UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

GABRIEL CICALESE BEVILAQUA

POLIÓIS: UM ESTUDO DA PRODUÇÃO BIOLÓGICA SIMULTÂNEA DE XILITOL E ARABITOL A PARTIR DE RESÍDUOS AGROINDUSTRIAIS

POLYOLS: A STUDY OF THE SIMULTANEOUS BIOLOGICAL PRODUCTION OF XYLITOL AND ARABITOL FROM AGROINDUSTRIAL WASTE

CAMPINAS
2023

GABRIEL CICALESE BEVILAQUA

POLIÓIS: UM ESTUDO DA PRODUÇÃO BIOLÓGICA SIMULTÂNEA DE XILITOL E ARABITOL A PARTIR DE RESÍDUOS AGROINDUSTRIAIS

POLYOLS: A STUDY OF THE SIMULTANEOUS BIOLOGICAL PRODUCTION OF XYLITOL AND ARABITOL FROM AGROINDUSTRIAL WASTE

Dissertação apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestre em Engenharia de Alimentos

Dissertation presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Food Engineering.

Supervisor/Orientador: Marcus Bruno Soares Forte
Co-supervisor/Coorientador: Francisco Maugeri Filho

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO/TESE DEFENDIDA PELO ALUNO GABRIEL CICALESE BEVILAQUA, E ORIENTADA PELO PROF. DR. MARCUS BRUNO SOARES FORTE

CAMPINAS

2023

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

Bevilaqua, Gabriel Cicalese, 1997-
B468p Polióis : um estudo da produção biológica simultânea de xilitol e arabitol a partir de resíduos agroindustriais / Gabriel Cicalese Bevilaqua. – Campinas, SP : [s.n.], 2023.

Orientador: Marcus Bruno Soares Forte.
Coorientador: Francisco Maugeri Filho.
Dissertação (mestrado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Fermentação de pentoses. 2. Biorrefinaria. 3. Edulcorantes naturais. 4. Bagaço de cana. 5. Álcoois de açúcar. I. Forte, Marcus Bruno Soares. II. Maugeri Filho, Francisco. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações Complementares

Título em outro idioma: Polyols : a study of the simultaneous biological production of xylitol and arabitol from agroindustrial waste
Palavras-chave em inglês:
Pentose fermentation
Biorefinery
Natural sweeteners
Sugarcane bagasse
Sugar alcohols
Área de concentração: Engenharia de Alimentos
Titulação: Mestre em Engenharia de Alimentos
Banca examinadora:
Marcus Bruno Soares Forte [Orientador]
Ruann Janser Soares de Castro
Eliana Setsuko Kamimura
Data de defesa: 06-03-2023
Programa de Pós-Graduação: Engenharia de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: 0000-0002-2947-4213
- Currículo Lattes do autor: http://lattes.cnpq.br/2793963040311901

COMISSÃO EXAMINADORA

Prof. Dr. Marcus Bruno Soares Forte
Universidade Estadual de Campinas
Presidente

Prof. Dr. Ruann Janser Soares de Castro
Universidade Estadual de Campinas

Profa. Dra. Eliana Setsuko Kamimura
Universidade de São Paulo

A Ata de Defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertações/Teses e na Secretaria do Programa de Pós-Graduação

DEDICATÓRIA

Dedico este trabalho a todos que, de uma forma ou de outra,
me apoiaram nessa jornada

AGRADECIMENTOS

Agradeço inicialmente a meus pais, pois sem o apoio deles eu não seria capaz de estar aqui. Amplio o agradecimento ao meu irmão, Renato, e aos meus familiares e amigos de Recife, que me auxiliaram principalmente no período remoto, durante a pandemia de COVID-19.

Sou imensamente agradecido à professora Ana Maria, sem a introdução científica dela não sei como eu poderia definir minha vida acadêmica hoje. Junto a ela, agradeço às professoras Neila Cortez, uma das minhas parceiras acadêmicas, e Lia Pinheiro, que me deu coragem para fazer o mestrado na FEA.

Agradeço a meu orientador, Marcus Forte, pelo tempo disponibilizado, pela confiança, pelo apoio e pela paciência para lidar com meus anseios. Tenho aprendido muito com nosso tempo de parceria acadêmica. Ao meu coorientador, Francisco Maugeri Filho, pelas dicas necessárias.

Agradeço aos meus amigos de pós e laboratório: Paulo, pelo apoio inicial com os microrganismos; Allan, por sempre me ajudar (socorrer) com inúmeras questões; Dani Garcia, por toda a parceria estabelecida; Igor, pelas orientações, conselhos, conversas e ajudas; Marcelo e Isa, pela amizade, energia positiva e companheirismo no cotidiano, sou muito feliz de ter vocês como amigos; Bárbara e Enylson, por compartilharem as lamúrias de uma forma divertida; Marcos e Dani Matias, pelas conversas e descontrações; Pri, pelo auxílio sempre que necessário; e a todos os outros que de alguma forma conviveram comigo no laboratório durante esse ano e acabaram compartilhando experiências e momentos. Muito obrigado!

Agradeço em especial a Murilo, que me acompanhou durante boa parte do processo do mestrado, me apoiou sempre e foi muitas vezes quem me ajudou a não esquecer que também é importante ser feliz e desfrutar da vida.

Agradeço à Faculdade de Engenharia de Alimentos (FEA – Unicamp) e ao Programa de Pós-Graduação em Engenharia de Alimentos da Universidade Estadual de Campinas, por contribuírem à minha formação e possibilitar a realização desta dissertação. O presente trabalho foi realizado com o apoio financeiro, processo nº 2021/05508-9, da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001 e com o apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Código

de financiamento 439548/2018-7. Agradeço, portanto, aos órgãos de fomento que proporcionaram a execução desta pesquisa.

Por fim, agradeço também às instituições que forneceram materiais ou instalações que permitiram a realização da dissertação de mestrado: à Ingredion Brasil, por fornecer a água de maceração de milho; à Procelys por fornecer o hidrolisado de levedura NuCell; ao Laboratório Nacional de Biorrenováveis (LNBR – CNPEM) por ceder as instalações para a produção de hidrolisado hemicelulósico e ao Laboratório de Genômica e Proteômica do Instituto de Biologia (LGE – IB) da Unicamp por ceder a cepa de *Candida tropicalis*.

RESUMO

O xilitol e o arabitol são poliálcoois estereoisômeros que possuem propriedades e aplicações similares e despertam o interesse mercadológico. Mesmo que a via química seja atualmente mais empregada na produção desses compostos, a produção por meio de processos fermentativos utiliza condições brandas e é ambientalmente menos danosa. Apesar disso, a via biotecnológica apresenta obstáculos como o baixo rendimento e a viabilidade econômica do processo. Nesse contexto, estudos de otimização da produção destacam-se como forma de tornar o processo mais rentável, utilizando hidrolisados hemicelulósicos de biomassas lignocelulósicas como fontes de carbono, e oportunidades ainda não exploradas podem ser desenvolvidas, como a aplicação simultânea dos dois poliálcoois como um único produto, o que dispensaria a separação dos mesmos. Este trabalho buscou otimizar a coprodução biológica de xilitol e arabitol a partir do hidrolisado hemicelulósico de bagaço de cana-de-açúcar e fontes alternativas de nitrogênio. Para isso, verificou-se a possibilidade de utilização de *Candida tropicalis* para conversão de pentoses em poliálcoois e sua respectiva adaptação ao hidrolisado hemicelulósico destoxificado de bagaço de cana-de-açúcar. Em seguida, definiu-se que o hidrolisado de levedura foi a fonte mais promissora para ser adicionada ao meio e permitir uma maior produção dos compostos alvo. A partir de estratégias de delineamento experimental (DOE), foi possível definir uma composição e condições favoráveis para o processo fermentativo com inserção de água de maceração de milho como fonte de nitrogênio adicional ao processo, com a condição mais promissora para a co-produção sendo composta de hidrolisado hemicelulósico diluído para 75 g/L de xilose inicial, pH inicial de 6,5 e adição de 2,2 g/L de nitrogênio, sendo 80 % de hidrolisado de levedura e 20 % de água de maceração de milho. A partir da ampliação de escala em biorreatores foi possível obter concentrações de xilitol (52,1 g/L) e arabitol (4,2 g/L) 65 e 3,7 vezes maiores, respectivamente, em relação a processos similares de coprodução encontrados na literatura, apresentando produtividades e rendimentos de 0,24 g/L.h e 0,74 g/g para o xilitol e 0,02 g/L.h e 0,43 g/g para o arabitol. Esses resultados mostram o potencial biotecnológico para a obtenção simultânea desses dois álcoois de açúcar a partir da fermentação de hidrolisado hemicelulósico de bagaço de cana de açúcar por *C. tropicalis*.

ABSTRACT

Xylitol and arabitol are stereoisomeric polyalcohols that have similar properties and applications and arouse market interest. Even though the chemical route is currently more used in the production of these compounds, production through fermentation processes uses mild conditions and is less environmentally harmful. Despite this, the biotechnological route presents difficulties such as the low yield and the economic viability of the process. In this context, production optimization studies stand out as a way to make the process more profitable, using hemicellulosic hydrolysates of lignocellulosic biomass as carbon sources, for example, and opportunities not yet explored can be developed, such as the simultaneous use of the two polyalcohols, which would dispense with the process of separating them. This project sought to optimize the biological coproduction of xylitol and arabitol from the hemicellulosic hydrolysate of sugarcane bagasse and alternative nitrogen sources. For this, the possibility of using *Candida tropicalis* for the conversion of pentoses into polyalcohols and their respective adaptation to the detoxified hemicellulosic hydrolysate of sugarcane bagasse was verified. Then, it was defined that the yeast hydrolysate was the most promising source to be added to the medium and allow a greater production of the target compounds. From experimental design strategies (DOE), it was possible to define a composition and favorable conditions for the fermentation process with the insertion of corn steep water as a source of additional nitrogen to the process, with the most promising condition for co-production being composed of hemicellulosic hydrolysate diluted to 75 g/L of initial xylose, initial pH of 6.5 and addition of 2.2 g/L of nitrogen, being composed 80% by yeast hydrolysate and 20% by corn steep liquor. From the scale-up in bioreactors it was possible to obtain concentrations of xylitol (52.1 g/L) and arabitol (4.2 g/L) which are 65 and 3.7 higher, respectively, in relation to similar processes of co -production found in the literature, showing productivities and yields of 0.24 g/L.h and 0.74 g/g for xylitol and 0.02 g/L.h and 0.43 g/g for arabitol. These results show the biotechnological potential for simultaneously obtaining these two sugar alcohols from the fermentation of sugarcane bagasse hemicellulosic hydrolysate by *C. tropicalis*.

SUMÁRIO

INTRODUÇÃO

O xilitol e o arabitol são poliálcoois de cinco carbonos produzidos naturalmente por diferentes organismos vivos em pequenas quantidades ou como composto intermediário no metabolismo celular (HERNÁNDEZ-PÉREZ *et al.*, 2019; KORDOWSKA-WIATER, 2015). Nos mamíferos, por exemplo, o xilitol é gerado pelas células hepáticas, mas é liberado apenas em quantidades traço no organismo (KALU; MASORO, 1986). Das diferentes propriedades desses dois compostos, a de maior interesse para o meio comercial é a capacidade de atuar como edulcorante, substituindo o açúcar e fornecendo um menor valor calórico ao produto final (HERNÁNDEZ-PÉREZ *et al.*, 2019; KORDOWSKA-WIATER, 2015).

Esses poliálcoois são produzidos a partir de fontes renováveis, sendo obtidos a partir de pentoses (xilose e arabinose) por vias químicas ou biotecnológicas. Apesar da via química ser amplamente utilizada, a mesma é composta por etapas dispendiosas e que impactam negativamente o meio ambiente. Nesse sentido, a via biotecnológica, fundamentada na conversão bioquímica, principalmente por leveduras em condições brandas, é mais sustentável e vem ganhando destaque, sendo o xilitol biotecnológico já produzido, inclusive, em escala industrial. Apesar disso, há desafios relativos ao custo do processamento e à otimização dos parâmetros operacionais como forma de tornar a produção por via biológica tão rentável quanto a via química (KORDOWSKA-WIATER, 2015; XU *et al.*, 2019).

Nesse sentido, o uso de resíduos agroindustriais como substrato dos processos fermentativos é uma forma de reduzir custos e promover a sustentabilidade. Resíduos vegetais, como o bagaço de cana de açúcar, já vêm sendo utilizados como uma fonte para a obtenção de hidrolisado hemicelulósico, fornecendo pentoses e hexoses necessárias para a ocorrência do processo biológico (ALVES *et al.*, 2021; DE MEDEIROS *et al.*, 2020). Apesar disso, outros resíduos agroindustriais também podem ser utilizados a fim de atuarem como fontes de nutrientes necessários ao crescimento e manutenção das leveduras, reduzindo ainda mais os custos associados às matérias-primas na etapa de fermentação. Ressalta-se que o uso de extrato de levedura como fonte de nitrogênio é um dos principais obstáculos a serem superados para a viabilização de processos

biotecnológicos em larga escala, tendo em vista o seu custo elevado inclusive na produção de xilitol (SADH; DUHAN; DUHAN, 2018; SALGADO *et al.*, 2009).

Após a etapa de produção biotecnológica do xilitol ou do arabitol, são necessários processos dispendiosos de separação e purificação a fim torná-los aptos à comercialização. Ressalta-se que o xilitol e o arabitol são isômeros e, devido à similaridade química, há dificuldade no processo de separação (HERNÁNDEZ-PÉREZ *et al.*, 2019; KORDOWSKA-WIATER, 2015; SAHA; KENNEDY, 2020). Nesse contexto, o arabitol é geralmente tratado como um composto indesejável no processo produtivo, tendo em vista que o xilitol atualmente possui um mercado mais expressivo (GRAND VIEW RESEARCH, 2022b; SAHA; KENNEDY, 2020). Apesar disso, questiona-se o porquê de o arabitol e o xilitol, devido à similaridade de propriedades e aplicações, não poderem ser tratados como coprodutos, reduzindo custos de separação e otimizando o aproveitamento das matérias primas.

De Medeiros e colaboradores (2020), a partir de hidrolisado de bagaço de sisal com a levedura *Debaryomyces hansenii*, e Araújo e colaboradores (2021), a partir de hidrolisados hemicelulósicos alternativos e a levedura *Komagataella pastoris*, indicaram a possibilidade de produção simultânea de xilitol e arabitol. Contudo, o quantitativo dos poliálcoois obtido nos dois casos foi bastante inferior ao reportado na literatura para tais compostos separadamente. Além disso, não foram encontrados estudos que avaliem parâmetros de produção para a otimização do processo, a ampliação de escala ou a alteração do modo de operação para biorreatores, revelando que ainda existem lacunas quanto ao conhecimento associado à produção conjunta de tais compostos a partir de hidrolisados hemicelulósicos.

Nesse contexto, o presente trabalho buscou avaliar os parâmetros que influenciam o processo fermentativo para a produção simultânea dos álcoois de açúcar, xilitol e arabitol, como possibilidade de reaproveitamento de resíduos agroindustriais e viabilização do processo biotecnológico em maiores escalas.

OBJETIVOS

Este trabalho objetivou avaliar a produção simultânea de xilitol e arabitol a partir de hidrolisado hemicelulósico de bagaço de cana-de-açúcar por uma cepa de *Candida tropicalis* e, para isso, teve como objetivos específicos:

i. Verificar a capacidade de *C. tropicalis* consumir os açúcares presentes no hidrolisado hemicelulósico;
ii. Selecionar a fonte nitrogenada que permita maior produção dos poliálcoois;
iii. Avaliar a influência das condições de cultivo, tais como temperatura e composição do meio, no rendimento e produtividade do processo biotecnológico;
iv. Verificar a possibilidade de alteração do modo de operação para biorreatores de bancada.

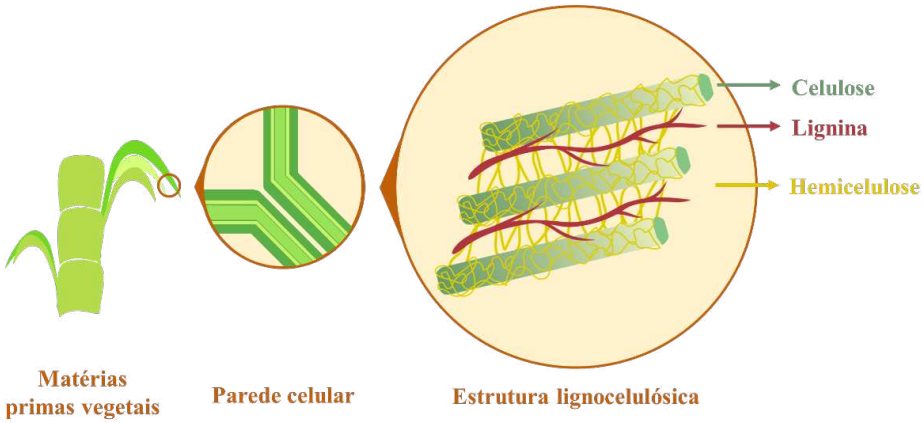
REVISÃO BIBLIOGRÁFICA

i. Biomassas lignocelulósicas e hidrolisado hemicelulósico

Lignocelulose pode ser definida como a estrutura polimérica tridimensional que está presente na parede celular de vegetais, sendo um componente estrutural que confere, de forma geral, o aspecto estrutural das matérias-primas de origem vegetal. A biomassa lignocelulósica pode ser caracterizada como a mais abundante disponível no planeta, constituindo um recurso renovável e sendo relevante para o desenvolvimento sustentável principalmente por corresponder a diversos resíduos urbanos e agroindustriais, como a espiga de milho e o bagaço de cana-de-açúcar. Por se tratar de resíduos que muitas vezes não podem ser devidamente aproveitados para a alimentação humana ou animal, tais materiais despertam o interesse também por evitarem a competição entre matérias primas destinadas à produção de energia e biocompostos *versus* à alimentação (BANWELL *et al.*, 2021; FARIAS *et al.*, 2022).

Em relação à composição, a lignocelulose é constituída, além de componentes presentes em menores quantidades, como proteínas, compostos extrativos e minerais, de três estruturas poliméricas principais: lignina, celulose e hemicelulose (FARIAS *et al.*, 2022), como apresentado no esquema da Figura 1. A composição em termos de cada um desses componentes pode variar de acordo com a espécie, porém, tem-se, em média, de 10 a 20 % de lignina, 20 a 40 % de hemicelulose e 30 a 50 % de celulose (MUSSATTO; DRAGONE, 2016).

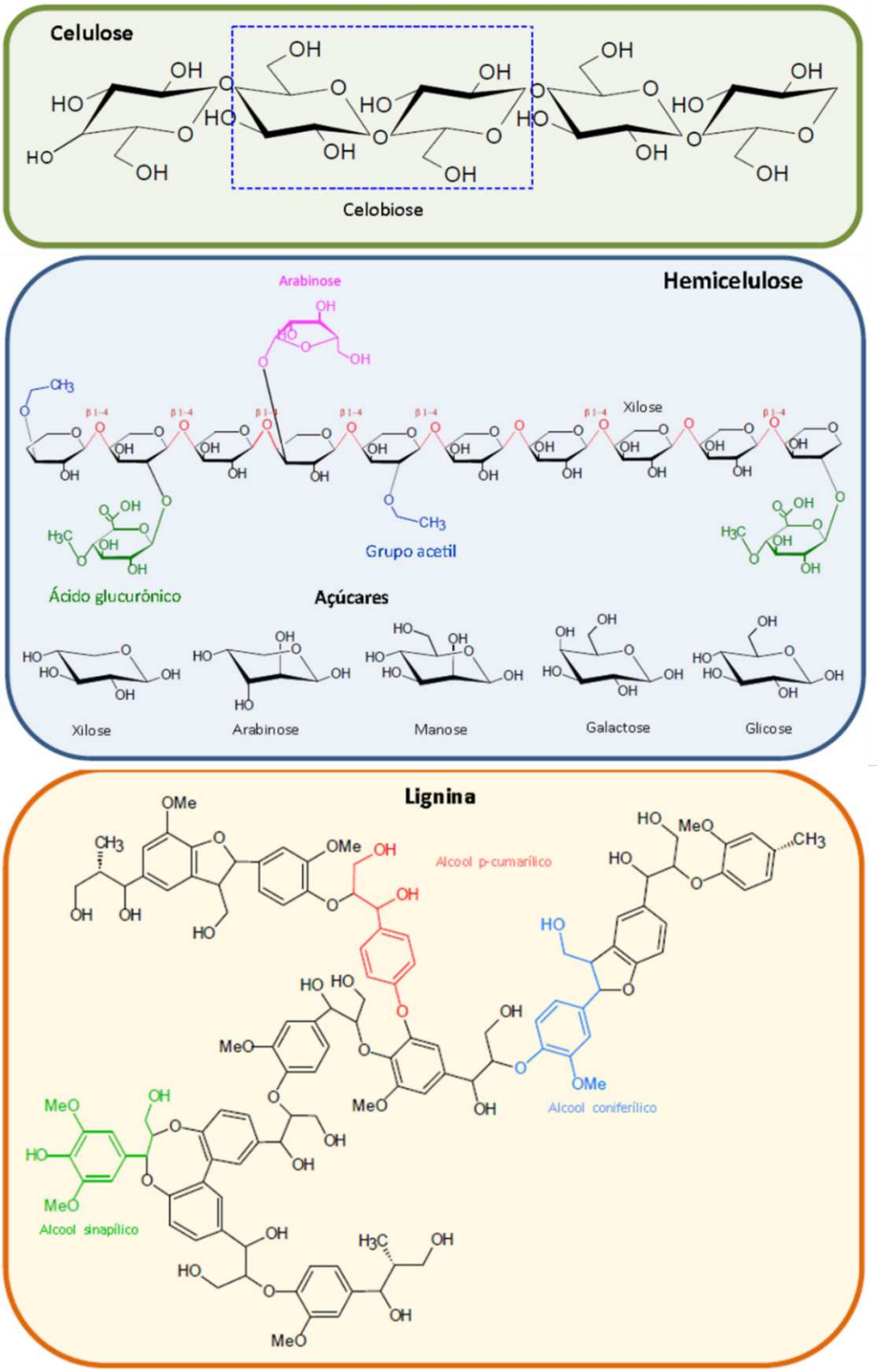
Figura 1. Esquema representativo da biomassa lignocelulósica,



Fonte: elaboração própria.

A estrutura química generalizada dos principais componentes da biomassa lignocelulósica é apresentada na Figura 2.

Figura 2. Estrutura química geral de celulose, hemicelulose e lignina.



Fonte: adaptado de Mussatto e Dragone (2016)

O principal polímero constituinte da lignocelulose é a celulose, formada por moléculas de glicose ligadas por ligações glicosídicas β-1,4, formando fibras rígidas conectadas por ligações de hidrogênio intra e intermoleculares. Microscopicamente há regiões amorfas, menos ordenadas, e cristalinas, altamente ordenadas e que dificultam a hidrólise enzimática ou química da celulose para a liberação de glicose ou oligossacarídeos (FARIAS *et al.*, 2022; MUSSATTO; DRAGONE, 2016)

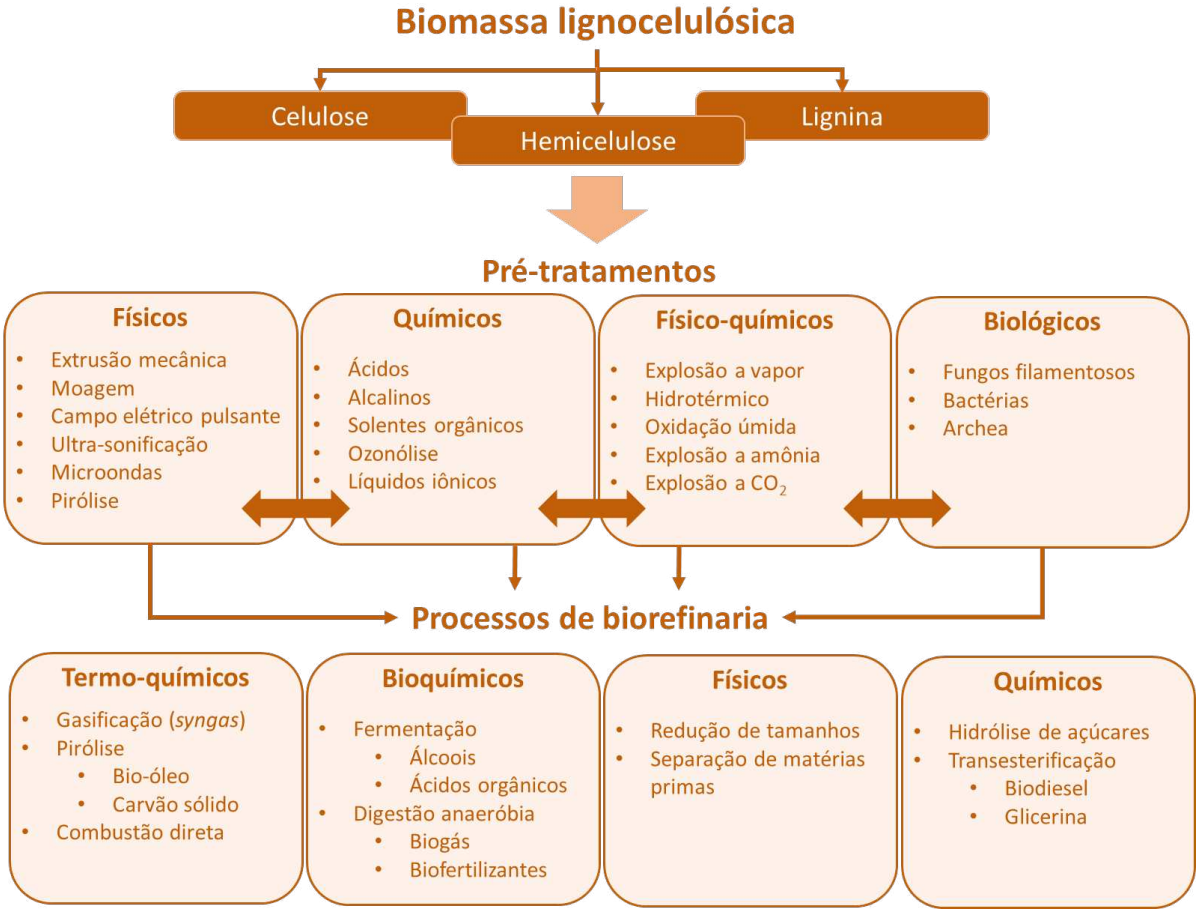
Quanto à lignina, apesar de estar presente em menores quantidades, é o maior responsável pela recalcitrância da biomassa lignocelulósica. Constitui-se de uma estrutura polimérica de fenil propanóides, como o ácido coniferilíco e os álcoois sinapílico e p-cumarílico, conectados por diferentes tipos de ligações e inclusive conectado às estruturas de celulose e hemicelulose por ligações covalentes. Essa estrutura covalente complexa e amorfa confere a união e a rigidez da biomassa (MUSSATTO; DRAGONE, 2016)

Em relação à hemicelulose, trata-se de um dos biopolímeros mais abundantes no mundo, atrás apenas da celulose. Esta pode ser definida como os polissacarídeos não celulósicos que compõem a estrutura da biomassa lignocelulósica. Possuem estrutura amorfa e heterogênea, sendo constituída de pentoses, hexoses e grupos ácidos, ligadas à celulose por ligações de hidrogênio e à lignina por ligações covalentes. As principais cadeias presentes na hemicelulose são compostas por xilose, chegando a 90 %, e L-arabinose, com cerca de 10 %, podendo variar de acordo com a matéria-prima (MUSSATTO; DRAGONE, 2016; XIE *et al.*, 2020).

No contexto de biorrefinarias, ou seja, do aproveitamento de biomassas renováveis para a produção de combustíveis, compostos químicos e materiais, a biomassa lignocelulósica é introduzida como um elemento fundamental (CHANDEL *et al.*, 2021). Nesse sentido, a mesma pode ser submetida a diferentes pré-tratamentos físicos, químicos, físico-químicos, biológicos e combinações desses como forma de romper a estrutura lignocelulósica e obter diferentes compostos que atuarão como substrato para diferentes processos integrados, como a produção de carvão, a produção de biodiesel e a fermentação para a produção de polióis (CHANDEL *et al.*, 2021; WAGLE *et al.*, 2022). A Figura 3 sumariza os diferentes

pré-tratamentos que podem ser realizados e os produtos que podem ser obtidos a partir da biomassa lignocelulósica.

Figura 3. Visão geral dos diferentes pré-tratamentos que podem ser aplicados e produtos que podem ser obtidos a partir da biomassa lignocelulósica.



Fonte: adaptado de Wagle et al. (2022)

Os pré-tratamentos são processos aplicados como forma de tornar a lignocelulose mais disponível para os processos hidrolíticos, podendo atuar, por exemplo, aumentando a área superficial, diminuindo a cristalinidade da celulose e separando a hemicelulose. A aplicação de tais processos, que podem variar de acordo com a biomassa e com o objetivo desejado, possibilitam a realização dos processos de biorrefinaria de forma mais eficiente, para, a partir daí, obter os produtos desejados do processo (WAGLE *et al.*, 2022).

Em relação especificamente à hemicelulose, diferente da celulose, sua estrutura pode ser facilmente hidrolisada por diferentes processos, como o uso de

ácido diluído, o uso de compostos alcalinos, explosão a vapor, processos hidrotérmicos e a aplicação de líquidos iônicos (CHANDEL *et al.*, 2021; FARIAS *et al.*, 2022). Dependendo da aplicação desejada e das tecnologias disponíveis, diferentes pré-tratamentos podem ser mais adequados. A utilização de solventes alcalinos, por exemplo, promove a quebra das ligações de hidrogênio entre a hemicelulose e a celulose assim como as covalentes entre a hemicelulose e a lignina. Isso faz com que seja obtida uma hemicelulose de maior pureza com as moléculas de xilose apresentadas principalmente na forma polimérica de xilana, que pode ser interessante, por exemplo, para a produção de xilooligossacarídeos, compostos prebióticos (BANWELL *et al.*, 2021)

O processo que utiliza ácido diluído, por outro lado, é um dos mais bem estabelecidos comercialmente em larga escala. Em tal processo aplicam-se soluções de ácidos diluídos, como o sulfúrico, o nítrico, clorídrico ou o fluorídrico, em combinação com temperaturas que podem variar entre 120 e 140 ºC. Nesse processo, as ligações entre a hemicelulose e a celulose são quebradas e é promovida a solubilização e hidrólise da própria hemicelulose. Nesse contexto, é promovida uma maior quebra da estrutura polissacarídica hemicelulósica, levando à liberação de unidades de açúcares, como a xilose, a glicose e a arabinose, e ácidos, como o acético e o glucurônico (BANWELL *et al.*, 2021; CHANDEL *et al.*, 2021; FARIAS *et al.*, 2022).

Os açúcares liberados constituem um grande interesse para a utilização do hidrolisado hemicelulósico ácido em processos de biorrefinaria fermentativos, já que os monossacarídeos podem ser melhor aproveitados pelos microrganismos para a formação de diferentes bioprodutos. Por outro lado, compostos como os fenólicos e o ácido acético, liberados na quebra da estrutura lignocelulósica e da cadeia hemicelulósica, e furânicos, furfural e 5-hidroximetilfurfural, subprodutos gerados a partir da desidratação, respectivamente, das pentoses e hexoses pelo tratamento térmico ácido, devem ser adequadamente monitorados, tendo em vista que os mesmos possuem potencial de inibir do metabolismo microbiano (CHOUDHARY *et al.*, 2021; FARIAS *et al.*, 2022; YE *et al.*, 2021).

Nesse sentido, metodologias de remoção desses compostos podem ser desenvolvidas a fim de obter um hidrolisado hemicelulósico com menores concentrações de inibidores, ou seja, destoxificados. Uma das metodologias bem

estabelecidas para essa remoção é o *liming*, processo que consiste em elevar o pH até a neutralidade do hidrolisado hemicelulósico ácido seguido de redução até o pH desejado para o processo fermentativo. Assim, é promovida a precipitação de compostos que são instabilizados pela alteração do pH. Esse processo pode ser seguido de métodos adicionais, como a adsorção em carvão ativado para aumentar a eficiência do processo. Apesar de ocorrerem perdas da concentração inicial dos açúcares presentes no hidrolisado hemicelulósico, essa metodologia é bastante eficiente, principalmente na remoção de compostos inibidores aromáticos, como é o caso do furfural e do 5-hidroximetilfurfural (PALLADINO *et al.*, 2021; RAVELLA *et al.*, 2012).

Dessa maneira, podem ser obtidos hidrolisados hemicelulósicos ricos em açúcares, apresentando baixas concentrações de compostos inibidores e com potencial para a utilização em processos biotecnológicos. Ressalta-se que é possível utilizar as pentoses e hexoses liberadas como substratos para processos químicos e bioquímicos (fermentativos ou enzimáticos) como forma de gerar produtos como o xilitol, o arabitol, o etanol, o 2,3-butanodiol, butanol, ácidos orgânicos e biopolímeros (ARCAÑO *et al*., 2020; KUMAR *et al*., 2018).

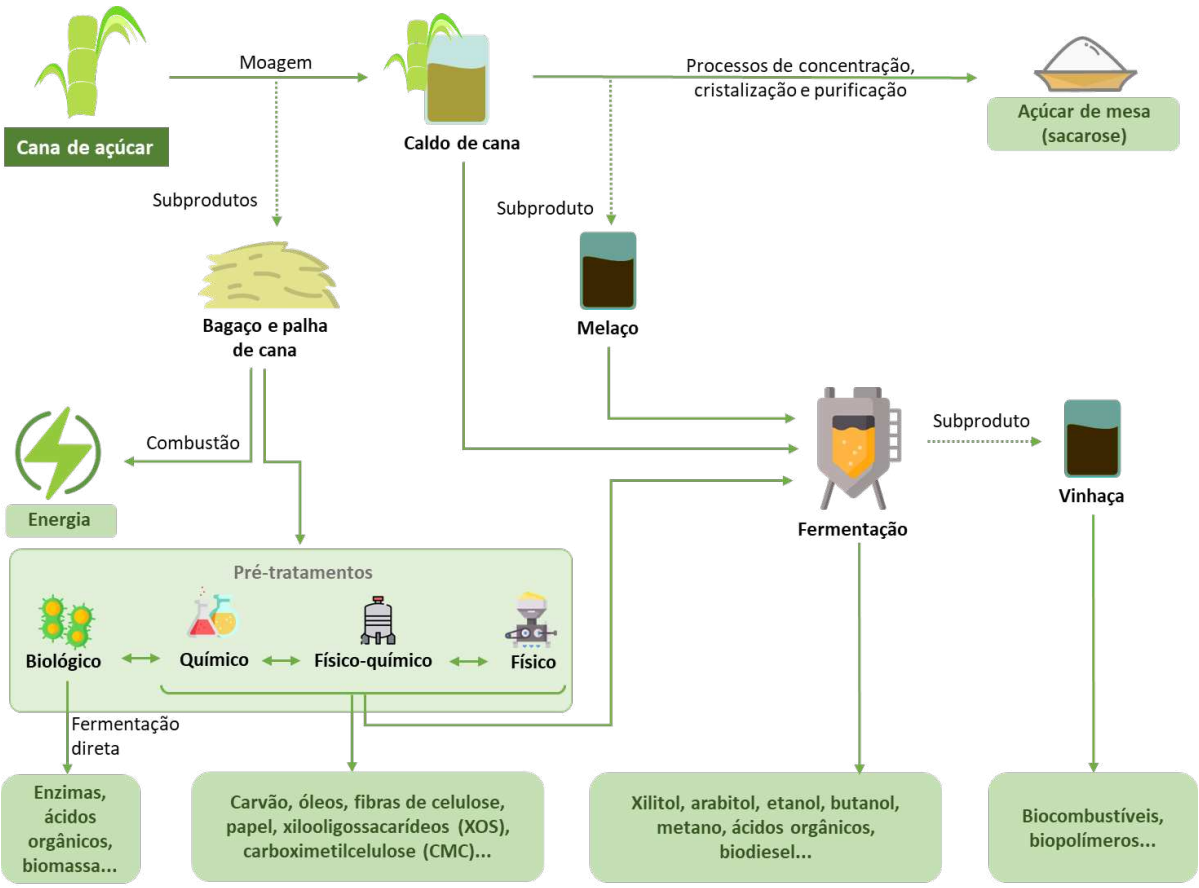
ii. Hidrolisado hemicelulósico de bagaço de cana-de-açúcar

Avaliando o contexto brasileiro, a cana-de-açúcar continua apresentando destaque como biomassa lignocelulósica. Isso acontece porque a cana-de-açúcar continua sendo a principal matéria-prima utilizada para a produção de bioetanol, além de poder ser processada para a obtenção do açúcar de mesa. A partir de tais processos, o bagaço da cana-de-açúcar pode ser considerado como um dos principais resíduos gerados, sendo muitas vezes destinado para a combustão ou a geração de energia térmica nas próprias usinas (FARIAS *et al.*, 2022; REENA *et al.*, 2022; SHABBIRAHMED *et al.*, 2022).

Nesse contexto, o reaproveitamento do bagaço de cana-de-açúcar permite a integração, na própria usina, dos processos de produção de etanol de segunda geração e outros bioprodutos. Assim, a ideia de desenvolvimento de uma biorrefinaria integrada para a produção de bioprodutos, biocombustíveis e energia desponta como uma possibilidade no reaproveitamento integral dessa biomassa

(FARIAS *et al.*, 2022). A Figura 4 esquematiza o processo de aproveitamento e possibilidades de metodologias e produtos a partir da cana-de-açúcar.

Figura 4. Esquema de aproveitamento da cana-de-açúcar e seu bagaço em processos integrados para a geração de bioprodutos, combustíveis e energia.



Fonte: elaboração própria

O uso de biomassa lignocelulósica oriunda de resíduos agroindustriais para a produção biotecnológica de diferentes compostos promove o conceito de *biobased economy*. Isso acontece porque esse tipo de aplicação induz a redução do uso de fontes fósseis, não renováveis, como o petróleo, para a utilização de um recurso renovável e de mais baixo custo na produção de combustíveis e químicos de alto valor agregado, reduzindo os impactos ambientais e promovendo a sustentabilidade (MUSSATTO; DRAGONE, 2016).

Nesse contexto, o hidrolisado hemicelulósico do bagaço de cana-de-açúcar, fonte de pentoses e hexoses que pode ser obtido a partir desse resíduo, representa um substrato em potencial para processos biotecnológicos. A Tabela 1

mostra a composição de uma amostra de hidrolisado hemicelulósico de bagaço de cana de açúcar caracterizado no Laboratório de Engenharia Metabólica e Bioprocessos da Universidade Estadual de Campinas.

Tabela 1. Composição de hidrolisado hemicelulósico de bagaço de cana de açúcar.

Componente	**Composição ($g.L^{-1}$)**	**Composição (%)**
Xilose	48,209	79,53
Glicose	5,197	8,57
Arabinose	5,179	8,54
Ácido acético	1,948	3,21
Furfural	0,043	0,07
Hidroximetilfurfural	0,038	0,06

Fonte: baseado em Travália (2020)

Percebe-se que a xilose foi o principal constituinte, mas as concentrações de glicose e arabinose, apesar de menos presentes que a xilose, também apresentaram percentuais significativos, acima de 8 %. Tais aspectos tornam essa matéria prima um substrato potencial para a utilização por leveduras assimiladoras de pentoses, como é o caso das capazes de produzir xilitol e arabitol. Ressalta-se que as proporções de hemicelulose na biomassa lignocelulósica e a composição da própria hemicelulose podem variar de acordo com a espécie, estágio de desenvolvimento, condições de crescimento e as partes da planta que originaram a biomassa. Além disso, o hidrolisado hemicelulósico é obtido de um processo de solubilização em ácido diluído seguido de evaporação, logo, a concentração dos componentes pode variar dependendo das condições em que os processos são realizados, principalmente a evaporação (HERNÁNDEZ-PÉREZ; DE ARRUDA; FELIPE, 2016; SHRESTHA *et al.*, 2021).

iii. Xilitol e arabitol: definições, aplicações e mercado

O xilitol é um poliálcool composto de cinco carbonos que tem ocorrência na natureza e possui a capacidade de atuar como composto adoçante de alimentos e bebidas, tendo propriedades de doçura similares à sacarose comercial, mas sendo

40% menos calórico (XU *et al.*, 2019). Esse composto é obtido exclusivamente a partir de biomassa, especificamente da D-xilose presente nela, e vem sendo utilizado comercialmente como substituto da sacarose. Aplica-se o xilitol, por exemplo, em alimentações especiais, como a de diabéticos, por seu metabolismo independente da insulina e efeito de saciedade, e na nutrição parenteral, por ser inerte a aminoácidos e, portanto, mais estável em tais produtos (ALBUQUERQUE *et al.*, 2014; XU *et al.*, 2019).

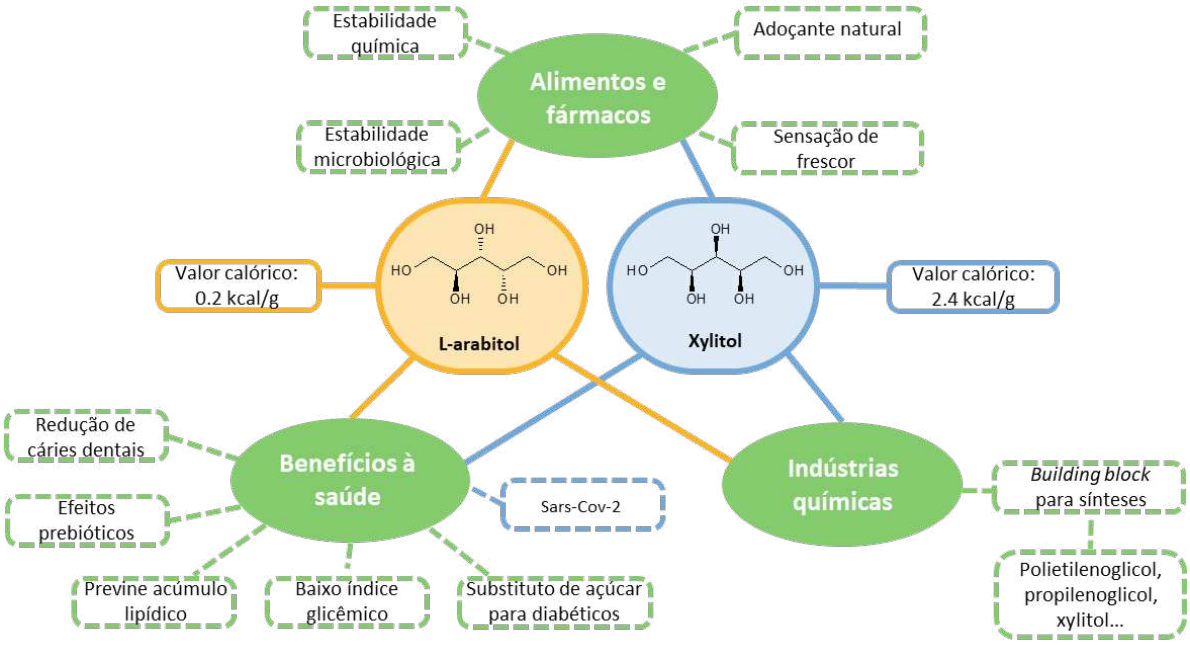
Em relação aos seus efeitos benéficos à saúde, estudos indicam que o xilitol atua como um prebiótico, reduzindo o pH fecal, além de contribuir na prevenção da osteoporose e na proteção dos dentes contra a formação de cáries e a desmineralização. Tais características, junto à capacidade de retenção de umidade e à sensação de frescor, causada na boca pelo calor de dissolução endotérmico do xilitol, fazem com que o produto seja amplamente utilizado em gomas de mascar sem açúcar e em cremes dentais. Além disso, o xilitol pode ser utilizado como matéria prima na indústria química para a produção de outros compostos, como propilenoglicol, polietilenoglicol e ácido xilárico (ALBUQUERQUE *et al.*, 2014; ARCAÑO *et al.*, 2020; XU *et al.*, 2019).

Em relação ao L-arabitol, trata-se de um poliálcool de cinco carbonos, que é um estereoisômero do xilitol. Além das semelhanças nas estruturas químicas, esses dois compostos compartilham de propriedades similares. O arabitol possui capacidade de atuar como composto adoçante, com doçura 70% menor que o xilitol, mas possuindo um teor calórico de 0,2 kcal/g, que é 12 vezes menor que o do xilitol e 20 vezes menor que o da sacarose, fazendo com que, mesmo que adicionado em maior quantidade para um mesmo dulçor, apresente menor valor calórico (AWUCI; ECHETA, 2019; ZHENG *et al.*, 2020).

Devido ao calor de dissolução endotérmico, o arabitol gera a sensação de frescor na boca e, também de maneira similar ao xilitol, possui potencial para a aplicação em produtos de saúde bucal por propriedades anticariogênicas, podendo ser utilizado em alimentações para diabéticos por seu metabolismo independente da insulina. Do mesmo modo, esse composto pode auxiliar na manutenção de bactérias probióticas no organismo e pode ser utilizado pela indústria química para a síntese de fármacos e outros compostos como xilitol, propilenoglicol e etilenoglicol, sendo apontado como um composto alvo para o desenvolvimento de pesquisas em

biotecnologia industrial (ERICKSON; NELSON; WINTERS, 2012; KORDOWSKA-WIATER, 2015; KORDOWSKA-WIATER; KUBIK-KOMAR; TARGOŃSKI, 2013). O esquema da Figura 5 sumariza as principais aplicações em potencial e propriedades de interesse do xilitol e do arabitol.

Figura 5. Principais propriedades e aplicações potenciais do xilitol e do arabitol

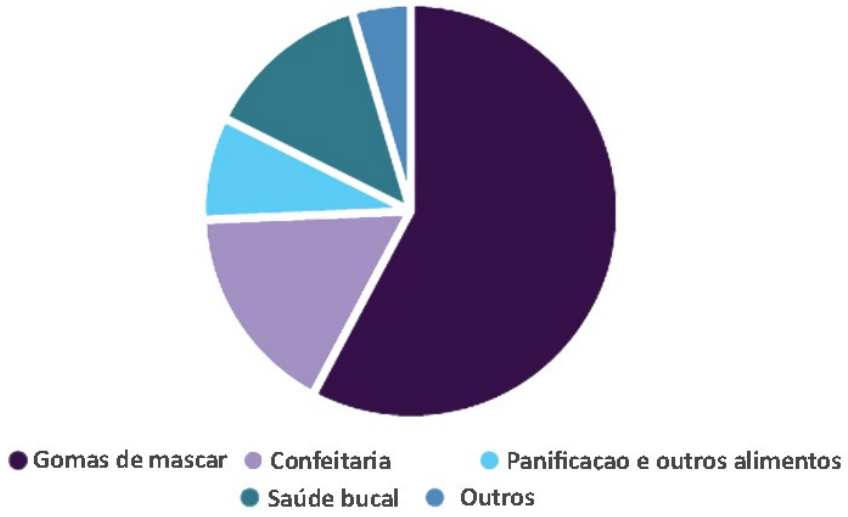


Fonte: adaptado de Farias et al. (2022).

Com o aumento da demanda por produtos mais saudáveis e funcionais, o mercado de álcoois de açúcar está em constante expansão, tendo sido estimado em 3.42 bilhões de dólares em 2021 e com previsão de aumento para até 5.14 bilhões até 2028 (GRAND VIEW RESEARCH, 2022b). Especificamente em relação ao xilitol, o mesmo foi responsável pela circulação de 880 milhões de dólares em 2019, com previsão de aumento da circulação no setor para valores superiores a 1 bilhão de dólares até 2026 (GLOBAL MARKET INSIGHTS, 2020).

A Figura 6 apresenta a distribuição do mercado de xilitol por área de aplicação. Percebe-se, como ressaltado anteriormente, que a principal aplicação atual no mercado para o xilitol está no setor de gomas de mascar, correspondendo a mais de 50 %. Além disso, o setor de alimentos apresenta um grande destaque, sendo responsável por mais de 75 % do mercado de xilitol.

Figura 6. Mercado de xilitol por aplicações, baseado no mercado de $ 447,9 milhões avaliados em 2019



Fonte: adaptado de Grand View Research (2022a)

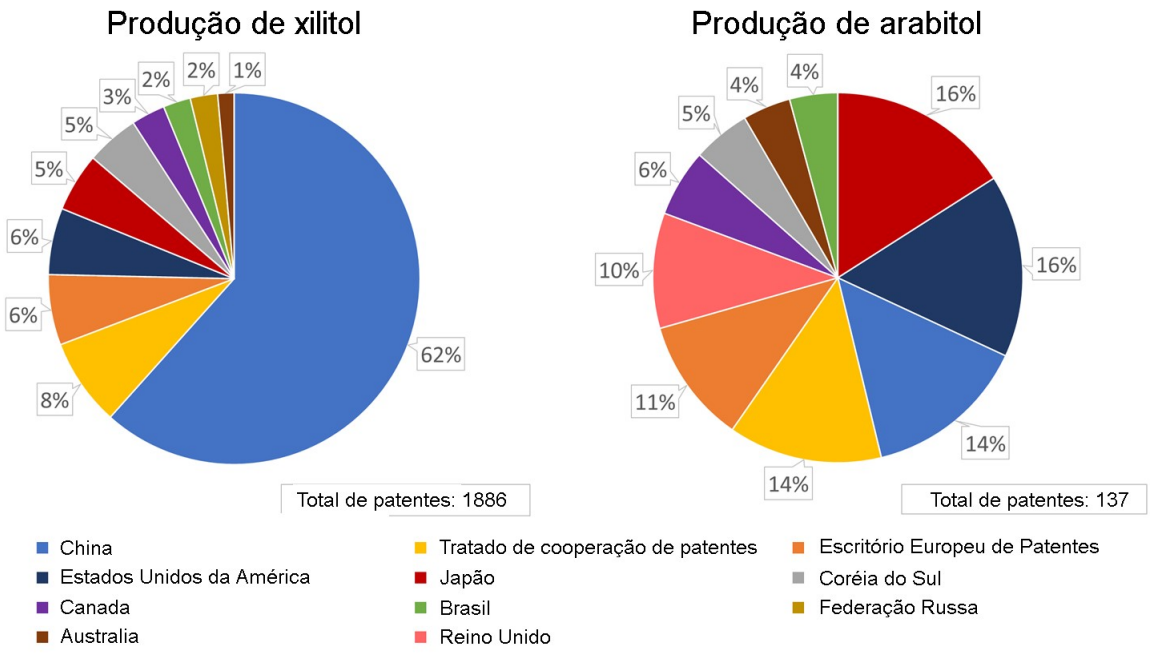
Quanto ao arabitol, apesar do mercado não ser tão expressivo, evidencia-se que, caso o custo de produção do arabitol fosse reduzido, o mesmo teria potencial para concorrer diretamente com o xilitol, por possuir propriedades semelhantes associadas a um menor valor calórico (LOMAN, A. A.; ISLAM; JU, 2018; LOMAN, ABDULLAH Al; JU, 2013). Além disso, uma das principais lacunas associadas às indústrias que utilizam xilitol é a não disponibilidade de uma alternativa viável de substituição do mesmo (GLOBAL MARKET INSIGHTS, 2020).

Em relação ao conhecimento de produção de xilitol e arabitol pelo mercado, a Figura 7 apresenta a distribuição de patentes associadas a tais conteúdos por países. Nota-se que há um número mais de dez vezes menor de patentes associadas à produção de arabitol em comparação com o xylitol, apesar disso, as patentes associadas ao arabitol apresentam ampla distribuição, indicando oportunidades em diferentes regiões geográficas.

Quanto ao xilitol, percebe-se que há uma relação entre os principais mercados produtores e o domínio regional de patentes, tendo em vista que a China é um dos principais produtores de xilitol, já tendo sido responsável por mais de 50 % da produção mundial em 2012 e, consequentemente, detém historicamente o maior número de patentes. Além disso, outro país que tem aumentado o potencial

produtivo de xilitol é os Estados Unidos, que passaram a explorar a biomassa lignocelulósica de eucalipto para tal fim. No cenário atual, portanto, as principais companhias responsáveis pela produção de xilitol são a DuPont, cuja sede está localizada nos Estados Unidos, e a Futaste Pharmaceutical, cuja sede está localizada na China (GLOBAL MARKET INSIGHTS, 2020; RAVELLA *et al.*, 2012).

Figura 7. Distribuição de patentes associadas à produção de xilitol ou arabitol por países em 2021.

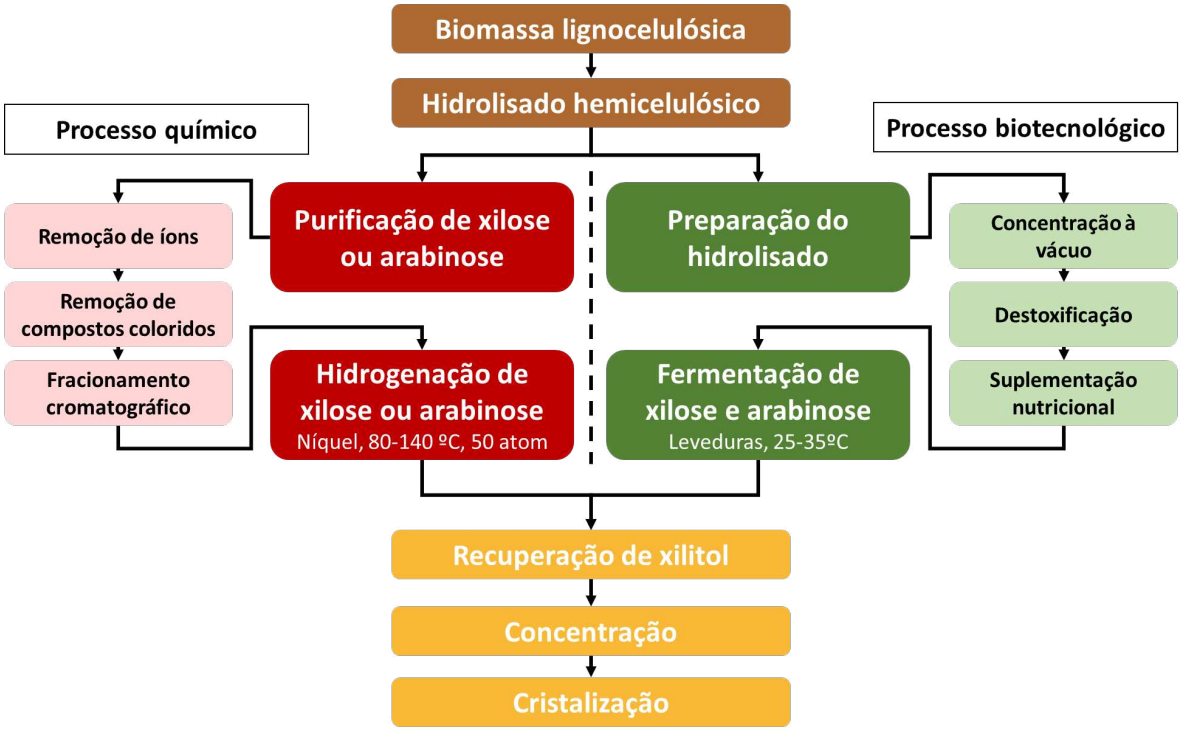


Fonte: adaptado de Farias et al. (2022)

iv. Produção química e biotecnológica de xilitol e arabitol

O xilitol e o arabitol podem ser produzidos por vias químicas e biotecnológicas, como mostrado no fluxograma comparativo da Figura 8. Apesar disso, comercialmente, tanto o xilitol como o arabitol vêm sendo produzidos majoritariamente por meio de processamentos químicos (XU *et al.*, 2019; ZHENG *et al.*, 2020).

Figura 8. Comparação entre os processos de produção química e biotecnológica para a obtenção do xilitol.



Fonte: adaptado de Hernández-Pérez et al. (2019).

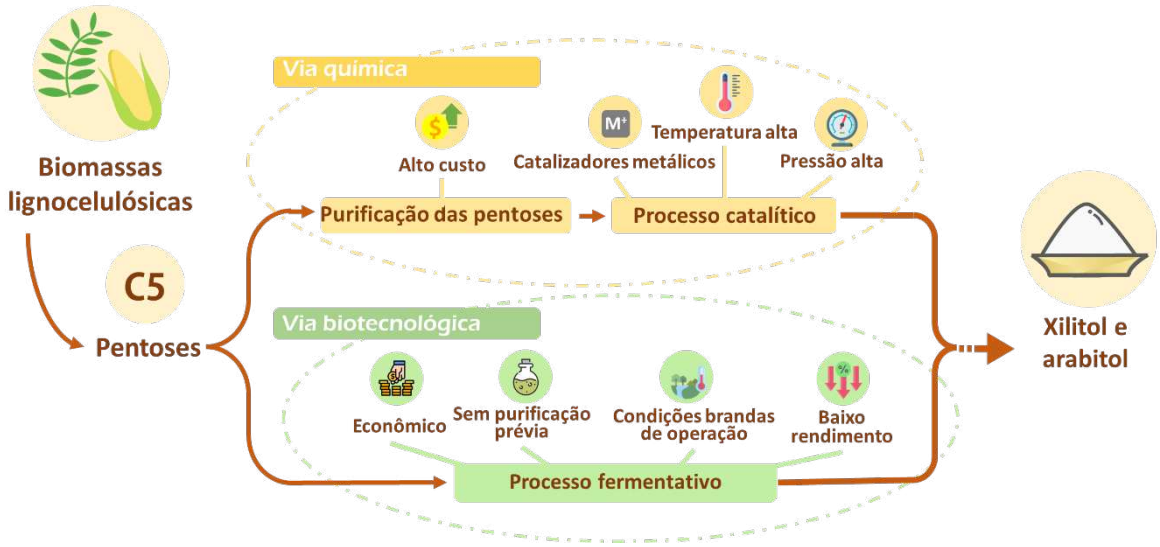
No processo químico de obtenção do xilitol, após o tratamento da biomassa lignocelulósica a fim de obter o hidrolisado hemicelulósico, é necessária uma onerosa etapa de purificação da xilose, uma vez que a presença de impurezas pode reduzir a eficiência do processo de catálise química. Esse processo é constituído, então, de diferentes etapas que incluem, inclusive, o fracionamento cromatográfico até a obtenção da xilose purificada. A partir daí, é promovida uma reação por meio de catalisadores metálicos, como ligas de níquel e alumínio, em temperaturas entre 70 e 140 ºC e pressões acima de 40 atm (HERNÁNDEZ-PÉREZ *et al.*, 2019; MUSSATTO; ROBERTO, 2004; ZHANG *et al.*, 2013). Em relação ao arabitol, ele é quimicamente é obtido a partir de lactonas do ácido arabinônico, em um processo similar ao do xilitol, em que etapas de purificação inicial são necessárias, com o uso posterior de um catalisador metálico em temperaturas de cerca de 100 ºC (KORDOWSKA-WIATER, 2015; KUMDAM; MURTHY; GUMMADI, 2013). Tanto em relação ao xilitol quanto ao arabitol, são necessárias etapas

posteriores de separação e purificação a fim de obter o produto final separado do excedente de reagentes (HERNÁNDEZ-PÉREZ *et al.*, 2019).

Percebe-se que o processamento químico dos dois produtos demanda condições drásticas (elevadas pressão e temperatura), aplicam metais onerosos como catalisadores e possuem etapas de separação e purificação com elevados custos. Esses fatores são responsáveis por tornar o processo mais dispendioso, com elevados consumo de energia e agressão ao meio ambiente, não contribuindo para a sustentabilidade do planeta (XU *et al.*, 2019; ZHENG *et al.*, 2020).

Nesse contexto, vem ganhando destaque a via de produção biotecnológica, que utiliza o metabolismo de microrganismos, como a levedura *Candida tropicalis*, para converter açúcares como a xilose e a arabinose, presentes no hidrolisado hemicelulósico, em xilitol e arabitol. O processo biológico desperta o interesse por utilizar condições de operação brandas, gerando um menor impacto ambiental, além de ser biosseletivo, ou seja, o hidrolisado hemicelulósico pode ser utilizado de forma concentrada e destoxificada, mas dispensando etapas de purificação da xilose ou da arabinose, pois os microrganismos são capazes de assimilar as pentoses de interesse no próprio meio complexo. Apesar disso, algumas dificuldades também são evidenciadas, como o elevado custo dos meios de cultivo, o baixo rendimento do processo e os custos e eficiência não satisfatórios dos processos de separação e purificação (XU et al. 2019; ALVES et al. 2021). O esquema da Figura 9 sumariza as diferenças e desafios entre os processamentos por via química ou biotecnológica para a produção de xylitol ou arabitol.

Figura 9. Esquema comparativo entre as vias química e biotecnológica de produção de xilitol e arabitol.



Fonte: elaboração própria.

Em relação ao alto custo associado aos meios de cultivo, trata-se de uma consequência tanto do preparo do hidrolisado hemicelulósico, quando este é utilizado, quanto da adição de outros componentes ao mosto fermentativo. Nesse sentido, estudos de otimização de composição do meio nesses processos biotecnológicos são relevantes como forma de promover o menor custo inicial com as matérias primas. Em relação à composição de nitrogênio, por exemplo, há trabalhos que avaliam o efeito de diferentes fontes do nutriente no rendimento do processo, inclusive de fontes alternativas, como o farelo de arroz, como forma de reduzir ainda mais os custos atrelados à produção (KORDOWSKA-WIATER, 2015; LU *et al.*, 1995; MORAIS JUNIOR *et al.*, 2019; PALLADINO *et al.*, 2021; RACHWAŁ *et al.*, 2020; WU *et al.*, 2018). Além da redução de custos, o uso de resíduos agroindustriais em bioprocessos pode contribuir com a diminuição dos impactos do homem ao meio ambiente e agrega valor a subprodutos, tornando as cadeias produtivas mais sustentáveis (SADH; DUHAN; DUHAN, 2018).

Quanto ao rendimento do processo, diversos fatores permitem a obtenção de uma maior quantidade de bioprodutos, como o aperfeiçoamento da composição do meio e a otimização das condições de fermentação, como a temperatura e a oxigenação do mosto, fatores relacionados à manutenção das vias metabólicas dos microrganismos (CHENG *et al.*, 2014; LOMAN, A. A.; ISLAM; JU, 2018; MORAIS JUNIOR *et al.*, 2019).

Ressalta-se que, devido à similaridade química dos compostos, é obtida uma mistura de difícil e onerosa separação, em que o arabitol é geralmente tratado como uma impureza do xilitol (SAHA & KENNEDY, 2020). Apesar disso, as propriedades e aplicabilidades bastante equivalentes desses bioprodutos indicam uma possibilidade de utilização simultânea dos mesmos, embora não haja citações na literatura quanto a tal uso. Metodologias de produção e separação vêm sendo estudadas e, principalmente em relação ao arabitol, a literatura necessita de progresso em relação aos processos aplicados desde a obtenção do bioproduto (KORDOWSKA-WIATER, 2015; XU *et al.*, 2019).

v. Vias metabólicas de assimilação de pentoses

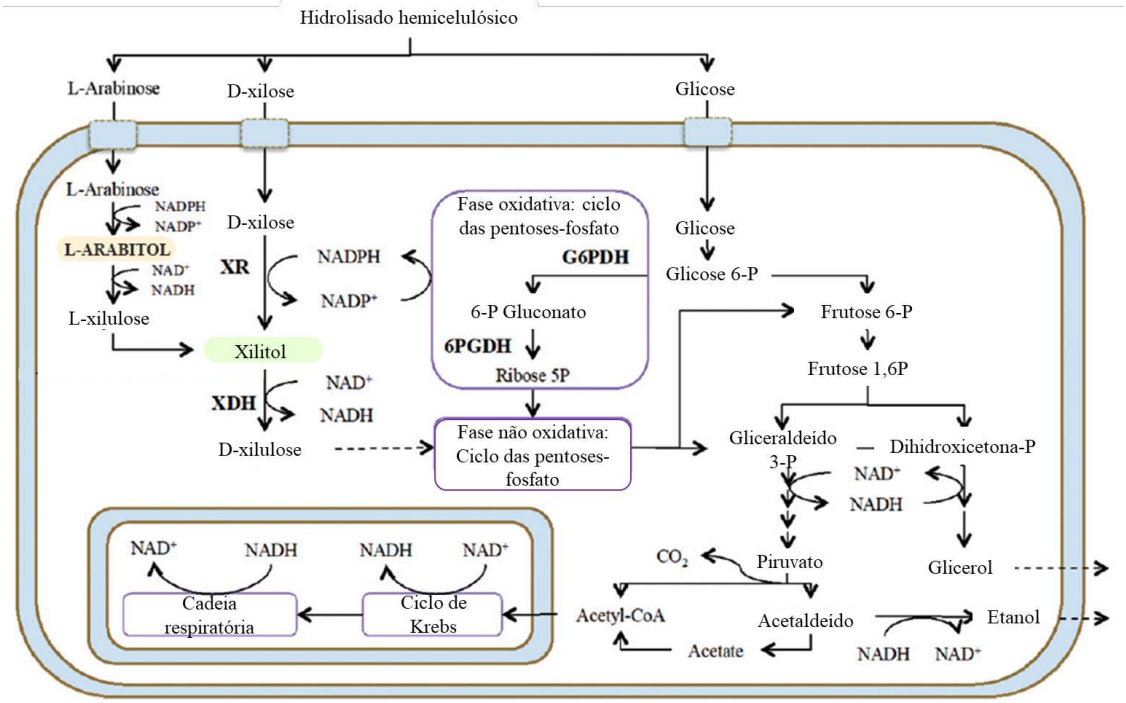
O xilitol e o arabitol, quando produzidos a partir da xilose e da arabinose por fungos filamentosos e leveduras, são gerados no meio celular como intermediários em vias metabólicas energéticas, de autorregulação e de aumento da biomassa celular dos microrganismos (FARIAS *et al.*, 2022; FRANCOIS; ALKIM; MORIN, 2020; HERNÁNDEZ-PÉREZ *et al.*, 2019). A Figura 10 esquematiza as vias metabólicas de assimilação dos açúcares de hidrolisados hemicelulósicos por leveduras e a Figura 11 particulariza a etapa de síntese do xilitol e do arabitol.

Em relação a *Eubacteria*, como *Escherichia coli*, apesar de serem capazes de assimilar pentoses, as vias metabólicas não geram xilitol ou arabitol, seguindo por vias catalisadas por L-arabinose e D-xilose isomerases (Figura 11), com raras exceções, como *Mycobacterium smegmatis*.

Na via metabólica dos fungos filamentosos e leveduras assimiladoras de pentoses (Figura 11), a assimilação das pentoses ocorre por processos de oxirredução, dependentes dos cofatores $NAD(P)^+$ (oxidante) e NAD(P)H (redutor). Após a assimilação das pentoses para o meio intracelular por estruturas transportadoras de açúcar na membrana, uma enzima aldose redutase NADPH dependente (L-arabitol ou D-xilose redutase) reduz L-arabinose ou D-xilose a, respectivamente, L-arabitol ou xilitol. Em seguida, o L-arabitol ou o xilitol sintetizados podem ser oxidados a L-xilulose ou D-xilulose por ação das enzimas NAD^+ dependentes L-arabitol desidrogenase ou xilitol desidrogenase, respectivamente (BETTIGA *et al.*, 2009; FARIAS *et al.*, 2022; JAGTAP; RAO, 2018). A L-xilulose produzida a partir do L-arabitol é então reduzida a xilitol pela enzima NADPH

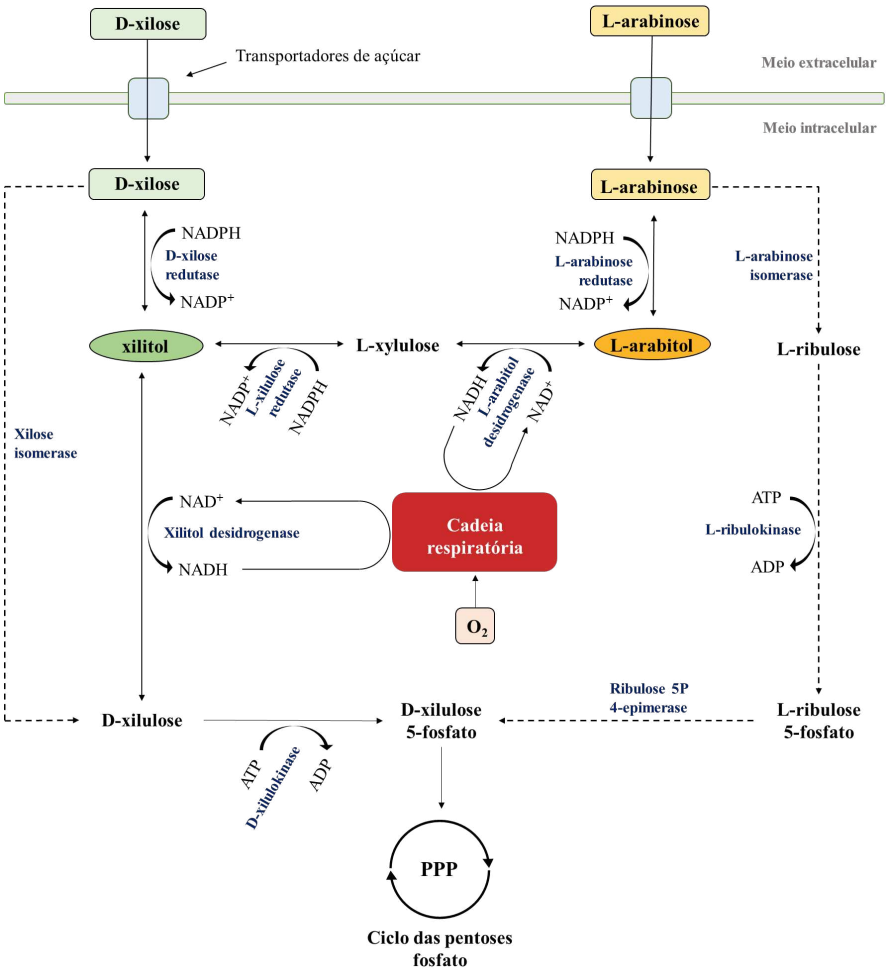
dependente L-xilulose redutase e, então, como descrito anteriormente, o xilitol é oxidado a D-xilulose. A D-xilulose produzida pelos processos de assimilação de L-arabinose e D-xilose pode, então, ser fosforilada em um processo irreversível que consome ATP, a D-xilulose 5-fosfato, molécula que será introduzida no Ciclo das Pentoses Fosfato (PPP) para a continuidade do metabolismo celular (FARIAS *et al.*, 2022; FRANCOIS; ALKIM; MORIN, 2020; GUO *et al.*, 2019; SEIBOTH; METZ, 2011).

Figura 10. Esquema do metabolismo envolvido na assimilação dos principais carboidratos do hidrolisado hemicelulósico.



Fonte: adaptado de Hernández-Pérez et al. (2019).

Figura 11. Via metabólica de assimilação de D-xilose e L-arabinose por bactérias (linha tracejada) e por fungos filamentosos e leveduras com geração intermediária de xilitol (linha cheia).



Fonte: adaptado de Farias et al. (2022).

Destaca-se que, através da via respiratória, com o consumo de oxigênio, o NAD^+ é constantemente reconstituído a partir do NADH, promovendo a continuidade da via metabólica. Isso faz com que o L-arabitol e o xilitol sintetizados sejam consumidos pelas vias enzimáticas, como explicado anteriormente. Entretanto, em condições de baixa oxigenação, a regeneração de NADH a NAD^+ é dificultada, fazendo com que o NADH seja acumulado nas células. Assim, as enzimas que utilizam o NAD^+ como cofator, como as desidrogenases do xilitol e do L-arabitol, têm a ação reduzida, fazendo com que o L-arabitol e o xilitol não sejam convertidos a D-xilulose e, portanto, sejam acumulados no meio intracelular e

excretados pelas células (BETTIGA *et al.*, 2009; FARIAS *et al.*, 2022; HERNÁNDEZ-PÉREZ *et al.*, 2019; JAGTAP; RAO, 2018).

Percebe-se, portanto, que a disponibilidade de oxigênio durante o processo fermentativo pode determinar se o xilitol e o arabitol permanecerão no meio ou se serão utilizados para a obtenção de energia e crescimento da biomassa celular, fazendo com que a quantidade de oxigênio seja um fator chave no controle do bioprocesso (ARCAÑO *et al.*, 2020; BETTIGA *et al.*, 2009; JAGTAP; RAO, 2018). Ressalta-se que há estudos indicando que leveduras assimiladoras de pentoses, como *Candida tropicalis*, são capazes de sobreviver em anaerobiose, mas a quantidade de xilitol gerado no meio nesse caso é inferior ao obtido com o fornecimento controlado de oxigênio, pois nesses contextos a via de assimilação das pentoses é limitada, sendo este um fator chave na produção biotecnológica desses poliálcoois (CHENG *et al.*, 2014; HERNÁNDEZ-PÉREZ *et al.*, 2019; KORDOWSKA-WIATER, 2015).

vi. Xilitol e arabitol por *Candida tropicalis* e outras leveduras

Candida sp. é uma espécie de levedura que apresenta diversas espécies conhecidas por serem capazes de produzir compostos de interesse biotecnológico. *Candida tropicalis*, apesar de ser um microrganismo oportunista de classe de risco 2, é relatada como uma espécie capaz de sintetizar diferentes ácidos carboxílicos, além de assimilar xilose e arabinose, convertendo-as em xilitol e arabitol. Em relação à produção dos poliálcoois a partir de pentoses, principalmente quanto ao xilitol, *C. tropicalis* é relatada como uma levedura que possui um elevado rendimento de conversão, com valores superiores a 90 % (BERNARD *et al.*, 1981; DALLI; PATEL; RAKSHIT, 2017; JAGTAP; RAO, 2018; MCMILLAN; BOYNTON, 1994; SCHÖRKEN; KEMPERS, 2009).

Em trabalhos prévios do grupo de pesquisa do Laboratório de Engenharia Metabólica e Bioprocessos da Faculdade de Engenharia de Alimentos da Unicamp, especificamente na produção biológica do xilitol a partir de hidrolisado hemicelulósico de bagaço de cana de açúcar com a levedura *C. tropicalis*, percebeu-se que, após 86 h de cultivo, toda a arabinose do meio havia sido consumida (ALVES, 2018; ALVES *et al.*, 2021). Esse fator é um indicativo de que o arabitol

pode estar sendo produzido no meio a partir da arabinose, gerando um meio com presença simultânea de xilitol e arabitol.

Tal aspecto vem sendo explorado pelo grupo e pode representar um potencial no reaproveitamento do bagaço de cana de açúcar. Na sequência, foi identificada a produção simultânea de xilitol e arabitol na presença de xilose e arabinose pela mesma levedura (dados não mostrados). No entanto, um estudo visando a otimização do meio e das condições do processo fermentativo para a coprodução de xilitol e arabitol por essa levedura, principalmente em relação à oxigenação, ainda não foi realizada pelo grupo e é necessária.

A Tabela 2 sumariza estudos de produção biotecnológica de xilitol e arabitol a partir de hidrolisados hemicelulósicos de biomassas assim como a partir de arabinose, xilose e glicose na forma purificada.

Em relação à produção de xilitol, nota-se que há uma grande variedade de modos de operação e escalas, indo de erlenmeyers a biorreatores, assim como de microrganismos, geneticamente modificados ou não, e biomassas lignocelulósicas. Além disso, os resultados atuais levam a valores de produtividade de até 7,9 g/L.h e rendimentos de até 90 % do valor teórico (0,917 g/g) (DOS SANTOS *et al.*, 2011; FARIAS *et al.*, 2022; OU *et al.*, 2020; SASAKI *et al.*, 2010; SU *et al.*, 2015). Ressalta-se também que um dos maiores rendimentos identificados foi obtido a partir de *C. tropicalis* em hidrolisado de bagaço de cana de açúcar, 0,86 g/g (MORAIS JUNIOR *et al.*, 2019).

Em relação à produção de arabitol, percebe-se que a diversidade de estudos não é tão ampla quanto a de xilitol, com os trabalhos limitados, em sua maioria, à produção em Erlenmeyers e utilizando pentoses purificadas como substrato. Nesse contexto, foi possível atingir valores elevados de rendimento, praticamente equivalentes ao teórico (1,01 g/g) a partir de *C. tropicalis* (MCMILLAN; BOYNTON, 1994). Em relação à produção a partir de hidrolisados hemicelulósicos, destacou-se a produção do hidrolisado de farinha de soja, com a maior produtividade encontrada na literatura, de 0,90 g/L.h (LOMAN, A. A.; ISLAM; JU, 2018).

Tabela 2. Estudos de produção de xilitol e arabitol a partir de xilose, arabinose e hidrolisados de biomassas lignocelulósicas.

Microorganismo	Substrato	Concentração de açúcares	Tipo de processo	Xol, Arol (g/L)	Y_{ps} (g/g)	Pr (g/L.h)	Referência
Produção de xylitol							
Candida boidinii	Hidrolisado de bagaço de azeitona	24,0 g/L de xilose	Batelada, Erlenmeyers de 100 mL	6,0	0,43	0,07	López-Linares et al. (2020)
Candida guilliermondii	Hidrolisado de palha de trigo	58,0 g/L de xilose	Batelada, Erlenmeyers de 250 mL	41,0	0,71	0,94	Saravanan et al.(2021)
Candida guilliermondii	Hidrolisado de madeira de choupo	50,0 g/L de xilose	Batelada, Biorreatores de 1 L	46,0	0,92	0,88	Dalli et al. (2017)
Candida tropicalis	Hidrolisado de bagaço de cana-de-açúcar	41,0 g/L de xilose	Batelada, Erlenmeyers de 1 L	19,3	0,55	0,21	Alves et al. (2021)
Candida tropicalis	Hidrolisado de bagaço de cana-de-açúcar	177,0 g/L de xilose	Batelada, Erlenmeyers de 125 mL	109,5	0,86	2,81	Morais Junior et al. (2019)
Candida tropicalis	Hidrolisado de palha de arroz	45,0 g/L de xilose	Biorreator contínuo de 5 L com unidade de microfiltração	31,0	0,78	0,21	Zahed et al. (2016)
Candida tropicalis	Hidrolisado de espiga de milho	57,2 g/L de xilose	Batelada, Erlenmeyers de 500 mL com células imobilizadas	41,0	0,73	0,43	Yewale et al. (2016)
Debaryomyces hansenii var *hansenii*	Hidrolisado de palha de arroz	60,0 g/L de xilose	Batelada, Erlenmeyers de 250 mL	40,0	0,72	0,83	Saravanan et al. (2021)
Kluyveromyces marxianus	Hidrolisado de espiga de milho	32,0 g/L de xilose	Batelada, Biorreatores de 5 L	24,2	0,82	0,4	Du et al. (2020)
Pachysolen tannophilus	Hidrolisado de espiga de milho	60,0 g/L de xilose	Batelada, Erlenmeyers de 250 mL	49,0	0,81	1,02	Saravanan et al. (2021)
MG *Candida tropicalis*	Xilose purificada	300,0 g/L	Batelada alimentada, Biorreatores de 5 L	260,0	0,92	5,09	Lee et al. (2003)
MG *Saccharomyces cerevisiae*	Oat and soybean hull hydrolysates	18,0 g/L de xilose	Batelada, Biorreator de 2 L	8,2	0,45	0,08	Cortivo et al. (2018)
MG *Ashbya gossypii*	Xilose purificada	20,0 g/L	Batelada, Erlenmeyers	22,6	0,8	0,14	Díaz-Fernández et al. (2017)
Produção de arabitol							
Candida parapsilosis	L-arabinose purificada	20,0 g/L	Batelada, Erlenmeyers de 100 mL	10,7	0,53	0,15	Kordowska-Wiater et al. (2017)
Candida tropicalis	L-arabinose purificada	15,5 g/L	Batelada, Erlenmeyers de 250 mL	8,0	1,02	0,09	McMillan & Boynton (1994)
Debaryomyces hansenii	Hidrolisado de bagaço de sisal	2,9 g/L de xilose	Batelada, Erlenmeyers de 1 L	1,1	0,99	0,01	de Medeiros et al. (2020)
Pichia manchurica	L-arabinose purificada	150,0 g/L	Batelada, Erlenmeyers de 250 mL	13,7	0,09	0,11	Sundaramoorthy & Gummadi (2019)
Pichia stiptis	L-arabinose purificada	15,0 g/L	Batelada, Erlenmeyers de 250 mL	8,2	0,57	0,09	McMillan & Boynton (1994)
MG karyoductant of *S,cerevisiae* and *Pichia stipites*	L-arabinose purificada	32,5 g/L	Batelada, Erlenmeyers de 100 mL	18,9	0,58	0,39	Kordowska-Wiater et al. (2012)
MG *Saccharomyces cerevisiae*	L-arabinose, glicose e xilose purificadas	20,0 g/L de cada	Batelada, Erlenmeyers de 1 L	17,5	0,95	0,145	Sanchez et al. (2010)

Arol – arabitol; MG: Modificado geneticamente; Pr – Produtividade; xol – xilitol; Y_{ps} – rendimento de substrato em produto.
Fonte: adaptado de Farias et al. (2022)

Em relação à produção simultânea de xilitol e arabitol, apenas dois estudos recentes foram identificados reportando tal coprodução a partir de hidrolisados hemicelulósicos. De Medeiros e colaboradores (2020) observaram a produção de xilitol e arabitol pela levedura *Debaryomyces hansenii* em hidrolisado de sisal e, apesar de terem identificado valores elevados para o rendimento de arabitol, a concentração inicial dos açúcares foi de apenas 2,84 g/L de xilose e 1,14 g/L de arabinose na composição, levando a valores de 1,1 g/L de arabitol e 0,1 g/L de arabitol.

Um segundo estudo, proposto por Araújo e colaboradores (2021), também promoveu a produção de xilitol e arabitol por *Komagataella pastoris* a partir de hidrolisados hemicelulósicos de casca de banana, de resíduos de grãos de cervejarias, de talos de uva, de espiga de milho, de serragem e de bagaço de uva. Apesar de terem avaliado diferentes hidrolisados como potencial para a coprodução de xilitol e arabitol, as concentrações máximas obtidas foram de 4,0 g/L de xilitol e 0,9 g/L de arabitol.

Nesse contexto, nota-se que o presente estudo é o primeiro a propor o estudo da produção simultânea de xilitol e arabitol a partir de fermentação de hidrolisado de bagaço de cana pela levedura *Candida tropicalis*, principalmente no que tange à otimização considerando a introdução de subprodutos industriais como fonte de nitrogênio para o microrganismo, visando a otimização do processo fermentativo.

vii. Água de maceração de milho

No processamento do milho, uma das possíveis etapas realizadas é a moagem úmida. Esse processo consiste em umedecer os grãos secos em uma solução contendo ácido lático e sulfitos por diferentes combinações de tempo e temperatura. Com isso, o amido presente no grão absorve a umidade, amolecendo-o, e a matriz proteica que protege o grão é rompida. Um dos principais objetivos desta etapa é remover as proteínas solúveis do grão, que migram para a solução, e facilitar os processos subsequentes de moagem para extração do amido (SELIM et al., 2021; ZHOU et al., 2022).

A água utilizada nesse processo que, portanto, carrega consigo diversos sólidos solúveis presentes nos grãos de milho, é definida como água de maceração

de milho (*corn steep water* – CSW), um dos principais resíduos das indústrias de processamento desse grão. Devido principalmente a fatores como a presença de grãos danificados, sejam brocados ou danificados pelo calor, a água de maceração de milho pode conter também a presença de açúcares redutores em diferentes concentrações. A sua composição pode variar de acordo com a variedade do grão e o tipo e a etapa do processamento, mas, de uma forma geral, define-se que esse efluente é composto principalmente de proteínas, aminoácidos, minerais, ácidos orgânicos, vitaminas, açúcares redutores e enzimas, o que o caracteriza como um substrato em potencial para o crescimento microbiano (HULL et al., 1996; SELIM et al., 2021; ZHOU et al., 2022).

Uma das principais aplicações atuais de CSW é para a ração animal, já que ele pode ser concentrado e misturado a sólidos, glúten e materiais fibrosos. Contudo, CSW já vem sendo aplicado também em pequenas quantidades como fonte de nutrientes de baixo custo em processos fermentativos diversos, para a produção de, por exemplo, enzimas, etanol, penicilina, ácido glutâmico e ácido lático (SELIM et al., 2021; ZHOU et al., 2022). Adicionalmente, CSW tem recebido destaque recente devido à capacidade de gerar, a partir de processos fermentativos, biossurfactantes, moléculas com aplicações diversas, que apresentam inclusive potencial antimicrobiano, e que podem substituir os surfactantes de origem química (LÓPEZ-PRIETO et al., 2019; MARTÍNEZ-ARCOS; MOLDES; VECINO, 2021).

Selim e colaboradores (2021), em uma caracterização de CSW, identificaram a presença de vitaminais e íons inorgânicos, com destaque para fósforo, magnésio e cálcio, e concentrações de 11,3 g/L de proteínas e 10,7 g/L de aminoácidos livres, estando presentes 17 tipos de aminoácidos distintos, diversidade relevante para o crescimento microbiano. Apesar disso, detectou-se também um teor elevado de carboidratos, chegando a 250 g/L. Hull e colaboradores (1996) realizaram a caracterização de CSW em diferentes etapas do processamento e obtiveram concentrações de aminoácidos totais variando de 8 a 62 g/L e, para carboidratos, de 5,6 a 17,2 g/L. Nota-se que há uma grande variação dependendo do processamento realizado com os grãos e, portanto, o uso de CSW exige cautela, tendo em vista que ele pode conter concentrações relevantes de açúcares ou compostos inibidores diversos que podem alterar o comportamento esperado dos microrganismos.

ARTIGO - SIMULTANEOUS PRODUCTION OF XYLITOL AND ARABITOL BY *Candida tropicalis* FERMENTATION IMPROVING AGRO-INDUSTRIAL WASTES VALORIZATION

Artigo experimental submetido, aceito e publicado na revista “Food and Bioproducts processing”, revista com fator de impacto de 5.581 e A1 na área de avaliação “Ciência de Alimentos” na classificação de periódicos quadriênio 2017-2020 da Qualis Periódicos CAPES.

O artigo encontra-se sob o DOI https://doi.org/10.1016/j.fbp.2023.04.006 e foi disponibilizado online no dia 25 de abril de 2022.

As autorizações para uso integral do artigo nesta tese encontram-se no Anexo IV.

Simultaneous production of xylitol and arabitol by *Candida tropicalis* fermentation improving agro-industrial wastes valorization

Gabriel Cicalese Bevilaqua[a], Francisco Maugeri Filho[a], Marcus Bruno Soares Forte[a]*

[a] *Postal Code: 13083-862, Monteiro Lobato Street, 80, Bioprocess and Metabolic Engineering Laboratory (LEMeB), Faculty of Food Engineering (FEA), University of Campinas (UNICAMP), Campinas, SP, Brazil.*
**corresponding author: forte@unicamp.br*

Abstract

Xylitol and arabitol are sugar alcohols with similar properties and applications. Although the biotechnological production of xylitol is already being explored, its coproduction with arabitol is still in the early stages. This study aimed to improve the coproduction of xylitol and arabitol by *Candida tropicalis* from sugarcane bagasse hemicellulosic hydrolysate. The most promising results were achieved with 2.2 g L^{-1} nitrogen, obtained by mixing 80% yeast hydrolysate and 20% corn steep water. For fermentations conducted in a bioreactor, the concentrations of xylitol (52.1 g L^{-1}) and arabitol (4.2 g L^{-1}) were 65 and 3.7 times higher, respectively, than those reported in the literature for similar processes, with productivities and yields of 0.24 g L^{-1} h^{-1} and 0.74 g g^{-1} for xylitol and 0.02 g L^{-1} h^{-1} and 0.43 g g^{-1} for arabitol. These results show promise for the coproduction of sugar alcohols from sugarcane bagasse hemicellulosic hydrolysate by C *tropicalis*.

Keywords

biorefinery, fermentation, pentose-assimilating yeasts, polyols, sugar substitutes

Highlights

i. Optimum media for sugar alcohols production reduced media costs by 10 times

ii. Corn steep water can substitute 20 % of yeast hydrolysate as nitrogen source

iii. Optimum media improved the production of xylitol by 450 % and arabitol by 250 %

iv. Bioreactor fermentation produced 52.1 g L^{-1} of xylitol and 4.2 g L^{-1} of arabitol

Abbreviations

CSW – Corn steep water

SBHH – Sugarcane bagasse hemicellulosic hydrolysate

YH - Yeast hydrolysate

YE – Yeas extract

YPMG - Yeast peptone malt glucose

YP – Yeast peptone

PB12 – Plackett-Burman

DOE – Design of experiments

$Y_{xol,xyl}$ – Xylitol yield

$Y_{arol,arab}$ – Arabitol yield

$Y_{x,s}$ – Biomass yield

P_{xyl} – Xylitol productivity

P_{arol} – Arabitol productivity

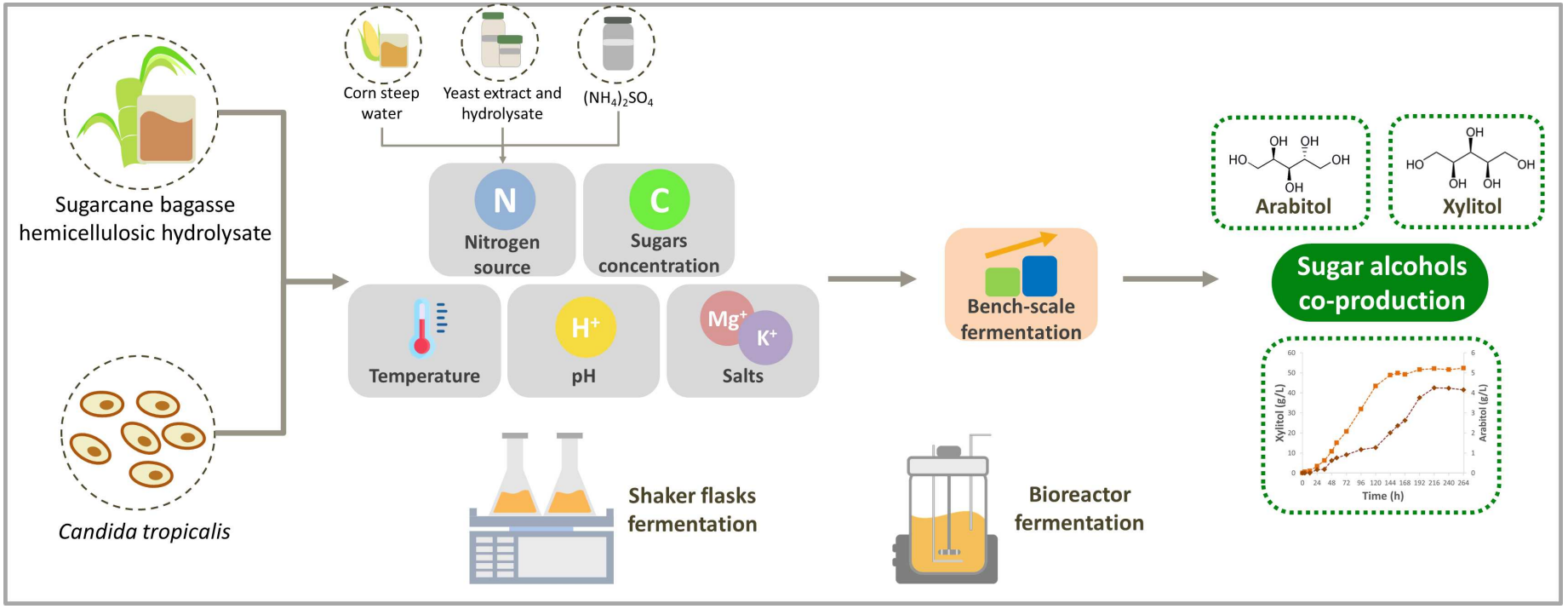
ε_{xyl} – Xylose consumption

ε_{arab} - Arabinose consumption

C_{xol} – Xylitol concentration

C_{arol} – Arabitol concentration

Graphical abstract



1. Introduction

The sugar alcohol market is under constant expansion, forecast to increase from US$3.42 billion in 2021 to US$5.14 billion in 2028 (Grand View Research 2022). Xylitol and arabitol are sugar alcohol stereoisomers that can be used as sweetening agents in foods and beverages (Farias et al., 2022).

Xylitol has a sweetening potency similar to that of sucrose but with 40 % less calories (2.4 kcal g^{-1}). Additionally, it can help protect teeth against cavities and impart a fresh sensation to the mouth, with major application in chewing gums (Farias et al., 2022; Xu et al., 2019). Regarding arabitol, even though it has a sweetening power of 70% to that of sucrose, its caloric value is 20 times lower (0.2 kcal g^{-1}), having also properties similar to xylitol, such as sensation of freshness and protection against teeth cavities. Furthermore, arabitol, like xylitol, is classified as one of the 12 biomass-derivable chemicals designated for further research within biotechnology (Kordowska-Wiater et al., 2017; Farias et al., 2022).

Arabitol, despite having similar properties, is not widely used as a food additive, which consequently affects its cost in the market. While xylitol is marketed for less than US$ 0.04 per gram, arabitol, a molecule with similar synthesis and application, is marketed only in its purified form for more than US$ 60 per gram, which increases the interest in the production of this molecule. It should be noted, however, that if its production costs were reduced, arabitol would compete directly with xylitol in food applications or could be applied together for possible synergistic effects not yet explored (Loman and Ju 2013; Loman et al. 2018; Xu et al. 2019; Arcaño et al. 2020, Farias et al., 2022).

Xylitol and arabitol can be chemically synthesized via processes that are both expensive and environmentally harmful (Xu et al. 2019; Zheng et al. 2020). On the other hand, biotechnological production is an ecofriendly route using microorganisms, such as the yeast *Candida tropicalis*. This species has been applied to metabolize pentoses into sugar alcohols, but current methods are hampered by drawbacks such as expensive growth media and low yields (Xu et al. 2019).

The main substrates used in the bioproduction of sugar alcohols are hemicellulosic hydrolysates derived from lignocellulosic biomasses, such as sugarcane bagasse, which serve as sources of glucose, xylose, and arabinose (Antunes et al. 2019). Xylitol and arabitol co-production is also a way of promoting the full use of pentoses present in lignocellulosic biomass, since xylose can be converted to xylitol and arabinose to arabitol (Murzin et al., 2020; Farias et al., 2022). Also, valuing the application of a co-product of xylitol and arabitol would reduce purification costs, since the separation of these molecules is potentially not cost effective (Saha and Kennedy, 2020).

In these fermentative processes, the requirement for medium enrichment with nitrogen is an important additional cost. Studies assessed the effects of different nitrogen sources on biological fermentation, including rice bran and other industrial wastes (Kordowska-Wiater 2015; Morais Junior et al. 2019; Palladino et al. 2021). However, no studies were found with corn steep water (CSW) in association with hemicellulosic hydrolysates for coproducing polyalcohols.

CSW is an effluent resulting from corn milling. Its composition may vary but it can be characterized as a source of nitrogen and other nutrients and is commonly applied in animal feed. Due to the presence of sulfites and organic acids, CSW should not be used directly in human food (Zhou et al., 2022; Xiao et al., 2012). Despite this, this byproduct has been applied in processes of interest to food industries that undergo further purification processes, such as the growth of probiotics (Wu et al., 2020) and carotenoids (Rodrigues et al., 2019), polysaccharides (Lee et al., 2018) and biosurfactants (Almeida et al., 2021) production. It should be noted that valorization of agro-industrial wastes via bioprocessing contributes to environmental sustainability and favors the development of a circular economy.

Studies on the optimization of xylitol bioproduction are being developed, including by our workgroup, also addressing the bioproduct purification stages, separating it from residual sugars, colored compounds, organic acids and generated co-products, such as ethanol, in which the optimization of the

fermentation is also highlighted as a way to improve xylitol purification (Alves et al., 2021; Cardoso and Forte, 2021). However, there is limited research on the simultaneous production of arabitol. Even though both polyalcohols are produced from pentoses present in the medium, arabitol is either not analyzed or treated as a contaminant in xylitol production (Saha and Kennedy 2020). However, xylitol and arabitol production through integral utilization of hemicellulosic hydrolysates as substrates allows for combined application of these compounds. Coproduction from synthetic media has been reported, and recent studies have underscored the possibility of producing both sugar alcohols from hemicellulosic hydrolysates (De Medeiros et al. 2020; Araújo et al. 2021). Nevertheless, there is still a gap in our understanding of the possibility of operating in bioreactors, scaling up the process and of the contribution of process variables to fermentative coproduction, especially regarding *C. tropicalis*.

In view of the above, this study aimed to investigate and optimize the simultaneous production of xylitol and arabitol from sugarcane bagasse hemicellulosic hydrolysate (SBHH) using a *C. tropicalis* strain isolated from Brazilian sugarcane crops. The possibility of reducing process costs through the use of less expensive protein sources, such as CSW, was evaluated. Fermentations were evaluated at the bioreactor level, the first step of the scale-up, and conversion parameters were optimized to ensure the feasibility of the biotechnological process.

2. Materials and methods

Fig. 1 presents a flowchart illustrating the steps adopted in this research.

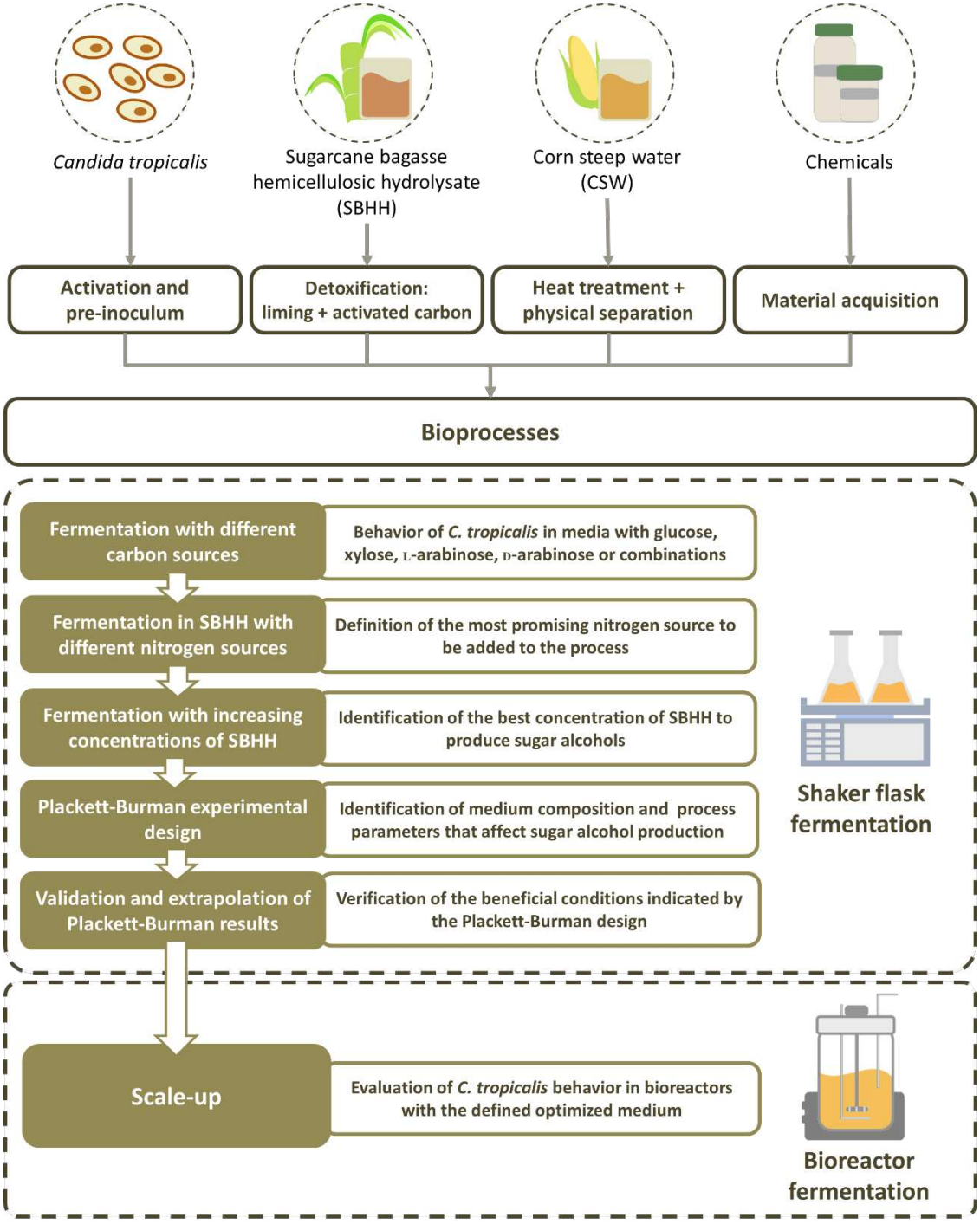


Fig. 1. Flowchart illustrating the methods used in the study

2.1. Raw materials

CSW was kindly provided by Ingredion Brasil. The raw material was heat treated (121 °C, 20 min), centrifuged (Andreas Hettich GmbH & Co, Rotanta 460r, Germany) (5000 rpm, 10 min), and filtered. Yeast hydrolysate (YH) (NuCell, Procelys) was kindly provided by Procelys by Lesaffre. Yeast extract (YE) was obtained commercially from Neogen®.

SBHH was produced by treatment of dried sugarcane bagasse with sulfuric acid (0.5% v/v) at 121 °C for 20 min, followed by vacuum evaporation. Two batches of SBHH (hereafter referred to as batches A and B) were obtained at the facilities of the Brazilian Biorenewables National Laboratory (LNBR). Crude SBHH was detoxified by overliming and activated carbon adsorption, according to a method adapted from Antunes (2019). The process was carried out in three stages, and, after each stage, the mixture was centrifuged at 5000 rpm for 15 min. In step 1, the pH was raised from 0.5 to 7.0 by adding sodium hydroxide solution (45% w/v). In step 2, the pH was reduced from 7.0 to 5.5 with concentrated phosphoric acid. Finally, in step 3, activated carbon (2.5% w/v) was added, and the mixture was incubated in a rotary shaker/incubator (New Brunswick Scientific Co., Innova 4430, United States) at 30 °C and 200 rpm for 1 h.

2.2. Yeast strain

The *C. tropicalis* strain, isolated from Brazilian sugarcane crops, was kindly provided by the Laboratory of Genomics and Expression of the University of Campinas, Institute of Biology, Campinas, Brazil. The microorganism was activated on Yeast Peptone Malt Glucose (YPMG) medium (3 g L^{-1} YE, 5 g L^{-1} peptone, 3 g L^{-1} malt extract, 10 g L^{-1} glucose, and 10 g L^{-1} bacteriological agar) in a bacteriological incubator at 30 °C for 48 h. For use in fermentation processes, pre-inocula were prepared in 300 mL of Yeast Peptone (YP) media (10 g L^{-1} yeast extract and 20 g L^{-1} peptone) added of 30 g L^{-1} xylose to 1 L shaker flasks in a rotary shaker/incubator (30 °C, 200 rpm, 24 h).

2.3. Fermentations using different carbon sources

Fermentative processes were carried out in YP media supplemented with one of the following carbon sources at 30 g L^{-1}: glucose, xylose, L-arabinose, D-arabinose, glucose + L-arabinose (1:2), and glucose + D-arabinose (1:2). Fermentations were performed in 125 mL shaker flasks containing 50 mL of medium in a rotary shaker/incubator (30 °C, 200 rpm, 72 h).

2.4. Fermentations in SBHH supplemented with different nitrogen sources

The growth of *C. tropicalis* on different nitrogen sources was studied by carrying out fermentative processes in diluted detoxified SBHH media (batch A, 30 g L^{-1} xylose) added of KH_2PO_4 (2.7 g L^{-1}) and $MgSO_4$ (0.3 g L^{-1}) at an initial pH of 6.0. Four nitrogen sources were tested: (i) YE, (ii) YE + ammonium sulfate ($(NH_4)_2SO_4$), (iii) YH, and (iv) CSW. The concentration of all media was equivalent to 0.8 g L^{-1} total nitrogen (Morais Júnior et al., 2019; Antunes et al., 2021). YE was treated as control because it is conventionally added to hemicellulosic hydrolysates. The YE + $(NH_4)_2SO_4$ process contained 0.6 g L^{-1} nitrogen from YE and 0.2 g L^{-1} nitrogen from $(NH_4)_2SO_4$. Fermentations were performed in 1 L flasks containing 300 mL of medium in a rotary shaker/incubator (35 °C, 200 rpm, 120 h).

2.5. Fermentations using increasing SBHH concentrations

Fermentative processes were performed using detoxified SBHH media at different concentrations (batch A, 30, 55, 75, and 85 g L^{-1} xylose) added of KH_2PO_4 (2.7 g L^{-1}), $MgSO_4$ (0.3 g L^{-1}), salts conventionally added to this culture medium (Narisetty et al., 2021; Morais Júnior et al., 2019), and 0.8 g L^{-1} nitrogen from the nitrogen source selected in the previous experiment (YH), at an initial pH of 6.0. Fermentations were performed in 1 L shaker flasks containing 300 mL of medium in a rotary shaker/incubator (35 °C, 200 rpm, 120 h). An additional fermentation was carried out in a 1 L baffled flask containing medium at an initial xylose concentration of 75 g L^{-1}.

2.6. Plackett–Burman experimental design

The effects of fermentation conditions and medium composition on sugar alcohol production were assessed using a Plackett–Burman (PB12) experimental design with 12 runs, 6 factors at high (+1) and low (−1) levels, and 4 replications of the center point (0), totaling 16 tests (Rodrigues and Iemma, 2014), in order to simultaneously and multivariately identify the effect of medium composition parameters and define the most appropriate process conditions for the production of sugar alcohols from SBHH. The six factors were initial nitrogen concentration (x_1), initial KH_2PO_4 concentration (x_2), initial $MgSO_4$ concentration (x_3), temperature (x_4), initial pH (x_5), and CSW percentage in the nitrogen source (x_6). Factor levels and runs are detailed in Section 3.5. Fermentative processes were performed using detoxified SBHH (Batch A) at an average concentration of 75 g L^{-1} xylose in 1 L shaker flasks containing 300 mL of medium in a rotary shaker/incubator (200 rpm, 96 h). The main nitrogen source was YH. Data were analyzed using the online tool Protimiza Experimental Design (experimental-design.protimiza.com.br).

2.7. Validation and extrapolation of Plackett–Burman results

For validation (V-I to V-IV) of the most productive and least expensive conditions, fermentations were performed under the beneficial conditions identified from the results of the Plackett–Burman design. Fermentation processes were carried out using detoxified SBHH (batch B) at an average concentration of 75 g L^{-1} xylose in a rotary shaker/incubator (200 rpm, 96 h). Fermentation conditions and medium compositions are described in Section 3.6. An additional test (CP) was performed under center point conditions using crude (undetoxified) SBHH to assess the feasibility of eliminating the detoxification step in subsequent reactions.

2.8. Bioreactor fermentations

After the best conditions were defined in the Plackett–Burman validation and extrapolation step, a bench scale fermentation process was carried out using 1 L of diluted detoxified SBHH culture medium (batch B, 75 g L^{-1} xylose) added of CSW (39.3 g L^{-1}) and YH (16.0 g L^{-1}), resulting in a nitrogen concentration of 2.2 g L^{-1}, at an initial pH of 6.5. Fermentations were performed for 264 h in a 2 L

bioreactor without baffles (New Brunswick BioFlo 3000 Fermentor, Canada) under compressed air aeration (0.7 L min^{-1}/0.7 vvm) and constant agitation (250 rpm) provided by a flat-blade turbine-type stirrer. Antifoam C emulsion (Sigma, A8011) was added as needed to prevent excessive foaming.

2.9. Analysis

Medium components and fermentation products were quantified using a high-performance liquid chromatography (HPLC) system (Thermo Fisher Scientific Inc., Accela, United States) equipped with a refractive index detector (Thermo Fisher Scientific Inc., Accela, United States). Xylose, arabinose, glucose, ethanol, xylitol, and arabitol were separated, with the methodology adapted from de Medeiros et al. (2020), by elution on an Agilent Hi-Plex Ca column at 65 °C using ultrapure water at a flow rate of 0.4 mL min^{-1}. Acetic acid, hydroxymethylfurfural, and furfural were separated by elution on a Bio-Rad Aminex HPX-87h column at 45 °C using H_2SO_4 solution (pH 2.6) at a flow rate of 0.6 mL min^{-1}.

Total nitrogen quantification of nitrogen sources (YE, YH, and CSW) was performed using a PerkinElmer 2400 CHN elemental analyzer.

C. tropicalis suspensions were quantified by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer (Beckman DU-640). Cell mass was estimated by correlating gravimetric measurements of the same fermentative processes with OD_{600} values.

2.10. Fermentative process parameters

Product and biomass yields were calculated as the ratio of product formed to substrate consumed. Xylitol ($Y_{xol,xyl}$) and arabitol ($Y_{arol,arab}$) yields were calculated considering xylose and arabinose as substrates, respectively. Biomass yield ($Y_{x,s}$) was calculated from total sugars (glucose, xylose, and arabinose). Xylitol (P_{xyl}) and arabitol (P_{arol}) productivities at specific times were calculated as the ratio of the quantity of product generated to the respective time. Xylose (ε_{xyl}) and arabinose (ε_{arab}) consumptions were calculated as the percentage of substrate consumed in relation to its initial concentration.

3. Results and discussion

3.1. Characterization of raw materials

Characterization of SBHH (Table 1) revealed variations in sugar concentration between SBHH batches. This result was expected, given that acid hydrolysis was followed by evaporation, which can lead to different sugar concentrations depending on variations in operating conditions, especially evaporation time. Despite this, sugar proportions were similar, with glucose and arabinose corresponding from 9% to 12% of the total sugars' concentration.

It was observed that the detoxification process efficiently removed furanic compounds (furfural and hydroxymethylfurfural) from crude hydrolysate, resulting in concentrations lower than 0.01 g L^{-1} in detoxified SBHH. This result is relevant because furanic compounds, generated during acid hydrolysis of hemicellulose, are potent inhibitors of microbial growth. Additionally, it was noted that detoxification caused a sugar loss of up to 20%, which was within the expected range for an overliming process followed by activated carbon adsorption (Antunes et al. 2019).

Acetic acid, generated during acid hydrolysis, remained at concentrations greater than 4 g L^{-1} even after detoxification. This result raises some concerns, because acetic acid may negatively affect the pentose metabolism of yeasts, especially at more acidic pH (lower than its pKa, 4.76). It should be noted, however, that the inhibitory effect of acetic acid is likely reduced by SBHH dilution during preparation of the culture medium (Morais Junior et al. 2019; Singh et al. 2022).

Table 1. Sugars, acetic acid, furfural and 5-hydroxymethylfurfural concentrations in raw and detoxified sugarcane bagasse hemicellulosic hydrolysate.

Raw material	C_{xyl} (g L^{-1})	C_{arab} (g L^{-1})	C_{glu} (g L^{-1})	$C_{Ac\ acid}$ (g L^{-1})	C_{HMF} (g L^{-1})	C_{FUR} (g L^{-1})
SBHH-r (batch a)	114.82 ± 1.95	15.33 ± 0.34	14.16 ± 0.27	5.06 ± 0.12	0.57 ± 0.05	1.00 ± 0.07
SBHH -r (batch b)	153.22 ± 1.38	21.86 ± 1.56	20.60 ± 1.48	4.56 ± 0.21	0.06 ± 0.02	2.64 ± 0.08
SBHH-d (batch a)	92.74 ± 3.49	12.16 ± 0.25	11.45 ± 0.43	4.23 ± 0.23	< 0.01	< 0.01
SBHH d (batch b)	137.73 ± 3.49	17.52 ± 0.25	18.64 ± 0.43	4.20 ± 0.91	< 0.01	< 0.01

r - Raw sugarcane bagasse hemicellulosic hydrolysate

d - Detoxified sugarcane bagasse hemicellulosic hydrolysate

Ac acid – Acetic acid; Arab – Arabinose; FUR – Furfural; Glu – Glucose; HMF – 5-hydroxymethylfurfural; C – concentration

As for nitrogen sources, the results showed that YH and YE had similar concentrations of total nitrogen (0.10 and 0.11 g g^{-1}, respectively). YE is a source of nitrogen and nutrients commonly used in culture media, being produced from yeast growth, cell lysis and nutrient purification processes. YH is a less purified variation of conventional YE with the aim of being applied on a large scale in the food industry. Consequently, it has a reduced cost: while YE is sold for, on average in the Brazilian market, US$ 150.00 kg^{-1}, YH can be acquired for 6.8 times less, US$ 22 kg^{-1}.

CSW had a lower nitrogen concentration (0.01 g g^{-1}) but also some advantages over the other nitrogen substrates. CSW is a liquid substrate with an average density of 1.02 g cm^{-3}, which translates into a nitrogen content of about 9.80 g L^{-1}, confirming its potential as a nitrogen source. CSW is considered an effective substrate for microbial growth because it contains several amino acids and inorganic compounds required for cell growth. However, its composition may vary according to the industry of origin and processing method (Hull et al. 1996; Selim et al. 2021). It is also noteworthy that xylose (1.2 g L^{-1}), arabinose (5.7 g L^{-1}), and acetic acid (2.2 g L^{-1}) were detected in CSW, but these compounds were likely to have had only a minimal effect on the reaction because the substrate was diluted with SBHH, a more concentrated carbon source used in greater amounts. Therefore, sugars derived from SBHH have a more significant contribution to medium composition.

3.2. Fermentations using different carbon sources

By analyzing the growth of *C. tropicalis* in synthetic media containing different carbon sources (Table S1, Supplementary material), it was found that the yeast was able to metabolize all the evaluated carbon sources. However, consumption was minimal (ε_{arab} = 3.8% ± 1.4%) in medium containing D-arabinose only, as evidenced by comparison with medium containing glucose, which resulted in 100% consumption. Interestingly, D-arabinose consumption was more expressive (ε_{arab} = 20.0% ± 1.0%) in medium containing both glucose and D-arabinose, possibly owing to the increase in biomass growth provided by

glucose metabolism (100% consumption). The potential of obtaining products from the metabolization of D-arabinose, due to it being a rarer sugar in nature, is not yet fully explored (Ruchala and Sibirny 2021). There are studies in butanetriol production (Wang et al., 2022) and identification of aldose reductase, converting d-arabinose to arabitol in *Pichia stipitis* (Watanabe et al., 2016). However, no reports on its metabolization by *C. tropicalis* were found.

Analysis of product formation showed that the medium containing only glucose led to the production of ethanol, confirming that *C. tropicalis* is able to metabolize hexoses. Media containing xylose, L-arabinose, or D-arabinose resulted in the production of xylitol and arabitol. This fact attests to the interrelation between metabolic routes for xylose and arabinose assimilation; that is, regardless of the pentose used as substrate, *C. tropicalis* can generate these two polyols.

It was possible to observe that xylose consumption was directed toward xylitol formation and that L-arabinose consumption was directed toward arabitol formation, given that these polyalcohols were the first to be formed in the metabolic route of each respective substrate. In pentose assimilation routes of *C. tropicalis*, xylose is directly converted to xylitol and L-arabinose is directly converted to L-arabitol by the action of arabinose reductase enzymes with preferably NADPH as cofactor. Then, for arabitol, two other enzymatic processes can occur: the conversion of L-arabitol to L-xylulose and the reduction of L-xylulose to xylitol. The xylitol formed by these metabolic pathways can then be dehydrogenated to D-xylulose, which will be phosphated and consumed via the pentose-phosphate pathway for cellular metabolism (Ruchala and Sibirny 2021; Farias et al., 2022; Soares et al., 2022).

Table S1. Parameters of xylitol, arabitol and ethanol production by *C. tropicalis* after 72 h of fermentation (30 °C, 200 rpm) with different carbon sources (30 g L^{-1}) in the composition of the synthetic medium (yeast extract – 10 g L^{-1}, peptone – 20 g L^{-1}).

Carbon source	ε_{glu} (%)	ε_{xyl} or ε_{arab} (%)	C_{Etoh} (g L^{-1})	$Y_{etoh,glu}$ (g g^{-1})	C_{gol} (g L^{-1})	$Y_{gol,glu}$ or $Y_{gol,xyl}$ (g g^{-1})	C_{xol} (g L^{-1})	$Y_{xol,xyl}$ or $Y_{xol,arab}$ (g g^{-1})	C_{arol} (g L^{-1})	$Y_{arol,xyl}$ or $Y_{arol,arab}$ (g g^{-1})
Glu	100.0 ± 0.0		5.81 ± 0.82	0.26 ± 0.03	0.35 ± 0.10	0.02 ± 0.01	-	-	-	-
Xyl	-	100.0 ± 0.0	-	-	0.93 ± 0.03	0.04 ± 0.01	15.11 ± 0.75	0.57 ± 0.03	0.21 ± 0.11	0.01 ± 0.01
L-arab	-	59.1 ± 11.2	-	-	-	-	0.99 ± 0.42	0.06 ± 0.04	5.27 ± 0.19	0.32 ± 0.04
D-arab	-	3.8 ± 1.4	-	-	-	-	0.06 ± 0.05	0.29 ± 0.16	0.06 ± 0.10	0.06 ± 0.10
Glu + L-arab (1:2)	100.0 ± 0.0	59.7 ± 5.6	-	-	-	-	0.72 ± 0.04	0.06 ± 0.01	5.38 ± 0.03	0.45 ± 0.01
Glu + D-arab (1:2)	100.0 ± 0.0	20.0 ± 1.0	-	-	-	-	0.15 ± 0.04	0.13 ± 0.07	0.18 ± 0.02	0.05 ± 0.03

Arab – arabinose; arol – arabitol; etoh – ethanol; glu - glucose; xol – xylitol; xyl – xylose; Y – yield; ε – substrate conversion; C – concentration.

As a direct consequence of metabolic pathways, xylitol production was highest in the medium containing xylose only (C_{xol} = 15.11 ± 0.75 g L^{-1}, $Y_{xol,xyl}$ = 0.57 ± 0.03 g g^{-1}), with 100.0% xylose consumption. Similarly, arabitol production was highest in the medium containing glucose and L-arabinose (C_{arol} = 5.38 ± 0.03 g L^{-1}, $Y_{xol,xyl}$ = 0.45 ± 0.01 g g^{-1}), with 100% glucose consumption and 59.7% ± 5.6% arabinose consumption (Table S1, Supplementary material). It should be noted that L-arabinose is the isomer of arabinose that is mostly present in the hemicellulosic hydrolysate (Kordowska-Wiater et al., 2017; Palladino et al., 2021).

The results confirmed the ability of *C. tropicalis* to convert pentoses into polyalcohols, demonstrating its potential application in fermentation reactions with hemicellulosic hydrolysates, which are renewable, low-cost sources of xylose, glucose, and arabinose, contributing to the economic and environmental feasibility of xylitol and arabitol production.

3.3. Fermentations in SBHH supplemented with different nitrogen sources

The kinetic profile of SBHH fermentative processes with different nitrogen sources was used to determine xylitol (P_{xol}) and arabitol (P_{arol}) productivities. For the four nitrogen sources evaluated, the maximum combined productivity (P_{xol} + P_{arol}) occurred after 56 h of reaction. The parameters of compound production and yeast growth in different nitrogen sources were therefore assessed at this reaction time (Table S2, Supplementary material) (Fig. 2).

Table S2. Parameters of xylitol and arabitol production by *C. tropicalis* after 56 h of fermentation (35 °C, 200 rpm) with different nitrogen sources (0.8 g L^{-1} of total nitrogen) in the medium composition using diluted sugarcane bagasse hemicellulosic hydrolyzate as carbon source (30 g L^{-1} of xylose).

Nitrogen source	C_{xol} (g L^{-1})	C_{arol} (g L^{-1})	$Y_{xol.xyl}$ (g g^{-1})	$Y_{arol.arab}$ (g g^{-1})	P_{xol} (g L^{-1} h^{-1})	P_{arol} (g L^{-1} h^{-1})	$Y_{x,s}$ (g g^{-1})	ε_{xyl} (%)	ε_{arab} (%)
YE	9.80 ± 1.19a	1.23 ± 0.30a	0.40 ± 0.02a	0.41 ± 0.13ab	0.18 ± 0.01a	0.02 ± 0.01a	0.23 ± 0.05a	84.9 ± 10.1a	59.1 ± 8.6a
YE + NH	9.62 ± 1.17a	1.63 ± 0.28a	0.35 ± 0.07a	0.49 ± 0.06a	0.17 ± 0.01a	0.03 ± 0.01a	0.19 ± 0.08a	96.7 ± 3.0ab	78.0 ± 19.4ab
YH	15.17 ± 1.73b	1.80 ± 0.15a	0.53 ± 0.06b	0.46 ± 0.06ab	0.27 ± 0.03b	0.03 ± 0.01a	0.21 ± 0.07a	100.0 ± 0.1b	82.2 ± 5.9ab
CSW	4.86 ± 1.53c	1.33 ± 0.21a	0.19 ± 0.01c	0.33 ± 0.06b	0.09 ± 0.02c	0.02 ± 0.01a	0.23 ± 0.04a	100.0 ± 0.1b	0.92 ± 0.6b

Different letters in the same column indicate a statistically significant difference by the Tukey Test at the 5% level.
xol – xylitol; xyl – xylose; arol – arabitol; arab – arabinose; x – biomass; s – total sugars (glicose, xylose and arabinose); Y – yield; P – productivity; ε – substrate conversion; C - concentration

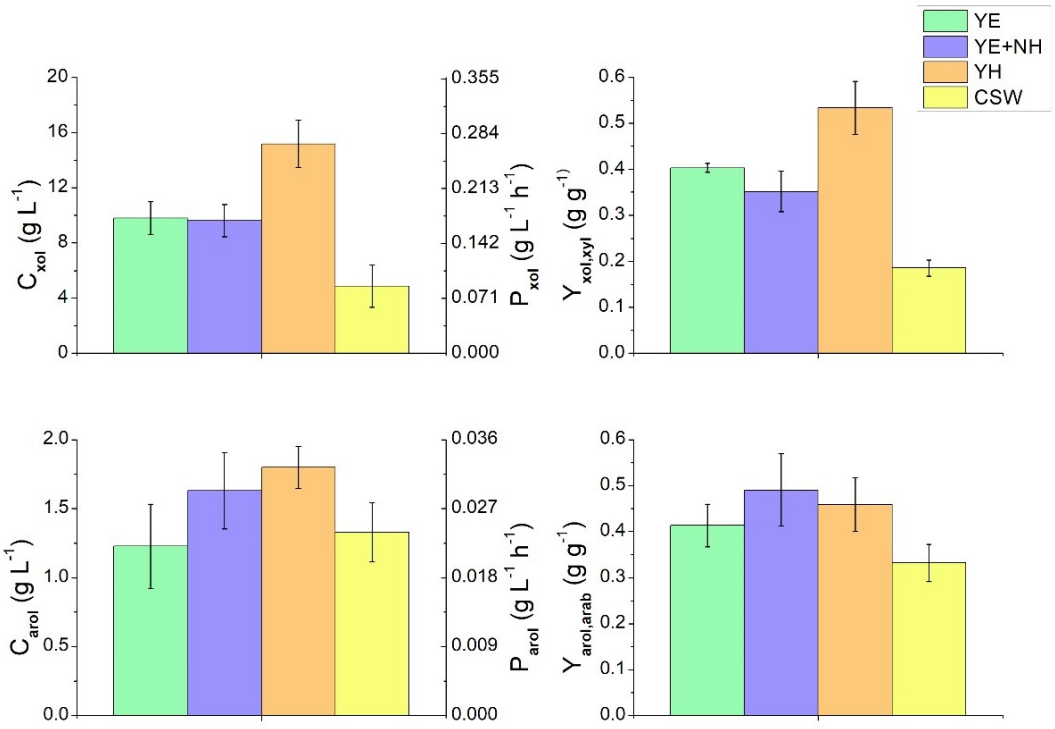


Fig. 2. Xylitol and arabitol production from different nitrogen sources (0.8 g L^{-1} total nitrogen) after 56 h of fermentation (35 °C, 200 rpm) by *C. tropicalis* in media containing diluted sugarcane bagasse hemicellulose hydrolysate (30 g L^{-1} xylose) as carbon source. YE, yeast extract; YE + NH, yeast extract + ammonium sulfate (80:20); YH, yeast hydrolysate; CSW, corn steep water; xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; *C*, concentration; *Y*, yield; *P*, productivity

YE and YE + $(NH_4)_2SO_4$ assays did not differ statistically ($p > 0.05$) in any parameter, revealing that partial replacement of yeast extract with ammonium sulfate (inorganic nitrogen source) did not significantly affect conversion factors or substrate consumption. Arabitol concentration, yield, and productivity were not significantly influenced by nitrogen source. Therefore, any of the four evaluated nitrogen sources can be used under the appropriate fermentation conditions.

Xylitol production, on the other hand, was influenced by nitrogen source. The use of CSW as the sole nitrogen source, although providing cost benefits because of the material's low cost, was the least attractive in terms of production, affording the lowest xylitol yield and productivity. The final xylitol concentration was 50% lower than that of the control (YE). YH provided the highest xylitol concentration, productivity, and yield (C_{xol} = 15.17 ± 1.73 g L^{-1}, P_{xol} = 0.27 ± 0.03 g L^{-1} h^{-1}, $Y_{xol,xyl}$ = 0.53 ± 0.06 g g^{-1}), being about 1.4 times

higher than the values obtained with the control nitrogen source (YE). Furthermore, xylitol productivity and yield were 2.8 and 2.4 times higher, respectively, than that obtained with CSW. In this context, YH was used as the main nitrogen source in subsequent experiments.

These findings support that not only total concentration but also nitrogen source is relevant in the production of sugar alcohols by yeasts. Nitrogen assimilation can be facilitated or hindered depending on the nitrogen source. Some microorganisms have preferences for inorganic sources, others for organic sources, such as *C. tropicalis* (Morais Junior et al. 2019). Regarding organic sources, their assimilation is made possible at the cellular level from molecules of low molecular weight, such as amino acids. The amino acid profile can affect the growth of the microorganism and, if the medium contains peptides and soluble proteins, proteases must first be released to promote hydrolysis and subsequent assimilation (Hofer et al., 2018; Zhu et al., 2019). In the studied context, CSW may present a higher fraction of nitrogen in molecules of higher molecular weight, including lipopeptides and phospholipids (Rodríguez-Lopez et al., 2020; Zhou et al., 2022), therefore, the growth of microorganisms may be hindered using it as the only nitrogenous source when compared to the other evaluated sources.

It was possible to achieve xylitol concentrations, yields, and productivities in SBHH medium that were statistically equivalent to those obtained in synthetic medium containing 30 g L^{-1} xylose. Arabitol concentration and productivity in SBHH medium containing YH (C_{arol} = 1.80 ± 0.15 g L^{-1}, P_{arol} = 0.032 ± 0.003 g L^{-1} h^{-1}) were lower than those obtained in synthetic medium. This result was expected given that the initial arabinose concentration in diluted SBHH was four times lower than the initial concentration in synthetic medium. Nevertheless, arabitol yield did not differ between complex ($Y_{arol,arab}$ = 0.46 ± 0.02 g g^{-1}) and synthetic media. These results demonstrate the good adaptability of the microorganism to the complex medium, which has SBHH as carbon source. Similar results of polyalcohol production from pentoses were obtained in media with synthetic carbon source.

3.4. Fermentations using increasing SBHH concentrations

An increase in the initial concentration of the carbon source resulted in a longer time to reach maximum productivity (Fig. 3) (Table S3, Supplementary material). The yeast requires more time to convert a greater mass of substrates. In medium containing more than 45 g L^{-1} xylose, the biomass yield remained below 0.10, without significant differences between runs. Biomass yield was highest ($Y_{x,s}$ = 0.21 ± 0.07 g g^{-1}) at the lowest initial SBHH concentration (30 g L^{-1} xylose). This result possibly occurred because of the increased substrate consumption (ε_{xyl} = 100.0%, ε_{arab} = 82.2% ± 5.9%), which might be associated with the reduced concentration of inhibitory compounds, such as acetic acid and phenolics (Soares et al., 2022).

Arabitol concentration and yield did not differ according to initial SBHH concentration. Productivity, however, decreased with increasing initial SBHH concentrations, attributed to the increase in time needed to convert the same amount of arabitol. Arabinose consumption was less than 50% at the highest substrate concentration, although it can be expected to increase with time, increasing arabitol formation.

With the increase in initial SBHH concentration from 30 to 75 g L^{-1} xylose, xylitol concentration and productivity increased, but yield was not significantly influenced. Xylitol productivity was lower at 85 g L^{-1} xylose compared with 75 g L^{-1} xylose, given the increase in the time needed for substrate conversion. This factor further confirms the possibility that compounds derived from SBHH inhibit fermentation and that such effects are potentiated at higher initial SBHH concentrations.

Moreover, different studies report substrate inhibition in xylitol production (Winkelhausen et al., 1998; Bedo et al., 2021). Regarding *C. tropicalis*, a study in synthetic medium with initial xylose concentration between 15 and 300 g L^{-1} found substrate inhibition in both cell growth and xylitol formation. Optimal concentrations were obtained between 60 and 80 g/L of xylose, similar to our study. It was also observed that, beyond this range, a greater proportion of the

substrate was directed towards cellular respiration and ATP formation, balancing the high osmotic pressure associated with higher substrate concentration (Tamburini et al., 2015)

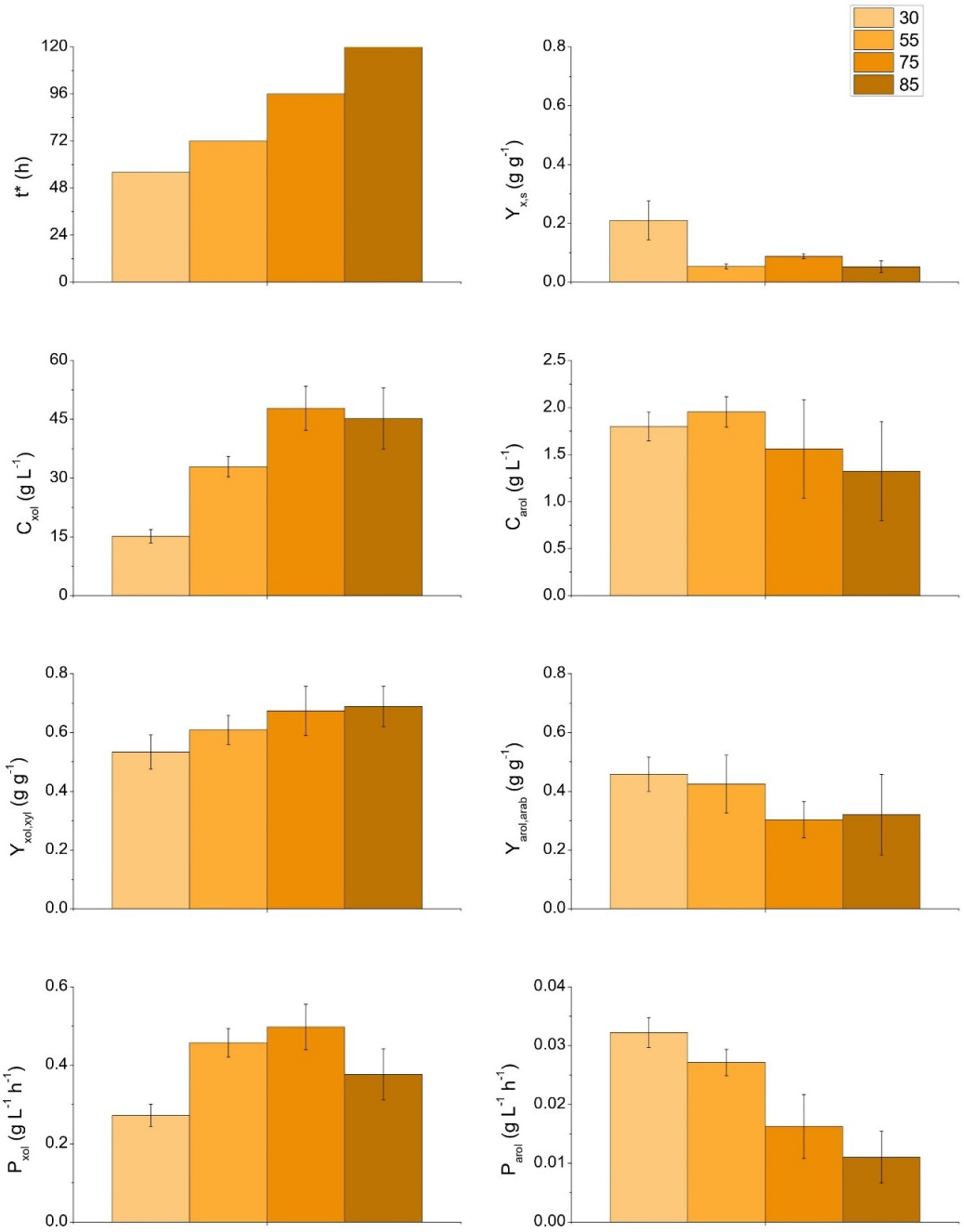


Fig. 3. Xylitol and arabitol production by *C. tropicalis* from fermentations (35 °C, 200 rpm) in media containing different initial concentrations of sugarcane bagasse hemicellulose hydrolysate (30, 55, 75, or 85 g L^{-1} xylose) at the time of maximum productivity (*t**). xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; x, biomass; s, total sugars (glucose, xylose, and arabinose); *C*, concentration; *Y*, yield; *P*, productivity

Table S3. Parameters of xylitol and arabitol production by *C. tropicalis* in fermentations (35 °C, 200 rpm) with different concentrations of sugarcane bagasse hemicellulosic hydrolysate as carbon source.

Initial C_{xyl} ($g\ L^{-1}$)	t*	C_{xol} ($g\ L^{-1}$)	C_{arol} ($g\ L^{-1}$)	$Y_{xol,xyl}$ ($g\ g^{-1}$)	$Y_{arol,arab}$ ($g\ g^{-1}$)	P_{xol} ($g\ L^{-1}\ h^{-1}$)	P_{arol} ($g\ L^{-1}\ h^{-1}$)	$Y_{x,s}$ ($g\ g^{-1}$)	ε_{xyl} (%)	ε_{arab} (%)
30	56	15.17 ± 1.73a	1.80 ± 0.15a	0.53 ± 0.06a	0.46 ± 0.06a	0.27 ± 0.03a	0.03 ± 0.01a	0.21 ± 0.07a	100.0 ± 0.1a	82.2 ± 5.9a
55	72	32.92 ± 2.61b	1.95 ± 0.16a	0.61 ± 0.05a	0.42 ± 0.10a	0.46 ± 0.04bc	0.03 ± 0.01a	0.05 ± 0.01b	96.6 ± 0.9a	49.7 ± 6.8b
75	96	47.79 ± 5.59c	1.56 ± 0.52a	0.67 ± 0.08a	0.30 ± 0.06a	0.50 ± 0.06c	0.02 ± 0.01b	0.09 ± 0.01b	93.1 ± 4.9a	42.6 ± 13.2b
85	120	45.18 ± 7.84bc	1.32 ± 0.53a	0.69 ± 0.07a	0.32 ± 0.14a	0.38 ± 0.06ab	0.01 ± 0.01b	0.05 ± 0.02b	77.6 ± 19.4a	36.0 ± 18.1b

Different letters in the same column indicate a statistically significant difference by the Tukey Test at the 5% level.
arab – arabinose; arol – arabitol; s – total sugars (glicose, xylose and arabinose); t* – time of maximum productivity; xol – xylitol; xyl – xylose; x – biomass; P – productivity; Y – yield; ε – substrate conversion; C - concentration

In view of the greater effect of xylitol concentration on combined productivity (P_{xol} + P_{arol}), we chose to use an initial SBHH concentration of 70–80 g L^{-1} xylose in subsequent experiments. It should be noted that these changes to substrate concentration led to 3.1 and 1.8 times increase in xylitol concentration and productivity (C_{xol} = 47.79 ± 5.59 g L^{-1}, P_{xol} = 0.50 ± 0.06 g L^{-1} h^{-1}) compared to the less concentrated media (xylose 30 g L ^{1}).

After definition of the initial SBHH concentration, the influence of baffles, if present, and, consequently, of a higher oxygen transfer rate, was tested in baffled flasks. Biomass yield was higher in baffled flasks ($Y_{x,s}$ = 0.34 ± 0.07 g g^{-1}), being up to 6.8 times higher than under the other conditions.

It should be noted that pentose metabolism by *C. tropicalis* is closely associated with medium oxygenation. As highlighted earlier, xylitol and L-arabitol produced by the initial assimilation of pentoses are converted to D-xylulose or L-xylulose by dehydrogenase enzymes, following the yeast metabolic pathway. These dehydrogenase enzymes require the cofactor NAD^+, which is consumed and converted to NADH. Under conditions of high oxygenation, the generated NADH is constantly regenerated to NAD^+ in the respiratory chain, favoring the enzymatic reaction for the consumption of polyalcohols. In oxygen limitation, the regeneration of NADH to NAD+ is impaired, generating an imbalance of NAD+/NADH. This imbalance means that, despite being produced, xylitol and arabitol are not preferentially consumed by the following metabolic stages. So, the higher the oxygenation, the greater the utilization of xylose and arabinose for cellular metabolism and growth. On the other hand, under low oxygen conditions, there is a reduction in the activity of enzymes that metabolize the sugar alcohols, consequently, xylitol and arabitol are produced but only partially metabolized, leading to their accumulation (Farias et al., 2022).

Therefore, high biomass growth and low polyalcohol accumulation are expected in baffled flasks. This hypothesis was confirmed by the reduction in xylitol concentration (C_{xol} = 23.00 ± 0.45 g L^{-1}), yield ($Y_{xol,xyl}$ = 0.32 ± 0.45 g g^{-1}), and productivity (P_{xol} = 0.32 ± 0.01 g L^{-1} h^{-1}) in baffled flasks compared with

normal flasks under the same conditions. By contrast, arabitol concentration (C_{arol} = 2.66 ± 0.07 g L^{-1}) and yield ($Y_{arol,arab}$ = 0.62 ± 0.04 g g^{-1}) were higher in baffled runs. These findings may be due to the greater use of glucose and xylose for biomass production and cellular metabolism, with greater regeneration of NADPH, necessary for the conversion of arabinose to arabitol (Farias et al., 2022). Possibly, given enough time, the arabitol formed would also be consumed for biomass and metabolism. Considering the effects of process parameters on the combined production of xylitol and arabitol and the higher concentration of xylitol in the medium, it was decided to use baffle-free flasks in subsequent assays.

3.5. Plackett–Burman experimental design

The influence of the factors initial nitrogen concentration (from 0.8 to 3.6 g L^{-1}) (x_1), initial KH_2PO_4 concentration (from 0.4 to 5 g L^{-1}) (x_2), initial $MgSO_4$ concentration (from 0.1 to 0.5 g L−1) (x_3), temperature (from 28 to 36 °C) (x_4), initial pH (from 6 to 7) (x_5), and CSW percentage in the nitrogen source (from 0 to 20 %) (x_6) in the fermentation process of *C. tropicalis* aiming to produce xylitol and arabitol was studied using Design of Experiments (DOE) with Plackett-Burman (PB12) experimental design. It is worth clarifying that, even though CSW did not provide the best results as a nitrogen source, it was included in the experimental design because of its low cost and environmental friendliness. The results of tests carried out according to the DOE PB12 are described in Tables 2 and 3 and Table S4 (Supplementary material). A reaction time of 96 h was adopted, because this period corresponded to the highest combined productivity for medium containing an initial xylose concentration of 75 g L^{-1} (as described in Section 3.4). Due to this, the effects related to "concentration" and "productivity" responses are similar, therefore, only the effects related to productivity are presented

Center point runs (runs 13–16, Table 2) compared with the other runs, afforded the highest concentrations, yields, and productivities for xylitol parameters and intermediate values for arabitol. Runs 11 and 12, which had high nitrogen source concentrations and lacked or not 40% CSW, respectively,

afforded the highest arabitol concentrations (Table 2). Furthermore, xylitol concentration and productivity of runs 11 and 12 were close to those of center point runs, with higher xylose and arabinose conversions.

Runs carried out at pH (6.0) and nitrogen (0.8 g L^{-1}) levels provided the least promising results for combined production of sugar alcohols (runs 5, 6, and 10). In runs 6 and 10, there was no substrate consumption or significant product or biomass formation. These tests, in addition to having low nitrogen concentration and pH, were carried out at high temperatures (36 °C). These combined factors might have potentiated the action of inhibitors, derived from SBHH, particularly acetic acid, which remained even after detoxification.

It is noteworthy that acetic acid is a weak acid (pKa 4,756 at 25 ºC) and lower pH favors the balance to keep it in the undissociated form. In this chemical form, acetic acid, like other organic acids, is more permeable to the cell membrane and, when in contact with the cytoplasm with a pH generally closer to neutrality, it dissociates, reducing the intracellular pH (Palmqvist and Hahn-Hägerdal, 2000; Chaves et al., 2021). The presence of intracellular acetic acid mainly affects the conformation of protein structures. This can structurally and functionally alter cytoplasmic organelles and cause a reduction in the activity of enzymes, such as xylose reductase, which converts xylose to xylitol and is inhibited at relatively low concentrations of acetic acid (5.4 g L^{-1}) (Rafiqul et al., 2015; Morais Júnior et al., 2019; Chaves et al., 2021). Moreover, increasing the temperature may favor the effect of acetic acid, even at low concentrations. An increase of 30 to 40 ºC reduces the acidity constant (Ka) of this acid (Paabo et al., 1965), favoring its undissociated form and entry into cells. The combination of acetic acid in concentrations below 4.8 g L^{-1} with higher temperatures (37-40 ºC) can also cause the fragmentation of mitochondrial membranes, affecting cellular respiration and resulting in the so-called high enthalpy cell death (Chaves et al., 2021).

Table 2. Plackett–Burman experimental design for assessing the effects of medium composition and process variables on xylitol and arabitol production from sugarcane bagasse hemicellulose hydrolysate (75 g L^{-1} initial xylose) fermentation (96 h, 200 rpm) by *Candida tropicalis*.

Run	N (g L^{-1}) (x_1)	KH_2PO_4 (g L^{-1}) (x_2)	$MgSO_4$ (g L^{-1}) (x_3)	T (°C) (x_4)	pH (x_5)	CSW (%) (x_6)	C_{xol} (g L^{-1})	C_{arol} (g L^{-1})	$Y_{xol,xyl}$ (g g^{-1})	$Y_{arol,arab}$ (g g^{-1})	P_{xol} (g L^{-1} h^{-1})	P_{arol} (g L^{-1} h^{-1})	ε_{xyl} (%)	ε_{arab} (%)	$Y_{x,s}$ (g g^{-1})
1	0.8 (−1)	5 (1)	0.1 (−1)	36 (1)	7 (1)	40 (1)	43.810	1.540	0.611	0.156	0.456	0.016	97.2	58.5	0.074
2	0.8 (−1)	0.4 (−1)	0.5 (1)	28 (−1)	7 (1)	40 (1)	12.258	2.442	0.300	0.323	0.128	0.025	56.7	45.7	0.286
3	3.6 (1)	0.4 (−1)	0.1 (−1)	36 (1)	6 (−1)	40 (1)	42.520	1.900	0.575	0.249	0.443	0.020	94.2	51.6	0.055
4	0.8 (−1)	5 (1)	0.1 (−1)	28 (−1)	7 (1)	0 (−1)	24.612	0.576	0.410	0.061	0.256	0.006	74.4	54.2	0.145
5	0.8 (−1)	0.4 (−1)	0.5 (1)	28 (−1)	6 (−1)	40 (1)	11.860	0.900	0.371	0.217	0.124	0.009	40.0	36.0	0.192
6	0.8 (−1)	0.4 (−1)	0.1 (−1)	36 (1)	6 (−1)	0 (−1)	0.000	0.000	0.000	0.000	0.000	0.000	0.0	0.0	0.000
7	3.6 (1)	0.4 (−1)	0.1 (−1)	28 (−1)	7 (1)	0 (−1)	45.246	2.088	0.670	0.234	0.471	0.022	88.7	51.0	0.151
8	3.6 (1)	5 (1)	0.1 (−1)	28 (−1)	6 (−1)	40 (1)	21.420	1.970	0.665	0.392	0.223	0.021	38.8	30.6	0.131
9	3.6 (1)	5 (1)	0.5 (1)	28 (−1)	6 (−1)	0 (−1)	41.940	2.440	0.573	0.306	0.437	0.025	87.6	48.4	0.110
10	0.8 (−1)	5 (1)	0.5 (1)	36 (1)	6 (−1)	0 (−1)	0.000	0.000	0.000	0.000	0.000	0.000	0.0	0.0	0.000
11	3.6 (1)	0.4 (−1)	0.5 (1)	36 (1)	7 (1)	0 (−1)	46.660	3.750	0.626	0.301	0.486	0.039	100.0	73.4	0.072
12	3.6 (1)	5 (1)	0.5 (1)	36 (1)	7 (1)	40 (1)	43.280	3.240	0.591	0.274	0.451	0.034	97.5	70.1	0.088
13	2.2 (0)	2.7 (0)	0.3 (0)	32 (0)	6.5 (0)	20 (0)	50.960	1.620	0.759	0.182	0.531	0.017	84.5	54.7	0.071
14	2.2 (0)	2.7 (0)	0.3 (0)	32 (0)	6.5 (0)	20 (0)	53.000	2.200	0.736	0.234	0.552	0.023	90.3	57.2	0.081
15	2.2 (0)	2.7 (0)	0.3 (0)	32 (0)	6.5 (0)	20 (0)	44.090	1.970	0.702	0.182	0.459	0.021	86.1	64.1	0.097
16	2.2 (0)	2.7 (0)	0.3 (0)	32 (0)	6.5 (0)	20 (0)	48.040	1.960	0.726	0.223	0.500	0.020	88.4	53.8	0.057

N, added nitrogen concentration; T, temperature; CSW, corn steep water percentage in the nitrogen source; xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; x, biomass; s, total sugars (glucose, xylose, and arabinose); *C*, concentration; *Y*, yield; *P*, productivity; *ε*, substrate conversion. Theoretical $Y_{xol,xtl}$ = 0.917 g g^{-1} and $Y_{arol,arab}$ = 1.01 g g^{-1}.

Tabela S4. Statistical parameters per response from the Plackett-Burman experimental design for the study of medium composition and process variables on xylitol and arabitol production from sugarcane bagasse hemicellulose hydrolysate (75 g L^{-1} initial xylose) fermentation (96 h, 200 rpm) by *Candida tropicalis*.

Response	Variables	Effect	Standard error	calculated t	p-value
P_{xol}	**Mean**	0.290	0.033	8.817	< 0.001
	Curvature	0.442	0.131	3.363	0.010
	Nitrogen source (x_1)	0.258	0.066	3.925	0.004
	KH_2PO_4 (x_2)	0.029	0.066	0.434	0.676
	$MgSO_4$ (x_3)	-0.037	0.066	-0.566	0.587
	Temperature (x_4)	0.033	0.066	0.500	0.631
	Initial pH (x_5)	0.170	0.066	2.591	0.032
	CSW (x_6)	0.029	0.066	0.444	0.669
$Y_{xol,xyl}$	**Mean**	0.449	0.033	13.725	< 0.001
	Curvature	0.563	0.131	4.298	0.003
	Nitrogen source (x_1)	0.335	0.065	5.111	0.001
	KH_2PO_4 (x_2)	0.051	0.065	0.784	0.456
	$MgSO_4$ (x_3)	-0.078	0.065	-1.196	0.266
	Temperature (x_4)	-0.098	0.065	-1.492	0.174
	Initial pH (x_5)	0.171	0.065	2.607	0.031
	CSW (x_6)	0.139	0.065	2.123	0.067
ε_{xyl}	**Mean**	64.592	6.908	9.350	< 0.001
	Curvature	45.467	27.634	1.645	0.139
	Nitrogen source (x_1)	39.750	13.817	2.877	0.021
	KH_2PO_4 (x_2)	2.650	13.817	0.192	0.853
	$MgSO_4$ (x_3)	-1.917	13.817	-0.139	0.893
	Temperature (x_4)	0.450	13.817	0.033	0.975
	Initial pH (x_5)	42.317	13.817	3.063	0.016
	CSW (x_6)	12.283	13.817	0.889	0.400
$Y_{arol,arab}$	**Mean**	0.209	0.015	14.080	< 0.001
	Curvature	-0.008	0.059	-0.140	0.892
	Nitrogen source (x_1)	0.166	0.030	5.597	0.001
	KH_2PO_4 (x_2)	-0.023	0.030	-0.756	0.471
	$MgSO_4$ (x_3)	0.055	0.030	1.843	0.103
	Temperature (x_4)	-0.092	0.030	-3.098	0.015
	Initial pH (x_5)	0.031	0.030	1.037	0.330
	CSW (x_6)	0.118	0.030	3.973	0.004
P_{arol}	**Mean**	0.018	0.001	15.092	< 0.001
	Curvature	0.004	0.005	0.904	0.392
	Nitrogen source (x_1)	0.017	0.002	7.302	< 0.001
	KH_2PO_4 (x_2)	-0.002	0.002	-0.904	0.392
	$MgSO_4$ (x_3)	0.008	0.002	3.269	0.011
	Temperature (x_4)	< 0.001	0.002	0.070	0.946
	Initial pH (x_5)	0.011	0.002	4.660	0.002
	CSW (x_6)	0.006	0.002	2.295	0.051

Tabela S4. (continued).

Response	Variables	Effect	Standard error	calculated t	p-value
ε_{arab}	**Mean**	-0.468	-0.027	-17.135	< 0.001
	Curvature	-0.212	-0.109	-1.942	0.088
	Nitrogen source	0.147	-0.055	2.683	0.028
	KH_2PO_4 (x_2)	-0.002	-0.055	-0.038	0.971
	$MgSO_4$ (x_3)	0.037	-0.055	0.679	0.516
	Temperature (x_4)	0.051	-0.055	0.927	0.381
	Initial pH (x_5)	0.239	-0.055	4.377	0.002
	CSW (x_6)	0.038	-0.055	0.694	0.507
$Y_{x,s}$	**Mean**	0.109	0.005	23.849	< 0.001
	Curvature	-0.064	0.018	-3.530	0.008
	Nitrogen source	-0.015	0.009	-1.646	0.138
	KH_2PO_4 (x_2)	-0.035	0.009	-3.804	0.005
	$MgSO_4$ (x_3)	0.032	0.009	3.512	0.008
	Temperature (x_4)	-0.121	0.009	-13.278	< 0.001
	Initial pH (x_5)	0.055	0.009	5.999	< 0.001
	CSW (x_6)	0.058	0.009	6.365	< 0.001

arab – arabinose; arol – arabitol; CSW – corn steep water; P – productivity; s – total sugars (glicose, xylose and arabinose); xol – xylitol; xyl – xylose; x – biomass; Y – yield; ε – substrate conversion.

In assessing the effects of process parameters on production variables (Table 3), in the range studied and considering 90 % of significance ($p < 0.10$), it was found that the results of previous assays were confirmed: nitrogen source concentration (x_1) had a positive effect on all parameters except $Y_{x,s}$ and the most significant effect was exerted on sugar alcohol yield and productivity. It was observed that the curvature had a significant effect on most of the evaluated responses, showing that inflection points (maximum or minimum) are included between the lower and upper levels studied (Rodrigues and Iemma, 2014), indicating, in this case, adequate ranges for the study. As demonstrated by the significant positive effect of the curvature, center point runs provided superior results of xylitol production.

Xylitol variables were not significantly influenced by factors x_2, x_3 or x_4. In other words, added salt concentration and process temperature did not significantly influence xylitol yield, xylitol productivity, or xylose consumption. All variables, except for x_2, significantly influenced at least one of the arabitol parameters ($Y_{arol,arab}$, P_{arol}, and ε_{arab}). Based on these effects, it can be inferred that the insertion of CSW in the nitrogen source can be beneficial for the

production of both sugar alcohols; the variation in KH_2PO_4 concentration did not have significant effects; the high temperature can negatively affect the production of arabitol and the central point presented the best results for the production of xylitol.

Table 3. Summary of the standardized effects (calculated t) of medium composition and process variables on xylitol and arabitol production from sugarcane bagasse hemicellulose hydrolysate (75 g L^{-1} initial xylose) fermentation (96 h, 200 rpm) by *C. tropicalis*.

Variable	P_{xol}	$Y_{xol,xyl}$	ε_{xyl}	P_{arol}	$Y_{arol,arab}$	ε_{arab}	$Y_{x,s}$
N (x_1)	+	++	+	+++	+	+	
KH_2PO_4 (x_2)							-
$MgSO_4$ (x_3)				+			+
T (x_4)					-		----
pH (x_5)	+	+	+	++		++	+
CSW (x_6)		+		+	+		+
Curvature	+	++				-	-

N, added nitrogen concentration; T, temperature; CSW, corn steep water percentage in the nitrogen source; xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; x, biomass; s, total sugars (glucose, xylose, and arabinose); *Y*, yield; *P*, productivity; *ε*, substrate conversion.

Statistical significance was set at $p < 0.10$. Significant effects are indicated in green (positive) or red (negative). Gray cells represent non-significant effects.

Biomass yield was negatively affected by factors. The main factor positively influencing sugar alcohol production, x_1, had no significant effect on biomass yield. Center point conditions did not favor biomass yield. These results confirm the expectation that the higher the value of $Y_{x,s}$, the greater the amount of polyalcohols consumed and directed toward metabolic pathways of cell growth (Hernández-Pérez et al. 2019).

This experiment provided a similar xylitol concentration and productivity to the initial SBHH concentration experiment (Section 3.4) as well as higher xylose conversion (up to 7.4% higher), arabitol concentration and productivity (up to 2.4 times higher), and arabinose consumption (up to 72.3% higher).

3.6. Validation and extrapolation of Plackett–Burman results

Fermentation conditions and medium compositions for validation of Plackett-Burmann design experiments are presented in Table 4 and Table 5 presents its results. Center point runs conducted with different SBHH batches (CP-a and CP-b) did not differ significantly in arabitol variables or xylitol concentration and

productivity. There were, however, differences in xylitol yield and xylose consumption. Such differences between batches can be attributed to differences in substrate composition regarding micronutrients, organic acids, and phenolic compounds. These factors may affect yeast metabolism.

We found no significant differences between experiments carried out using the same SBHH batch (CP-b, V-I, V-II, V-III, and V-IV), demonstrating that any of the culture media conditions can be applied for sugar alcohol production.

The V-I run was conducted under the same conditions as that of the center point of the Plackett–Burman design but without addition of KH_2PO_4 or $MgSO_4$ salts. These salts are commonly added to culture media for xylitol production from hemicellulosic hydrolysates (Kordowska-Wiater et al. 2017; Morais Junior et al. 2019). CSW is known to contain different minerals, such as phosphorus, magnesium, calcium, zinc, manganese, and aluminum, with phosphorus being one of the major minerals (Selim et al. 2021). These minerals, together with mineral components already present in YH, might have contributed to meeting the micronutrient demands of yeast, precluding the need for potassium and magnesium salt addition for bioprocess optimization.

Table 4. Fermentations carried out to explore different processing conditions based on the results of the Plackett–Burman experimental design.

Run	N (g L^{-1})	KH_2PO_4 (g L^{-1})	$MgSO_4$ (g L^{-1})	T (°C)	pH	CSW (%)
CP	2.2 (0)	2.7 (0)	0.3 (0)	32 (0)	6.5 (0)	20 (0)
V-I	2.2 (0)	0 (-)	0 (-)	32 (0)	6.5 (0)	20 (0)
V-II	3.6 (+1)	0.4 (−1)	0.5 (+1)	32 (0)	7 (+1)	40 (+1)
V-III	5 (+2)	0.4 (−1)	0.5 (+1)	32 (0)	7 (+1)	40 (+1)
V-IV	3.6 (+1)	0 (-)	0.5 (+1)	32 (0)	7 (+1)	20 (0)

N, added nitrogen concentration, T, temperature; CSW, corn steep water percentage in the nitrogen source; CP, center point.

The center point run was performed using batches A and B of sugarcane bagasse hemicellulosic hydrolysate. Coded values of the Plackett–Burman experimental design are indicated in parentheses. Level +2 represents a higher concentration than that used in the original experiment. A hyphen (-) indicates absence of the nutrient in the culture medium.

Table 5. Validation and exploration of Plackett–Burman experimental results for assessing the effects of medium composition and process variables on xylitol and arabitol production from sugarcane bagasse hemicellulose hydrolysate fermentation (96 h, 200 rpm) by *C. tropicalis*.

Run	C_{xol} (g L^{-1})	C_{arol} (g L^{-1})	$Y_{xol,xyl}$ (g g^{-1})	$Y_{arol,arab}$ (g g^{-1})	P_{xol} (g L^{-1} h^{-1})	P_{arol} (g L^{-1} h^{-1})	$Y_{x,s}$ (g g^{-1})	ε_{xyl} (%)	ε_{arab} (%)
CP (batch A)	49.02 ± 3.87a	1.94 ± 0.24a	0.73 ± 0.02a	0.21 ± 0.03b	0.51 ± 0.04[a]	0.020 ± 0.002a	0.07 ± 0.01b	87.3 ± 2.5b	57.5 ± 4.7a
CP (batch B)	38.70 ± 4.34ab	2.11 ± 0.79a	0.56 ± 0.07b	0.27 ± 0.09ab	0.40 ± 0.05ab	0.022 ± 0.008a	0.15 ± 0.04[a]	100.0 ± 0.00a	65.7 ± 9.0a
V-I	39.09 ± 4.90ab	2.61 ± 0.14a	0.55 ± 0.09b	0.40 ± 0.10a	0.41 ± 0.05ab	0.027 ± 0.001a	0.13 ± 0.04ab	100.0 ± 0.00a	67.8 ± 15.1a
V-II	37.38 ± 0.58b	2.57 ± 0.43a	0.52 ± 0.01b	0.26 ± 0.03ab	0.39 ± 0.01b	0.027 ± 0.004a	0.16 ± 0.03[a]	100.0 ± 0.00a	68.2 ± 2.0a
V-III	34.75 ± 5.81b	2.96 ± 0.25a	0.45 ± 0.07b	0.33 ± 0.02ab	0.36 ± 0.06b	0.031 ± 0.003a	0.11 ± 0.01ab	100.0 ± 0.00a	68.1 ± 4.7a
V-IV	43.56 ± 3.29ab	2.39 ± 0.86a	0.61 ± 0.06b	0.22 ± 0.05b	0.45 ± 0.03ab	0.025 ± 0.009a	0.06 ± 0.01b	100.0 ± 0.00a	71.1 ± 13.6a

N, added nitrogen concentration; T, temperature; CSW, corn steep water percentage in the nitrogen source; xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; x, biomass; s, total sugars (glucose, xylose, and arabinose); *C*, concentration; *Y*, yield; *P*, productivity; *ε*, substrate conversion; CP, center point.

Different letters in the same column indicate significant differences at $p < 0.05$ by Tukey's test

Runs V-II and V-III, as compared with V-I, despite having higher CSW addition (40%), had higher total nitrogen concentration. In V-IV, the percentage addition of CSW was 20%, but there was also an increase in total nitrogen concentration. In these runs, therefore, it was necessary to add more YH to the medium, increasing process costs. Given the statistical equivalence of these runs with CP-b, it can be said that the increase in total nitrogen concentration at the evaluated levels did not contribute significantly to production parameters. Furthermore, runs V-II, V-III, and V-IV were performed at pH 7, indicated as positive for arabitol production. However, as the results did not differ from those of run VI, pH was found not to influence arabitol production parameters.

The medium conditions used in run V-I (2.2 g L^{-1} of nitrogen with 20 % of CSW, 32 ºC, initial pH 6.5 and no $MgSO_4$ or KH_2PO_4) were selected for the subsequent experiment. This run combines the most economical and environmentally friendly conditions, namely non-addition of salts, low YH concentration (16 g L^{-1}), and addition of CSW to the medium (20% total nitrogen, 39.3 g L^{-1}). It is noteworthy that, in a cost estimate based on the Brazilian market, this improved medium is equivalent to US$ 0.35 L^{-1}, almost 10 times less than a standard medium with MgSO4, KH2PO4 and YE as the only nitrogen source, which would cost US$ 3.58 L^{-1}.

A further test was conducted using crude SBHH under center point conditions. In this assay, substrate consumption or product formation did not occur, demonstrating the need for hydrolysate detoxification for successful fermentation by *C. tropicalis*. The high concentrations of inhibitory compounds in crude SBHH inhibited microbial growth.

3.7. Bioreactor fermentations

Fermentation was conducted in a 2 L bioreactor with 1 L working volume using medium composition defined in the previous experiment, namely SBHH detoxified with about 75 g L^{-1} xylose and 2.2 g L^{-1} total nitrogen (80% YH and 20% CSW). The kinetic behaviors are depicted in Fig. 4.

Glucose was completely consumed in 12 h of fermentation, leading to the formation of ethanol and biomass. During this period, xylose and arabinose

concentrations also declined. Preferential glucose consumption at the beginning is important for biomass growth and generation of NADPH, a coenzyme regenerated from the pentose phosphate pathway that will act in the synthesis of xylitol and arabitol with aldose reductase enzymes (Yuan et al., 2021; Farias et al., 2022). Studies with *C. tropicalis* report that there is Carbon Catabolite Repression (CCR) by glucose, so glucose in the medium reduces the metabolism of pentoses due to repression of gene regulation and decreased activity of pentose reductase enzymes (Oktaviani et al., 2021; Chattopadhyay et al., 2020). This repression can occur with glucose, xylose, and arabinose because they share common sugar transport systems in the cell and glucose is more effective for cellular energy generation (Fonseca et al., 2007; Oktaviani et al., 2021). Despite this, during these first 12 h period, xylose and arabinose concentrations slightly declined.

After 24 h, xylose continued to be consumed by microorganisms, affording xylitol, but arabinose concentration remained practically constant, with low arabitol formation. Therefore, there was CCR in the metabolism of arabinose due to the presence of xylose, a prioritized pentose that is assimilated in fewer steps in the metabolic pathway compared to arabinose (Farias et al., 2022). Similar xylose repression behavior was identified by Oktaviani group (2021) evaluating xylitol production by *C. tropicalis*.

From 156 h onward, with xylose tending toward depletion, the rate of xylitol formation decreased and arabinose consumption increased, leading to arabitol formation. At this time, there was greater biomass growth, possibly due to the occurrence of arabinose consumption concomitantly with sugar alcohol formation for metabolism and cell growth.

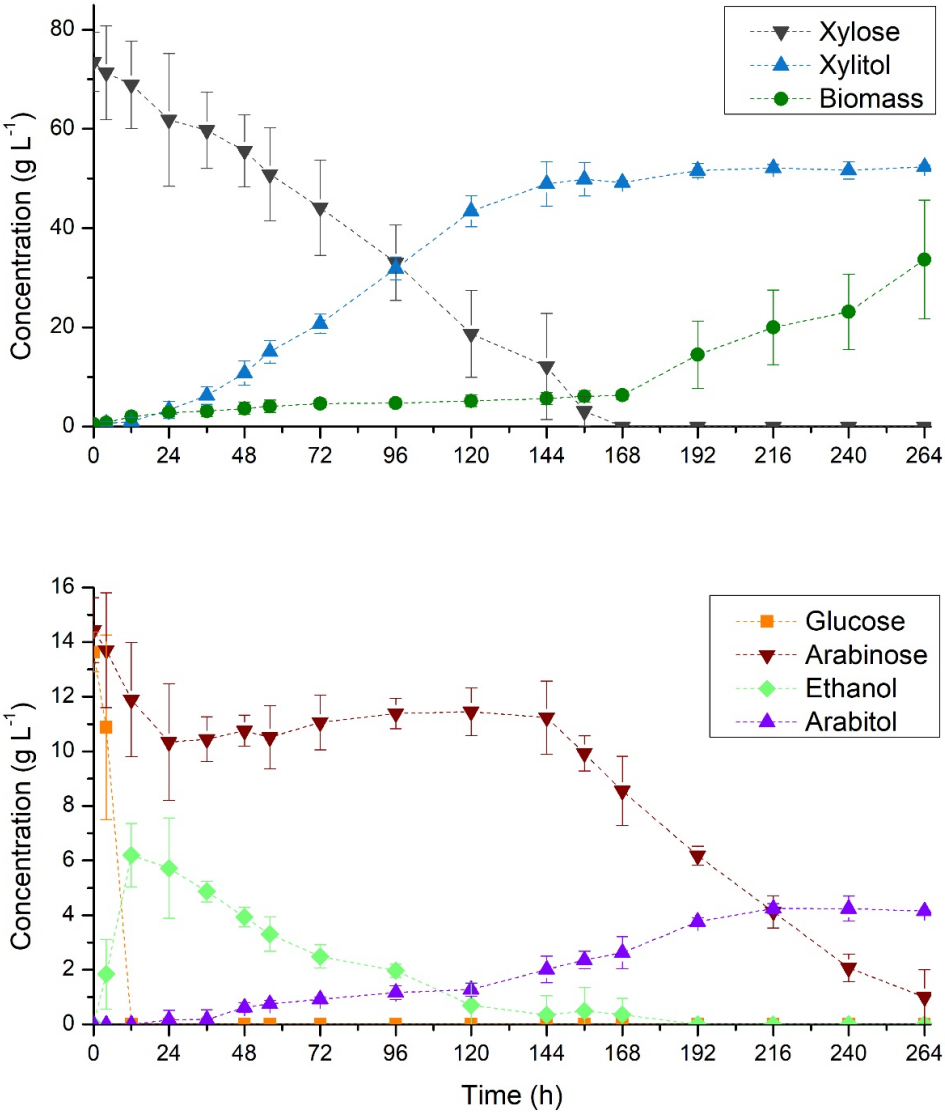


Fig. 4. *C. tropicalis* fermentation (0.7 L min^{-1}, 250 rpm) in a bioreactor using diluted hemicellulosic hydrolysate as carbon source (75 g L^{-1} initial xylose).

Glucose depletion in the first hours of the reaction and subsequent prioritization of xylose consumption have also been reported in previous studies using different yeasts for xylitol production in hemicellulosic hydrolysate (Antunes et al. 2021; Narisetty et al. 2021), highlighting the possibility of optimizing the process in two stages: consumption of glucose for cell growth and consumption of pentoses for the production of sugar alcohols. This is the first identified report of arabinose consumption and arabitol formation in hemicellulosic hydrolysates with high initial concentrations of glucose, xylose, and arabinose.

It was possible to observe that, in experiments carried out in the bioreactor, a longer time was required to obtain greater substrate conversion and xylitol and arabitol formation than in experiments carried out in shaker flasks. Such a finding is evidenced by the maximum productivity, which, for xylitol, was reached after 144 h (P_{xol} = 0.34 ± 0.03 g L^{-1} h^{-1}) and, for arabitol, only after 216 h (P_{arol} = 0.020 ± 0.001 g L^{-1} h^{-1}). Such productivities were lower than those obtained with the same culture medium in shaker flasks, possibly due to the use of agitation and aeration conditions that did not adequately represent the oxygen transfer rate of the shaker flasks.

Of note, some bioreactor parameters, such as agitation and aeration, were not assessed. These factors affect the oxygen transfer rate of the process, which, as previously highlighted, is fundamental for pentose metabolism and polyalcohol accumulation by yeasts (Manjarres-Pinzón et al. 2022). These operating parameters may hold potential for increasing the combined productivity of xylitol and arabitol production in bioreactors and may have caused the reduction in bioreactor productivity compared to shaker flasks. The bioreactor experiment afforded the highest arabitol concentration (C_{arol} = 4.25 ± 0.18 g L^{-1}) as well as a high xylitol concentration (C_{xol} = 52.12 ± 3.87 g L^{-1}) after 216 h of fermentation compared with all other experiments conducted in the current study, being 450 % for xylitol, and 250 %, for arabitol, higher than the concentrations obtained in the first experiments with standard nitrogen source and SBHH (Section 3.3).

Fig. 5 compares the results obtained in standard and improved culture media in shaker flask and bioreactor through carbon mass balance using a calculation basis of 100 kg of total sugars from SBHH. The determination of CO2 generated was performed from stoichiometric balances of growth, respiration and product formation by cells (each carbon mol for biomass, sugar alcohols, ethanol and respiration generating, respectively, 0.095, 0.111, 0.500 and 1.000 mols of CO_2) (Converti et al., 2002; Bonan et al., 2021). The improved processes did not promote an increase in the mass conversion of arabinose to arabitol, despite this, there was a reduction in the volume required for the process, which leads to a more concentrated product, a relevant factor

for downstream purification processes (Marques Júnior and Rocha, 2021). For xylitol, there was an increase in mass conversion of 57% with the change in the medium and an additional 23% with the change in the mode of operation for bioreactors.

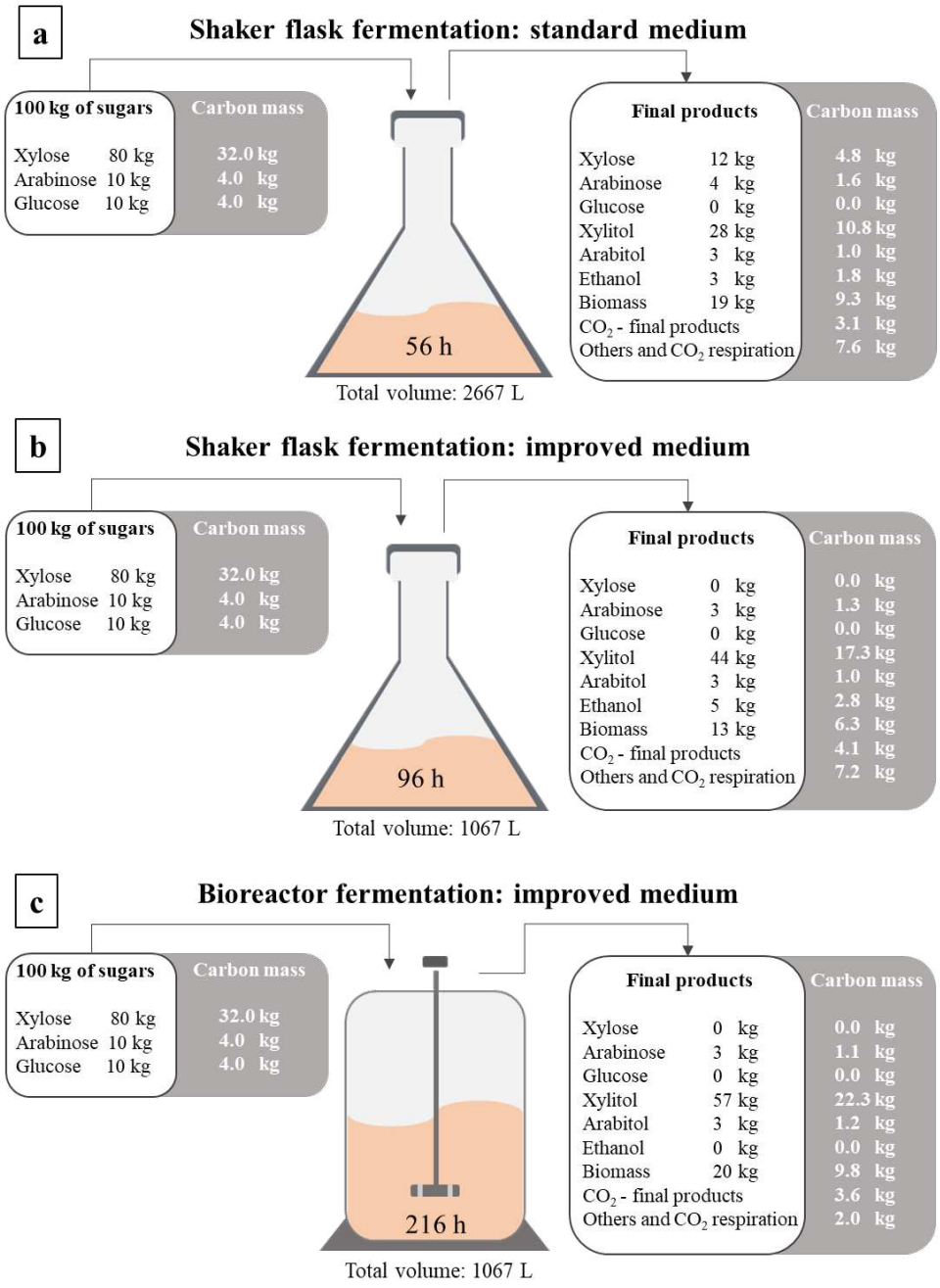


Fig. 5. Carbon mass balance of xylose, arabinose, glucose, xylitol, arabitol, ethanol, biomass (dry cell weight) and CO_2 with 100 kg of total sugars as basis of calculation for fermentations with *C. tropicalis* in SBHH with different media composition and operation modes. **a.** Standard medium with YE, $MgSO_4$ and KH_2PO_4 **b.** Media with YH + CSW as nitrogen source **c.** Same media as "b" in bioreactor operation mode.

Comparing the conversions in terms of carbon mass, in the process with standard media 30% of the initial carbon was converted to xylitol and arabitol. In the same process with improved media this carbon conversion was 46%, while in bioreactors it reached 59%. The increased conversion of carbon to products reveals the better targeting of initial carbon sources for the production of sugar alcohols. This fact can also be evidenced by the carbon mass expended for other metabolites and in the generation of CO_2 by cellular respiration, which reduced from 7.6 kg to 2.0 kg in the process in bioreactors. A similar behavior was evidenced by the Tamburini group (2015) who observed a reduction in CO_2 used in cellular respiration of approximately 30% to 5% under conditions that optimized *C. tropicalis* adaptation and xylitol production.

Table 6 presents a comparison of the results obtained in shaker flasks after 96 h of fermentation, bioreactor after 216 h of fermentation, and previous studies reported in the literature conducted under similar conditions. Other studies reported superior results, but the experiments were conducted with addition of pure xylose or L-arabinose to the medium, a factor that can elevate production costs, particularly when low conversion rates are obtained.

Palladino et al. (2021) investigated the effect of introducing an alternative nitrogen source, rice bran, to SBHH for the production of xylitol by *Cyberlindnera xylosilytica*. In the current study, addition of CSW as an alternative nitrogen source in bioreactor processes afforded a 17% higher $Y_{xol,xyl}$ and 20% higher P_{xol}. Although shaker flask experiments had lower yields, productivities were about 50% higher.

Arruda et al. (2017) produced xylitol under similar conditions in bioreactors but without arabitol formation. In our study, we obtained comparable but superior results for C_{xol} and $Y_{xol,xyl}$ (44% and 14% higher, respectively).

Table 6. Comparison of the results of the current study with similar works assessing xylitol and arabitol production by yeasts.

Reference	Feedstock	Microorganism	Reactor type	C_{xol} (g L^{-1})	C_{arol} (g L^{-1})	$Y_{xol,xyl}$ (g g^{-1})	$Y_{arol,arab}$ (g g^{-1})	P_{xol} (g L^{-1} h^{-1})	P_{arol} (g L^{-1} h^{-1})	ε_{xyl} (%)	ε_{arab} (%)
This work[a]	SBHH	*Candida tropicalis*	1 L shaker flasks	39.1	2.61	0.55	0.40	0.41	0.027	100.0	57.5
This work[b]	SBHH	*Candida tropicalis*	2 L bioreactor	52.1	4.25	0.74	0.43	0.24	0.020	100.0	70.3
Alves et al. (2021)	SBHH	*Candida tropicalis*	1 L shaker flasks	19.3	ND	0.55	ND	0.21	ND	100.0	100.0
Antunes et al. (2021)	SBHH	*Candida tropicalis*	125 mL shaker flasks	12.0	ND	0.61	ND	0.12	ND	100.0	ND
Morais Júnior et al. (2019)	SBHH + xylose	*Candida tropicalis*	125 mL shaker flasks	109.5	ND	0.86	ND	2.81	ND	55.4	ND
Arruda et al. (2017)	SBHH	*Candida guilliermondii*	2.4 L bioreactor	36.0	ND	0.65	ND	0.28	ND	92.0	71.5
Palladino et al. (2021)	SBHH	*Cyberlindnera xylosilytica*	125 mL shaker flasks	14.1	ND	0.63	ND	0.20	ND	ND	ND
Kordowska-Wiater et al. (2017)	L-Arabinose	*Candida parapsilosis*	100 mL shaker flasks	ND	10.70	ND	0.53	ND	0.007	ND	100.0
Kordowska-Wiater et al. (2013)	L-Arabinose	*Candida parapsilosis*	500 mL shaker flasks	ND	17.90	ND	0.48	ND	0.372	ND	100.0
McMillan and Boynton (1994)	L-Arabinose and xylose	*Candida tropicalis*	250 mL shaker flasks	6.0	8.00	0.35	1.02	0.06	0.087	97.0	21.0
Medeiros et al. (2020)	Sisal bagasse hydrolysate	*Debaryomyces hansenii*	1 L shaker flasks	0.1	1.14	0.04	0.96	0.01	0.01	100	100.0
Araújo et al. (2021)	Grape stalks hydrolysate	*Komagataella pastoris*	250 mL shaker flaks	0.8	0.34	0.25	0.85	0.006	0.003	56.4	21.0

[a] Fermentation in shaker flasks (V-I) for 96 h.
[b] Fermentation in a bioreactor for 216 h.
xol, xylitol; xyl, xylose; arol, arabitol; arab, arabinose; *C*, concentration; *Y*, yield; *P*, productivity; *ε*, substrate conversion; SBHH, sugarcane bagasse hemicellulosic hydrolysate; ND, not determined

Only two recent studies highlighted the potential for simultaneous production of xylitol and arabitol from hemicellulosic hydrolysates using different yeasts (Table 5) (de Medeiros et al. 2020; Araújo et al. 2021). High values of $Y_{arol,arab}$ were obtained, but the scale of production was low, being limited to shaker flasks; consequently, substrate consumption, as well as product formation, was lower than those found in our study. In general, C_{xol}, P_{xol}, and $Y_{xol,xyl}$ were up to 65, 24, and 3 times higher, respectively, and C_{arol} and P_{arol} were up to 3.7 and 2 times higher, respectively, in our bioreactor experiment than in literature reports. Furthermore, in 216 h, $Y_{xol,xyl}$ and $Y_{arol,arab}$ corresponded to, respectively, 80.7% and 42.6% of the theoretical yields of 0.917 g g^{-1} for xylitol (Farias et al., 2022) and 1.01 g g^{-1} for arabitol (McMillan and Boynton, 1994).

It can be concluded that, even if optimization studies can be carried out to achieve higher conversion parameters, the results for xylitol production alone were superior to those reported in the literature, and, regarding coproduction of xylitol and arabitol from hemicellulosic hydrolysate, our findings are the most promising to date. A unique factor of our study was that processes were also carried out on a benchtop bioreactor scale.

4. Conclusions

It was verified that xylitol and arabitol can be coproduced by *C. tropicalis* using SBHH as substrate and increasing the initial concentration of SBHH from 30 to 75 g L^{-1} of xylose contributed to the formation of coproducts. Analysis of the culture medium and the effects of variables showed that the nitrogen source and the added amount of it represented the greatest influence on sugar alcohol production. In addition, it was possible to define improved culture medium conditions in terms of economic and environmental feasibility using YH as the main nitrogen source, without adding salts and using 20 % of total nitrogen from CSW, enabling an almost 10-fold reduction in the cost of culture medium. It was also possible to perform coproduction in a bioreactor reaching concentrations of xylitol and arabitol 65 and 3.7 times higher, respectively, than those reported in the literature for similar processes. These results contribute to the viability of the bioproduction of xylitol and arabitol and show promise for the coproduction of sugar alcohols from sugarcane bagasse hemicellulosic hydrolysate by *C. tropicalis.*

Acknowledgements

This work was supported by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) [Finance code 001], the São Paulo Research Foundation (FAPESP) [grant #2021/05508-9] and the Brazilian National Council for Scientific and Technological Development (CNPq) [grant #439548/2018-7]. The authors would also like to thank the Brazilian Biorenewables National Laboratory (LNBR) for allowing the use of their facilities, Ingredion Brasil and Procelys for the materials provided and Laboratory of Genomics and Expression of the University of Campinas for providing the yeast strain.

References

Almeida, D.G., Silva, R. de C.F.S. da, Meira, H.M., Brasileiro, P.P.F., Silva, E.J., Luna, J.M., Rufino, R.D., Sarubbo, L.A., 2021. Production, Characterization and Commercial Formulation of a Biosurfactant from *Candida tropicalis* UCP0996 and Its Application in Decontamination of Petroleum Pollutants. Processes 9, 1–18. https://doi.org/https://doi.org/10.3390/pr9050885.

Alves, Y.P.C., Fernandes Antunes, F.A., Silverio da Silva, S., Forte, M.B.S., 2021. From by- to bioproducts: selection of a nanofiltration membrane for biotechnological xylitol purification and process optimization. Food Bioprod. Process. 125, 79–90. https://doi.org/10.1016/j.fbp.2020.10.005

Antunes FAF, Santos JC, Chandel AK, et al (2019) Repeated batches as a feasible industrial process for hemicellulosic ethanol production from sugarcane bagasse by using immobilized yeast cells. Cellulose 26:3787–3800. https://doi.org/10.1007/s10570-019-02341-z

Antunes FAF, Thomé LC, Santos JC, et al (2021) Multi-scale study of the integrated use of the carbohydrate fractions of sugarcane bagasse for ethanol and xylitol production. Renew Energy 163:1343–1355. https://doi.org/10.1016/j.renene.2020.08.020

Araújo D, Costa T, Freitas F (2021) Biovalorization of lignocellulosic materials for xylitol production by the yeast komagataella pastoris. Appl Sci 11:. https://doi.org/10.3390/app11125516

Arcaño YD, García ODV, Mandelli D, et al (2020) Xylitol: A review on the progress and challenges of its production by chemical route. Catal Today 344:2–14. https://doi.org/10.1016/j.cattod.2018.07.060

Arruda P, dos Santos JC, de Cássia Lacerda Brambilla Rodrigues R, et al (2017) Scale up of xylitol production from sugarcane bagasse hemicellulosic hydrolysate by Candida guilliermondii FTI 20037. J Ind Eng Chem 47:297–302. https://doi.org/10.1016/j.jiec.2016.11.046

Awuci CG, Echeta KC (2019) Current developments in sugar alcohols: chemistry, nutrition, and health concerns of sorbitol, xylitol, glycerol, arabitol, inositol, maltitol, and lactitol. Int J Adv Acad Res 5:2488–9849

Bedő, S., Fehér, A., Khunnonkwao, P., Jantama, K., Fehér, C., 2021. Optimized

bioconversion of Xylose derived from pre-treated crop residues into Xylitol by using Candida Boidinii. Agronomy 11. https://doi.org/10.3390/agronomy11010079

Bonan, C.I.D.G., Tramontina, R., dos Santos, M.W., Biazi, L.E., Soares, L.B., Pereira, I.O., Hoffmam, Z.B., Coutouné, N., Squina, F.M., Robl, D., Ienczak, J.L., 2022. Biorefinery Platform for Spathaspora passalidarum NRRL Y-27907 in the Production of Ethanol, Xylitol, and Single Cell Protein from Sugarcane Bagasse. Bioenergy Res. 15, 1169–1181. https://doi.org/10.1007/s12155-021-10255-7

Cardoso, B.S., Forte, M.B.S., 2021. Purification of biotechnological xylitol from Candida tropicalis fermentation using activated carbon in fixed-bed adsorption columns with continuous feed. Food Bioprod. Process. 126, 73–80. https://doi.org/10.1016/j.fbp.2020.12.013

Chaves SR, Rego A, Martins VM, et al (2021) Regulation of cell death induced by acetic acid in yeasts. Front Cell Dev Biol 9:. https://doi.org/10.3389/fcell.2021.642375

Converti, A., Perego, P., Sordi, A., Torre, P., 2002. Effect of starting xylose concentration on the microaerobic metabolism of Debaryomyces hansenii: The use of carbon material balances. Appl. Biochem. Biotechnol. - Part A Enzym. Eng. Biotechnol. 101, 15–29. https://doi.org/10.1385/ABAB:101:1:15

Farias, D, de Mélo, AHF, da Silva, MF, Bevilaqua, GC, Ribeiro, DG, Goldbeck, R, Forte, MBS, Maugeri-Filho, F (2022) New biotechnological opportunities for C5 sugars from lignocellulosic materials. Bioresour. Technol. Reports 17, 100956. https://doi.org/10.1016/j.biteb.2022.100956

Grand View Research (2022) Sugar Alcohol Market Size, Share & Trends Analysis Report By Contain (Sorbitol, Mannitol, Xylitol, Maltitol), By Form (Powder & Crystal, Liquid & Syrup), By Industry Vertical, And Segment Forecasts, 2022 – 2028

Hofer, A., Hauer, S., Kroll, P., Fricke, J., Herwig, C., 2018. In-depth characterization of the raw material corn steep liquor and its bioavailability in bioprocesses of Penicillium chrysogenum. Process Biochem. 70, 20–28. https://doi.org/10.1016/j.procbio.2018.04.008

Hull SR, Peters E, Cox C, Montgomery R (1996) Composition of Corn Steep Water during Experimental Steeping. J Agric Food Chem 44:3521–3527. https://doi.org/10.1021/jf960320v

Kordowska-Wiater M (2015) Production of arabitol by yeasts: Current status and future prospects. J Appl Microbiol 119:303–314. https://doi.org/10.1111/jam.12807

Kordowska-Wiater M, Kuzdraliński A, Czernecki T, et al (2017) The production of arabitol by a novel plant yeast isolate Candida parapsilosis 27RL-4. Open Life Sci 12:326–336. https://doi.org/10.1515/biol-2017-0038

Lee, S.J., Lee, H.S., Kim, S.Y., Shin, K.S., 2018. Immunostimulatory and anti-metastatic activity of polysaccharides isolated from byproducts of the corn starch industry. Carbohydr. Polym. 181, 911–917. https://doi.org/10.1016/j.carbpol.2017.11.060

Loman AA, Islam SMM, Ju LK (2018) Production of arabitol from enzymatic

hydrolysate of soybean flour by Debaryomyces hansenii fermentation. Appl Microbiol Biotechnol 102:641–653. https://doi.org/10.1007/s00253-017-8626-5

Manjarres-Pinzón K, Mendoza-Meza D, Arias-Zabala M, et al (2022) Effects of agitation rate and dissolved oxygen on xylose reductase activity during xylitol production at bioreactor scale. Food Sci Technol 42:1–7. https://doi.org/10.1590/fst.04221

Marques Júnior, J.E., Rocha, M.V.P., 2021. Development of a purification process via crystallization of xylitol produced for bioprocess using a hemicellulosic hydrolysate from the cashew apple bagasse as feedstock. Bioprocess Biosyst. Eng. https://doi.org/10.1007/s00449-020-02480-9

McMillan JD, Boynton BL (1994) Arabinose utilization by xylose-fermenting yeasts and fungi. Appl Biochem Biotechnol 45–46:569–584. https://doi.org/10.1007/BF02941831

Medeiros LL, da Silva FLH, de Queiroz ALM, et al (2020) Structural-chemical characterization and potential of sisal bagasse for the production of polyols of industrial interest. Brazilian J Chem Eng 37:451–461. https://doi.org/10.1007/s43153-020-00049-3

Morais Junior WG, Pacheco TF, Trichez D, et al (2019) Xylitol production on sugarcane biomass hydrolysate by newly identified Candida tropicalis JA2 strain. Yeast 36:349–361. https://doi.org/10.1002/yea.3394

Murzin, D.Y., Daigue, E., Slotte, R., Sladkovskiy, D.A., Salmi, T., 2020. Techno-Economic Analysis for Production of L-Arabitol from L-Arabinose. Chem. Eng. Technol. 43, 1260–1267. https://doi.org/10.1002/ceat.202000125

Narisetty V, Castro E, Durgapal S, et al (2021) High level xylitol production by Pichia fermentans using non-detoxified xylose-rich sugarcane bagasse and olive pits hydrolysates. Bioresour Technol 342:126005. https://doi.org/10.1016/j.biortech.2021.126005

Paabo, M., Bates, R.G., Robinson, R.A., 1965. in Aqueous Solution and Related Isotope Effects from 0 to 50 ° 1147, 540–543.

Palladino F, Rodrigues RCLB, Cadete RM, et al (2021) Novel potential yeast strains for the biotechnological production of xylitol from sugarcane bagasse. Biofuels, Bioprod Biorefining 15:690–702. https://doi.org/10.1002/bbb.2196

Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour. Technol. 74, 25–33. https://doi.org/10.1016/S0960-8524(99)00161-3

Rafiqul, I.S.M., Sakinah, A.M.M., Zularisam, A.W., 2015. Inhibition by toxic compounds in the hemicellulosic hydrolysates on the activity of xylose reductase from Candida tropicalis. Biotechnol. Lett. 37, 191–196. https://doi.org/10.1007/s10529-014-1672-5

Rodrigues, MF, Iemma, AF (2014) Experimental Design and Process Optimization,CRC Press, New York.

Rodrigues, T.V.D., Amore, T.D., Teixeira, E.C., Burkert, J.F. de M., 2019. Carotenoid production by Rhodotorula mucilaginosa in Batch and Fed-Batch fermentation using agroindustrial byproducts. Food Technol. Biotechnol. 57, 388–398. https://doi.org/10.17113/ftb.57.03.19.6068

Ruchala J, Sibirny AA (2021) Pentose metabolism and conversion to biofuels and high-value chemicals in yeasts. FEMS Microbiol Rev 45:1–44. https://doi.org/10.1093/femsre/fuaa069

Saha BC, Kennedy GJ (2020) Production of xylitol from mixed sugars of xylose and arabinose without co-producing arabitol. Biocatal Agric Biotechnol 29:101786. https://doi.org/10.1016/j.bcab.2020.101786

Selim, MT, Salem SS, Fouda A, et al (2021) Use of corn-steep water effluent as a promising substrate for lactic acid production by enterococcus faecium strain wh51-1. Fermentation 7:1–18. https://doi.org/10.3390/fermentation7030111

Singh AK, Pandey AK, Kumar M, et al (2022) Improved xylitol production by the novel inhibitor-tolerant yeast Candida tropicalis K2. Environ Technol (United Kingdom) 1–15. https://doi.org/10.1080/09593330.2022.2095227

Soares, L.B., da Silveira, J.M., Biazi, L.E., Longo, L., de Oliveira, D., Furigo Júnior, A., Ienczak, J.L., 2022. An overview on fermentation strategies to overcome lignocellulosic inhibitors in second-generation ethanol production using cell immobilization. Crit. Rev. Biotechnol. 1–22. https://doi.org/10.1080/07388551.2022.2109452

Tamburini, E., Costa, S., Marchetti, M.G., Pedrini, P., 2015. Optimized production of xylitol from xylose using a hyper-acidophilic Candida tropicalis. Biomolecules 5, 1979–1989. https://doi.org/10.3390/biom5031979

Winkelhausen, E., Kuzmanova, S., 1998. Microbial conversion of D-xylose to xylitol. J. Ferment. food Eng. 86, 1–14.

Wu, W., Pang, B., Yang, R.R., Liu, G., Ai, C., Jiang, C., Shi, J., 2020. Improvement of the probiotic potential and yield of Lactobacillus rhamnosus cells using corn steep liquor. Lwt 131, 109862. https://doi.org/10.1016/j.lwt.2020.109862

Xiao, X., Hou, Y., Du, J., Liu, Yang, Liu, Yanjie, Dong, L., Liang, Q., Wang, Y., Bai, G., Luo, G., 2012. Determination of main categories of components in corn steep liquor by near-infrared spectroscopy and partial least-squares regression. J. Agric. Food Chem. 60, 7830–7835. https://doi.org/10.1021/jf3012823

Xu Y, Chi P, Bilal M, Cheng H (2019) Biosynthetic strategies to produce xylitol: an economical venture. Appl Microbiol Biotechnol 103:5143–5160. https://doi.org/10.1007/s00253-019-09881-1

Zheng S, Jiang B, Zhang T, Chen J (2020) Combined mutagenesis and metabolic regulation to enhance d-arabitol production from Candida parapsilosis. J Ind Microbiol Biotechnol 47:425–435. https://doi.org/10.1007/s10295-020-02278-4.

Zhou, K., Yu, J., Ma, Y., Cai, L., Zheng, L., Gong, W., Liu, Q. ai, 2022. Corn Steep Liquor: Green Biological Resources for Bioindustry. Appl. Biochem. Biotechnol. 194, 3280–3295. https://doi.org/10.1007/s12010-022-03904-w

Zhu, L., Zhao, Y., Zhang, W., Zhou, H., Chen, X., Li, Y., Wei, D., Wei, Z., 2019. Roles of bacterial community in the transformation of organic nitrogen toward enhanced bioavailability during composting with different wastes. Bioresour. Technol. 285, 121326. https://doi.org/10.1016/j.biortech.2019.121326

.

CONCLUSÃO

Neste trabalho, a proposta de coprodução biotecnológica de xylitol e arabitol a partir de *C. tropicalis* usando hidrolisado hemicelulósico de bagaço de cana de açúcar como substrato foi validada. A partir da análise dos efeitos de diferentes variáveis sobre a produção dos sugar alcohols, foi possível desenvolver condições mais econômicas e ambientalmente amigáveis para o meio de cultivo. Para isso, utilizou-se um meio sem adição de sais e com a fonte nitrogenada composta por um hidrolisado de levedura menos dispendioso associado à inserção de água de maceração de milho, um resíduo agroindustrial.

Destaca-se, também, que foi possível manter parâmetros de coprodução de xylitol e arabitol a partir de hidrolisados hemicelulósicos em biorreatores de bancada, passo inicial para a ampliação de escala do bioprocesso. Com isso, foram encontrados valores de concentração e produtividade conjuntas dos sugar alcohols superiores ao evidenciado na literatura para processos fermentativos similares. Nesse contexto, este trabalho é mais um passo no desenvolvimento de processos sustentáveis que favorecem o reaproveitamento de resíduos agroindustriais com redução do impacto ambiental gerado, promovendo também os conceitos associados à economia circular.

REFERÊNCIAS

ALBUQUERQUE, T. L. De; DA SILVA, I. J.; DE MACEDO, G. R.; ROCHA, M. V. P. Biotechnological production of xylitol from lignocellulosic wastes: A review. **Process Biochemistry**, vol. 49, no. 11, p. 1779–1789, 2014. DOI 10.1016/j.procbio.2014.07.010. Available at: http://dx.doi.org/10.1016/j.procbio.2014.07.010.

ALVES, Y. P. C. Produção e purificação de xilitol a partir de bagaço de cana-de-açúcar utilizando nanofiltração. **Dissertação - Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas**, 2018. .

ALVES, Y. P. C.; FERNANDES ANTUNES, F. A.; SILVERIO DA SILVA, S.; FORTE, M. B. S. From by- to bioproducts: selection of a nanofiltration membrane for biotechnological xylitol purification and process optimization. **Food and Bioproducts Processing**, vol. 125, p. 79–90, 2021. DOI 10.1016/j.fbp.2020.10.005. Available at: https://doi.org/10.1016/j.fbp.2020.10.005.

ARAÚJO, D.; COSTA, T.; FREITAS, F. Biovalorization of lignocellulosic materials for xylitol production by the yeast komagataella pastoris. **Applied Sciences (Switzerland)**, vol. 11, no. 12, 2021. https://doi.org/10.3390/app11125516.

ARCAÑO, Y. D.; GARCÍA, O. D. V.; MANDELLI, D.; CARVALHO, W. A.; PONTES, L. A. M. Xylitol: A review on the progress and challenges of its production by chemical route. **Catalysis Today**, vol. 344, p. 2–14, 2020. DOI 10.1016/j.cattod.2018.07.060. Available at: https://doi.org/10.1016/j.cattod.2018.07.060.

AWUCI, C. G.; ECHETA, K. C. Current developments in sugar alcohols: chemistry, nutrition, and health concerns of sorbitol, xylitol, glycerol, arabitol, inositol, maltitol, and lactitol. **International Journal of Advanced Academic Research**, vol. 5, no. 11, p. 2488–9849, 2019. .

BANWELL, M. G.; POLLARD, B.; LIU, X.; CONNAL, L. A. Exploiting Nature's Most Abundant Polymers: Developing New Pathways for the Conversion of Cellulose, Hemicellulose, Lignin and Chitin into Platform Molecules (and Beyond). **Chemistry - An Asian Journal**, vol. 16, no. 6, p. 604–620, 2021. https://doi.org/10.1002/asia.202001451.

BERNARD, E. M.; CHRISTIANSEN, K. J.; TSANG, S. F.; KIEHN, T. E.; ARMSTRONG, D. Rate of arabinitol production by pathogenic yeast species. **Journal of Clinical Microbiology**, vol. 14, no. 2, p. 189–194, 1981. https://doi.org/10.1128/jcm.14.2.189-194.1981.

BETTIGA, M.; BENGTSSON, O.; HAHN-HÄGERDAL, B.; GORWA-GRAUSLUND, M. F. Arabinose and xylose fermentation by recombinant Saccharomyces cerevisiae expressing a fungal pentose utilization pathway. **Microbial Cell Factories**, vol. 8, p. 1–12, 2009. https://doi.org/10.1186/1475-2859-8-40.

CHANDEL, A. K.; FORTE, M. B. S.; GONÇALVES, I. S.; MILESSI, T. S.; ARRUDA, P. V.; CARVALHO, W.; MUSSATTO, S. I. Brazilian biorefineries from second generation biomass: critical insights from industry and future perspectives. **Biofuels, Bioproducts and Biorefining**, vol. 15, no. 4, p. 1190–1208, 2021. https://doi.org/10.1002/bbb.2234.

CHENG, K. K.; WU, J.; LIN, Z. N.; ZHANG, J. A. Aerobic and sequential anaerobic fermentation to produce xylitol and ethanol using non-detoxified acid pretreated corncob. **Biotechnology for Biofuels**, vol. 7, no. 1, p. 1–9, 2014. https://doi.org/10.1186/s13068-014-0166-y.

CHOUDHARY, A.; KUMAR, V.; KUMAR, S.; MAJID, I.; AGGARWAL, P.; SURI, S. 5-Hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: recent developments. **Toxin Reviews**, vol. 40, no. 4, p. 545–561, 2021. DOI 10.1080/15569543.2020.1756857. Available at: https://doi.org/10.1080/15569543.2020.1756857.

CORTIVO, P. R. D.; HICKERT, L. R.; HECTOR, R.; AYUB, M. A. Z. Fermentation of oat and soybean hull hydrolysates into ethanol and xylitol by recombinant industrial strains of Saccharomyces cerevisiae under diverse oxygen environments. **Industrial Crops and Products**, vol. 113, no. January, p. 10–18, 2018. DOI 10.1016/j.indcrop.2018.01.010. Available at: https://doi.org/10.1016/j.indcrop.2018.01.010.

DALLI, S. S.; PATEL, M.; RAKSHIT, S. K. Development and evaluation of poplar hemicellulose prehydrolysate upstream processes for the enhanced fermentative

production of xylitol. **Biomass and Bioenergy**, vol. 105, p. 402–410, 2017. DOI 10.1016/j.biombioe.2017.08.001. Available at: http://dx.doi.org/10.1016/j.biombioe.2017.08.001.

DE MEDEIROS, L. L.; DA SILVA, F. L. H.; DE QUEIROZ, A. L. M.; DE OLIVEIRA, Y. S. L.; DE SOUZA JUNIOR, E. F.; MADRUGA, M. S.; DA CONCEIÇÃO, M. M. Structural-chemical characterization and potential of sisal bagasse for the production of polyols of industrial interest. **Brazilian Journal of Chemical Engineering**, vol. 37, no. 3, p. 451–461, 2020. DOI 10.1007/s43153-020-00049-3. Available at: https://doi.org/10.1007/s43153-020-00049-3.

DÍAZ-FERNÁNDEZ, D.; LOZANO-MARTÍNEZ, P.; BUEY, R. M.; REVUELTA, J. L.; JIMÉNEZ, A. Utilization of xylose by engineered strains of Ashbya gossypii for the production of microbial oils. **Biotechnology for Biofuels**, vol. 10, no. 1, p. 1–12, 2017. https://doi.org/10.1186/s13068-016-0685-9.

DOS SANTOS, D. T.; SOTELO, A. F.; SARROUH, B. F.; SALAUE, L. M.; PALÁCIOS, N. R.; SILVA, S. S. Tecnologia supercrítica como uma alternativa para purificar xilitol biotecnológico. **Semina:Ciencias Agrarias**, vol. 32, no. 2, p. 621–632, 2011. https://doi.org/10.5433/1679-0359.2011v32n2p621.

DU, C.; LI, Y.; ZONG, H.; YUAN, T.; YUAN, W.; JIANG, Y. Production of bioethanol and xylitol from non-detoxified corn cob via a two-stage fermentation strategy. **Bioresource Technology**, vol. 310, no. March, p. 123427, 2020. DOI 10.1016/j.biortech.2020.123427. Available at: https://doi.org/10.1016/j.biortech.2020.123427.

ERICKSON, B.; NELSON, J. E.; WINTERS, P. Perspective on opportunities in industrial biotechnology in renewable chemicals. **Biotechnology Journal**, vol. 7, no. 2, p. 176–185, 2012. https://doi.org/10.1002/biot.201100069.

FARIAS, D.; DE MÉLO, A. H. F.; DA SILVA, M. F.; BEVILAQUA, G. C.; RIBEIRO, D. G.; GOLDBECK, R.; FORTE, M. B. S.; MAUGERI-FILHO, F. New biotechnological opportunities for C5 sugars from lignocellulosic materials. **Bioresource Technology Reports**, vol. 17, p. 100956, Feb. 2022. DOI 10.1016/j.biteb.2022.100956. Available at: https://linkinghub.elsevier.com/retrieve/pii/S2589014X22000135.

FRANCOIS, J. M.; ALKIM, C.; MORIN, N. Engineering microbial pathways for production of bio-based chemicals from lignocellulosic sugars: Current status and perspectives. **Biotechnology for Biofuels**, vol. 13, no. 1, p. 1–23, 2020. DOI 10.1186/s13068-020-01744-6. Available at: https://doi.org/10.1186/s13068-020-01744-6.

GLOBAL MARKET INSIGHTS. Xylitol Market Size By Application (Chewing Gum, Confectionary, Food, Personal Care, Pharmaceuticals, Nutraceuticals), Downstream Application Potential (Xylaric Acid, Ethylene Glycol, Propylene Glycol), Industry Analysis Report, Regional Outlook, Industry. 2020. Available at: https://www.gminsights.com/industry-analysis/xylitol-market.

GRAND VIEW RESEARCH. **GVR Report cover Xylitol Market Size, Share & Trends Analysis Report By Application (Chewing Gum, Oral Care, Confectionery), By Form (Powder, Liquid), By Region (Europe, Asia Pacific, North America, MEA), And Segment Forecasts, 2020 - 2028**. [*S. l.*: *s. n.*], 2022a.

GRAND VIEW RESEARCH. **Sugar Alcohol Market Size, Share & Trends Analysis Report By Contain (Sorbitol, Mannitol, Xylitol, Maltitol), By Form (Powder & Crystal, Liquid & Syrup), By Industry Vertical, And Segment Forecasts, 2022 - 2028**. [*S. l.*: *s. n.*], 2022b. Available at: https://www.grandviewresearch.com/industry-analysis/sugar-alcohol-market-report/toc#.

GUO, Q.; ZABED, H.; ZHANG, H.; WANG, X.; YUN, J.; ZHANG, G.; YANG, M.; SUN, W.; QI, X. Optimization of fermentation medium for a newly isolated yeast strain (Zygosaccharomyces rouxii JM-C46) and evaluation of factors affecting biosynthesis of D-arabitol. **Lwt**, vol. 99, no. September 2018, p. 319–327, 2019. DOI 10.1016/j.lwt.2018.09.086. Available at: https://doi.org/10.1016/j.lwt.2018.09.086.

HERNÁNDEZ-PÉREZ, A. F.; DE ARRUDA, P. V.; FELIPE, M. das G. de A. Sugarcane straw as a feedstock for xylitol production by Candida guilliermondii FTI 20037. **Brazilian Journal of Microbiology**, vol. 47, no. 2, p. 489–496, 2016. https://doi.org/10.1016/j.bjm.2016.01.019.

HERNÁNDEZ-PÉREZ, A. F.; DE ARRUDA, P. V.; SENE, L.; DA SILVA, S. S.; KUMAR CHANDEL, A.; DE ALMEIDA FELIPE, M. das G. Xylitol bioproduction: state-

of-the-art, industrial paradigm shift, and opportunities for integrated biorefineries. **Critical Reviews in Biotechnology**, vol. 39, no. 7, p. 924–943, 2019. https://doi.org/10.1080/07388551.2019.1640658.

HULL, S. R.; PETERS, E.; COX, C.; MONTGOMERY, R. Composition of Corn Steep Water during Experimental Steeping. **Journal of Agricultural and Food Chemistry**, vol. 44, no. 11, p. 3521–3527, 1996. https://doi.org/10.1021/jf960320v.

JAGTAP, S. S.; RAO, C. V. Microbial conversion of xylose into useful bioproducts. **Applied Microbiology and Biotechnology**, vol. 102, no. 21, p. 9015–9036, 2018. https://doi.org/10.1007/s00253-018-9294-9.

KALU, D. N.; MASORO, E. J. Metabolic and nutritional aspects of aging. **Gerodontics**, vol. 2, no. 4, p. 121–126, 1986. .

KARP, S. G.; BURGOS, W. J. M.; VANDENBERGHE, L. P. S.; DIESTRA, K. V.; TORRES, L. A. Z.; WOICIECHOWSKI, A. L.; LETTI, L. A. J.; PEREIRA, G. V. M.; THOMAZ-SOCCOL, V.; RODRIGUES, C.; DE CARVALHO, J. C.; SOCCOL, C. R. Sugarcane: A Promising Source of Green Carbon in the Circular Bioeconomy. **Sugar Tech**, vol. 24, no. 4, p. 1230–1245, 2022. https://doi.org/10.1007/s12355-022-01161-z.

KORDOWSKA-WIATER, M. Production of arabitol by yeasts: Current status and future prospects. **Journal of Applied Microbiology**, vol. 119, no. 2, p. 303–314, 2015. https://doi.org/10.1111/jam.12807.

KORDOWSKA-WIATER, M.; KUBIK-KOMAR, A.; TARGOŃSKI, Z. Application of response surface methodology for the optimization of arabinose biotransformation to arabitol by Candida parapsilosis. **Central European Journal of Biology**, vol. 8, no. 9, p. 835–842, 2013. https://doi.org/10.2478/s11535-013-0196-z.

KORDOWSKA-WIATER, M.; KUBIK-KOMAR, A.; TARGOŃSKI, Z. Optimization of arabitol production by karyoductant SP-K 7 of S. cerevisiae V30 and P. stipitis CCY 39501 using response surface methodology. **Polish Journal of Microbiology**, vol. 61, no. 4, p. 291–297, 2012. https://doi.org/10.33073/pjm-2012-039.

KORDOWSKA-WIATER, M.; KUZDRALIŃSKI, A.; CZERNECKI, T.; TARGOŃSKI, Z.;

FRAC, M.; OSZUST, K. The production of arabitol by a novel plant yeast isolate Candida parapsilosis 27RL-4. **Open Life Sciences**, vol. 12, no. 1, p. 326–336, 2017. https://doi.org/10.1515/biol-2017-0038.

KUMDAM, H.; MURTHY, S. N.; GUMMADI, S. N. Production of ethanol and arabitol by Debaryomyces nepalensis: Influence of process parameters. **AMB Express**, vol. 3, p. 1–12, 2013. https://doi.org/10.1186/2191-0855-3-23.

LEE, J. K.; KOO, B. S.; KIM, S. Y. Cloning and Characterization of the xyl1 Gene, Encoding an NADH-Preferring Xylose Reductase from Candida parapsilosis, and Its Functional Expression in Candida tropicalis. **Applied and Environmental Microbiology**, vol. 69, no. 10, p. 6179–6188, 2003. https://doi.org/10.1128/AEM.69.10.6179-6188.2003.

LOMAN, A. A.; ISLAM, S. M. M.; JU, L. K. Production of arabitol from enzymatic hydrolysate of soybean flour by Debaryomyces hansenii fermentation. **Applied Microbiology and Biotechnology**, vol. 102, no. 2, p. 641–653, 2018. https://doi.org/10.1007/s00253-017-8626-5.

LOMAN, A. Al; JU, L. K. Purification of arabitol from fermentation broth of Debaryomyces hansenii using glycerol as substrate. **Journal of Chemical Technology and Biotechnology**, vol. 88, no. 8, p. 1514–1522, 2013. https://doi.org/10.1002/jctb.3995.

LÓPEZ-LINARES, J. C.; RUIZ, E.; ROMERO, I.; CASTRO, E.; MANZANARES, P. Xylitol production from exhausted olive pomace by candida boidinii. **Applied Sciences (Switzerland)**, vol. 10, no. 19, p. 1–16, 2020. https://doi.org/10.3390/app10196966.

LÓPEZ-PRIETO, A.; VECINO, X.; RODRÍGUEZ-LÓPEZ, L.; MOLDES, A. B.; CRUZ, J. M. A multifunctional biosurfactant extract obtained from corn steep water as bactericide for agrifood industry. **Foods**, vol. 8, no. 9, 2019. https://doi.org/10.3390/foods8090410.

LU, J.; TSAI, L. B.; GONG, C. S.; TSAO, G. T. Effect of nitrogen sources on xylitol production from D-xylose by Candida Sp. L-102. **Biotechnology Letters**, vol. 17, no. 2, p. 167–170, 1995. https://doi.org/10.1007/BF00127982.

MARTÍNEZ-ARCOS, A.; MOLDES, A. B.; VECINO, X. Adding value to secondary streams of corn wet milling industry. **CYTA - Journal of Food**, vol. 19, no. 1, p. 675–681, 2021. DOI 10.1080/19476337.2021.1965661. Available at: https://doi.org/10.1080/19476337.2021.1965661.

MCMILLAN, J. D.; BOYNTON, B. L. Arabinose utilization by xylose-fermenting yeasts and fungi. **Applied Biochemistry and Biotechnology**, vol. 45–46, no. 1, p. 569–584, 1994. https://doi.org/10.1007/BF02941831.

MORAIS JUNIOR, W. G.; PACHECO, T. F.; TRICHEZ, D.; ALMEIDA, J. R. M.; GONÇALVES, S. B. Xylitol production on sugarcane biomass hydrolysate by newly identified Candida tropicalis JA2 strain. **Yeast**, vol. 36, no. 5, p. 349–361, 2019. https://doi.org/10.1002/yea.3394.

MUSSATTO, S. I.; DRAGONE, G. M. **Biomass Pretreatment, Biorefineries, and Potential Products for a Bioeconomy Development**. [*S. l.*]: Elsevier Inc., 2016. DOI 10.1016/B978-0-12-802323-5.00001-3. Available at: http://dx.doi.org/10.1016/B978-0-12-802323-5.00001-3.

MUSSATTO, S. I.; ROBERTO, I. C. Optimal Experimental Condition for Hemicellulosic Hydrolyzate Treatment with Activated Charcoal for Xylitol Production. **Biotechnology Progress**, vol. 20, no. 1, p. 134–139, 2004. https://doi.org/10.1021/bp034207i.

OU, C. M.; MA, T. Y.; TU, W. L.; CHAO, Y.; GUO, G. L. Xylitol production from non-detoxified Napiergrass hydrolysate using a recombinant flocculating yeast strain. **BioResources**, vol. 15, no. 4, p. 9575–9583, 2020. .

PALLADINO, F.; RODRIGUES, R. C. L. B.; CADETE, R. M.; BARROS, K. O.; ROSA, C. A. Novel potential yeast strains for the biotechnological production of xylitol from sugarcane bagasse. **Biofuels, Bioproducts and Biorefining**, vol. 15, no. 3, p. 690–702, 2021. https://doi.org/10.1002/bbb.2196.

RACHWAŁ, K.; WAŚKO, A.; GUSTAW, K.; POLAK-BERECKA, M. Utilization of brewery wastes in food industry. **PeerJ**, vol. 8, p. 1–28, 2020. https://doi.org/10.7717/peerj.9427.

RAVELLA, R. S.; J., G.; S., F.; R.S., P. Overview on Commercial Production of Xylitol, Economic Analysis and Market Trends. *In*: S., da S.; A., C. (eds.). **D-Xylitol**. [*S. l.*]: Springer Berlin Heidelberg, 2012. p. 344. https://doi.org/https://doi.org/10.1007/978-3-642-31887-0_13.

REENA, R.; ALPHY, M. P.; RESHMY, R.; THOMAS, D.; MADHAVAN, A.; CHATURVEDI, P.; PUGAZHENDHI, A.; AWASTHI, M. K.; RUIZ, H.; KUMAR, V.; SINDHU, R.; BINOD, P. Sustainable valorization of sugarcane residues: Efficient deconstruction strategies for fuels and chemicals production. **Bioresource Technology**, vol. 361, no. August, p. 127759, 2022. DOI 10.1016/j.biortech.2022.127759. Available at: https://doi.org/10.1016/j.biortech.2022.127759.

SADH, P. K.; DUHAN, S.; DUHAN, J. S. Agro-industrial wastes and their utilization using solid state fermentation: a review. **Bioresources and Bioprocessing**, vol. 5, no. 1, p. 1–15, 2018. DOI 10.1186/s40643-017-0187-z. Available at: https://doi.org/10.1186/s40643-017-0187-z.

SAHA, B. C.; KENNEDY, G. J. Production of xylitol from mixed sugars of xylose and arabinose without co-producing arabitol. **Biocatalysis and Agricultural Biotechnology**, vol. 29, no. September, p. 101786, 2020. DOI 10.1016/j.bcab.2020.101786. Available at: https://doi.org/10.1016/j.bcab.2020.101786.

SALGADO, J. M.; RODRÍGUEZ, N.; CORTÉS, S.; DOMÍNGUEZ, J. M. Development of cost-effective media to increase the economic potential for larger-scale bioproduction of natural food additives by lactobacillus rhamnosus, debaryomyces Hansenll, and aspergillus niger. Journal of Agricultural and Food Chemistry, vol. 57, no. 21, p. 10414–10428, 2009. https://doi.org/10.1021/jf901911c.

SANCHEZ, R. G.; KARHURMAA, K.; FONSECA, C.; NOGUÉ, V. S.; ALMEIDA, J. R.; LARSSON, C. U.; BENGTSSON, O.; BETTIGA, M.; HAHN-HÄGERDAL, B.; GORWA-GRAUSLUND, M. F. Improved xylose and arabinose utilization by an industrial recombinant. **Biotechnology for Biofuels**, vol. 3, no. 13, 2010. Available at: http://www.biotechnologyforbiofuels.com/content/3/1/13.

SARAVANAN, P.; RAMESH, S.; JAYA, N.; JABASINGH, S. A. Prospective

evaluation of xylitol production using Dabaryomyces hansenii var hansenii, Pachysolen tannophilus, and Candida guillermondii with sustainable agricultural residues. **Biomass Conversion and Biorefinery**, 2021. https://doi.org/10.1007/s13399-020-01221-y.

SASAKI, M.; JOJIMA, T.; INUI, M.; YUKAWA, H. Xylitol production by recombinant Corynebacterium glutamicum under oxygen deprivation. **Applied Microbiology and Biotechnology**, vol. 86, no. 4, p. 1057–1066, 2010. https://doi.org/10.1007/s00253-009-2372-2.

SELIM, M. T.; SALEM, S. S.; FOUDA, A.; EL-GAMAL, M. S.; ABDEL-RAHMAN, M. A. Use of corn-steep water effluent as a promising substrate for lactic acid production by enterococcus faecium strain wh51-1. **Fermentation**, vol. 7, no. 3, p. 1–18, 2021. https://doi.org/10.3390/fermentation7030111.

SCHÖRKEN, U.; KEMPERS, P. Lipid biotechnology: Industrially relevant production processes. **European Journal of Lipid Science and Technology**, vol. 111, no. 7, p. 627–645, 2009. https://doi.org/10.1002/ejlt.200900057.

SEIBOTH, B.; METZ, B. Fungal arabinan and L-arabinose metabolism. **Applied Microbiology and Biotechnology**, vol. 89, no. 6, p. 1665–1673, 2011. https://doi.org/10.1007/s00253-010-3071-8.

SHABBIRAHMED, A. M.; HALDAR, D.; DEY, P.; PATEL, A. K.; SINGHANIA, R. R.; DONG, C. Di; PURKAIT, M. K. Sugarcane bagasse into value-added products: a review. **Environmental Science and Pollution Research**, , p. 62785–62806, 2022. DOI 10.1007/s11356-022-21889-1. Available at: https://doi.org/10.1007/s11356-022-21889-1.

SHRESTHA, S.; KOGNOU, A. L. M.; ZHANG, J.; QIN, W. Different Facets of Lignocellulosic Biomass Including Pectin and Its Perspectives. **Waste and Biomass Valorization**, vol. 12, no. 9, p. 4805–4823, 2021. DOI 10.1007/s12649-020-01305-w. Available at: https://doi.org/10.1007/s12649-020-01305-w.

SU, B.; WU, M.; ZHANG, Z.; LIN, J.; YANG, L. Efficient production of xylitol from hemicellulosic hydrolysate using engineered Escherichia coli. **Metabolic Engineering**, vol. 31, p. 112–122, 2015. DOI 10.1016/j.ymben.2015.07.003.

Available at: http://dx.doi.org/10.1016/j.ymben.2015.07.003.

SUNDARAMOORTHY, B. A.; GUMMADI, S. N. Screening of new yeast Pichia manchurica for arabitol production. **Journal of Basic Microbiology**, vol. 59, no. 3, p. 256–266, 2019. https://doi.org/10.1002/jobm.201800366.

TRAVÁLIA, B. M. **Destoxificação de hidrolisado hemicelulósico de bagaço de cana-de-açúcar por adsorção em hidróxidos duplos lamelares em reatores de mistura e tubular**. 2020. Universidade Estadual de Campinas, 2020.

WAGLE, A.; ANGOVE, M. J.; MAHARA, A.; WAGLE, A.; MAINALI, B.; MARTINS, M.; GOLDBECK, R.; RAJ PAUDEL, S. Multi-stage pre-treatment of lignocellulosic biomass for multi-product biorefinery: A review. **Sustainable Energy Technologies and Assessments**, vol. 49, no. September 2021, p. 101702, 2022. DOI 10.1016/j.seta.2021.101702. Available at: https://doi.org/10.1016/j.seta.2021.101702.

WU, J.; HU, J.; ZHAO, S.; HE, M.; HU, G.; GE, X.; PENG, N. Single-cell Protein and Xylitol Production by a Novel Yeast Strain Candida intermedia FL023 from Lignocellulosic Hydrolysates and Xylose. **Applied Biochemistry and Biotechnology**, vol. 185, no. 1, p. 163–178, 2018. https://doi.org/10.1007/s12010-017-2644-8.

XIE, Y.; GUO, X.; MA, Z.; GONG, J.; WANG, H.; LV, Y. Efficient extraction and structural characterization of hemicellulose from sugarcane bagasse pith. **Polymers**, vol. 12, no. 3, p. 1–13, 2020. https://doi.org/10.3390/polym12030608.

XU, Y.; CHI, P.; BILAL, M.; CHENG, H. Biosynthetic strategies to produce xylitol: an economical venture. **Applied Microbiology and Biotechnology**, vol. 103, no. 13, p. 5143–5160, 2019. https://doi.org/10.1007/s00253-019-09881-1.

YE, L.; HAN, Y.; WANG, X.; LU, X.; QI, X.; YU, H. Recent progress in furfural production from hemicellulose and its derivatives: Conversion mechanism, catalytic system, solvent selection. **Molecular Catalysis**, vol. 515, no. September, p. 111899, 2021. DOI 10.1016/j.mcat.2021.111899. Available at: https://doi.org/10.1016/j.mcat.2021.111899.

YEWALE, T.; PANCHWAGH, S.; RAJAGOPALAN, S.; DHAMOLE, P. B.; JAIN, R.

Enhanced xylitol production using immobilized Candida tropicalis with non-detoxified corn cob hemicellulosic hydrolysate. **3 Biotech**, vol. 6, no. 1, p. 1–10, 2016. https://doi.org/10.1007/s13205-016-0388-8.

ZAHED, O.; JOUZANI, G. S.; ABBASALIZADEH, S.; KHODAIYAN, F.; TABATABAEI, M. Continuous co-production of ethanol and xylitol from rice straw hydrolysate in a membrane bioreactor. **Folia Microbiologica**, vol. 61, no. 3, p. 179–189, 2016. DOI 10.1007/s12223-015-0420-0. Available at: http://dx.doi.org/10.1007/s12223-015-0420-0.

ZHANG, J.; LI, S.; XU, H.; ZHOU, P.; ZHANG, L.; OUYANG, P. Purification of xylitol dehydrogenase and improved production of xylitol by increasing XDH activity and NADH supply in gluconobacter oxydans. **Journal of Agricultural and Food Chemistry**, vol. 61, no. 11, p. 2861–2867, 2013. https://doi.org/10.1021/jf304983d.

ZHENG, S.; JIANG, B.; ZHANG, T.; CHEN, J. Combined mutagenesis and metabolic regulation to enhance d-arabitol production from candida parapsilosis. **Journal of Industrial Microbiology and Biotechnology**, vol. 47, no. 4–5, p. 425–435, 2020. doi 10.1007/s10295-020-02278-

ZHOU, K.; YU, J.; MA, Y.; CAI, L.; ZHENG, L.; GONG, W.; LIU, Q. ai. Corn Steep Liquor: Green Biological Resources for Bioindustry. **Applied Biochemistry and Biotechnology**, vol. 194, no. 7, p. 3280–3295, 2022. DOI 10.1007/s12010-022-03904-w. Available at: https://doi.org/10.1007/s12010-022-03904-w.

ANEXO I – REALIZAÇÕES ACADÊMICAS NO PERÍODO

a. Participação do “Programa de Estágio Docente” das disciplinas “TA332 – Fundamentos de Cálculo em Processos” em 2022.1 e “TA736 – Engenharia de Bioprocessos” em 2023.1, disciplinas do curso de graduação em Engenharia de Alimentos da Universidade Estadual de Campinas

b. Coautoria em artigo de revisão bibliográfica “New biotechnological opportunities for C5 sugars from lignocellulosic materials” publicado na revista científica “Bioresource Technology Reports” classificada como B1 em “Ciência de Alimentos” no quadriênio 2017-2020 pela Qualis Periódicos CAPES.

Doi: https://doi.org/10.1016/j.biteb.2022.100956

Bioresource Technology Reports 17 (2022) 100956

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

New biotechnological opportunities for C5 sugars from lignocellulosic materials

Daniele Farias [a,b,*], Allan H.F. de Mélo [a], Marcos Fellipe da Silva [a], Gabriel Cicalese Bevilaqua [a], Danielle Garcia Ribeiro [a], Rosana Goldbeck [a], Marcus Bruno Soares Forte [a], Francisco Maugeri-Filho [a,*]

[a] Bioprocess and Metabolic Engineering Laboratory, Department of Food Engineering, School of Food Engineering, University of Campinas, Campinas, SP, Brazil
[b] Toulouse Biotechnology Institute (TBI), University of Toulouse, 135 Rangueil Avenue, Toulouse, France

ARTICLE INFO

Keywords:
Pentose
Hemicellulose
Xylitol
Arabitol
Xylo-oligosaccharides
Protein
Bioprocesses

ABSTRACT

The transition to sustainable development with the replacement of gasoline and petroleum-based products with biofuels and green chemicals is a common goal for current concepts of the bioeconomy. In this sense, biorefinery integration appears as a promising route to replace chemical technologies. Moreover, there is the possibility of using existing first-generation (1G) bioethanol plants facilities as the host for the development of new processes using the huge amount of lignocellulosic biomasses available in the world. In order to maximize profitability, all sugars released after biomass pre-treatment and hydrolyses must be converted into target products. Regarding the pentose fraction, sugar conversion by microorganisms is not optimally performed, compared to glucose, to give rise to the production of bioproducts in a sustainable and competitive manner. Thus, this review focuses on the recent progresses and emerging strategies aiming towards pentose utilization, efficient assimilation and conversion into industrially relevant bioproducts.

c. Apresentação do trabalho “Xylitol and arabitol: biotechnological trends for the reuse of lignocellulosic biomass” na modalidade pôster no evento XXIII SINAFERM/XIV SHEB/XIV ENZITEC realizado de 28 a 31 de agosto de 2022

d. Apresentação do trabalho “Identification of the biotechnological production potential of sugar alcohols by *Candida tropicalis*” na modalidade pôster no evento 2nd International Congress on Bioactive Compounds realizado de 9 a 10 de novembro de 2022.

ANEXO II – CADASTRO NA PLATAFORMA SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A49D57A

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A49D57A**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Candida tropicalis

Título da Atividade:	**Produção e purificação de produtos biotecnológicos**

Equipe

Marcus Bruno Soares Forte	**UNICAMP**
Priscila Hoffmann Carvalho	**UNICAMP**
Beatriz Medeiros Travália	**UNICAMP**
Marcelo D'Elia Feliciano	**UNICAMP**
Felipe Augusto Ferrari	**UNICAMP**
Yara Pereira Cerceau Alves	**UNICAMP**
Igor Severo Gonçalves	**UNICAMP**
Davi de Oliveira Emirandetti	**UNICAMP**
Paulo Henrique Vieira Lima	**UNICAMP**

Bernardo Soares Cardoso	UNICAMP
Sílvio Silvério da Silva	USP
Rosana Goldbeck	UNICAMP
Francisco Maugeri Filho	UNICAMP
Maria Isabel Rodrigues	UNICAMP
Fátima Aparecida de Almeida Costa	UNICAMP
Felipe Antonio F. Antunes	USP
Ederson Paulo Xavier Guilherme	UNICAMP
Danielle Garcia Ribeiro	UNICAMP
Francisco Lucas Chaves Almeida	UNICAMP
Gabriel Cicalese Bevilaqua	UNICAMP
Giovanna Totti Bullo	UNICAMP

Parceiras Nacionais

46.068.425/0001-33 / Universidade Estadual de Campinas

63.025.530/0001-04 / Universidade de São Paulo

Data do Cadastro: **26/10/2021 12:04:36**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético

Situação cadastral conforme consulta ao SisGen em **12:05** de **26/10/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

ANEXO III – LICENÇAS DE AUTORIZAÇÃO DE USO DE IMAGENS

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jan 31, 2023

This Agreement between Universidade Estadual de Campinas -- Gabriel Bevilaqua ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5479430680672
License date	Jan 31, 2023
Licensed Content Publisher	Elsevier
Licensed Content Publication	Elsevier Books
Licensed Content Title	Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery
Licensed Content Author	S.I. Mussatto,G.M. Dragone
Licensed Content Date	Jan 1, 2016
Licensed Content Pages	22
Start Page	1
End Page	22
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	electronic
Are you the author of this Elsevier chapter?	No
Will you be translating?	Yes, without English rights
Number of languages	1
Title	POLIÓIS: UM ESTUDO DA PRODUÇÃO SIMULTÂNEA DE XILITOL E ARABITOL A PARTIR DE RESÍDUOS AGROINDUSTRIAIS

Institution name	Universidade Estadual de Campinas
Expected presentation date	Mar 2023
Portions	Figure 1.1
Specific Languages	Português
Requestor Location	Universidade Estadual de Campinas Rua Monteiro Lobato, 80 Campinas, São Paulo 13083862 Brazil Attn: Universidade Estadual de Campinas
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD

Terms and Conditions

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier's permissions helpdesk here). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this

licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. **For journal authors**: the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
  - via their non-commercial person homepage or blog
  - by updating a preprint in arXiv or RePEc with the accepted manuscript
  - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
  - directly by providing copies to their students or to research collaborators for their personal use
  - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
  - via non-commercial hosting platforms such as their institutional repository
  - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier's posting policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

19. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our open access license policy for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by-nc-nd/4.0. Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article

- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. **Other Conditions**:

v1.10

ELSEVIER LICENSE
TERMS AND CONDITIONS

Jan 31, 2023

This Agreement between Universidade Estadual de Campinas -- Gabriel Bevilaqua ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5479430934346
License date	Jan 31, 2023
Licensed Content Publisher	Elsevier
Licensed Content Publication	Sustainable Energy Technologies and Assessments
Licensed Content Title	Multi-stage pre-treatment of lignocellulosic biomass for multi-product biorefinery: A review
Licensed Content Author	Aditi Wagle,Michael J. Angove,Asmita Mahara,Amrita Wagle,Bandita Mainali,Manoela Martins,Rosana Goldbeck,Shukra Raj Paudel
Licensed Content Date	Feb 1, 2022
Licensed Content Volume	49
Licensed Content Issue	n/a
Licensed Content Pages	1
Start Page	101702
End Page	0
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	Yes, without English rights

Number of languages	1
Title	POLIÓIS: UM ESTUDO DA PRODUÇÃO SIMULTÂNEA DE XILITOL E ARABITOL A PARTIR DE RESÍDUOS AGROINDUSTRIAIS
Institution name	Universidade Estadual de Campinas
Expected presentation date	Mar 2023
Portions	Figure 3
Specific Languages	Português
Requestor Location	Universidade Estadual de Campinas Rua Monteiro Lobato, 80 Campinas, São Paulo 13083862 Brazil Attn: Universidade Estadual de Campinas
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD

Terms and Conditions

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier

Ltd. (Please contact Elsevier's permissions helpdesk here). No modifications can be made to any Lancet figures/tables and they must be reproduced in full.

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article.

16. **Posting licensed content on any Website**: The following terms and conditions apply as follows: Licensing material from an Elsevier journal: All content posted to the web site must maintain the copyright information line on the bottom of each image; A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com; Central Storage: This license does not include permission for a

scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

Licensing material from an Elsevier book: A hyper-text link must be included to the Elsevier homepage at http://www.elsevier.com . All content posted to the web site must maintain the copyright information line on the bottom of each image.

Posting licensed content on Electronic reserve: In addition to the above the following clauses are applicable: The web site must be password-protected and made available only to bona fide students registered on a relevant course. This permission is granted for 1 year only. You may obtain a new license for future website posting.

17. **For journal authors**: the following clauses are applicable in addition to the above:

Preprints:

A preprint is an author's own write-up of research results and analysis, it has not been peer-reviewed, nor has it had any other value added to it by a publisher (such as formatting, copyright, technical enhancement etc.).

Authors can share their preprints anywhere at any time. Preprints should not be added to or enhanced in any way in order to appear more like, or to substitute for, the final versions of articles however authors can update their preprints on arXiv or RePEc with their Accepted Author Manuscript (see below).

If accepted for publication, we encourage authors to link from the preprint to their formal publication via its DOI. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help users to find, access, cite and use the best available version. Please note that Cell Press, The Lancet and some society-owned have different preprint policies. Information on these policies is available on the journal homepage.

Accepted Author Manuscripts: An accepted author manuscript is the manuscript of an article that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and editor-author communications.

Authors can share their accepted author manuscript:

- immediately
  - via their non-commercial person homepage or blog
  - by updating a preprint in arXiv or RePEc with the accepted manuscript
  - via their research institute or institutional repository for internal institutional uses or as part of an invitation-only research collaboration work-group
  - directly by providing copies to their students or to research collaborators for their personal use
  - for private scholarly sharing as part of an invitation-only work group on commercial sites with which Elsevier has an agreement
- After the embargo period
  - via non-commercial hosting platforms such as their institutional repository
  - via commercial sites with which Elsevier has an agreement

In all cases accepted manuscripts should:

- link to the formal publication via its DOI
- bear a CC-BY-NC-ND license - this is easy to do
- if aggregated with other manuscripts, for example in a repository or other site, be shared in alignment with our hosting policy not be added to or enhanced in any way to appear more like, or to substitute for, the published journal article.

Published journal article (JPA): A published journal article (PJA) is the definitive final record of published research that appears or will appear in the journal and embodies all value-adding publishing activities including peer review co-ordination, copy-editing, formatting, (if relevant) pagination and online enrichment.

Policies for sharing publishing journal articles differ for subscription and gold open access articles:

Subscription Articles: If you are an author, please share a link to your article rather than the full-text. Millions of researchers have access to the formal publications on ScienceDirect, and so links will help your users to find, access, cite, and use the best available version.

Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal

publications on ScienceDirect.

If you are affiliated with a library that subscribes to ScienceDirect you have additional private sharing rights for others' research accessed under that agreement. This includes use for classroom teaching and internal training at the institution (including use in course packs and courseware programs), and inclusion of the article for grant funding purposes.

Gold Open Access Articles: May be shared according to the author-selected end-user license and should contain a CrossMark logo, the end user license, and a DOI link to the formal publication on ScienceDirect.

Please refer to Elsevier's posting policy for further information.

18. **For book authors** the following clauses are applicable in addition to the above: Authors are permitted to place a brief summary of their work online only. You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version. **Posting to a repository:** Authors are permitted to post a summary of their chapter only in their institution's repository.

19. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for Proquest/UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission. Theses and dissertations which contain embedded PJAs as part of the formal submission can be posted publicly by the awarding institution with DOI links back to the formal publications on ScienceDirect.

Elsevier Open Access Terms and Conditions

You can publish open access with Elsevier in hundreds of open access journals or in nearly 2000 established subscription journals that support open access publishing. Permitted third party re-use of these open access articles is defined by the author's choice of Creative Commons user license. See our open access license policy for more information.

Terms & Conditions applicable to all Open Access articles published with Elsevier:

Any reuse of the article must not represent the author as endorsing the adaptation of the article nor should the article be modified in such a way as to damage the author's honour or reputation. If any changes have been made, such changes must be clearly indicated.

The author(s) must be appropriately credited and we ask that you include the end user license and a DOI link to the formal publication on ScienceDirect.

If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source it is the responsibility of the user to ensure their reuse complies with the terms and conditions determined by the rights holder.

Additional Terms & Conditions applicable to each Creative Commons user license:

CC BY: The CC-BY license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article and to make commercial use of the Article (including reuse and/or resale of the Article by commercial entities), provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. The full details of the license are available at http://creativecommons.org/licenses/by/4.0.

CC BY NC SA: The CC BY-NC-SA license allows users to copy, to create extracts, abstracts and new works from the Article, to alter and revise the Article, provided this is not done for commercial purposes, and that the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, indicates if changes were made and the licensor is not represented as endorsing the use made of the work. Further, any new works must be made available on the same conditions. The full details of the license are available at http://creativecommons.org/licenses/by-nc-sa/4.0.

CC BY NC ND: The CC BY-NC-ND license allows users to copy and distribute the Article, provided this is not done for commercial purposes and further does not permit distribution of the Article if it is changed or edited in any way, and provided the user gives appropriate credit (with a link to the formal publication through the relevant DOI), provides a link to the license, and that the licensor is not represented as endorsing the use made of the work. The

full details of the license are available at http://creativecommons.org/licenses/by-nc-nd/4.0. Any commercial reuse of Open Access articles published with a CC BY NC SA or CC BY NC ND license requires permission from Elsevier and will be subject to a fee.

Commercial reuse includes:

- Associating advertising with the full text of the Article
- Charging fees for document delivery or access
- Article aggregation
- Systematic distribution via e-mail lists or share buttons

Posting or linking by commercial companies for use by customers of those companies.

20. **Other Conditions**:

v1.10

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gmail

Gabriel Cicalese <gabriel.cicalese@gmail.com>

ibty20:Xylitol bioproduction: state-of-the-art, industrial paradigm shift, and opportunities for integrated biorefineries

1 message

Taylor & Francis Journal Permissions Support <taylorandfrancissupport@tandf.co.uk> Tue, Jan 31, 2023 at 2:42 PM
Reply-To: journalpermissions@tandf.co.uk
To: "gabriel.cicalese@gmail.com" <gabriel.cicalese@gmail.com>

Taylor & Francis Group
an informa business

Our Ref: ibty/02921493

1/31/2023

Dear Requester,

Thank you for your correspondence requesting permission to reproduce content from a Taylor & Francis Group journal content in your thesis to be posted on your university's repository.

We will be pleased to grant free permission on the condition that your acknowledgement must be included showing article title, author, full Journal title, and **© copyright # [year]**, reprinted by permission of Informa UK Limited, trading as Taylor & Taylor & Francis Group, http://www.tandfonline.com

This permission does not cover any third party copyrighted work which may appear in the article by permission. Please ensure you have checked all original source details for the rights holder and if need apply for permission from the original rightsholder.

Please note that this license **does not allow you to post our content on any other third-party websites.**

Please note permission does not provide access to our article, if you are affiliated to an institution and your institution holds a subscription to the content you are requesting you will be able to view the article free of charge, if your institution does not hold a subscription or you are not affiliated to an institution that has a subscription then you will need to purchase this for your own personal use as we do not provide our articles free of charge for research.

Thank you for your interest in our Journal.

With best wishes,

Taylor & Francis Journal Permissions
Web: www.tandfonline.com
4 Park Square, Milton Park, Abingdon, OX14 4RN
(+44 (0)20 8052 0600

Disclaimer: T&F publish Open Access articles in our subscription priced journals, please check if the article you are interested in is an OA article and if so, which license was it published under.
Before printing, think about the environment.

ref:_00D0Y35IJI._5007THoH6G:ref

Permissions Request

Type of use: Academic
Article title: Xylitol bioproduction: state-of-the-art, industrial paradigm shift, and opportunities for integrated biorefineries
Article DOI: 10.1080/07388551.2019.1640658
Author name: Andrés Felipe Hernández-Pérez, Priscila Vaz de Arruda, Luciane Sene, Silvio Silvério da Silva, Anuj Kumar Chandel & Maria das Graças de Almeida Felipe
Journal title: Critical Reviews in Biotechnology
Volume number: 39
Issue number: 7
Year of publication: 2019
Name: Gabriel C Bevilaqua
Street address: Rua Monteiro Lobato, 80
Town: Campinas
Postcode/ZIP code: 13083860
Country: Brazil
Email: gabriel.cicalese@gmail.com
Telephone: 81996775842
Intended use: To be used in your Thesis?
Are you requesting the full article?: No
Extract and number of words: 0
Details of figure/table: Figure 1; Figure 2
Which University?: Campinas State University
Title of your Thesis?:
University repository URL:
Is this a "Closed" or "Open" deposit?: open
Additional comments:

UNICAMP

Gabriel Cicalese Bevilaqua <g215654@dac.unicamp.br>

RE: Gabriel, Xylitol Market

1 mensagem

Radhika from Grand View Research INC <radhika@grandviewresearch.com> 13 de fevereiro de 2023 às 10:10
Para: g215654@dac.unicamp.br

Dear Gabriel,

Greeting for the day!

As requested, please find attached the sample report on **Xylitol Market.**

The sample might not represent the full scope of the study due to masking and data trimming. Moreover, since each segment is individually assessed and then collated to form the whole market, the study can be tailor-made to fit your exact requirements.

With reference to your interest in the market data for **"Xylitol Market Analysis"**, please note that you can certainly use the data available on the website or from the attached sample report, with proper citation of the source as **'Grand View Research'.**

If you have any further queries or requirements, please get back to me & I would be glad to assist.

Have a great day ahead.

Thanks & Regards,

Radhika Bhonsle | Executive – Academics

GRAND VIEW RESEARCH

ESOMAR member

Office +1 415 349 0058

E-Mail radhika@grandviewresearch.com

www.grandviewresearch.com

---------- Forwarded message ----------
From: **Gabriel Cicalese Bevilaqua** <g215654@dac.unicamp.br>
Date: Fri, Feb 10, 2023 at 6:50 PM
Subject: Request for image use
To: <sales@grandviewresearch.com>

ANEXO IV – LICENÇA DE AUTORIZAÇÃO DE USO DE ARTIGO PUBLICADO

Simultaneous production of xylitol and arabitol by Candida tropicalis fermentation improving agro-industrial wastes valorization

Author: Gabriel Cicalese Bevilaqua,Francisco Maugeri Filho,Marcus Bruno Soares Forte

Publication: Food and Bioproducts Processing
Publisher: Elsevier
Date: Available online 25 April 2023

Author rights

The below table explains the rights that authors have when they publish with Elsevier, for authors who choose to publish either open access or subscription. These apply to the corresponding author and all co-authors.

Author rights in Elsevier's proprietary journals	Published open access	Published subscription
Retain patent and trademark rights	√	√
Retain the rights to use their research data freely without any restriction	√	√
Receive proper attribution and credit for their published work	√	√
Re-use their own material in new works without permission or payment (with full acknowledgement of the original article): 1. Extend an article to book length 2. Include an article in a subsequent compilation of their own work 3. Re-use portions, excerpts, and their own figures or tables in other works.	√	√
Use and share their works for scholarly purposes (with full acknowledgement of the original article): 1. In their own classroom teaching. Electronic and physical distribution of copies is permitted 2. If an author is speaking at a conference, they can present the article and distribute copies to the attendees 3. Distribute the article, including by email, to their students and to research colleagues who they know for their personal use 4. Share and publicize the article via Share Links, which offers 50 days' free access for anyone, without signup or registration 5. Include in a thesis or dissertation (provided this is not published commercially) 6. Share copies of their article privately as part of an invitation-only work group on commercial sites with which the publisher has a hosting agreement	√	√
Use and share their works for scholarly purposes (with full acknowledgement of the original article): 1. In their own classroom teaching. Electronic and physical distribution of copies is permitted 2. If an author is speaking at a conference, they can present the article and distribute copies to the attendees 3. Distribute the article, including by email, to their students and to research colleagues who they know for their personal use 4. Share and publicize the article via Share Links, which offers 50 days' free access for anyone, without signup or registration 5. Include in a thesis or dissertation (provided this is not published commercially) 6. Share copies of their article privately as part of an invitation-only work group on commercial sites with which the publisher has a hosting agreement	√	√
Publicly share the preprint on any website or repository at any time.	√	√
Publicly share the accepted manuscript on non-commercial sites	√	√ using a CC BY-NC-ND license and usually only after an embargo period (see Sharing Policy for more information)
Publicly share the final published article	√ in line with the author's choice of end user license	×
Retain copyright	√	×

https://www.elsevier.com/about/policies/copyright#Author-rights

ELSEVIER

Rights and Access

Simultaneous production of xylitol and arabitol by Candida tropicalis fermentation improving agro-industrial wastes valorization

Corresponding author	Prof. Marcus Bruno Soares Forte
E-mail address	forte@unicamp.br

Journal	Food and Bioproducts Processing
Our reference	FBP1676
PII	S0960-3085(23)00047-0
DOI	10.1016/j.fbp.2023.04.006

Order Confirmation

Thank you for taking the time to complete the Rights and Access form.

Order number	OACSRFBP16760
Order date	27 April 2023

Research Funders

FAPESP
Grant numbers: 2021/05508-9

CAPES
Grant numbers: Finance code 001

CNPq
Grant numbers: 439548/2018-7

Publishing Option Subscription

Publishing Agreement

- I am one author signing on behalf of all co-authors of the manuscript

I may post the accepted manuscript in my institutional repository and make this public after an embargo period. To ensure the sustainability of peer-reviewed research in journal publications, I may not share the final article publicly, for example on ResearchGate or Academia.edu.
Further details on Elsevier Sharing Policy here.

Based on information provided the embargo period/end date is 24 months.