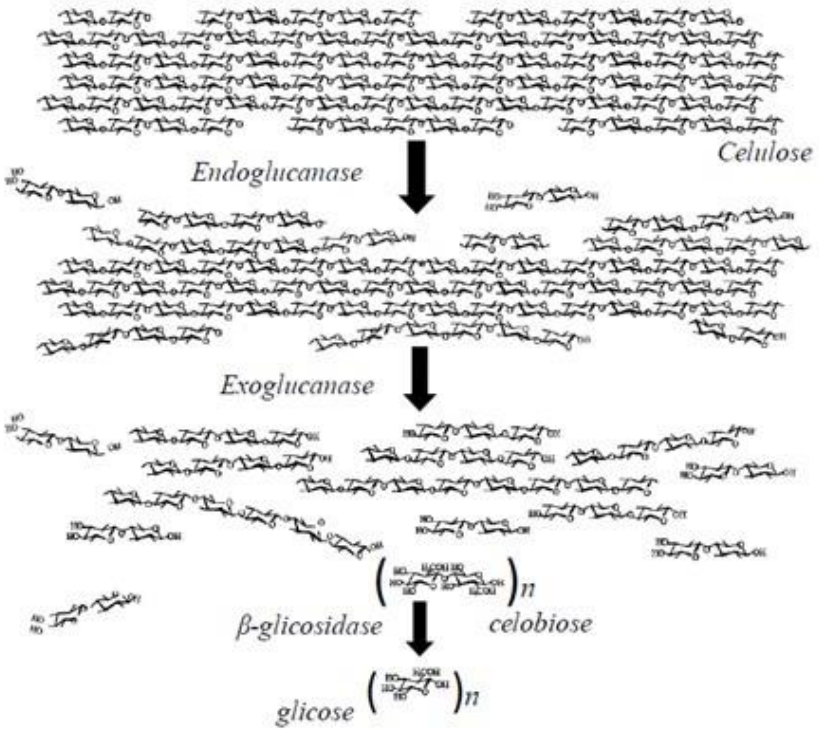

Figura 1.10. Modo de ação das enzimas do complexo celulolítico de *Trichoderma reesei.* Martins et al. (72).

Os principais desafios na eficiência da hidrólise enzimática, além do alto custo das enzimas, são difusão da enzima da solução aquosa para as partículas do substrato e adsorção produtiva da enzima na molécula de celulose. O tamanho e formato das partículas e dos poros das superfícies da biomassa também influencia na área de superfície do substrato e quanto

maior a área acessível, melhor a adsorção da enzima e a eficiência de hidrólise enzimática (73).

A adsorção não produtiva da enzima à lignina reduz a atividade da enzima uma vez que a lignina inibe o acesso da enzima à celulose formando uma barreira física restringindo a acessibilidade da celulose às celulases (74).

A alta carga de sólidos (alta consistência) é muito utilizada devido a alguas vantagens, como se segue: redução no consumo de energia, baixo consumo de água e diminuição na geração de resíduos, porém, apresenta limitações relacionadas à efeitos de restrição de água, inibição pelo produto final, fatores reológicos que dificultam a mistura adequada e transferência de massa durante a hidrólise do substrato (73).

A melhora na eficiência de conversão de carboidratos presentes no material lignocelulósico pode ser alcançada variando-se parâmetros operacionais como carga enzimática, consistência (razão sólido/líquido), velocidade de agitação, presença de aditivos e tempo de hidrólise (75).

OBJETIVO

Geral

Otimizar o pré-tratamento hidrotérmico (PTH) dos subprodutos de cana-de-açúcar (BCA e PCA) utilizando a metodologia de superfície de resposta, visando à produção de XOS e hidrolisado enzimático de glicose.

Específicos

- ✓ Identificar as condições ótimas de processo para selecionar as variáveis de operação como a temperatura, tempo e consistência para a produção máxima de XOS;

- ✓ Realizar os PTHs nas condições previamente otimizadas para validação dos modelos matemáticos e acúmulo das frações sólidas ricas em celulose;

- ✓ Hidrolisar enzimaticamente as frações celulósicas, obtidas anteriormente, visando à produção de hidrolisado de glicose em frascos tipo Erlenmeyer e biorreator.

Na sequência da tese de doutorado são apresentados os capítulos 1 e 2.

O capítulo 1 refere-se ao artigo científico publicado na íntegra na revista Biomass Conversion and Biorefinery intitulado “Experimental design, modeling, and optimization of production of xylooligosaccharides by hydrothermal pretreatment of sugarcane bagasse and straw” o qual corresponde as etapas de otimização da produção de XOS por PTH em BCA e PCA.

O capítulo 2 refere-se ao manuscrito em fase final de preparação intitulado “Efficiency of the enzymatic hydrolysis on sugarcane bagasse and straw thermotrated: Assessment in batch systems subject to high mass loads” o qual refre-se à avaliação da eficiência da hidrólise enzimática sob alta consistências de BCA e PCA previamente termotratadas em suas condições otimziadas.

O tópico 4 refere-se às considerações finais referentes aos capítulos 1 e 2 e o tópico 5 às referências citadas na introdução expandida (tópico 1).

2. CAPÍTULO 1

Experimental design, modeling, and optimization of production of xylooligosaccharides by hydrothermal pretreatment of sugarcane bagasse and straw

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Experimental design, modeling and optimization of production of xylooligosaccharides by hydrothermal pretreatment of sugarcane bagasse and straw

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ABSTRACT

Hydrothermal pretreatment (HP) of two by-products of sugarcane, bagasse (SB) and straw (SS), was optimized to produce xylooligosaccharides (XOS). A central composite rotational design (CCRD) in conjunction with response surface methodology was used to optimize the conditions for maximum XOS production. The developed mathematical models were statistically adequate to predict xylan conversion to XOS, and the by-products were promising for the production of XOS. The optimized conditions for the SB and SS were 161.9 and 177.2 °C °C for 75.3 and 64.8 min, respectively. For both by-products, the mass load in the optimal experiments was of 10%. The conversion of xylan to XOS was 24.8% for SB and 45.3% for SS, XOS yield of 53.3 and 96 mg.g-1, respectively. The SB produced more XOS with a greater degree of polymerization than SS. Sugarcane by-products are important in the production of XOS, which may be a valuable commercial product. Furthermore, cellulose and lignin enriched solids recovered following the optimized HP conditions can be fractionated to obtain other bioproducts of commercial interest.

KEYWORDS: Sugarcane by-products, Thermotreatment, Xylooligosaccharides production; Central composite rotational design; Response surface methodology.

INTRODUCTION

Sugarcane processing in the sugar-ethanol industry generates approximately 280 million metric tons of waste fibrous material annually. These by-products include bagasse (SB), the fibrous fraction resulting from the extraction of juice, and straw (SS), a by-product of the harvest that comprises leaves, tips, roots, and soil particles [1]. According to the National Supply Company [2], in the 2020/21 harvest, 665 million tons of sugarcane were obtained in Brasil. Brazilian ethanol production was over 29.8 billion liters in the same period. The processing of sugarcane generates 14% and 12.5% of SS and SB (dry weight), representing 82.4 and 73.6 million tons, respectively, of these by-products annually [3].

Currently, most SB are destined for burning to produce heat and energy (energy co-generation). The main components of SB and SS are cellulose, hemicellulose, and lignin and their content in SB ranges from 34.1 to 45.5%, 16.8 to 32.2%, and 17.1 to 32.4%, respectively, and their content in SS ranges from 33.6 to 40.8%, 26.2 to 30.8%, and 19.6 to 31.8%, respectively [3]. The high content of cellulose and hemicellulose polysaccharides present in these by-products make them promising for use in the production of value-added products in biorefining.

XOS selectively stimulate the growth and maintenance of beneficial intestinal bacteria. XOS comprising fewer than four monomer units promote the proliferation of beneficial bifidobacteria present in the human intestine that inhibit the growth of pathogenic bacteria. The described benefits of bifidobacteria include antiallergic and antioxidant properties, selective

cytotoxic activity, protective activities against cardiovascular disease and cancer, reduction of the levels of serum triglyceride, cholesterol, and low-density lipoprotein, and increased levels of high-density lipoprotein [4–7].

In addition to the numerous benefits of XOS to human and livestock health, the heat and pH stability, organoleptic properties, low-calorie value, sweetening power, and competitive pricing of the recommended dose price compared to other prebiotics are attributes that position XOS as a potential prebiotic food ingredient [8, 9].

Commercial xylan is an expensive substrate for XOS production that is difficult to obtain. Therefore, alternative production processes and the use of agroindustrial by-products rich in xylan as a raw material are potential strategies to decrease production costs and increase the commercial viability of XOS [9].

XOS can be obtained from a wide variety of feedstock sources through hydrothermal pretreatment (HP, also termed autohydrolysis) of the material. HP involves the use of compressed hot water delivered at high pressure and temperature at predetermined liquid/solid ratios. HP represents an efficient method of fractionation of hemicellulose-derived xylan for different types of feedstock. The approach using HP is an ecological and environmentally friendly process that requires only water as a reaction medium [10–13].

In the HP process, hemicellulose is mainly solubilized, generating a pretreated material that is rich in cellulose with potential value for bioethanol production. During the reaction, the high temperature and pressure conditions promote the cleavage of O-acetyl and uronic acids from hemicellulose,

producing acetic acid and other organic acids. These acids are responsible by the hydrolysis of this xylan into xylose and XOS. At more severe reaction conditions, the oxidation of these sugars to furfural and formic acid can occur [11, 13–15].

The production of sugar and ethanol in Brazil is relatively large compared to other nations globally. A considerable amount of by-products can be used for the production of higher value-added products such as XOS instead of disposal by burning, as is routinely performed in sugar-ethanol industries. Scaling up the industrial production of XOS from raw materials rich in xylan, such as sugarcane by-products, has become an important goal [5, 8].

The objective of this study is to optimize the HP of SB and SS using a 23 full factorial design with six additional axial experiments and with three replications at the central point, followed by response surface methodology to optimize the main response variable (xylan conversion to desired bioproducts, XOS).

MATERIAL AND METHODS

Feedstock

The SB and SS by-products were obtained at Seis Lagoas Distillery located in Brotas, São Paulo, Brazil (2015/2016 harvest). Dried SB and SS were milled in a knife mill with a 0.84 mm retaining screen. The material that passed through the screen was collected. Approximately 2 g (dry weight) of SB and SS were added to a paper cartridge filter and inserted in Soxhlet extractors coupled to a volumetric flask containing 1 L of heated 95% (v/v)

ethanol under continuous heating. The system was maintained for approximately 6 h. The paper cartridges filters including the extracted by-products were dried at 25 °C until the ethanol was completely evaporated and the mass reached a constant value [16, 17]. The percentage of extractives was calculated based on the difference in the initial and final mass.

Design of experiments

HP was carried out through the application of an extended factorial design (central composite rotational design, CCRD). This design included eight factorial experiments with normalized variables at combinations of levels +1 and -1, six axial experiments with a rotatability parameter $\alpha = 1.68$ ($\pm \alpha$, 0, 0; 0, $\pm \alpha$, 0; and 0, 0, $\pm \alpha$), and three experiments at the central point (variables normalized at level 0) to estimate the experimental error. The proposed design of experiments was performed with the objective of developing quadratic models capable of predicting the better conditions of HP that maximize the production of XOS (Table 2.1).

The independent variables investigated were temperature, time, and mass load (ratio of mass/water volume) because the kinetics of hydrolysis mainly depends upon these variables as follows. The temperature strongly affects the overall reaction rate, the time define the extent of reaction in batch systems, which can be pre-specified, and the mass load indirectly accounts for the effect of substrate concentration. Furthermore, time and temperature have been sometimes setted as independent variables in studies on HP of biomass using statistical methods of experimental design [18, 19].

The levels of temperature, time, and mass load were defined based on HP data for the production of XOS reported in the literature [13]. The unique response variable investigated in CCRD was the xylan conversion to XOS, which is an equivalent measure of the XOS production, given that these variabels are directly proportional. Moreover, since the xylan degradation during the autohydrolysis process leads to production of XOS and other wanted by-products by a set of series-parallel multiple reactions, the xylan conversion to XOS becomes the more appropriate response variable because it gives the percentage of initial xylan converted to desired products (XOS), being an indicator of the process selectivity.

Table 2.1. Experimental conditions outlined by the proposed CCRD for HP of the sugarcane by-products.

Test	Coded levels of variables			Actual levels of variables			$\log R_0^{**}$
	$x_1^{(*)}$	x_2	x_3	T (°C)	t (min)	C (%)	
1	−1	−1	−1	140	30	5	2.65
2	+1	−1	−1	180	30	5	3.83
3	−1	+1	−1	140	90	5	3.13
4	+1	+1	−1	180	90	5	4.31
5	−1	−1	+1	140	30	15	2.65
6	+1	−1	+1	180	30	15	3.83
7	−1	+1	+1	140	90	15	3.13
8	+1	+1	+1	180	90	15	4.31
9	−1.68	0	0	126.4	60	10	2.56
10	+1.68	0	0	193.6	60	10	4.53
11	0	−1.68	0	160	9.6	10	2.75
12	0	+1.68	0	160	110.4	10	3.81
13	0	0	−1.68	160	60	1.6	3.54
14	0	0	+1.68	160	60	18.4	3.54
15	0	0	0	160	60	10	3.54
16	0	0	0	160	60	10	3.54
17	0	0	0	160	60	10	3.54

$^{(*)}x_1$, x_2, and x_3 are the coded values of temperature (T), time (t) and mass load (C), calculated by the equations: $x_1=(T-160)/20$; $x_2=(t-60)/30$; $x_3=(C-10)/5$

$^{(**)}R_0$ is calculated by Eq. (1)

Hydrothermal pretreatment (HP)

HP was performed in fractions of approximately 10 g of SB and SS in their untreated forms (dry weight), just washed with distilled water. Each fraction was added to individual stainless steel reactors (TMRegmed, Osasco-São Paulo, Brazil). Each reactor was equipped with mechanical agitation, temperature, and pressure control devices, consisting of four digesters with a capacity of 1500 mL. Volumes of distilled water were used (625, 200, 100, 75, and 54.3 mL) to vary the mass/water ratio (mass load) of the by-products in the reaction medium (1.6, 5, 10, 15, and 18.4%, respectively). After the material was transferred, the reactors were closed and the temperature was

adjusted to 126.4, 140, 160, 180, or 193.6 °C for 9.6, 30, 60, 90, or 110.4 min, respectively. The agitation was set at 4 rpm for each experimental test (Table 2.1). Upon reaching the desired temperature (attained on average in 40 min), the reaction time was determined. At the end of the reactions, the equipment was turned off and cooled naturally for 24 h. The cooled pretreated material was filtered through 30 mL number 3 porosity sintered glass filters (Schott, Germany). The filtrates were collected and bubbled in nitrogen to eliminate excess oxygen. Each sample was stored in a −20 °C freezer until use. The material retained in the filters (recovered solids) was washed with distilled water until it reached neutral pH. The material was then removed from the filter and dried in an oven with air circulation at a temperature of 50 °C for 72 h. HP yields were calculated as the difference in the initial mass prior to HP and the final mass after HP. The material retained in the filters (recovered solids) and filtrates (hemicellulosic hydrolysate, HH) were chemically characterized [13].

Chemical characterization of sugarcane by-products

Lignin contents in SB and SS were analyzed as previously described by Ferraz et al. [17] and Masarin et al. [16]. For the determination of acid-insoluble lignin (Klason lignin), approximately 300 mg (dry weight) of SB and SS were hydrolyzed in 3 mL of 72% sulfuric acid (w/w) for 1 h at 30 °C. The contents of each test tube were transferred to a 250 mL Erlenmeyer flask along with 79 mL of distilled water. The flask was sealed with aluminum foil and autoclaved for 1 h at 121 °C to complete the hydrolysis. The mixture was cooled and filtered through 30 mL number 3 porosity sintered glass filter (Schott,

Germany), which had previously been oven dried at 105 °C for 1.5 h and weighed. The material retained in the filters was washed with two 5 mL portions of distilled water. Each filter with the insoluble fraction was dried in an oven until a constant mass was obtained. To determine the acid-soluble lignin, the filtrates were made-up to 100 mL in a volumetric flask with distilled water, and the absorbance of the filtrates was read using an ultraviolet-visible spectrophotometer at 205 nm. An extinction coefficient of 105 $L.g^{-1}.cm^{-1}$ was used to determine of soluble lignin.

The cellulose, xylan, arabinosyl, and acetyl groups contents were determined in the filtrates obtained by the Klason lignin method using high performance liquid chromatography (HPLC) as described previously [16, 17]. An aliquot was passed through a SEP-PAK C18 cartridge to retain phenolic compounds on the 0.45 µm filter. The analyses were performed in a NEXERA XR chromatography apparatus (SHIMADZU, Japan) equipped with an Aminex HPX-87H column (300 x 7.8 mm; Bio-Rad, USA), an oven temperature of 60 °C, and 5 mM H2SO4 as the mobile phase at a flow rate of 0.6 mL.min-1. The refractive index was detected at 60 °C using a model RID-20A device (SHIMADZU). The concentrations of carbohydrates and organic acids were determined using calibration series of analytical grade standards (glucose, xylose, arabinose, formic acid, and acetic acid) and dried under vacuum phosphorus pentoxide.

Hemicellulosic hydrolysates chemical characterization

The XOS in HH aliquots adjusted to pH 6.5 was determined using 1 M sodium hydroxide (NaOH) by filtering through a SEP-PAK C18 cartridge equipped with a 0.45 µm filter. The analyses were performed by HPLC in a model NEXERA XR (SHIMADZU) equipped with an Aminex HPX-87C column (300 x 7.8 mm; Bio-Rad) at an oven temperature of 80 °C, with an eluent of ultrapure water at a flow of 0.6 $mL.min^{-1}$. The index of refraction was detected using a model RID-20A device (SHIMADZU) at 60 °C. Chromatography used to determine the XOS utilized analytical grade standards of xylose (X_1), xylobiose (X_2), xylotriose (X_3), xylotetraose (X_4), xylopentaose (X_5), xylohexaose (X_6), xyloheptaose (X_7), xylooctaose (X_8), and xylonanoose (X_9) (Megazyme, Ireland). X_2, X_3, X_4, X_5 and X_6 were used for XOS with a lower degree of polymerization. X7, X8 and X9 were used to designate XOS with a great degree of polymerization (>X6). The XOS were comprised of $(>X_6)+(X_6+X_5)+(X_4)+(X_3)+(X_2)$ [20].

The amounts of glucose, arabinose, acetic acid, and formic acid determined by HPLC were the same as those used for the determination of carbohydrates and organic acids in the chemical characterization of the by-products.

Furfural was determined in aliquots of HH following passage through the 0.45 µm filter. The analysis was performed by HPLC using a model NEXERA XR (SHIMADZU) equipped with a 250 mm long ODS Hypersil column, 4.0 mm diameter filled with octadecyl -C18 as the stationary phase (5 µm particle diameter, 120 Å pore diameter; Thermo Fisher Scientific, USA),

oven temperature of 25 °C, and a mobile phase composed of acetonitrile:water (1:8) and 1% (v/v) acetic acid at a flow rate of 0.8 mL.min-1. The detector used was an ultraviolet absorption spectrophotometric detector ("Photodiode Array" model SPD -M20A; SHIMADZU) set to 276 nm and an oven temperature of 25 °C [13].

The conversion of xylan to desirable and undesirable products was calculated as described by Neto et al. [13]. Soluble aromatics were determined in HH aliquots by determining the absorbance in an ultraviolet-visible spectrophotometer at 280 nm (extinction coefficient of 20 L.g-1.cm-1) [16].

Statistical analysis

Statistical analysis of the experimental data obtained in the CCRD, including mathematical modeling of the response variable (xylan conversion to XOS) as dependent on the controlled variables (factors), was performed using Statistical software (Version 10). Tukey test was applied to verify if the chemical composition data were statiscally different themselves , and was performed using BioEstat 5.0 software. A significance level of 5% was adopted for statistical tests.

Determination of severity factor (R_0)

R_0 is a parameter that indicates the intensity of HP based on the combined effects of temperature and time. It is used to compare, evaluate, and predict the fractionation and solubilization of biomass components [11, 13, 21]. This parameter can be calculated assuming that the reaction is pseudo-

homogeneous, elementary, irreversible, and first order, with the reaction rate constant dependent on the temperature according to the Arrhenius equation. The severity factor, R_0, during nonisothermal/isothermal HP of biomass is defined as follows [22]:

$$R_0 = \int_0^{t_r} exp\left(\frac{T(t)-100}{14.75}\right)dt \xrightarrow{constant\ T} R_0 = t_r \cdot exp\left(\frac{T-100}{14.75}\right) \quad (1)$$

where t is reaction time (min), T is reaction temperature (°C), and 14.75 is the value of the arbitrary constant ω, which incorporates the activation energy of the reaction. The log value of R_0 provides a more appropriate severity factor to compare the effects of HP on sugarcane by-products.

RESULTS AND DISCUSSION

Hydrothermal pretreatment of sugarcane by-products and chemical composition

The raw by-products were chemically characterized to determine the amounts of cellulose, hemicellulose, lignin, extractives, and ash as a function of log R_0 (Figure 2.1).

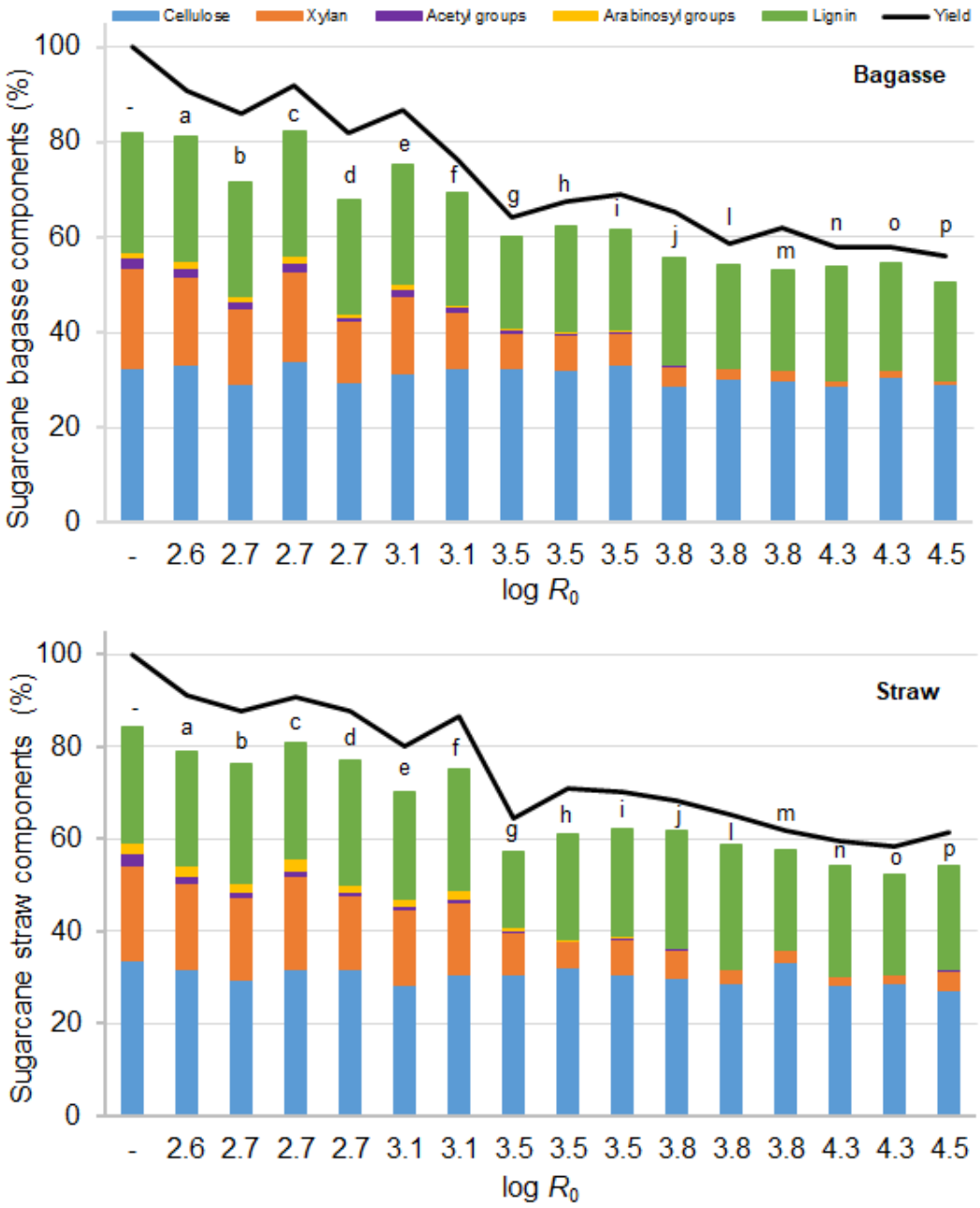

Figure 2.1. Components and yield of the insoluble fraction of SB and SS obtained by HP as dependent on the severity factor (log R_0). Components were presented as percentage in dry weight. The bars for each experiment are in increasing order of severity factor and follow the order 9, 1, 5, 11, 7, 3, 13, 14, mean of the central point (15, 16, and 17), 12, 6, 2, 8, 4 and 10 according to Table 2.1, which correspond to the letters “a” to “p” that are on the bars, respectively. The first bar corresponds to the by-product untreated.

SB presented levels of extractives, ash, total lignin, cellulose, xylan, arabinosyl groups and acetyl groups of 2.2%, 3.2%, 25.1%, 32.3%, 21.2%,

1.4% and 2.4%, respectively, resulting in a sum of 88.4 % (w/w, dry basis). In SS the same components were presented at levels of 2.8%, 3.3%, 25.3%, 33.8%, 20.4%, 2.2% and 2.7%, in that order, representing a total of 90.5% (w/w, dry weight) (Figure 2.1). The undetermined components (difference of 100%) could be assigned to the methyl-glucuronic acid molecule, which is commonly present in the hemicellulose chains of grasses and was not quantified in this study (76). Furthermore, the formation of sugar degradation by-products (including hydroxymethylfurfural, furfural, formic acid, and levulinic acid) generated in the acid hydrolysis stage comprises part of the undetermined components amounts (77). The levels of the polysaccharide and lignin in SB and SS were close to the values reported in the literature (78–84).

Visually, the insoluble fractions recovered after HP were darker than the untreated material. In the most severe operational conditions (180 °C, 90 min, log R_0=4.5), a more pronounced darkening was observed in both by-products compared to HP performed under milder conditions (140 °C, 30 min, log R_0=2.6). This darkening has also been described by other authors post HP of other agroindustrial by-products. This effect is assigned to the formation of derived from lignin on the cellulose surface, which is termed pseudo-lignin (12,60,82,85–87).

The levels of cellulose, hemicellulose (xylan, acetyl, and arabinosyl groups), and lignin were determined in the insoluble fraction of all HP conditions assessed (Figure 2.1). The fraction containing extracted material

was considered to be completely removed under all HP experimental conditions, and the ash content was considered negligible.

The yields of the insoluble fractions of SB and SS after HP declined owing to the increase in the severity factor (log R_0). The HP yields of SB and SS ranged from 56.7% to 81.8% and 58.1% to 81.1% (w/w, dry weight), respectively. The raw contents of cellulose, xylan, acetyl groups, arabinosyl groups, and lignin in the SB following HP ranged from 33.7% to 52.7%, 1.7% to 20.6%, 0.3% to 1.9%, 0.2% to 1.8%, and 27.9% to 41.6% (w/w, dry weight), respectively (data not shown). In SS, the raw contents in the same respective order following HP ranged from 32.4% to 53.8%, 2.8% to 21.9%, 0.1% to 1.4%, 0.2% to 2.9%, and 25.6% to 41.1% (w/w, dry weight) (data not shown).

For the material balance of the determined components of SB and SS, was compared the hydrothermally pretreated materials directly with the untreated by-products (Figure 2.1). The increase in the severity factor (log R_0) resulted in a reduction in the levels of hemicellulose (xylan, acetyl, and arabinosyl groups) for SB and SS, while cellulose and lignin showed minimal changes. The findings indicated the selectivity of HP for the hemicellulose removal. Furthermore, the more severe the HP (severity factor, log R_0), the greater was the removal efficiency of the hemicellulosic fraction (Figure 2.1).

The substantial removal of the xylan fraction was verified in the central conditions of the experimental design (160 °C, 60 min, and log R_0=3.5) resulting in an average removal of xylan of 64.3% and 62.5% (w/w, dry weight) for the SB and SS, respectively. The total removal of acetyl and arabinosyl groups in SB and SS after HP only occurred in the most severe conditions (log

R_0≥3.8). However, the fraction of xylan was not completely removed under these conditions, remaining in the order of 0.9-4.3% (w/w, dry weight) (Figure 2.1).

Cellulose did not show significant removal according to the Tukey test after the application of different HP protocols in SB and SS, except for the most severe condition of SS (log R_0=4.5), where removal of the cellulosic fraction averaged 19.2% (w/w, dry weight) (Figure 2.1).

In general, the lignin fraction was removed at severity factor values above log R_0≥3.5 according to the Tukey test. Removal of the lignin fraction in SS was significant (34.8%) using HP conditions of 160 °C, 60 min, and log R_0=3.5, in addition to the more severe HP conditions (log R_0=3.8 and log R_0=4.3). The removal of the lignin fraction reached 14.2%, for both conditions. In general, it was noted that HP was selective in the removal of the hemicellulosic fraction, especially under conditions of severity factor starting at log R_0=3.5.

Batista et al. (82) also reported an increase in the solubilization of the hemicellulosic fraction in HP on SS due to an increase in the severity factor. These authors obtained hemicellulose removals of 32.9%, 47.2%, and 85.4% for under HP conditions of 170 °C for 5 min (log R_0=3.2), 170 °C for 15 min (log R_0=3.4), and 195 °C for 10 min (log R_0=4.1), respectively. In the present study, hemicellulose removal was slightly higher (62.5%) at 160 °C for 60 min (log R_0=3.5).

Rocha et al. (88) presented the effect of HP on SB in the removal of xylan and lignin. An increase in the hemicellulosic fraction occurred due to an increase in the severity factor. These authors reported hemicellulose losses of

63.1% and 88.7% in HP with severities of log R_0=3.6 and log R_0=4.2, respectively. These results corroborate those found in the present study, with the removal of 64.3% of the hemicellulosic fraction in at conditions of 160 °C for 60 min (log R_0=3.5).

Chemical characterization of hemicellulosic hydrolysates obtained after hydrothermal pretreatments of sugarcane by-products

The HH displayed a yellow color, which was more evident due to the increase in the severity factor (log R_0) post HP. This appearance is assigned to the formation of phenolic or aromatic derived from lignin (e.g., formation of quinones), or to the formation of compounds derived from xylan and cellulose (11,12,85,89).

The HH were assessed for xylan conversion to XOS (desirable products) and degradation products (unwanted products) as dependent on the severity factor (log R_0). The total XOS corresponded to the sum of xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose (XOS with a low degree of polymerization) and >X_6 (XOS with a larger degree of polymerization). Their values are reported in Figure 2.2 and 3 as mg.g^{-1} of by-product (SB or SS, dry weight).

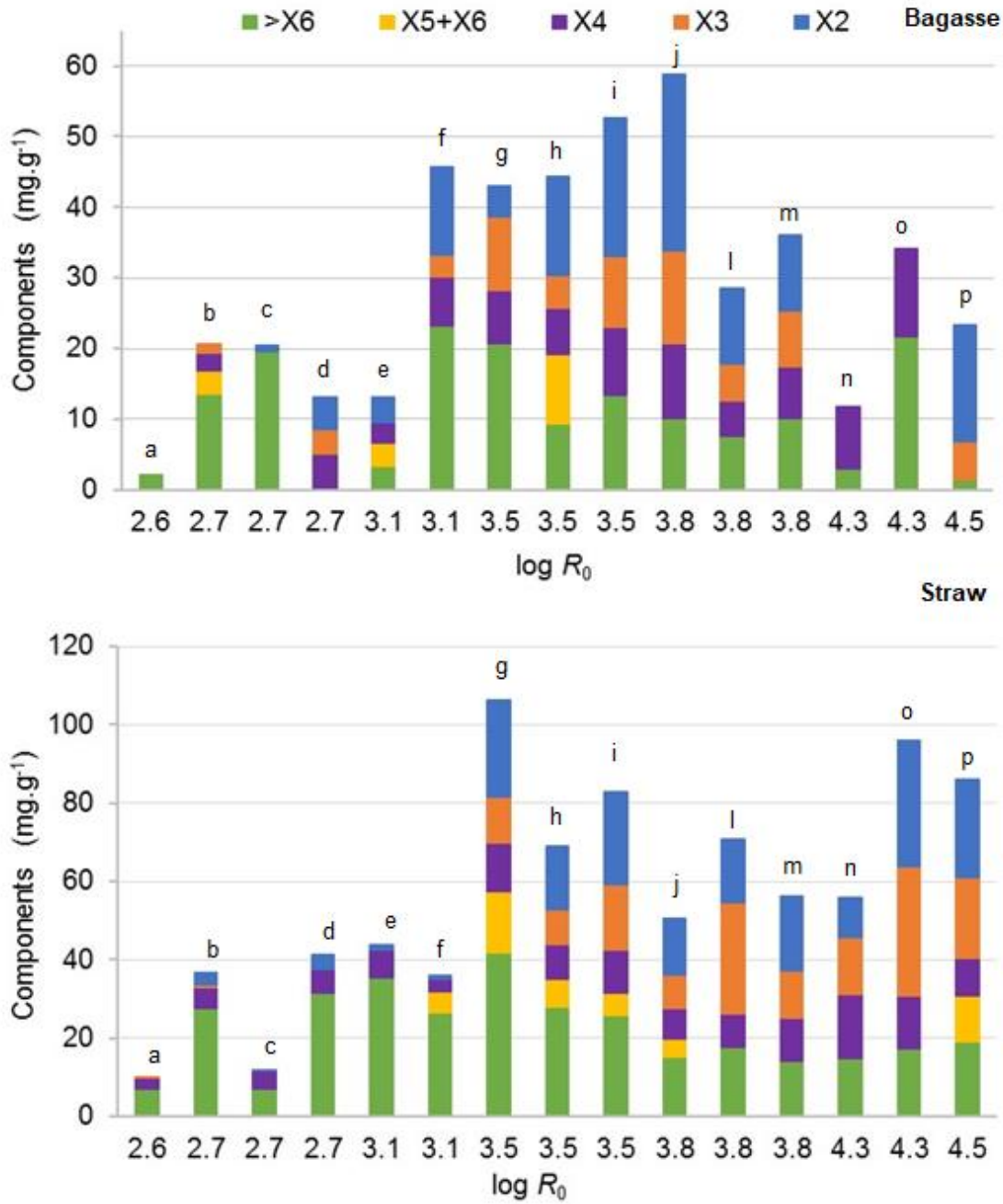

Figure 2.2. XOS contents in the SB and SS in HH obtained by HP as dependent on the severity factor (log R_0). The levels of components were presented in milligrams of product per gram of pretreated material (dry weight). The bars for each experiment are in increasing order of severity factor and follow the order 9, 1, 5, 11, 7, 3, 13, 14, mean of the central point (15, 16, and 17), 12, 6, 2, 8, 4 and 10 according to Table 2.1, which correspond to the letters “a” to “p” that are on the bars, respectively.

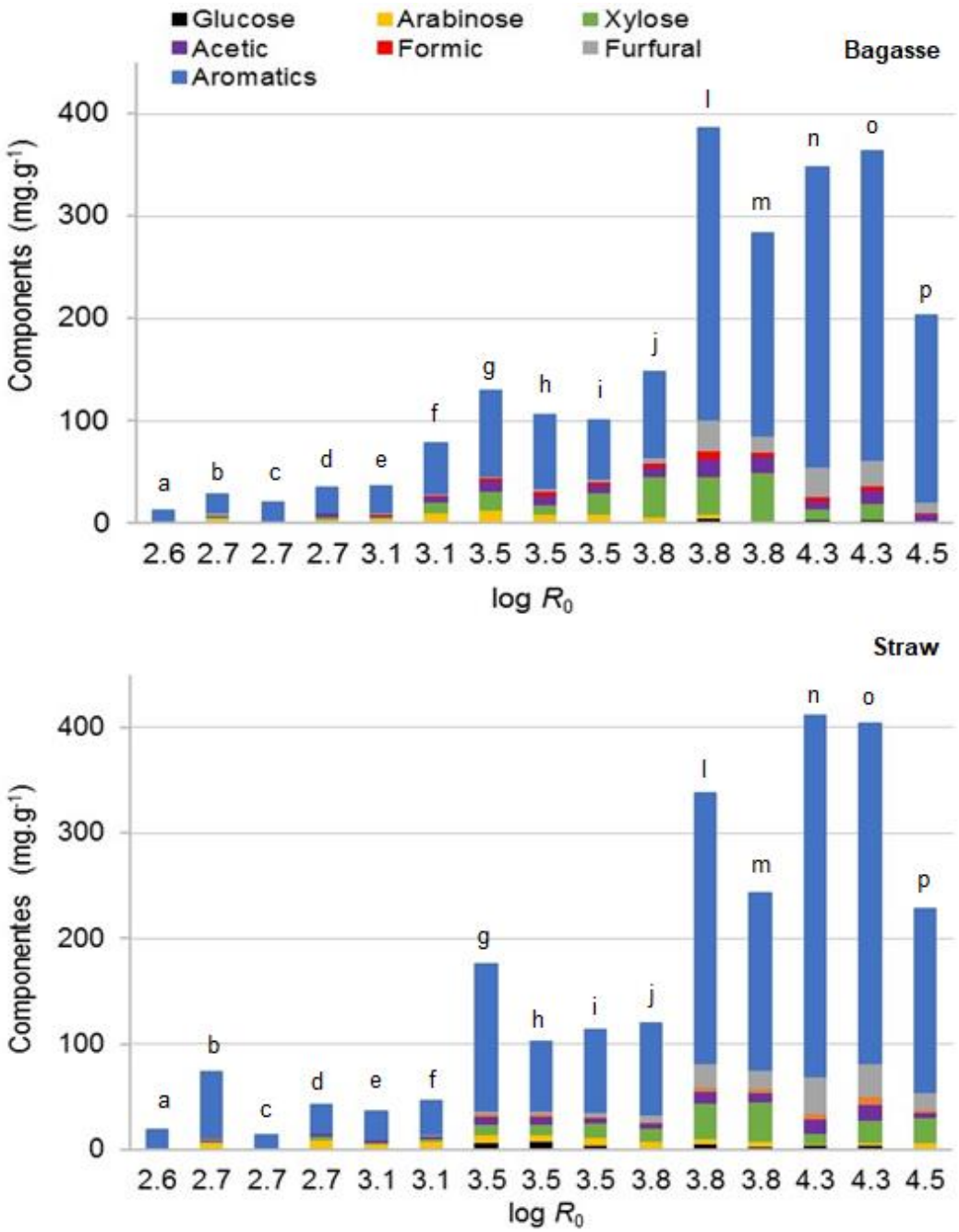

Figure 2.3. Unwanted products contents in the SB and SS in HH obtained by HPas dependent on the severity factor (log R_0). The levels of components were presented in milligrams of product per gram of pretreated material (dry weight). The bars for each experiment are in increasing order of severity factor and follow the order 9, 1, 5, 11, 7, 3, 13, 14, mean of the central point (15, 16, and 17), 12, 6, 2, 8, 4 and 10 according to Table 2.1, which correspond to the letters “a” to “p” that are on the bars, respectively.

The total XOS formed in the SB and SS HH ranged from 2.2 to 58.9 mg.g^{-1} and 9.9 to 106.48 mg.g^{-1} (dry weight), respectively. The intermediate conditions of SB and SS by-products (160 °C, 60 min, log R_0=3.5) resulted in a peak of XOS production of 52.6 and 78.7 mg.g^{-1} (dry weight), respectively. In the most severe conditions of SB (log R_0≥3.8), there was a decrease in the formation of XOS, resulting in an average of 23.1 mg.g^{-1} (dry weight). However, under drastic conditions of SS, the formation of XOS remained at the same level as the intermediate condition (average of 79.5 mg.g^{-1}, dry weight; Figure 2.2). The formation of monomeric sugars and mainly soluble aromatics in HH increased substantially under the most severe HP conditions (log R_0≥3.8) (Figure 2.3).

In general, all HP conditions promoted the formation of XOS, with a greater degree of polymerization (>X_6) for SB and SS. The range of XOS in SB and SS with the greatest degrees of polymerization (>X_6) was 1.3 to 23.3 mg.g^{-1} and 6.9 to 41.8 mg.g^{-1} (dry weight), respectively. The center point of experimental planning (conditions 15, 16, and 17; 160 °C, 60 min, log R_0=3.5; Table 2.1) averaged 13.3 and 25.8 mg.g $^{-1}$ (dry weight), respectively, with a high degree of polymerization (>X_6). The contents of XOS with a high degree of polymerization (>X_6) averaged 56% (for SB) and 72.5% (for SS) of the total XOS in the milder HP conditions (log R_0<3.5). However, in the intermediate and severe conditions (log R_0≥3.5), there was a greater formation of XOS with a lower degree of polymerization ((X_6 + X_5) + (X_4) + (X_3) + (X_2)) (Figure 2.2).

Hongdan et al. (90) reported XOS production ranging from 7.9 to 137.6 mg.g^{-1} using SB pretreated with hot water (HP). The study was performed in

conditions ranging from 160 °C for 20 min (log R_0=3.0) to 200 °C for 20 min (log R_0=4.2). The reported variation in XOS production exceeded than that observed in the present study. However, the aforementioned study used higher temperatures and shorter reaction times.

Zhang et al. (91) reported an XOS range of 40 to 146.8 mg.g^{-1} using SB when HP was performed using seawater at 165 °C for 10 min (log R_0=2.9) and 175 °C for 30 min (log R_0=3.6). These yields exceeded those observed in the present study. It could be relevant that the seawater contained elements such as iron, magnesium, and calcium, which would promote a weakly acidic environment for xylan degradation.

Rocha et al. (92) reported a variation of 1.2 to 122.5 mg.g^{-1} in the XOS production following HP of SS at temperatures ranging from 180 to 210 °C, for 5 to 40 min. These findings corroborate the results in the present study.

Brenelli et al. (93) investigated the production of XOS using mild deacetylation, followed by HP in SS at temperatures ranging from 170 to 210 °C, and times ranging from 5 to 25 min and reported a variation of 5.2 to 123.8 mg.g^{-1} in XOS production, corroborating the results reported in the present study.

Xylohexaose + xylopentaose (X_6 + X_5) were not detected in all HP conditions. However, they were formed in greater amounts in the 13th and 14th planned experimental conditions (Table 2.1), with levels of 9.9 mg.g^{-1} (dry weight) in condition 14 of SB, and 15.7 and 7.0 mg.g^{-1} (dry weight) in condition 13 and 14, respectively, for SS. The production of xylotetraose and xylotriose went up to 12.7 and 13.1 mg.g^{-1} in SB, respectively, and ranged from 2.9 to

16.4 $mg.g^{-1}$ and 0.1 to 33.0 $mg.g^{-1}$ (dry weight) in SS, respectively. At the center point of experimental planning, SB produced 9.6 and 10.0 $mg.g^{-1}$ and SS produced 11.1 and 16.4 $mg.g^{-1}$ (dry weight) of xylotetraose and xylotriose, respectively. Xylobiose was produced in quantities ranging from 0 to 25.1 $mg.g^{-1}$ (dry weight) in the SB, with production of 19.7 $mg.g^{-1}$ at the central point. These results corroborated those of Zhang et al. (91), who obtained a yield of 19 $mg.g^{-1}$ of xylobiose with HP from SB at 165 °C for 70 min (log R_0=3.7). In SS, xylobiose production ranged from 0 to 32.5 $mg.g^{-1}$ (dry weight) and the production was 24.0 $mg.g^{-1}$ (dry weight) at the central point. The main products formed in the HH were soluble aromatics (derived from lignin), xylose (derived from xylan), and furfural (derived from the degradation of monomeric sugars) for both SB and SS, and these products were mainly formed at major severity factors (log R_0>3.5) (Figure 2.3). The levels of soluble aromatics ranged from 10.6 to 285.5 $mg.g^{-1}$ (dry weight) in SB and from 12.1 to 343.4 $mg.g^{-1}$ (dry weight) in SS. At milder conditions (log R_0<3.5 of the center point of experimental planning), HH had limited generation of degraded products derived from hemicellulose and lignin, SB and SS produced 58.7 and 77.4 $mg.g^{-1}$ (dry weight) of soluble aromatics, respectively.

There was a tendency of increased formation of monomeric sugars, especially xylose and arabinose, due to the increase in the severity factor (log R_0) for both SB and SS. This trend was found mainly at the most severe values of the severity factor (log R_0≥3.5), corroborating the most pronounced removal of the xylan fractions, arabinosyl, and acetyl groups after HP. However, the increase in the severity factors (log R_0=4.3–4.5, 180-193.6 °C, 90 min) resulted

in appreciable degradation of monomeric sugars; resulting in an increased content of furfural and formic acid in HH. The respective levels of xylose and arabinose ranged from 1.3 to 47.2 $mg.g^{-1}$ and 0.2 to 10.8 $mg.g^{-1}$ (dry weight) in SB, and 0.3 to 36.9 $mg.g^{-1}$ and 0.5 to 7.4 $mg.g^{-1}$ (dry weight) in SS. At the center point of experimental planning of HP of the by-products, SB and SS produced 21 and 14.4 $mg.g^{-1}$ (dry weight) of xylose, respectively. Arabinose contents in this condition were 8.1 $mg.g^{-1}$ (dry weight) in SB and 7.1 $mg.g^{-1}$ (dry weight) in SS (Figure 2.3).

There was also a tendency of increased formation of acetic acid in HH (0.7-17.4 $mg.g^{-1}$ in SB and 0.9–14.8 $mg.g^{-1}$ in SS, dry weight) and formic acid (0.3-6.7 $mg.g^{-1}$ in SB and 1.0-7.8 $mg.g^{-1}$ in SS, dry weight) due to the increase in the severity factor (log R_0). The exception was in the most severe condition of reaction time and temperature (193.6 °C, 60 min, and log R_0=4.5). In this condition, a slight drop in the content of these organic acids and an increase in the formation of products that were not quantified were evident. These findings indicated a more advanced oxidation process (Figure 2.3) (89). At the center point of experimental planning, 7.1 and 3.2 $mg.g^{-1}$ (dry weight) of acetic acid and formic acid, respectively, were produced in SB, and 4.3 and 2.1 $mg.g^{-1}$ (dry weight), respectively, were produced in SS. The levels of furfural ranged from 0.1 to 30.8 $mg.g^{-1}$ (dry weight) in SB and 0.1 to 34.2 $mg.g^{-1}$ (dry weight) in SS. At the center point of experimental planning of HP of both by-products, SB and SS produced 2.1 and 4.1 $mg.g^{-1}$ (dry weight) of furfural, respectively (Figure 2.3).

The glucose levels ranged from 0.1 to 4.6 $mg.g^{-1}$ (dry weight) in SB and 0.3 to 8.3 $mg.g^{-1}$ (dry weight) in SS. At the center point of experimental planning, 1.3 $mg.g^{-1}$ (dry weight) of glucose was produced in SB and 7.1 $mg.g^{-1}$ was produced in SS (Figure 2.3). These results were consistent with the chemical characterization of the solids recovered from the SB and SS (Figure 2.1). The main component that was removed was the hemicellulosic fraction in intermediate and severe conditions (log R_0>3.5). After HP, glucose content in HH was minimal.

Rocha et al. (92) reported higher levels of acetic, formic acid, and furfural (22.1, 23.8, and 54 $mg.g^{-1}$, respectively) under conditions with higher process severity, such as log R_0=4.4 (210 °C for 15 min) post HP of SS.

Hongdan et al. (90) reported similar results for the levels of acetic acid and furfural. The levels of these compounds increased in SB as the severity of HP increased. The maximum levels were reached in the most severe condition of HP (log R_0=4.8, 220 °C, 20 min). At 190 °C for 20 min (log R_0=3.9), the furfural content was 50 $mg.g^{-1}$ in contrast to the 30.8 $mg.g^{-1}$ (dry weight) obtained in the present study in HP carried out at 180 °C for 30 min (log R_0=3.8).

Figure 2.4 shows xylan conversion to XOS and by-products (xylose, furfural, and formic acid) as dependent on the severity factor (log R_0). The data are expressed as % (w/w) dry weight.

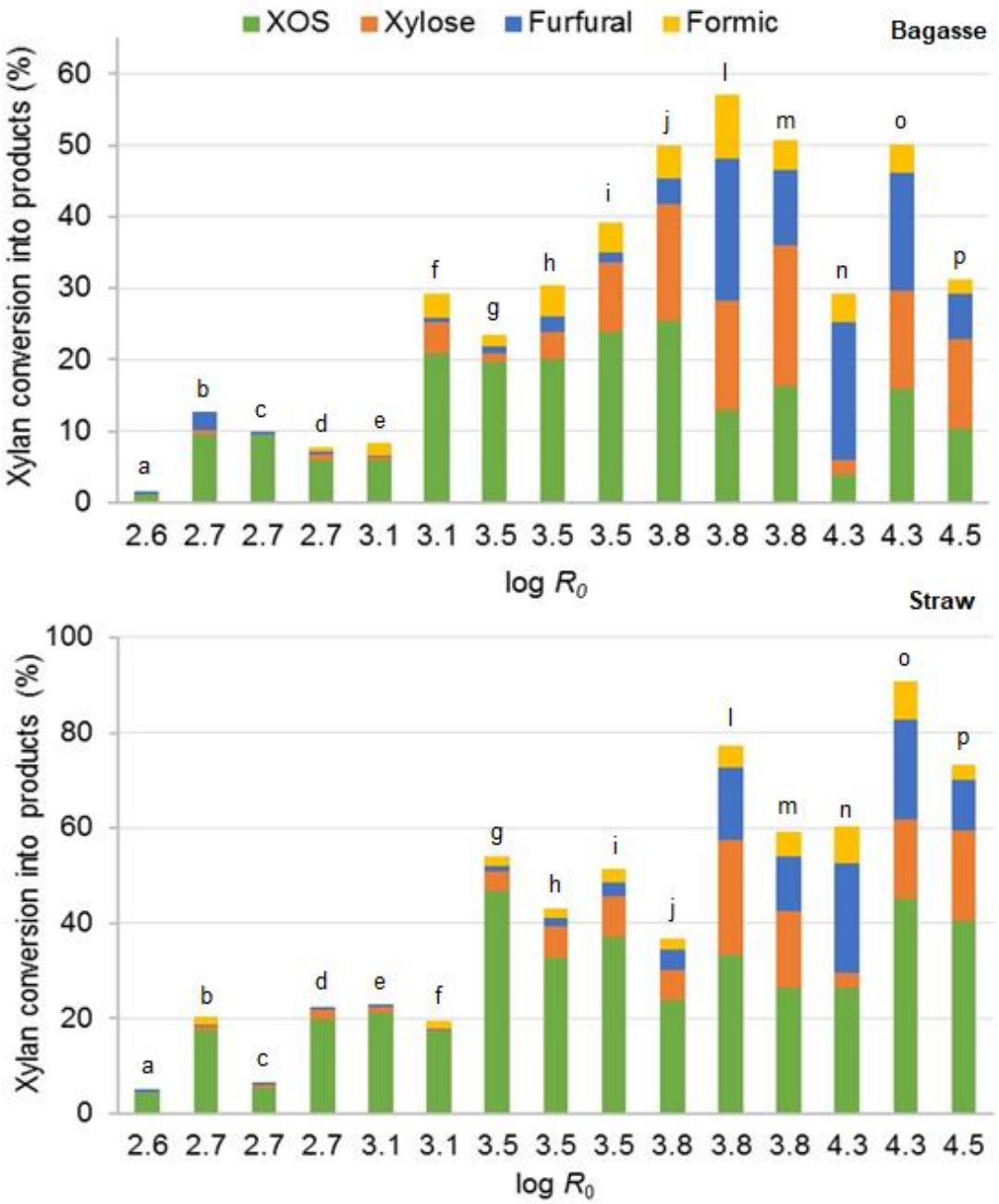

Figure 2.4. XOS and sugar degradated products in the HH obtained after HP under SB and SS as dependent on the severity factor (log R_0). Xylan conversion to products presented as percentage in dry weight. The bars for each experiment are in increasing order of severity factor and follow the order 9, 1, 5, 11, 7, 3, 13, 14, mean of the central point (15, 16 and 17), 12, 6, 2, 8, 4 and 10 according to Table 2.1, which correspond to the letters “a” to ”p” that are on the bars, respectively

In milder and intermediate conditions, there was more formation of XOS compared to that of the unwanted products. The average XOS at the center point of experimental planning was 23.9% (dry weight) for SB and 37.2% (dry weight) for SS. In this condition, xylose, furfural, and formic acid represented 9.9, 1.3, and 4.1% in SB, and 8.5, 2.7, and 2.7% in SS (dry weight), respectively. In the most drastic HP conditions (log R_0>3.8), the sum of degradation products exceeded that of XOS. In SB, the average production in drastic conditions (log R_0>3.8) were 10.1% (XOS), 9.4% (xylose), 14.1% (furfural), and 3.1% (formic acid); the respective production values in SS were 37.5, 12.8, 18.2, and 6.0% under the same conditions. Despite the relatively satisfactory production of XOS in the most drastic conditions of HP, mainly in SS, there was substantial formation of degradation products (Figure 2.4).

The mass recovery was performed with the objective of verifying whether the products formed and quantified in the HH constituted the entire biomass of the hemicellulosic fraction previously removed in HP. The percentage of xylan not converted to products was taken into account. These included the portion of xylan that remained in the solid fraction (hemicellulosic pulp), the products formed in the HH, and the unquantified products (unaccounted) (Figure 2.5).

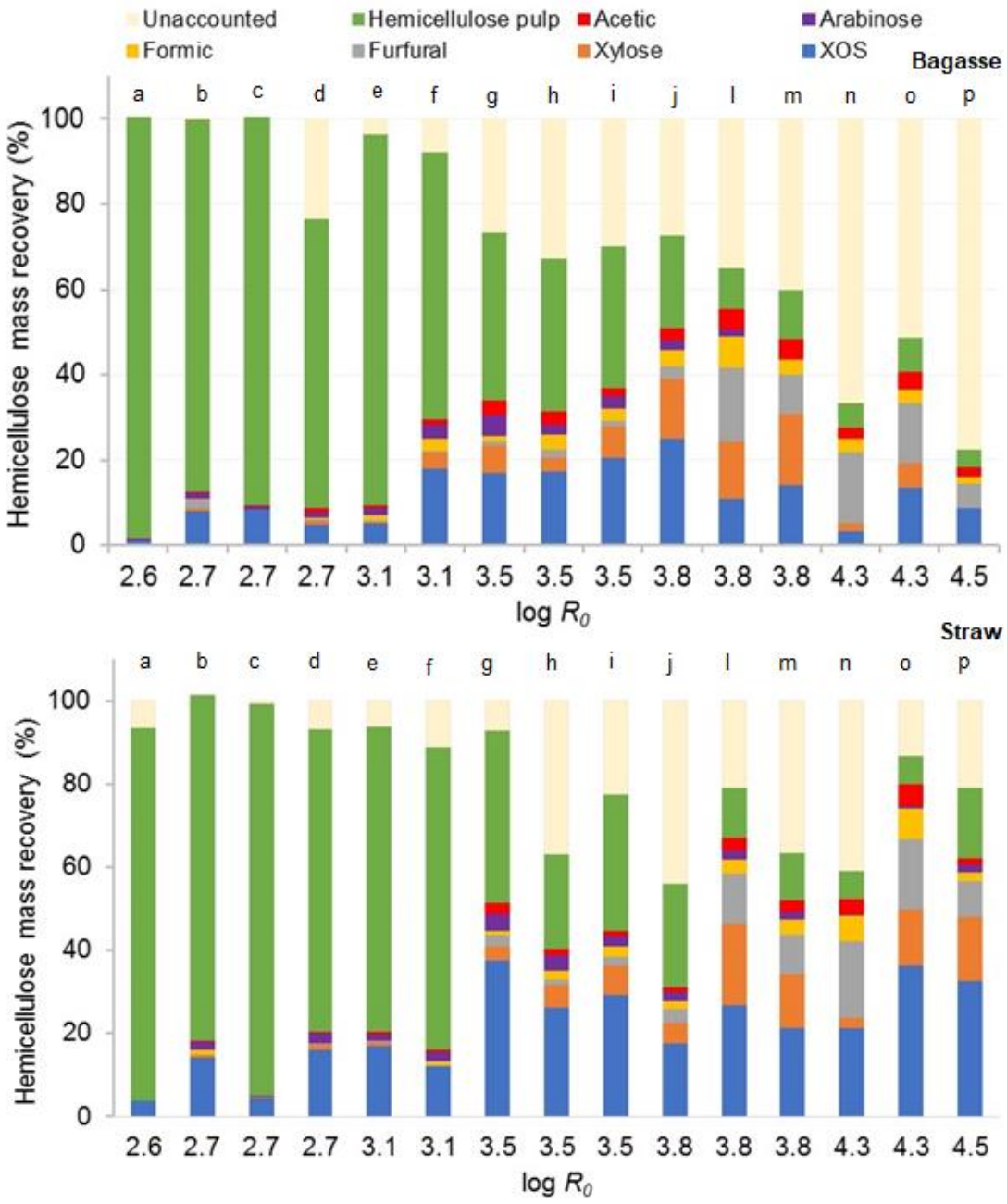

Figure 2.5. Mass recovery of the hemicellulosic fraction converted into products after the HP under SB and SS as dependent on the severity factor (log R_0). Components are presented as percentage in dry weight. The bars for each experiment are in increasing order of severity factor and follow the order 9, 1, 5, 11, 7, 3, 13, 14, mean of the central point (15, 16 and 17), 12, 6, 2, 8, 4 and 10 according to Table 2.1, which correspond to the letters “a” to “p” that are on the bars, respectively

Figure 2.5 shows that almost all products formed were quantified as the sum of the components came close to or reached 100% of the mass recovery

in the milder conditions of HP in SB and SS. In these conditions, a higher mass of the hemicellulose remained in the pulp and was not converted into products. In the HP of SB under the mildest conditions (log R_0<3.5), the mass of hemicellulosic pulp constituted 98.3 to 62.5% of the recovered mass of SB, and 89.4 to 72.8% of the recovered mass of SS.

In the intermediate and severe conditions of the experimental design (log R_0≥3.5), there was a decrease in the components recovered and in the hemicellulosic pulp. In contrast, there was an increase in the content of products formed in the HH, mainly XOS, in the intermediate conditions (log R_0=3.5) in both by-products. There was a greater formation of degradation products, especially xylose and furfural, under conditions whose severity exceeded log R_0=3.5. Thus, tendencies of decreased formation of XOS and increased amount of unaccounted products due to the increase in the severity factors (log R_0) were evident in both by-products. SB presented better xylan conversion to XOS under conditions where the severity varied from log R_0=3.1 to 3.8. An important loss of mass of unquantified products was apparent under severe HP conditions (log R_0>3.8), suggesting that the process of degradation products had become more advanced (94).

Xylan conversion to XOS and unaccounted products in SS were increased at severities above log R_0=3.1. However, in more severe HP conditions (log R_0>3.8) a decrease in the formation of XOS and an increase in the quantity of unaccounted products were not evident in SB.

Statistical analysis of the results from the central composite rotational design (CCRD) proposed for xylan conversion to xylooligosaccharides (XOS) by hydrothermal pre-treatments of sugarcane by-products

Tables 2.2 and 2.3 show the calculated values of the effects and coefficients of the mathematical models, as well as the corresponding standard deviations, *t* and *p* statistics for the CCRD applied to the HP of SB and SS, respectively, with xylan conversion to XOS as the response variable.

Table 2.2. Effects and coefficients of the model calculated for the CCRD having as response variable the xylan conversion to XOS after HP of SB.

Factor	Effect	SDE	τ	p	−95%	+95%	Coefficient	SDC
Mean	23.9956	1.484109	16.16836	0.003804	17.6100	30.38121	23.99560	1.484109
x_1	2.7130	1.394716	1.94518	0.191172	−3.2880	8.71395	1.35649	0.697358
x_1^2	−13.6531	1.536527	−8.88570	0.012430	−20.2643	−7.04197	−6.82656	0.768263
x_2	5.4531	1.394716	3.90984	0.059625	−0.5479	11.45410	2.72656	0.697358
x_2^2	−5.3303	1.536527	−3.46903	0.073992	−11.9414	1.28088	−2.66513	0.768263
x_3	−4.3603	1.394716	−3.12627	0.088886	−10.3612	1.64071	−2.18013	0.697358
x_3^2	−3.5661	1.536527	−2.32088	0.146047	−10.1772	3.04504	−1.78305	0.768263

SDE=standard deviation of the effect; τ=t-statistic of Student; p=p-value of significance probability; ±95%=confidence limits; SDC=standard deviation of the coefficient. X_1, X_2 e X_3 represent the coded temperature values (T), time (t) and consistency (C).

Table 2.3. Effects and coefficients of the model calculated for the CCRD having as response variable the xylan conversion to XOS after HP of SS.

Factor	Effect	SDE	τ	p	−95%	+95%	Coefficient	SDC
Mean	36.7731	2.852602	12.89108	0.001008	27.6949	45.85136	36.77311	1.484109
x_1	19.4282	3.093874	6.27957	0.008154	9.5821	29.27429	9.71410	0.697358
x_1^2	−11.2569	3.218079	−3.49802	0.039537	−21.4983	−1.01554	−5.62845	0.768263
x_2	4.0725	3.093874	1.31630	0.279609	−5.7736	13.91855	2.03623	0.697358
x_2^2	−12.5674	3.218079	−3.90526	0.029816	−22.8088	−2.32607	−6.28371	0.768263
x_3	−6.0435	3.093874	−1.95339	0.145791	−15.8896	3.80256	−3.02177	0.697358
x_3^2	0.8744	3.218079	0.27173	0.803457	−9.3669	11.11581	0.43722	0.768263

SDE=standard deviation of the effect; τ=t-statistic of Student; p=p-value of significance probability; ±95%=confidence limits; SDC=standard deviation of the coefficient. X_1, X_2 e X_3 represent the coded temperature values (T), time (t) and consistency (C).

In Table 2.2, the effects and model coefficients concerning xylan conversion to XOS after HP of SB show that the linear effect of temperature was not significant. This result was in contrast to the significant quadratic effect at a 95% confidence level. The variable time was marginally significant for both linear and quadratic effects. The main effects of mass load variable, and the effects of binary interactions were not significant (data not shown). The nonsignificant terms were excluded of the mathematical model. However, the linear and quadratic terms of temperature and time were maintained to allow the optimization of the response variable (95).

The statistical analysis of the results from CCRD for XOS production from xylan conversion by HP of SS showed that the linear and quadratic effects of temperature were significant at a 95% confidence level (Table 2.3). For the variable time, only the quadratic effect was significant. However, the linear term in x_2 was included in the model because the presence of the quadratic term required the inclusion of the corresponding linear term for optimization purposes, as previously discussed. Concerning the mass load variable, the linear and quadratic effects were not significant and were excluded from the mathematical model, as previously done for SB (95). The effects of binary interactions were also not significant.

According to Tables 2.2 and 2.3, the fitted model equations describing the percentage of xylan conversion to XOS for SB and SS as a function of the coded variables of temperature and time are:

$$XOS = b_0 + b_1x_1 + b_2x_1^2 + b_3x_2 + b_4x_2^2 \quad \textbf{(2)}$$

$$XOS = b'_0 + b'_1x_1 + b'_2x_1^2 + b'_3x_2 + b'_4x_2^2 \quad \textbf{(3)}$$

In Eqs. (2) and (3), x_1 and x_2 are the linear terms of temperature (°C) and time (min), respectively, and x_1^2 and x_2^2 are the respective quadratic terms for these variables. In Eq. (2), b_0 = 23.99560, b_1 = 1.35649, b_2 = -6.82656, b_3 = 2.72656, and b_4 = -2.66513. In Eq. (3), b_0 = 36.77311, b_1 = 9.71410, b_2 = -5.62845, b_3 = 2.03623, and b_4 = -6.28371.

Tables 2.4 and 2.5 present the analysis of variance (ANOVA) results for the model fit to the SB and SS data, respectively. The validity of the fitted models can be assessed using the *p* test and the determination coefficient (R^2). The lack of fit of the mathematical models was nonsignificant at the 95% confidence level, demonstrating that the developed models were statistically adequate to represent the experimental data obtained. Moreover, the models explained 66% of the variation around the mean (R^2=0.66) for SB and 84% (R^2=0.84) for SS.

Table 2.4. ANOVA for the model fit to the experimental data of xylan conversion from SB to XOS after HP

Factor	SS	df	MS = SS/df	*F*	*p*
x_1	25.107	1	25.1072	3.78373	0.191172
x_1^2	523.916	1	523.9158	78.95561	0.012430
x_2	101.437	1	101.4372	15.28687	0.059625
x_2^2	79.854	1	79.8537	12.03418	0.073992
x_3	64.853	1	64.8533	9.77358	0.088886
x_3^2	35.742	1	35.7425	5.38650	0.146047
Lack of fit	360.408	8	45.0510	6.78932	0.134669
Pure error	13.271	2	6.6356		
Total SS	1096.115	16			
$R^2 = 0.659$					

X_1, X_2 e X_3 represent the coded temperature values (T), time (t) and consistency (C).

Table 2.5. ANOVA for the model fit to the experimental data of xylan conversion from SS to XOS after HP.

Factor	SS	df	MS = SS/df	*F*	*p*
x_1	1287.574	1	1287.574	39.43302	0.008154
x_1^2	399.537	1	399.537	12.23614	0.039537
x_2	56.575	1	56.575	1.73264	0.279609
x_2^2	497.980	1	497.980	15.25104	0.029816
x_3	124.592	1	124.592	3.81572	0.145791
x_3^2	2.411	1	2.411	0.07384	0.803457
Lack of fit	332.170	8	41.521	1.27162	0.465604
Pure error	97.957	3	32.652		
Total SS	2727.137	17			
$R^2 = 0.842$					

X_1, X_2 e X_3 represent the coded temperature values (T), time (t) and consistency (C).

In Tables 2.2 and 2.3, the time and temperature variables had positive linear effects on xylan conversion to XOS. The quadratic effects of these variables were negative for this response. The findings indicated that increases in reaction time and temperature can, to a certain extent, lead to the maximum xylan conversion to XOS. Additional increases in these variables

beyond this maximum point would result in a loss of efficiency in the formation of XOS.

Figure 2.6 shows the response surfaces and contour curves generated by the mathematical models developed for the HP of SB and SS. The mass load variable was fixed at the value corresponding tothe center point of experimental planning (x_3=0) because it was not significant in the experimental range studied.

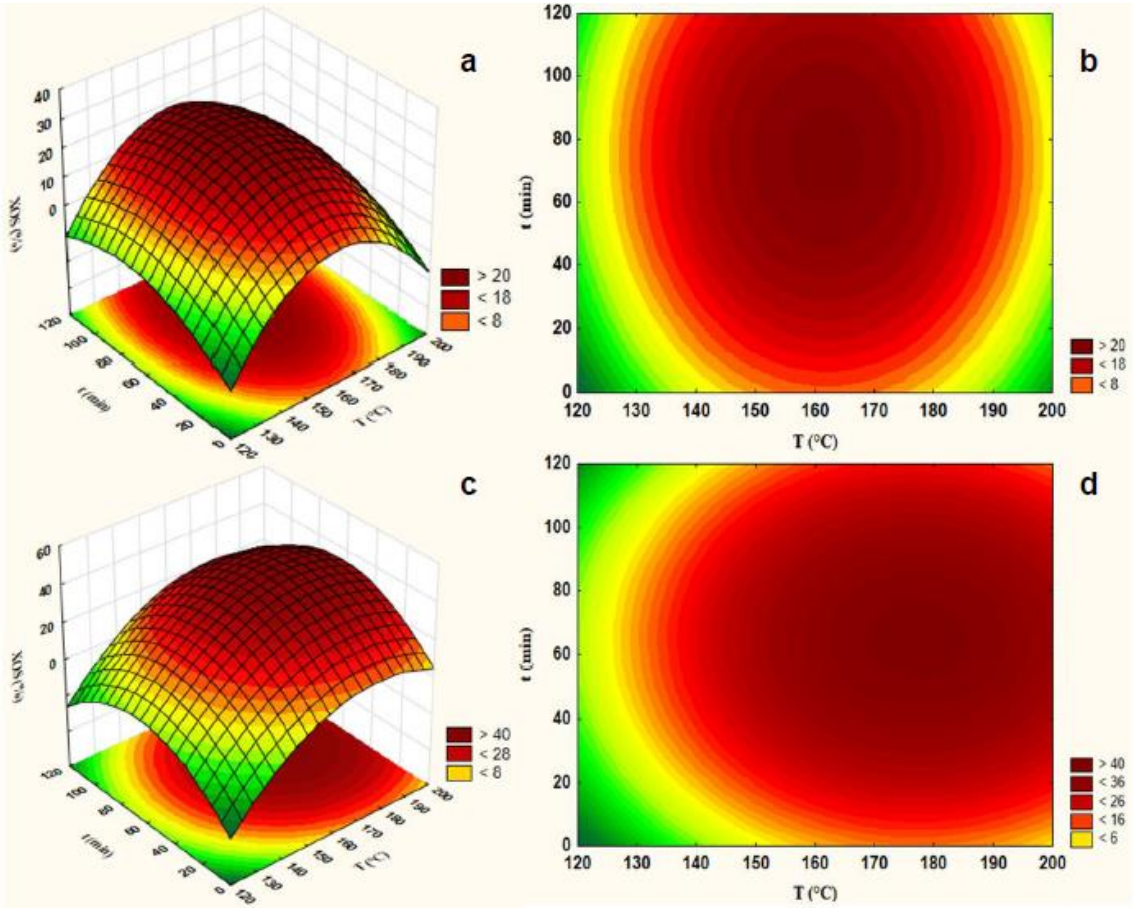

Figure 2.6. Response surfaces and contour curves for the xylan conversion to XOS from SB (ab) and SS (cd) as dependent on the temperature and time (mass load = 10%, w/v)

As expected, the response surface displayed a downward concavity and a point of maximum XOS production for both by-products. For SB, this point

was located at temperatures varying from160 to 170 °C, and reaction times of 60 to 80 min. For SS, maximum XOS conversion occurred at temperatures varying from 170 to 180° C, and reaction times of 60 to 80 min. The point of maximum conversion in XOS for the HP of both by-products was analytically determined by partially deriving Equations 2 and 3 with respect to variables x_1 and x_2. In making these derivatives equal to zero, two algebraic equations were obtained and solved in x_1 and x_2. Table 2.6 shows the coded and real values of time and temperature at the predicted point of maximum conversion into XOS for both by-products.

Table 2.6. Optimal values of the independent variables for the HP owing to maximization of XOS production from SB and SS.

By-product	Coordinates of the optimum points in coded values		
	X_1 (-)	X_2 (-)	X_3 (-)
Bagasse	0.098	0.51	0
Straw	0.86	0.16	0
	Coordinates of the optimum points in actual values		
	T (°C)	**t (min)**	**C (%)**
Bagasse	161.98	75.34	10.00
Straw	177.25	64.86	10.00

X_1, X_2 e X_3 represent the coded temperature values (T), time (t) and consistency (C). T=temperature; t=time and C=consistency.

Experimental validation of the mathematical models and chemical characterization of the obtained fractions

The optimum experimental conditions determined by the mathematical models for xylan conversion to XOS were evaluated experimentally for both by-products. Figure 2.7 shows the images of the recovered solids (insoluble fractions) and HH (soluble fractions) for SB and SS after HP under optimized conditions.

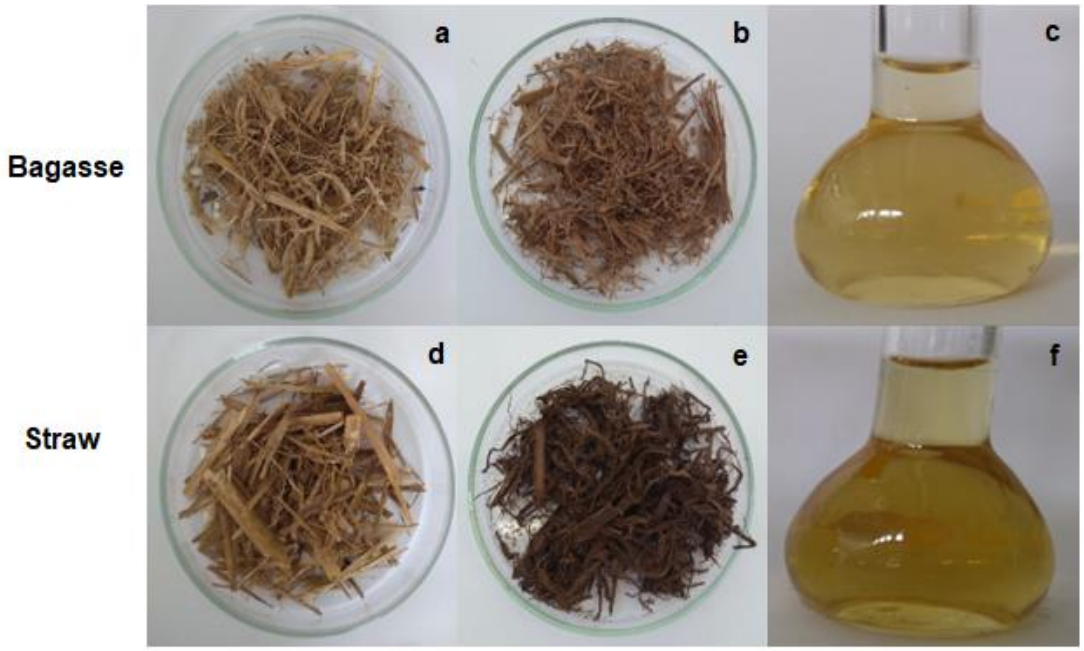

Figure 2.7. Untreated SB and SS (ad); Recovered solids after HP in SB and SS under optimized conditions (be); HH from SB and SS after HP under optimized conditions (cf), respectively

For both by-products, a darkening of the recovered solids was observed (Figure 2.7a, b, d, e). Darkening in the solids of SS was more intense. As previously discussed, this effect is assigned to the formation of derived from lignin on the cellulose surface (pseudo-lignin). The optimized conditions for SS resulted in a greater severity factor than that of SB (Table 2.7). As a consequence, higher contents of products derived from lignin and polysaccharide were present, which may explain the more intense browning. The HHs were yellow (Figure 2.7c, f), as previously discussed, this appearance is assigned to the formation of phenolic or aromatic generated from lignin (e.g., formation of quinones), or also to the formation of products originated from the decomposition of xylan and cellulose. These findings were similar to those reported for grasses and hardwood, as well as for recovered solids and HH obtained by HP from these materials (12,60,82,85–87).

The coded values of time and temperature referring to the optimal points were inputed in Equations 1 and 2 to calculate the xylan conversion to XOS calculated by the models. The mass load variable was fixed at the center point of the fatorial planning (10% w/v). The severity factor values calculated for the optimized conditions of time and temperature were log R_0=3.7 for SB and log R_0=4.0 for SS (Table 2.7). The experimental xylan conversion to XOS in the SB was 24.8%, identical to the value calculated by the mathematical model. The experimental xylan conversion to XOS for SS was 45.3%, with a predicted value of 41.1% (Table 2.7), resulting in an acceptable error of the mathematical model of 9.8%. Thus, the developed mathematical models proved to be adequate and valid to predict the xylan conversion to XOS after HP of the sugarcane by-products.

Table 2.7. Calculated and experimental values of xylan conversion to XOS for the HP of SB and SS under optimized conditions (data presented in percentage in dry weight).

By-product	Variables of PTH			Xylan conversion into XOS (%)		
	T **(°C)**	***t*** **(min)**	***C*** **(%)**	**Predicted XOS values (%)**	**Experimental XOS values (%)**	**Error (%)**
Bagasse	161.98	75.34	10.00	24.76	24.75	-
Straw	177.25	64.86	10.00	41.12	45.34	9.79

T=temperature; t=time and C=consistency.

The recovered solids from the SB and SS after HP under the optimized conditions were chemically characterized in terms of cellulose, xylan, arabinosyl groups, acetyl groups, and lignin (Table 2.8).

Table 2.8. Yield and chemical composition of SB and SS untreated, thermotreated under the optimized conditions and the HP mass balance in recovered solids. Components contents presented as percentage in dry weight.

	By-product	HP yield (%)	Components of material				
			Cellulose (%)	Xylan (%)	Arabinosyl groups (%)	Acetyl groups (%)	Lignin (%)
Untreated	**Bagasse**	-	32.3 ± 0.3	21.2 ± 0.2	1.3 ± 0.0	2.1 ± 0.2	25.1 ± 0.3
	Straw	-	33.8 ± 0.4	20.4 ± 0.3	2.2 ± 0.0	2.7 ± 0.3	25.3 ± 0.3
HP raw material	**Bagasse**	74.9	39.4 ± 2.1	12.6 ± 0.7	0.3 ± 0.0	0.7 ± 0.0	30.6 ± 0.7
	Straw	59.5	47.3 ± 6.0	6.9 ± 1.0	0.1 ± 0.0	0.1 ± 0.1	38.9 ± 1.1
HP mass balance	**Bagasse**	74.9	29.5 ± 1.6	9.5 ± 0.5	0.2 ± 0.0	0.5 ± 0.0	22.9 ± 0.5
	Straw	59.5	28.1 ± 3.6	4.1 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	23.1 ± 0.7

**Extractives in the untreated sugarcane bagasse and straw=2.8% (w/w, dry weight). The pretreated samples did not present significant extractive contents.*

The SB and SS yield was 74.9% and 59.5% (w/w, dry weight), respectively. After the HP, the removal of cellulose, xylan, arabinosyl, acetyl, and lignin in SB was 9.0, 55.2, 76.2, 84.6, and 8.8%, respectively. However, cellulose from SB did not show significant removals (Tukey test). The removal of cellulose, xylan, arabinosyl, acetyl and lignin in SS was 11.7, 77.9, 95.5, 96.3, and 8.7%, respectively.

After the HP of SS, there was greater solubilization of xylan compared to that in SB. These results are in agreement with the greater xylan conversion to products achieved by HP in SS more than that in SB, confirming the characterization results of the HH of the by-products (Figures 2.8 and 2.9). The higher hemicellulose removal in SS was possibly due to a lower pH during the HP resulting from the higher content of acetic acid present in the HH from straw (14.5 $mg.g^{-1}$) compared to the SB (6.5 $mg.g^{-1}$) (Figure 2.9). Hydronium ions of acid or water are released during HP, which promotes the depolymerization of the hemicellulosic fraction (96).

Figure 2.8 shows the distribution of XOS produced in the by-products under the optimized HP protocols. The total XOS production was 53.3 $mg.g^{-1}$ for SB and 96.3 $mg.g^{-1}$ for SS. The SB produced 4.6 $mg.g^{-1}$ (xylobiose), 4.8 $mg.g^{-1}$ (xylotriose), 9.1 $mg.g^{-1}$ (xylotetraose), and 34.8 $mg.g^{-1}$ ($>X_6$), resulting in a total of 53.3 $mg.g^{-1}$ (dry weight). The SS produced 22.3 $mg.g^{-1}$ (xylobiose), 40.9 $mg.g^{-1}$ (xylotriose), 16.8 $mg.g^{-1}$ (xylotetraose) and 16.2 $mg.g^{-1}$ ($>X_6$), for a total of 96.2 $mg.g^{-1}$ (dry weight). The SB produced more XOS with a greater degree of polymerization ($>X_6$) than SS, which produced more xylotriose and xylobiose. Both the by-products did not produce xylopentaose or xylohexaose (X_5+X_6).

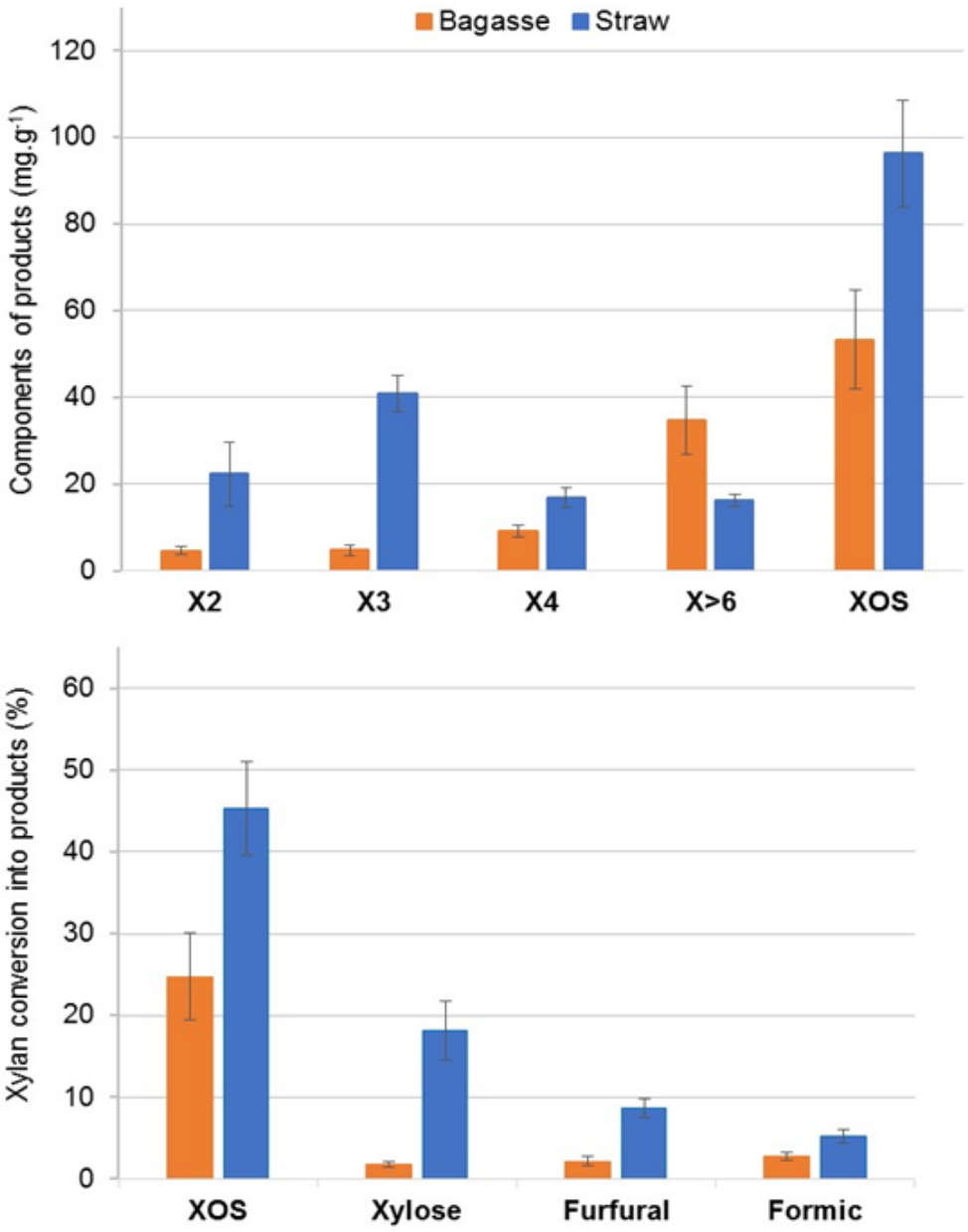

Figure 2.8. XOS contents in the SB and SS in HH after HP under optimized conditions. Xylan conversion to products presented as percentage in dry weight.

Figure 2.8 shows xylan conversion to XOS, xylose, furfural, and formic acid products. Xylan conversion to xylose, furfural and formic acid in SB was 1.8, 2.1, and 2.7%, respectively. In SS, the respective rates of xylan conversion to xylose, furfural and formic acid were 18.0, 8.7, and 5.2%, respectively. XOS

production in SS was superior (45.3%) compared to that in SB (24.8%). However, the content of degradation products was also considerably higher in SS compared to SB. The optimized conditions for SS resulted in a high severity factor (log R_0=4.0), where there was a higher production of degradation products compared to SB. In SB, where there was a low formation of degradation products, the severity factor (log R_0) approached the severity of the central point of the fatorial plan (log R_0=3.7).

In addition to the XOS content, the unwanted products (xylose, glucose, arabinose, acetic acid, formic acid, soluble aromatics, and furfural) in HH were chemically characterized (Figure 2.9).

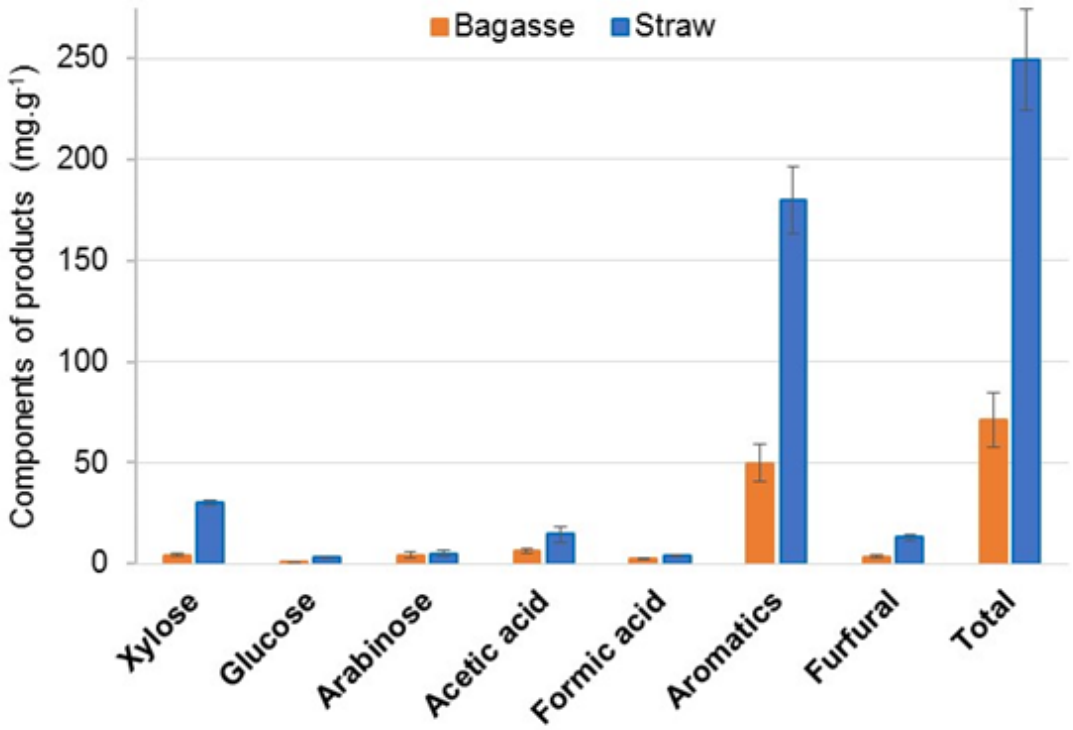

Figure 2.9. Unwanted products contents in the SB and SS in HH after HP under optimized conditions (data in milligrams of products per gram of raw material, dry weight). Total=xylose+glucose+arabinose+acetic acid+formic acid+aromatics+furfural.

The levels of the aforementioned unwanted products in the HH of SB after HP were 4.0, 0.6, 4.4, 6.5, 2.3, 49.9, and 3.4 $mg.g^{-1}$, respectively, representing a total of 71.1 $mg.g^{-1}$. The respective levels of these components in the HH of SS after HP were 30.1, 2.9, 5.1, 14.5, 3.9, 179.8, and 12.9 $mg.g^{-1}$, respectively, representing a total of 249.4 $mg.g^{-1}$. The findings indicate that more xylose, acetic acid, aromatics, and furfural were solubilized in SS (Figure 2.9).

Hongdan et al. (90) reported a 24% xylan conversion to XOS under HP in SB at 160 °C for 20 min (log R_0=3.1), which corresponds to 59 $mg.g^{-1}$ (mg of XOS per gram of substrate). The prior findings corroborated the result obtained in the present study. However, the HP reaction time for this study was 162 °C for 175 min (log R_0=3.7) (Figure 2.8).

Zhang et al. (91) reported 67% for xylan conversion to XOS (corresponding to 146.8 mg. g^{-1}) and 17.8 $mg.g^{-1}$ of xylose in HP with natural seawater for SB obtained under severe conditions of 175 °C for 30 min (log R_0=3.7). These results are higher than those obtained in the present study. The difference reflects bagasse catalysis by the chloride ion -metallic ion system (hydronium ion) that occurs naturally in seawater.

Under the same operational conditions as those in the present, but with fresh water, Zhang et al. (91) reported a 34.7% xylan conversion to XOS, which is close to that obtained in the present study with SB (24.8%, Figure 2.8).

Zhang et al. (97) reported a 31.4% xylan conversion to XOS (which corresponds to 27.9 $mg.g^{-1}$) from the acetyl-assisted automatic hydrolysis of SB performed at 160 °C for 70 min (log R_0=3.6). The contents of the individual

XOS were 5.9 mg.g^{-1} xylobiose (X_2), 6.4 mg.g^{-1} xylotriose (X_3), 5.3 mg.g^{-1} xylotetraose (X_4), and 10.5 mg.g^{-1} xylopentaose (X_5). The values corroborated the contents found in the present study.

Rocha et al. (92) reported a 59.5% xylan conversion to XOS (which corresponds to 119.1 mg.g^{-1}) in HP of SS at 180 °C for 40 min (log R_0=3.9); this reported value is slightly higher than the 41.1% XOS conversion (corresponding to 96.3 mg.g^{-1}) found in the present study with straw treated at 177 °C for 65 min (log R_0=4.1) (Figure 2.8). However, the analytical method reported by the previous authors was different from that used in the present study. In the study by Rocha et al. (92), the XOS calculation was performed as the difference between xylose contents present in HH from HP and in HH after complete acid hydrolysis. This method is less accurate than the method used in the present study. The unwanted products xylose, formic acid, acetic acid, and furfural reported previously (20.3, 9.4, 9.0, and 9.0 mg.g^{-1}, respectively) are only slightly higher or lower than those found in this study (Figure 2.8).

Brenelli et al. (93) reported a xylan conversion to XOS of 29.2% (corresponding to 91.3 mg.g^{-1}) for SS after alkaline deacetylation and HP under experimental conditions of 180 °C for 25 min (log R_0=3.7). The xylan conversion to XOS in this study resulted in a larger conversion at 177 °C for 65 min (log R_0=4.1), with a 41.1% of xylan conversion to XOS (Figure 2.8). Furthermore, previous utilized higher temperatures and shorter times, which could generate more degradation products.

These results were confirmed by the material balance data, in which the hemicellusic pulp mass represented the hemicellulosic fraction that was not

converted into products. These were added to the components quantified in the HH, which comprised the recovered mass, and the difference was the non-quantified components. In SB, almost half of the hemicellulose was not converted into products (49.8%). In SS, only 17.4% was not converted. Thus, XOS was produced in SS. However, more degradation products were also formed in SS, including xylose and furfural (Table 2.9).

Table 2.9. Mass recovery, hemicellulose mass in the pulp, products not found, degradation products and XOS in the hemicellulosic fraction of SB and SS under optimized HP conditions.

By-product	XOS (%)	Xylose (%)	Furfural (%)	Formic (%)	Arabinose (%)	Acetic acid (%)	Pulp hemicellulose mass (%)	Mass recovery (%)	Difference (%) - not found
Bagasse	21.3	2.2	1.6	2.4	1.8	2.1	49.8	81.1	18.9
Straw	38.7	14.6	7.0	3.9	2.0	3.7	17.4	87.3	12.7

CONCLUSION

The optimized conditions for maximum XOS production for SB and SS were temperature of 161.9 and 177.2°C for 75.3 and 64.8 min, respectively, with the mass load of 10% for both conditions. HP in SB and SS under these conditions produced 53.3 and 96.3 mg of XOS per gram of the by-products, respectively. In SB, production of XOS was greater with a greater degree of polymerization (>X_6), totaling 34.8 mg.g^{-1}. In SS, production of xylotriose and xylobiose was greater, adding 40.9 mg.g^{-1} and 22.3 mg.g^{-1}, respectively. Although SS produced more XOS than SB, it generated more soluble aromatics, xylose, and furfural, representing 179.8, 30.1 and 12.9 mg.g^{-1} compared to 49.9, 4.0 and 3.4 mg.g^{-1} produced by thermotreated bagasse, respectively. Thus, the HH of SB and SS must be refined further for future

bioformulations. Sugarcane by-products are strategic for the production of XOS, which has pronounced potential for commercialization and high-added value. Furthermore, the recovered solid fraction that is enriched in cellulose and lignin obtained from HP under optimized conditions can be fractionated and recovered to obtain bioproducts of commercial interest.

DECLARATIONS SECTION

List of abbreviations

(HPLC) High performance liquid chromatography system. (C_{18}) reverse phase column HYPERSIL C_{18}. (SB) Sugarcane bagasse. (SS) Sugarcane straw (XOS) Xylooligosaccharides. (HP) Hydrothermal pretreatment. (X_1) Xylose. (X_2) Xylobiose. (X_3) Xylotriose. (X_4) Xylotetraose. (X_5) Xylopentaose. (X_6) Xylohexaose. (X_7) Xyloheptaose. (X_8) Xylooctaose. (X_9) Xylonanose. (>X_6) Oligosaccharides with more than six repetitive units. (log R_0) Severity factor. (CCRD) Central composite rotational design. (HH) Hemicellulosic hydrolysate. (NaOH) Sodium hydroxide.

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

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Authors' contributions

LMD and FN performed HP in SB and SS and chemically characterized the recovered liquid and solid fractions. LMD, FN, MB, SCO and FM participated in the design of the experiments, data interpretation and mathematical modeling. LMD, FN, SCO, MB and FM participated in the reviewed the manuscript and data interpretation. All authors read and approved the final manuscript.

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3. CAPÍTULO 2

Efficiency of the enzymatic hydrolysis on sugarcane bagasse and straw thermotrated: Assessment in batch systems subject to high mass loads

This manuscript is in the final prepraration phase (final review of the discussion and English language).

Efficiency of the enzymatic hydrolysis on sugarcane bagasse and straw thermotrated: Assessment in batch systems subject to high mass loads

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ABSTRACT

Thermotreatment is a promising pretreatment because it is simple, low cost and environmentally friendly and facilitates the access of cellulases in the enzymatic hydrolysis step. Sugarcane bagasse (SB) was thermotreated at 161.9°C for 75.3 min and sugarcane straw (SS) at 177.2°C for 64.8 min, resulting in cellulose levels of 40.4 and 50.7%, respectively. Enzymatic hydrolysis of the thermotreated by-products resulted in higher cellulose conversions to glucose and concentrations than untreated by-products. Hydrolysis with an increase in mass load of 1, 5, 10, 20% were performed and SB and SS reached maximum conversions at consistency of 20% (63.6 and 73.1%) and maximum glucose concentration of 63.8 and 89.2 gL^{-1}, respectively. In assays with increased enzyme load of 15, 20 and 30 $FPU.g^{-1}$ the maximum cellulose conversions to glucose of SB and SS were 93.6 and 94.5%, resulting in glucose concentrations of 105.1 and 124.8 gL^{-1}, respectively. The analysis of the maximum rates of glucose formation estimated for the assays performed under different consistencies showed that there were significant differences between the tests, except between the assays performed at 1% and 5%, while the maximum rates of glucose formation estimated in the tests with varied enzymatic load showed that there were no significant differences, with the exception of an enzyme load of 30 FPU per gram of substrate (dry weight). The tests carried out in the bioreactor showed that there was rapid liquefaction of the biomass, both SB and SS, when compared to the Erlenmeyer flask. The maximum conversion and glucose concentration achieved were higher in the assay performed with the

SB in the bioreactor, but not with the SS, which may have occurred contamination, since sodium azide was not added to the bioreactor.

INTRODUCTION

The need to search for renewable sources for energy production in order to replace fossil fuels has been growing in recent decades and research for an ideal candidate has increased for this exchange. Lignocellulosic biomass is a promising feedstock to production of ethanol and replace fossil fuels, due to its abundance, versatility, low cost, low emission of greenhouse gases and is a renewable raw material (98).

Several types of lignocellulosic materials have been used for ethanol production and sugarcane is a variety widely used for first generation ethanol production (second-generation ethanol), which generates potentially promising by-products for conversion to cellulosic ethanol. The production of first-generation ethanol from sugarcane generates two types of waste: straw (SS) and bagasse (SB). After grinding the sugarcane stem to obtain the juice, SB is obtained, the residue of the stems after extracting the juice. SS is the dry or fresh leaves and tops (the part of the cane between the upper end and the last node of the stem) remaining in the field after harvest and has the function of preserving adequate soil cover. Part of the harvested SS can also be used as raw material for other industrial purposes (32,93).

Lignocellulosic biomass are renewable materials consisting mainly of polymer sugars; cellulose, hemicelluloses and lignin, which together form a complex structure naturally recalcitrant to enzymatic action. Thus, the biomass

conversion process for ethanol production requires an efficient pretreatment step before enzymatic hydrolysis and fermentation, to deconstruct the association between these polymers, disrupt the lignocellulosic structure and/or remove lignin (75,98,99).

Pretreatment is considered a crucial step in the cellulosic ethanol production process as it represents the main economic cost of the process and pretreatment methods include; chemical, physical, biological or the combination of these methods (100,101). The hydrothermal pretreatment is considered an environmentally friendly, green pretreatment, does not cause corrosion in equipment because it do not have chemical additives, the process uses water and lignocellulosic feedstocks as the only reagents, has simplicity of operation and a low cost in general (98,100). In the hydrothermal pretreatment the lignocellulosic biomass in predetermined water/solid proportions, is subjected to high temperatures and pressures for a certain time. Under these conditions, the water present in the medium forms hydronium ions that cleave the acetyl linkages forming acetic acid and the hydronium ions formed by the acetic acid promote the depolymerization of hemicellulose fraction, which is the main solubilized component and depending on the conditions of temperature, time and mass load, they promote the formation of xylooligosaccharides (XOS), monomeric sugars and aromatics, among other compounds (35). The solid residue generated, rich in cellulose, has potential value for the production of bioethanol, but needs to go through the enzymatic hydrolysis step to hydrolyze the β-1,4-D-glucan bonds of cellulose, releasing glucose, which can be directly fermentable (99).

As well as pretreatment, enzymatic hydrolysis is another step that hampers the viability of lignocellulosic ethanol production and an efficient cellulose conversion to glucose is crucial to make this process more achievable. Operational parameters such as enzyme load, mass load, shaking speed, additives added to the hydrolysate and hydrolysis time and temperature can be adjusted and optimized to achieve efficient conversion of carbohydrates to fermentable sugars (75). The increase in the mass load of lignocellulosic substrates (consistency) can be economically advantageous as it will result in a hydrolysate with a high concentration of glucose and consequently the final concentration of ethanol will be higher, not requiring an additional step of concentration of the hydrolysate. However, the increase in consistency can cause mass transfer problems, which can slow down the reaction, in addition to possible inhibition of cellulases by the high concentration of glucose. Adding additives has been a promising approach to improve enzymatic hydrolysis as it can minimize non-productive adsorption of enzymes to substrates (102–104).

Enzymatic hydrolysis can be performed in bioreactors with impellers more suitable for efficient homogenization of the reaction medium, preventing material from sticking to surfaces and minimizing mass transfer and enzymatic inhibition problems (103).

The present study aimed to evaluate different conditions of enzymatic hydrolysis, such as enzyme load and mass load, in a orbital shaker and in a stirred tank bioreactor in order to achieve the best production of fermentable sugars from thermotreated SB and SS sugarcane.

MATERIAL AND METHODS

Sugarcane bagasse and straw thermotreatment

The SB and SS by-products were obtained at a local sugar-alcohol plant, washed, oven dried at 45°C and stored until use. The thermotreatment was carried out in a 1.5 L reactor (AU/ E-20, ^{TM}Regmed), where 50 g (dry weight) of SB or SS were added with 500 mL of distilled water. For SB, the reactor operated at 161.9°C for 75.3 min and for SS, 177.2°C for 64.8 min, both at a stirring speed of 4 rpm (35). The material obtained was washed with water until reaching neutral pH, dried in an oven at 45°C and stored until use. The thermotreatment yields were calculated as the difference in the initial mass before and the final mass after pretreatment (gravimetry).

Chemical composition of sugarcane by-products

The chemical composition of untreated and thermotreated SB and SS was performed on the by-products milled in a knife mill with a 0.84 mm retention screen. Untreated by-products were subjected to extraction with 95% ethanol (v/v) for 6 h in *Soxhlet* extractor. The paper cartridges filters including the extracted by-products were dried until the ethanol was completely evaporated and the mass reached a constant value. The extractive content was determined by gravimetry (44). Structural carbohydrates and lignin were determined according to the methodology described by (44), where approximately 300 mg (dry weight) of SB and SS (that passed through the 0.84 mm screen) were hydrolyzed with 72% sulfuric acid (w/w) at 30°C for 1h and then with 4% H_2SO_4 (w/w) at 121°C for 1h. The hydrolysate was filtered on 30

mL number 3 porosity sintered glass filters (Schott, Germany), washed with 10 mL distilled water, dried and weighed. This material corresponds to insoluble lignin. Soluble lignin was determined by reading the absorbance of the filtrates at 205 nm, using an extinction coefficient of 105 $L.g^{-1}.cm^{-1}$ (44). The cellulose, xylan, arabinosyl, and acetyl groups contents were determined in the filtrates obtained by the Klason lignin method. An aliquot was passed through a 0.45 µm filter and a SEP-PAK C_{18} cartridge to retain phenolic compounds and injected in high-performance liquid chromatography (HPLC) system. The analyses were performed in a NEXERA XR chromatography apparatus (SHIMADZU, Japan) equipped with an Aminex HPX-87H column (300 x 7.8 mm; Bio-Rad, USA), at temperature of 60°C, and 0.005 M H_2SO_4 as the mobile phase at a flow rate of 0.6 $mL.min^{-1}$. The refractive index was detected at 60°C using a model RID-20A device (SHIMADZU). Calibration series of analytical grade standards (glucose, xylose, arabinose, formic acid, and acetic acid) dried under vacuum phosphorus pentoxide were used to calculate the concentrations of carbohydrates and organic acids.

Total cellulase enzyme activity assay

The measurement of total cellulase activities was performed according to the methodology described by Ghose (105), where a strip of Whatman N°1 filter paper was placed in tubes containing 1 mL of 0.05 M Na-citrate buffer at pH 4.8 and then added 0.5 mL of Cellic CTec 2 (^{TM}Novozymes) enzyme preparation diluted in citrate buffer. These tubes were placed in a water bath at 50°C for 60 min. After this time, 3 mL of 3,5-dinitrosalicylic acid reagent

(DNS) was added to the tubes and the tubes were boiled for 5 min to stop the reaction. Absorbance readings were taken in a spectrophotometer at 540 nm. Enzyme activity was calculated in filter paper units (FPU) and was used to calculate the enzyme load that should be used in SB and SS enzymatic hydrolysis assays.

Enzymatic hydrolysis of sugarcane by-products

Enzymatic hydrolysis was performed on untreated and thermotreated by-products using Cellic CTec 2 (^{TM}Novozymes). The activity of the enzyme was assayed to be 99 FPU.mL^{-1}. Enzymatic hydrolysis assays at a mass load of 1, 5, 10 and 20% (w/v) with an enzymatic load of 10 FPU by gram of substrate. These assays were performed in 125 mL Erlenmeyer flasks with 0.05 M sodium acetate buffer (pH 4.8) at 45°C and shaking at 120 rpm. Sodium azide (0.1%, w/v) was used in the medium to inhibit possible contamination (106). In addition, an assay was also carried out by adding the Tween 80 additive under the same operating conditions, with an enzymatic load of 10 FPU and consistency of 20% (w/v) (102). Afterwards, assays of range of the enzymatic load were carried out, where the thermotreated by-products were hydrolyzed with enzymatic load of 15, 20 and 30 FPU by gram of substrate, at a consistency of 20% (w/v), under the same operational conditions described. An assay was carried out in a benchtop bioreactor model TEC-BIO-7.5VI (Tecnal) at a consistency of 20% (200 g of by-product and 1000 mL of reaction medium), with an enzymatic load of 15 FPU by gram of substrate at 45 °C and 150 rpm. All assays were conducted up to 72h of hydrolysis (4, 8, 12, 24, 48

and 72 h). Sampling in assays in which the by-products liquefied was done by withdrawing about 1 mL of the reaction medium with a pipette. The sampling of the tests that did not liquefy was done by removing a small portion of the substrate and pressing with a spatula to extract the hydrolyzed liquid. The samples taken were boiled for 5 min and frozen until the moment of analysis. The analysis was performed on an HPLC system to determine and quantify monomeric sugars (item chemical composition of sugarcane by-products).

The cellulose conversion to glucose was calculated according to Equation 1 as follows:

$$\delta = \left[\frac{(glucose\ or\ xylose)\cdot f}{m}\right]\cdot 100\% \quad \textbf{(1)}$$

In Equation (1):

- δ = cellulose conversion to glucose (%);
- *glucose* = mass of glucose (g);
- *f* = hydrolysis factor of 0.9 ($g.g^{-1}$);
- *m* = initial dry mass of cellulose (g).

Kinetic parameter estimation

The glucose concentration data obtained during enzymatic hydrolysis was interpreted using a mathematical model applicable to the enzyme kinetics under study. This model was based on saturation kinetics, as the sugar concentration during enzymatic hydrolysis increases over time, reaching an asymptotic final value, as described by Equation (2) (107–110) as follows:

$$C = C_{max}(1 - e^{-kt}) \quad \textbf{(2)}$$

In Equation (2):

- C = sugar concentration ($g.L^{-1}$);
- C_{max} = asymptotic maximum sugar concentration ($g.L^{-1}$);
- k = kinetic constant of sugar accumulation (h^{-1});
- t = hydrolysis time (h).

Estimation of C_{max} and k parameters was performed using Origin software (OriginPro, 2017). The statistical difference among the C_{max} values estimated for each hydrolysis assay was evaluated by Tukey's test (BioEstat 5.0 software) with a significance level of 0.05. Although the value of the determination coefficient (R^2) is rigorously correct for quantifying the predictive capability of linear models, this indicator was used here, as first approximation, to evaluate the fit quality of the nonlinear mathematical model given by Equation (2).

RESULTS AND DISCUSSION

Chemical composition of thermotreated sugarcane by-products

The raw materials presented contents of 2.8% (extractives), 3.2% and 3.3% (ash), 25.1% and 25.3% (total lignin), 32.3% and 33.8 % (cellulose), 21.2% and 20.4% (xylan), 1.4% and 2.2% (arabinosyl groups) and 2.4% and 2.7% (acetyl groups; w/w, dry weight), for SB and SS, respectively. The sum of the components of SB and SS was 88.4% and 90.5% (w/w, dry weight),

respectively. Indeterminate components that were not quantified for the sum to reach 100% can be attributed to the presence of methyl glucuronic acid, commonly present in grass hemicellulose chains (111). The chemical composition of the raw by-products corroborates with SB and SS characterized by other authors (16,32,35,78,81–83,86,98). Some ranged may occur due to plant variety, location and climatic conditions of cultivation.

The thermotreated yields of SB and SS were 74.9% and 59.5% (dry weight, w/w), respectively. Thermotreatment SB and SS showed levels of 22.9% and 23.1% (total lignin), 30.3% and 30.2% (cellulose), 9.5% and 4.5% (xylan), 0.2% (arabinosil group) and 0.5% and 0.1% (acetyl group; w/w, dry weight), respectively. The thermotreatment caused mainly the depolymerization of hemicelluloses, solubilizing XOS. Cellulose and lignin were poorly solubilized. In the thermotreated SB, 55.2% of original xylan, 76.2% of original acetyl groups and 84.6% of original arabinosyl groups were solubilized; in SS, 79.9% of original xylan, 96.3% of original acetyl groups and 95.5% of original arabinosyl groups were solubilized. This solubilization shows the potential of hydrothermal pretreatment as a method for prepare the SB and SS for enzymatic hydrolysis, since hemicelluloses form a physical barrier that surrounds the cellulose fibers making the enzymatic attack difficult (35).

Effect of mass load range on enzymatic hydrolysis

The enzymatic hydrolysis step was carried out with the commercial preparation Cellic CTec 2 using the solid residues of each pretreated by-product. The first step of enzymatic hydrolysis was carried out at a consistency

of 1% (w/v) and 10 FPU enzyme load, in both by-products, raw and thermotreated, to evaluate the effect of thermotreatment on the cellulose conversion to glucose. It was observed that there was a greater cellulose conversion to glucose of both thermotreated by-products in relation to the raw ones, indicating that the thermotreatment improves the enzymatic conversion. Raw SB and SS achieved maximum cellulose to glucose conversions of 13.2% and 22.9%, respectively. The maximum conversion achieved by the thermotreated by-products was 68.7% and 83.1%, respectively.

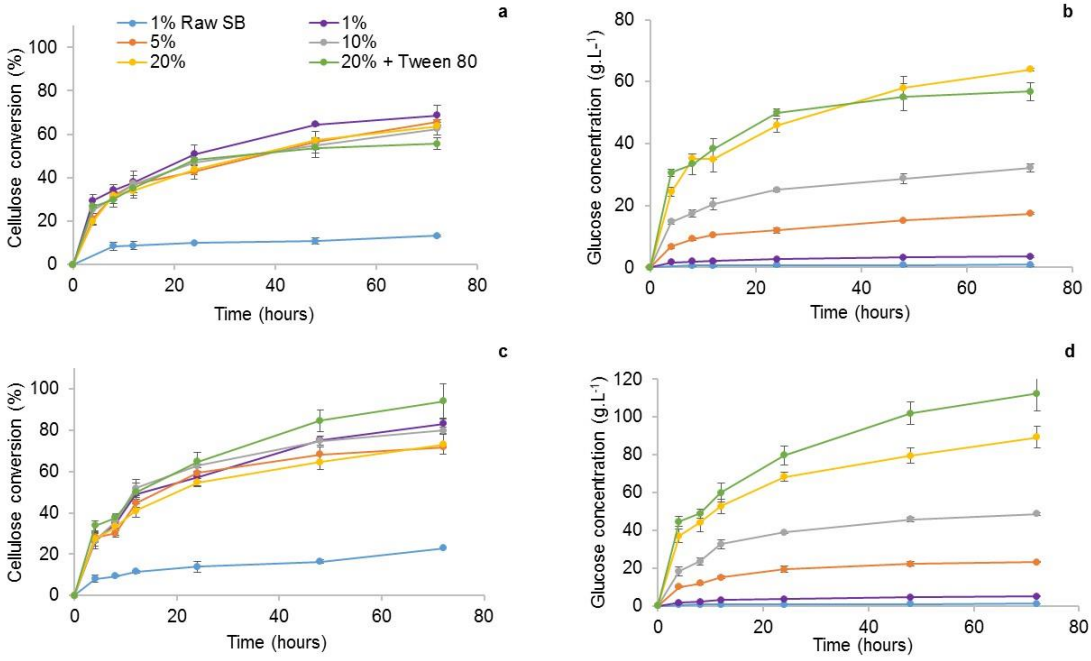

Figure 3.1. Enzymatic hydrolysis of thermotreated SB (a,b) and SS (c,d) under different consistencies or mass loads presented in percentage (w/v): (a,c) Cellulose conversion to glucose. (b,d) Glucose concentration. The error bars that are not visible are less than the own symbols.

Then, with the same enzymatic load of 10 FPU, assays were carried out with an increase in mass load of 5, 10 and 20% (w/v) only on the thermotreated by-products. In the thermotreated SB, the maximum cellulose conversion (72h

of hydrolysis) in the assays at 5, 10 and 20% (w/v) were 65.4%, 62.4% and 63.6% and in the SS 71.7, 79.8 and 73.1% (w/v), respectively (Figure 3.1). The ranged in consistency does not appear to have affected the hydrolysis efficiency, although the assays carried out at 20% on both by-products did not liquefy. The tests carried out at 1 and 5% on both by-products appeared in a liquefied appearance from the beginning of the hydrolysis. The hydrolysis carried out at 10% on both by-products was completely liquefied after 8 hours and the tests carried out at 20% remained solid throughout (72h) of the hydrolysis (Figure 3.2).

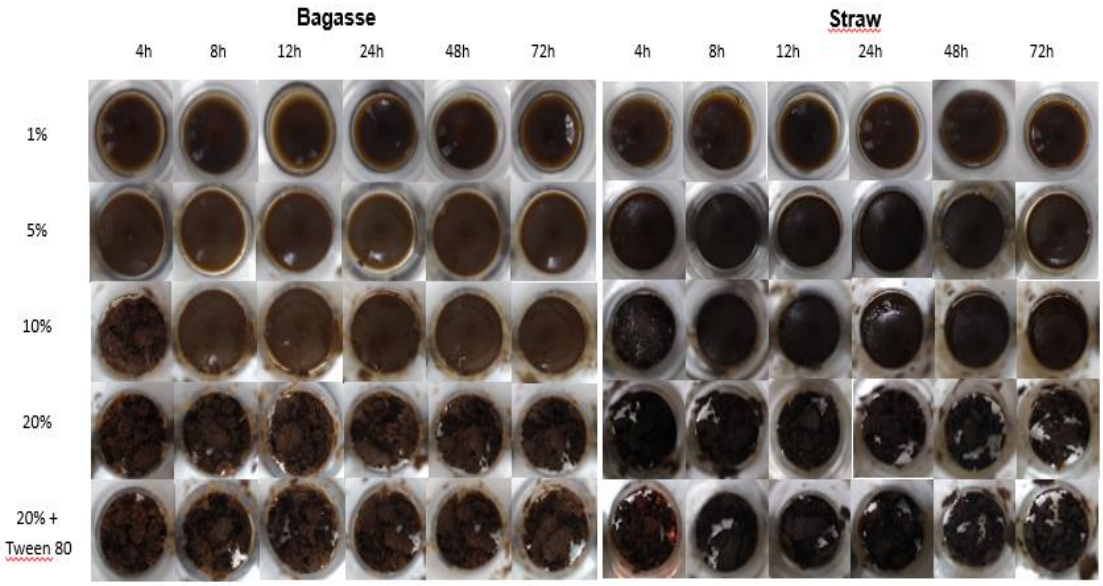

Figure 3.2. Visual aspect of the enzymatic hydrolysis tests of thermotreated sugarcane bagasse and straw at different mass loads.

Glucose concentration as a function of the hydrolysis time increased as the mass load increased. In the thermotreated SB, the maximum glucose concentrations were 3.50, 17.39, 32.15 and 63.86 $g.L^{-1}$, in consistencies 1, 5, 10 and 20% (w/v), respectively. In the thermotreated SS, at the same

consistencies, the maximum glucose concentrations as a function of the hydrolysis time were 5.07, 23.24, 48.64 and 89.20 $g.L^{-1}$, respectively (Figure 3.1).

Table 3.1 presents the values of the maximum glucose rate formation, the reaction constant (k) and maximum asymptotic glucose concentration (C_{max}) for enzymatic hydrolysis under different consistencies.

Table 3.1. Kinetic parameters of enzymatic hydrolysis of sugarcane bagasse (SB) and straw (SS) thermotreated under different consistency (w/v ratios).

By-product	Consistency (%, w/v)	*k* (h^{-1})	g_{max} (gL^{-1})	Maximum rate of products ($gL^{-1}h^{-1}$)	R^2
Sugarcane bagasse	20 + Tween 80	0.13 ± 0.00	54.07 ± 0.25	7.20 ± 0.24[a]	0.9393
	20	0.09 ± 0.01	59.17 ± 1.09	5.40 ± 0.66[b]	0.9438
	10	0.11 ± 0.01	29.31 ± 1.36	3.41 ± 0.39[c]	0.9394
	5	0.10 ± 0.01	15.50 ± 0.13	1.56 ± 0.14[d]	0.8779
	1	0.11 ± 0.01	3.15 ± 0.27	0.36 ± 0.00[d]	0.9169
Sugarcane straw	20 + Tween 80	0.07 ± 0.01	106.35 ± 8.67	8.13 ± 0.69[a]	0.9463
	20	0.09 ± 0.01	82.62 ± 1.58	7.96 ± 0.01[a]	0.9501
	10	0.10 ± 0.00	46.29 ± 0.98	4.67 ± 0.16[b]	0.9712
	5	0.08 ± 0.02	22.95 ± 0.92	1.97 ± 0.53[c]	0.9116
	1	0.08 ± 0.17	4.75 ± 0.17	0.49 ± 0.02[c]	0.9631

Data are mean ± standard deviation. In each column, the values with the same superscript letters do not differ among themselves at significance level of 0.05 (Tukey test).
Enzyme load of 10 $FPU.g^{-1}$. k=reaction rate constant (h^{-1}). g_{max}= asymptotic maximum glucose concentration ($g.L^{-1}$).

The kinetic profiles of enzymatic hydrolysis show that in both by-products there was no significant difference in the maximum rate of glucose formation in the assays performed with 1 and 5% consistency (w/v), but there were significant differences in the maximum rate of glucose formation when consistency was increased from 5, 10 and 20% (Table 3.1). In figure 3.2, the graph of glucose concentration in 72 h versus consistency confirms that there was a linear increase in maximum glucose concentration with increasing

consistency, corroborating the data obtained in the graphs of Figure 3.1 (b and d).

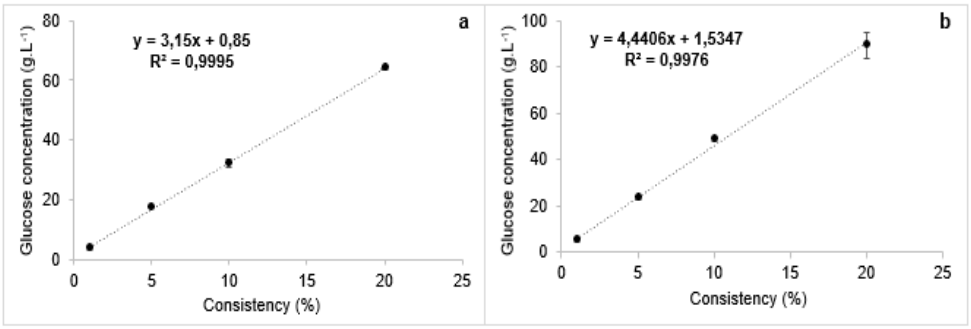

Figure 3.3. Glucose concentration in 72 h of enzymatic hydrolysis of thermotreated SB (a) and SS (b) under different consistencies or mass load.

Assays were also performed with the addition of Tween 80 at a consistency of 20% and an enzymatic load of 10 FPU. The maximum cellulose conversion to glucose in the assays with and without Tween 80 in SB were 63.6% and 55.6% and in SS they were 93.9% and 73.2%, respectively. The maximum glucose concentrations in the assays performed at consistency of 20% and 20% plus Tween 80 in SB were 63.8 and 56.7 $g.L^{-1}$, respectively. In SS, the maximum glucose concentrations were 89.2 and 112.4 $g.L^{-1}$, respectively (Table 3.1).

Table 3.1 shows that in SB there was a significant difference in the maximum rate of glucose formation between assays with and without Tween 80, while in SS the addition of Tween 80 had no influence on enzymatic hydrolysis. Controversies regarding the improvement of enzymatic conversion due to the addition of surfactants are found in the literature and therefore more studies are needed to better understand the mechanism of functioning of such additives (112).

Effect of enzymatic load range on hydrolysis

The hydrolysis of the thermotreated by-products was evaluated under range of the enzymatic load (15, 20 and 30 FPU.g^{-1}), without additives and at a consistency of 20% (w/v). Assays with ranged of enzymatic load were carried out at a consistency of 20%(w/v) because the ranged in consistency did not influence the cellulose conversion to glucose and hydrolysis carried out at high mass load does not require a hydrolysate concentration step, saving energy consumption.

In the thermotreated SB, the maximum cellulose conversion to glucose were 63.6, 78.7, 80.9 and 93.6% in assays performed at 10, 15, 20 and 30 FPU.g^{-1}, respectively. In the thermotreated SS, the maximum conversion to glucose were 73.1, 89.45, 94.5, 91.7%, in assays carried out at 10, 15, 20 and 30 FPU.g^{-1}, respectively (Figure 3.4). In SB assays performed with 10, 15, 20 and 30 FPU.g^{-1}, glucose concentrations were 63.8, 81.7, 87.4 and 105.11 g.L^{-1}, respectively. In SS, the concentrations in the respective enzyme loads were 89.2, 112.5, 121.6 and 124.8 g.L^{-1} (Figure 3.4).

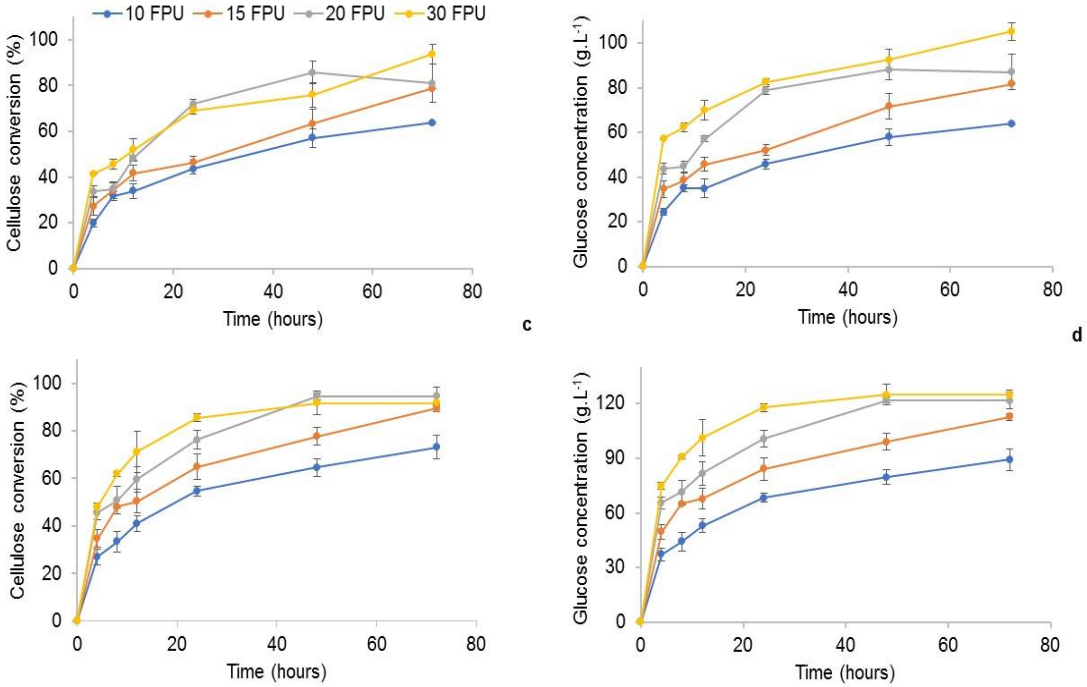

Figure 3.4. Enzymatic hydrolysis of thermotreated SB (a,b) and SS (c,d) under different enzymatic load presented in percentage (w/v): (a,c) Cellulose conversion to glucose. (b,d) Glucose concentration. The error bars that are not visible are less than the own symbols.

The visual aspect of SB and SS pretreated enzymatically hydrolyzed is shown in Figure 3.5. Note that the visual appearance of SB remained in solid state from start to finish in the three consistencies evaluated, while the SS liquefied in 72 h of hydrolysis in the assay performed at 30 FPU of enzymatic load.

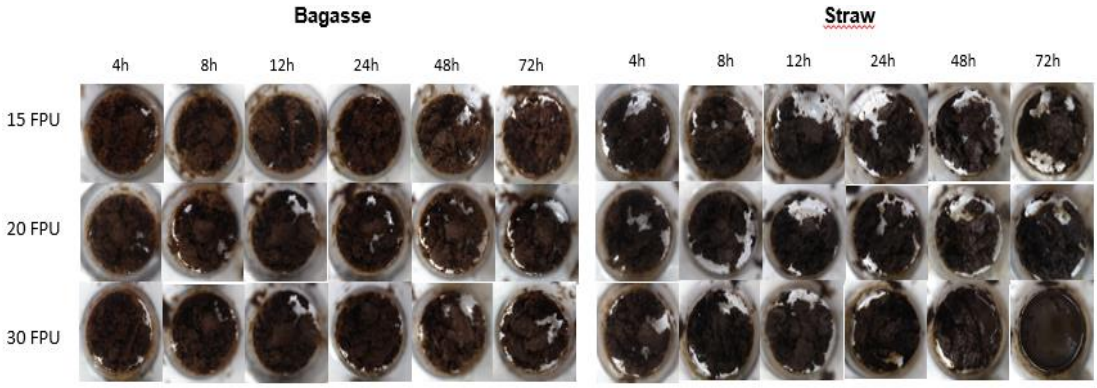

Figure 3.5. Visual aspect of enzymatic hydrolysis tests of thermotreated sugarcane bagasse and straw at different enzymatic loads.

According to Table 3.2, there were no statistically significant differences between assays performed at 10 and 15 FPU and between 15 and 20 FPU, but there were significant differences in maximal glucose formation rates between assays performed at 20 and 30 FPU enzyme loading on both by-products. This means that the maximum rate of glucose formation has no differences if hydrolysis is performed at 10, 15 or 20 FPU and increases with increasing enzyme load to 30 FPU.

Table 3.2. Kinetic parameters of enzymatic hydrolysis of sugarcane bagasse (SB) and straw (SS) thermotreated under different enzyme load.

By-product	Enzyme load (FPU.g^{-1})	k (h^{-1})	g_{max} (gL^{-1})	Maximum rate of products (gL^{-1}h^{-1})	R^2
Sugarcane bagasse	30	0.15 ± 0.01	92.90 ± 0.76	14.58 ± 1.01^{a}	0.9244
	20	0.09 ± 0.00	90.79 ± 0.00	9.01 ± 0.00^{b}	0.9697
	15	0.09 ± 0.03	73.62 ± 5.96	6.61 ± 1.55^{bc}	0.9033
	10	0.09 ± 0.01	59.17 ± 1.09	5.40 ± 0.66^{cd}	0.9438
Sugarcane straw	30	0.19 ± 0.00	121.03 ± 0.88	23.34 ± 0.77^{a}	0.9759
	20	0.13 ± 0.02	115.87 ± 0.98	15.09 ± 2.46^{b}	0.9401
	15	0.11 ± 0.00	100.98 ± 3.20	11.84 ± 0.21^{bc}	0.9360
	10	0.09 ± 0.01	82.62 ± 1.58	7.96 ± 0.01^{cd}	0.9501

Data are mean ± standard deviation. In each column, the values with the same superscript letters do not differ among themselves at significance level of 0.05 (Tukey test).
Consistency=20 (%, w/v). k=reaction rate constant (h^{-1}). g_{max}= asymptotic maximum glucose concentration (g.L^{-1}).

Silva et al. (113) hydrolyzed SB hydrothermally pretreated (190°C, 12 min) with different enzymatic preparations and range consistencies (5, 15 and 20%). In their trials with Cellic CTec2 at 20 FPU, these authors achieved maximum cellulose conversion to glucose of 86% at 5% (w/v) consistency, 68% at 15% (w/v) consistency assays, and 69% at 20% (w/v) consistency assay. Its conversion results were lower than those of SB in the present study (80.9%, at 20% of consistency and 20 FPU).

Gao et al. (114) pretreated SB with liquid hot water (LHW) (180°C, 20 min) and hydrolyzed the solid with Cellic Ctec2 at 5, 10, 20 and 30 FPU per gram of substrate, using a consistency 5% (w/v) in all experiments. These authors obtained conversions around 40% in their assays performed at 10 and 30 FPU and maximum glucose concentration of about 20 $g.L^{-1}$ in assay performed at 30 FPU. In the present study, we obtained a better cellulose conversion to glucose (65.4%) in an assay with the same consistency and lower enzymatic load (10 FPU). The maximum enzymatic concentration obtained in the present study corroborates those authors (17.39 $g.L^{-1}$); however, this conversion was obtained in an assay at 10 FPU of enzymatic load.

Batalha et al. (115) hydrothermally pretreated SB (180°C, 20 min) and hydrolyzed the solid with a mixture of Cellic CTec 2 and Cellic Htec 2 at 5 FPU per gram of substrate, at a consistency of 5% (w/v). These authors obtained cellulose conversion to glucose (64.4%) similar to the present study (65.4%) and a slightly higher maximum glucose concentration of 26 $g.L^{-1}$, compared to

17.4 gL^{-1} in assay with the same consistency and 10 FPU enzyme load as in the present study.

Dos Santos-Rocha et al. (116) hydrothermally pretreated SS (195°C, 10 min) and hydrolyzed the solid with Cellic CTec 2 at 10 FPU per gram of substrate, with consistencies ranging from 10 to 30% (w/v) solids loading. These authors obtained a maximum conversion of cellulose to glucose of 80.51% and 62.09% in the tests carried out with 10 and 20% solids loading (consistency). The maximum glucose concentrations reached was 48.9, 84.1 g/L, in the respective tested consistencies. The results corroborate those found in the present study, where in the tests at 10 and 20% of consistency and 10 FPU of enzyme load, a maximum conversion of cellulose to glucose of 79.8% and 73.2% and a concentration of 48.6 and 89.2 $g.L^{-1}$.

Effect of enzymatic hydrolysis of thermotreated bagasse and straw in a bioreactor

The thermotreated by-products were subjected to hydrolysis in a bench top stirred tank bioreactor, with temperature control and equipped with an impeller for purposes of comparison with the hydrolysis performed in Erlenmeyer flasks. The experiments were carried out at a consistency of 20% (w/v) and 15 FPU by gram of substrate.

It was found that the visual appearance of the hydrolysis carried out in the bioreactor differed from the hydrolysis carried out in the Erlenmeyer flask. In the bioreactor there was liquefaction of the by-products before 12h of

hydrolysis, which did not occur after 72h of hydrolysis in the Erlenmeyer (Figure 3.7).

In the bioreactor hydrolysis of SB, the maximum cellulose conversion to glucose was 90% compared to 78.8% in the same assay performed in the Erlenmeyer flask. In the hydrolysis of SS in a bioreactor, the maximum conversion was 59.4% and in the same assay in Erlenmeyer it was 89.4%. the maximum glucose concentrations in SB and SS were 81.7 and 112.5 $g.L^{-1}$ in the experiments carried out in the Erlenmeyer flask and 76.2 and 76.8 $g.L^{-1}$ in the experiments carried out in the bioreactor, respectively (Figure 3.6).

The maximum rates of glucose formation estimated in the hydrolysis of SB and SS in the bioreactor were 87.9 and 73.3 $g.L^{-1}.h^{-1}$, respectively, while in the Erlenmeyer flask were 73.6 and 100.9 $g.L^{-1}.h^{-1}$. The SS hydrolysis performed in the bioreactor had a lower conversion compared to SB, which contradicts the visual aspect observed in which there was liquefaction in the bioreactor in less than 12h of experiment, which was not observed in the hydrolysis in Erlenmeyer. An explanation for this observation may have been a possible contamination, since sodium azide was not added in the bioreactor hydrolysis (Figure 3.6).

Paz-Cedeno et al. (103) pretreated SB with Sulfite-NaOH (5 g NaOH+10 g Na_2SO_3) and hydrolyzed the solid with Cellic CTec 2 at 13,2 FPU per gram of substrate, with consistencie of 20% (w/v) solids loading in the same bioreactor used in the present study. These authors achieved a maximum conversion of cellulose to glucose of approximately 70% in 24 h of hydrolysis. The maximum glucose concentration reached was 80 $g.L^{-1}$ in 24 h

of hydrolysis. The maximum rates of glucose formation estimated in the bioreactor were 10.6 g L^{-1} h^{-1}. These authors obtained results similar to those performed in Erlenmeyer. In the present study, the maximum conversion of cellulose into glucose reached in SB thermotreated and hydrolyzed with 15 FPU at 20% consistency (w/v) was 90.0% in 72 hours of hydrolysis and the maximum concentration reached was 90, 6 g/L, corroborating the work of the respective authors. The maximum rate of glucose formation in the present study (87.9 g. L^{-1}.h^{-1}) was higher than the value reached by these authors (10.6 g. L^{-1}.h^{-1}).

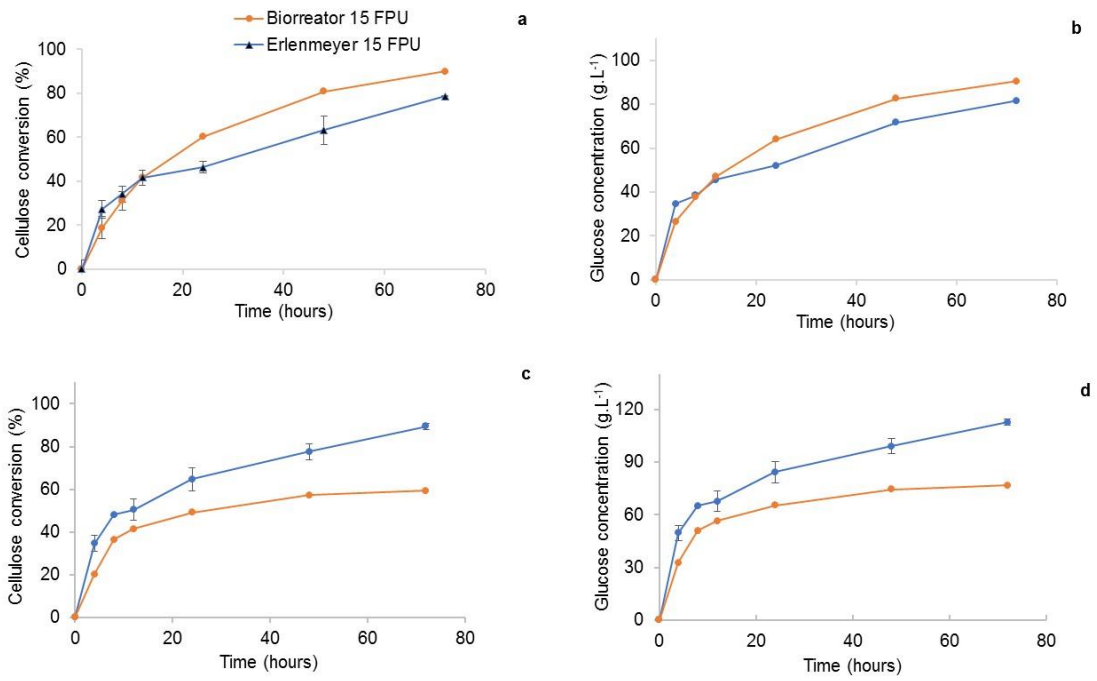

Figure 3.6. Enzymatic hydrolysis of thermotreated SB (a,b) and SS (c,d) under consistency of 20% (w/v) carried out in the Erlenmeyer and in the bioreactor: (a,c) Cellulose conversion to glucose. (b,d) Glucose concentration.

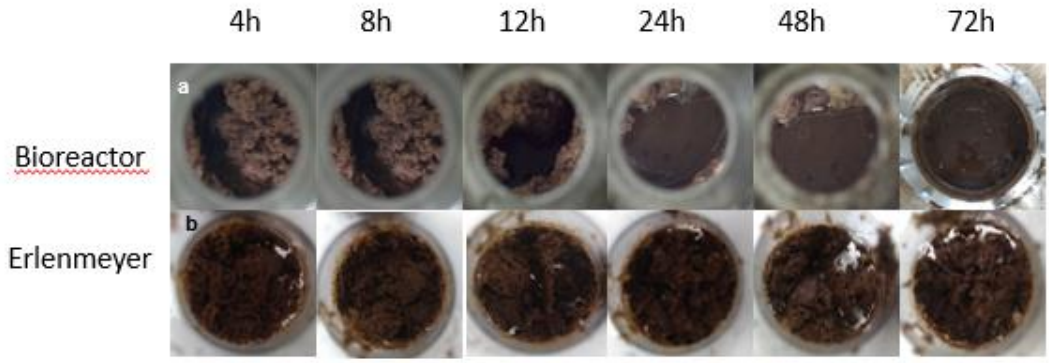

Figure 3.7. Visual aspect of the enzymatic hydrolysis assays of thermotreated sugarcane bagasse at a consistency of 20% (w/v) and enzymatic load of 15 FPU: (a) Hydrolysis carried out in a bioreactor. (b) Enzymatic hydrolysis performed in an Erlenmeyer flask.

CONCLUSION

The thermotreatment removed of the hemicellulose and improved the access of enzymes in enzymatic hydrolysis, improving the cellulose conversion to glucose, in SB and SS.

The increase in the mass load range (consistency) resulted in an increase in the concentration of glucose produced and increased a maximum cellulose conversion to glucose. At 20% (w/v) consistency, SB and SS reached conversions of 63.6% and 73.1%, resulting in hydrolysates with glucose concentrations of 63.8 and 89.2 $g.L^{-1}$, respectively.

The Increase in the enzyme load from 10 to 15 FPU and from 15 to 20 FPU was found to have no influence on hydrolysis efficiency but increasing it to 30 FPU resulted in improved hydrolysis efficiency and increased concentration of glucose produced.

The SB hydrolysis in the bioreactor showed better efficiency, resulting in a higher maximum rate of glucose formation compared to the assay

performed in Erlenmeyer flasks, corroborating with increased mass transfer. However, the hydrolysis of SS in the bioreactor showed a loss of efficiency and a drop in the maximum rate of glucose formation indicating that there was a problem associated with contamination by bacteria and fungi in the hydrolysis, because in the bioreactor sodium azide was not used in the reaction medium.

Finally, enzymatic hydrolysis carried out in loads of high solids content in a bioreactor have economic advantages as they do not require or reduce the time of the hydrolysate concentration step and consequently reduce energy consumption.

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4. CONSIDERAÇÕES FINAIS

O bagaço (BCA) e a palha (PCA) de cana-de-açúcar foram submetidos a pré-tratamentos hidrotérmicos (PTHs), variando-se o tempo, a consistência (relação massa/volume de água) e a temperatura em um planejamento fatorial completo (delineamento composto central rotacional, DCCR) a fim de se obter uma condição otimizada no qual houvesse a máxima produção de xilooligossacarídeos (XOS). Assim, aplicando-se esta metodologia foi possível obter e validar a melhor condição de tempo, temperatura e consistência de máxima produção de XOS em ambos os subprodutos.

As frações sólidas ricas em celulose obtidas após os PTHs do BCA e da PCA nas condições previamente otimizadas foram submetidas à hidrólise enzimática com preparado enzimático Cellic CTec 2, variando-se a carga mássica (consistência) e enzimática a fim de se obter as melhores condições de conversão de celulose em glicose para produção de hidrolisado rico em glicose .

Desta forma, foi possível obter através da metodologia de DCCR um excelente rendimento na produção de XOS, além disso, as frações sólidas ricas em celulose, resultantes dos PTHs, foram hidrolisadas enzimaticamente em glicose apresentndo potencial para produção de etanol de segunda geração (etanol celulósico).

Por fim, a estratégia de produção de XOS via PTH acoplado a hidrólise enzimática da fração rica em celulose visando à produção de bioetanol de segunda geração (bioetanol celulósico) é interessante, pois os XOS tem alto

valor agregado e o bioetanol é um combustível automotivo de origem renovável e tem um alto impacto na sociedade como um todo.

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