UNIVERSIDADE FEDERAL DE SÃO CARLOS
DEPARTAMENTO DE ENGENHARIA QUÍMICA
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA QUÍMICA

MARIA CAROLINA PEREIRA GONÇALVES

SÍNTESE DE ÉSTERES GRAXOS DE AÇÚCAR CATALISADA POR DERIVADOS IMOBILIZADOS-ESTABILIZADOS DE *CANDIDA ANTARCTICA* LIPASE B

SÃO CARLOS/SP

2023

FEDERAL UNIVERSITY OF SÃO CARLOS
DEPARTMENT OF CHEMICAL ENGINEERING
GRADUATE PROGRAM IN CHEMICAL ENGINEERING

MARIA CAROLINA PEREIRA GONÇALVES

SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY IMMOBILIZED-STABILIZED DERIVATIVES OF *CANDIDA ANTARCTICA* LIPASE B

SÃO CARLOS/SP

2023

MARIA CAROLINA PEREIRA GONÇALVES

SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY IMMOBILIZED-STABILIZED DERIVATIVES OF *CANDIDA ANTARCTICA* LIPASE B

Thesis presented to the Graduate Program in Chemical Engineering (Department of Chemical Engineering, Federal University of São Carlos) in order to obtain the title of Doctor in Chemical Engineering.

Supervisor: Prof. Dr. Paulo Waldir Tardioli

SÃO CARLOS/SP

2023

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Exatas e de Tecnologia
Programa de Pós-Graduação em Engenharia Química

Folha de Aprovação

Defesa de Tese de Doutorado da candidata Maria Carolina Pereira Gonçalves, realizada em 21/03/2023.

Comissão Julgadora:

Prof. Dr. Paulo Waldir Tardioli (UFSCar)

Profa. Dra. Gisella Maria Zanin (UEM)

Profa. Dra. Angélica Marquetotti Salcedo Vieira (UEM)

Prof. Dr. Ruy de Sousa Junior (UFSCar)

Prof. Dr. Antonio Jose Gonçalves da Cruz (UFSCar)

O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Engenharia Química.

This work was developed at the Laboratory of Enzymatic Technologies of the Federal University of São Carlos (Department of Chemical Engineering) and partly at the Bioprocess Engineering Laboratory of the University of Toronto (Biozone).

Financial support was provided by “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP) [2016/10636-8, 2019/23908-4, 2021/06525-4]; in part by Natural Sciences and Engineering Research Council of Canada (NSERC); “Ministerio de Ciencia e Innovación” (Spanish Government) [CTQ2017-86170-R]; “Consejo Superior de Investigaciones Científicas” (CSIC) [AEP045]; “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) [308212/2017-7, 315092/2020-3, 141111/2020-8]; “Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) [001].

AGRADECIMENTOS

Gostaria de agradecer a Deus, em primeiro lugar, pelas inúmeras oportunidades que ele vem me proporcionando ao longo da minha vida. Gostaria de agradecer imensamente aos meus pais (Márcia e Geraldo) e ao meu irmão (Lucas) pelo apoio desde à minha entrada no curso de graduação em Engenharia Química até o fim deste doutorado. Só nós sabemos o quanto foi difícil chegar até aqui. Tenho muito orgulho de vir de uma família humilde, que tira o seu sustento da agricultura familiar no sul de Minas Gerais, e que conseguiu fazer com que os seus filhos pudessem desfrutar de oportunidades que os pais não tiveram. Gostaria de agradecer ao meu marido (João Paulo) por todo o apoio e suporte durante os últimos 10 anos, por não desistir de mim mesmo nos momentos mais difíceis e por sempre me incentivar a fazer o que eu jamais imaginaria ser capaz. Quero agradecer também ao professor Paulo pela orientação e amizade nesses últimos 4 anos. Em especial ao meu amigo e orientador de mestrado professor Theo, que agora está ao lado de Deus, por me oferecer apoio quando eu mais precisei. Aos meus avós, aos meus familiares, aos amigos antigos e aos amigos novos que construí em São Carlos e em Toronto durante o período do doutorado. Ao professor Bradley, que foi meu orientador no Canadá, por todo o suporte e generosidade. Aos meus amigos da Biozone, Travis, Erica, Rachel, Susi e Márcia. Ao professor Ruy pela prontidão em me ajudar sempre que precisei. Agradeço à coordenação do Programa de Pós-graduação em Engenharia Química da UFSCar pela oportunidade e à FAPESP pelas bolsas (no país e no exterior). A todos do grupo de Engenharia Bioquímica do DEQ/UFSCar, aos técnicos pelos “socorros” prestados e em especial a todos do laboratório de tecnologias enzimáticas. Gostaria de reiterar aqui meus sinceros agradecimentos a todos as pessoas acima citadas e a todos que não foram lembrados neste texto, mas que, de alguma maneira, contribuíram para a minha formação e para a realização deste trabalho.

ACKNOWLEDGEMENTS

First, I would like to thank God for the countless opportunities he has provided me throughout my life. I would like to thank my parents (Márcia and Geraldo) and my brother (Lucas) for their support since my entry into the undergraduate course in Chemical Engineering until the end of this Ph.D. I would like to thank my husband (João Paulo) for all the support during the last 10 years, for not giving up on me even in the most difficult moments, and for always encouraging me to do what I would never have imagined being capable of. I also want to thank Professor Paulo for his guidance and friendship over the last 4 years. My friend and master's supervisor Professor Theo, who is now at God's side, for offering me support when I most needed it. My grandparents, my family, old friends, and new friends that I made in São Carlos and Toronto during the Ph.D. Professor Bradley, who was my supervisor in Canada, for all his support and generosity. Travis, Erica, Rachel, and Susi for the assistance at Biozone. Professor Ruy for his readiness to help me whenever I needed it. At last, I would like to thank the coordination of the Graduate Program in Chemical Engineering at UFSCar for the opportunity and FAPESP for the scholarships (in Brazil and abroad). I am also grateful to everyone in the Biochemical Engineering Group at the Department of Chemical Engineering (UFSCar), especially the technicians and my colleagues at the Laboratory of Enzymatic Technologies for the help provided. I would like to reiterate here my sincere thanks to all those abovementioned and to all those who were not mentioned in this text but somehow contributed to my formation and to the completion of this work.

RESUMO

A indústria biotecnológica está buscando cada vez mais desenvolver biocatalisadores estáveis e de alta produtividade para serem empregados na síntese de bioprodutos em escala comercial. Além disso, com o aquecimento global, os sistemas de produção enzimáticos que utilizam matérias-primas renováveis estão alinhados com os princípios da sustentabilidade e da química verde. Assim, os ésteres graxos de açúcar (EGAs) têm grande potencial de uso industrial como um bioproduto de valor agregado. Esses surfactantes não iônicos são amplamente utilizados em detergentes, produtos de higiene bucal, alimentos, cosméticos e na indústria farmacêutica. Geralmente são sintetizados a partir da esterificação de açúcares e ácidos graxos na presença de catalisadores químicos. No entanto, esta abordagem requer alto consumo de energia e apresenta baixa seletividade, tornando onerosas as etapas de separação/purificação dos produtos. Como alternativa, a síntese enzimática de EGAs tem sido estudada. Nesta tese foram usados princípios de mapeamento sistemático para escrever duas revisões de literatura, a primeira cobrindo pesquisas sobre a síntese de EGAs catalisada pela *Candida antarctica* lipase B (CALB), e a segunda cobrindo pesquisas sobre a produção e recuperação de açúcares lignocelulósicos (principalmente xilose) para sintetizar bioprodutos. Também se realizou a síntese do oleato de xilose catalisada pela Lipozyme® 435/Novozyme® 435 (L435/N435, CALB comercial imobilizada em resina acrílica) em metil etil cetona (MEC). Os resultados mostraram que um excesso de ácido oleico favoreceu significativamente a reação. O modelo cinético de Ping Pong Bi Bi ajustou-se aos dados experimentais e não houve evidências de inibição na faixa avaliada. O reuso da L435 promoveu uma redução de 48 e 19% nas conversões de xilose e ácido oleico, respectivamente, após 10 ciclos de 12 h. Essa redução significativa nas conversões ocorreu principalmente devido à dessorção da CALB do suporte na presença do produto da reação de esterificação. Na tentativa de minimizar esse problema, a próxima etapa consistiu no recobrimento da superfície da L435 com polietilenoimina (PEI) com foco no seu uso posterior como biocatalisador na síntese de laurato/palmitato de xilose em MEC. O revestimento da L435 com PEI 2 KDa evitou a lixiviação da enzima no éster de açúcar bruto produzido e proporcionou a maior estabilidade enzimática em diferentes meios (MEC e soluções tampão em diferentes pHs), além de promover um maior grau de modificação da xilose do que a enzima não revestida. Após 5 ciclos de reuso de 6 h com a L435 revestida com PEI, as conversões de xilose diminuíram apenas 10%, enquanto com o biocatalisador não revestido elas diminuíram 37%. Por fim, a síntese de EGAs a partir da biomassa lignocelulósica e ácido oleico em MEC foi catalisada pela N435 antes e após o seu revestimento com PEI. Após pré-tratamento por explosão a vapor e hidrólise enzimática, o extrato hidrolisado foi purificado e concentrado a uma razão mássica de xilose/glicose de ~3 para 1. Esses açúcares lignocelulósicos foram melhores fontes de carboidrato para a reação de esterificação (em termos de conversões de açúcar) quando comparados aos mesmos açúcares comerciais. O revestimento da N435 com PEI evitou a dessorção da enzima no meio de reação e produziu um aumento de 35% e 50% nas conversões de xilose e glicose, respectivamente. Após 6 ciclos de reutilização de 24 h com a N435 revestida com PEI, a conversão de xilose foi reduzida em 44%, enquanto uma redução de 65% foi observada com a lipase não revestida. Nos dois primeiros estudos, a análise de espectrometria de massas confirmou a formação de mono, di e triésteres de xilose. Neste último, foram encontrados apenas mono e diésteres de xilose e glicose. Em todos os casos, o produto purificado apresentou uma capacidade de emulsificação próxima à de um éster de açúcar comercial.

Palavras-chave: ésteres graxos de xilose, biomassa lignocelulósica, ácidos graxos, metil etil cetona, lipases imobilizadas.

ABSTRACT

The biotechnology industry is increasingly seeking to deploy high productivity and stable biocatalysts for commercial-scale production of bioproducts. In addition, with global warming, enzyme-based production systems using renewable feedstocks are aligned with the principles of sustainability and green chemistry. Considering this perspective, sugar fatty acid esters (SFAEs) have great potential for industrial use as high value bioproducts. These non-ionic surfactants are widely used in detergents, oral hygiene products, food, cosmetics, and the pharmaceutical industry. They are usually synthesized from the esterification of sugars and fatty acids in the presence of chemical catalysts. Nonetheless, this approach requires high energy consume and presents low selectivity, turning the products separation/purification steps costly. As an alternative, the enzymatic synthesis of SFAEs has been studied. In this thesis, we used principles of systematic mapping to write two literature reviews, the first covering research on the synthesis of SFAEs with *Candida antarctica* lipase B (CALB), and the second covering research on the production and recovery of sugars from lignocellulosics to synthesize bioproducts (mostly xylose-derived products). Then, we performed the synthesis of xylose oleate in methyl ethyl ketone (MEK) catalyzed by the Lipozyme® 435/Novozyme® 435 (L435/N435, commercial CALB immobilized on an acrylic resin). Results showed that an excess of oleic acid significantly favored the reaction. The predicted Ping Pong Bi Bi kinetic model fitted to the experimental data and there was no evidence of inhibitions in the range assessed. The L435 repeated use showed a reduction of 48 and 19% in the xylose and oleic acid conversions, respectively, after 10 12h-cycles. This significant decrease in the conversions mainly occurred due to the CALB desorption from the support in the presence of our reaction product. In an attempt to minimize this issue, the next step consisted of the L435 coating with polyethyleneimine (PEI) for use as biocatalyst in the synthesis of xylose laurate/palmitate in MEK. The L435 treatment with 2 KDa PEI prevented the enzyme leakage in the crude sugar ester product and produced the highest enzyme stability in different media (MEK and buffer solutions at different pHs), besides affording a higher xylose modification degree than the uncoated enzyme. After 5 6 h-reuse cycles with the PEI-coated L435, the xylose conversions only decreased by 10%, while with the non-treated biocatalyst they decreased by 37%. At last, the synthesis of SFAEs from lignocellulosic biomass and oleic acid was catalyzed by the uncoated and PEI-coated N435 in MEK medium. After steam-explosion pretreatment of mixed hardwoods and high solids enzymatic hydrolysis at 15%wt solids, the hydrolysate extract was purified and concentrated to a xylose/glucose mass ratio of ~3 to 1. These lignocellulosic sugars were superior to the same commercial sugars as the carbohydrate source for the esterification reaction in terms of sugar conversions. Coating the N435 with PEI prevented enzyme leakage into the reaction medium and produced 35% and 50% higher xylose and glucose conversions to SFAEs, respectively. After 6 24 h-reuse cycles with the PEI-coated N435, the xylose conversion decreased by 44%, while a 65% reduction was observed with the uncoated lipase. In the first two studies, mass spectrometry analysis confirmed the formation of xylose mono-, di-, and tri- esters. In this last one, only xylose and glucose mono- and di- esters were found. In all cases our purified product presented an emulsion capacity close to that of a commercial sugar ester.

Keywords: xylose fatty acid esters, lignocellulosic biomass, fatty acids, methyl ethyl ketone, immobilized lipases.

LIST OF FIGURES

REVIEWING RESEARCH ON THE SYNTHESIS OF CALB-CATALYZED SUGAR ESTERS INCORPORATING SYSTEMATIC MAPPING PRINCIPLES

Figure 3.1. Flow Diagram for the selection of studies.....30
Figure 3.2. Proportion of acyl acceptors (a) and donors (b) used as reagents in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.....32
Figure 3.3. Percentage of solvent occurrences used in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.....35
Figure 3.4. Network analysis of acyl acceptors (a) and acyl donors (b) addressed by our dataset as reagents in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.....40
Figure 3.5. Network analysis of solvents (a) and methodologies (b) addressed by our dataset in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.....43

SUPPLEMENTARY MATERIAL

Figure S1. Annual distribution of all records retrieved through sugar fatty acid esters bibliographic searching after duplicate removal.....177
Figure S2. Stages of the literature screening process.....178
Figure S3. Records eliminated after full-text analysis (a) and detailing of the category "others" in subcategories (b).....179

A REVIEW ON THE PRODUCTION AND RECOVERY OF SUGARS FROM LIGNOCELLULOSICS FOR USE IN THE SYNTHESIS OF BIOPRODUCTS

Figure 3.6. Flow Diagram for the selection of studies.....58
Figure 3.7. Pretreatment methods (a) and enzymatic activities (hydrolysis step) (b) used in the obtaining of xylose (and other sugars) from lignocellulosics.....61
Figure 3.8. Assortment of sugar recovery methods (physical separations to collect crude extracts and purification methods).....69
Figure 3.9. Xylose (and other sugars)-derived products.....71
Figure 3.10. Bibliometric analysis of pretreatment methods (a) and enzymatic activities (hydrolysis step) (b) used in the obtaining of xylose (and other sugars) from lignocellulosics.....73
Figure 3.11. Bibliometric analysis of separation/purification methods used to obtain xylose (and other sugars) from lignocellulosics (a) and sugar-derived products (b)...76

LIPOZYME 435-MEDIATED SYNTHESIS OF XYLOSE OLEATE IN METHYL ETHYL KETONE

Figure 5.1. Optimization of the xylose: oleic acid molar ratio in the synthesis of xylose oleate in methyl ethyl ketone.....101
Figure 5.2. Activity profile as a function of the temperature for L435.....105
Figure 5.3. Plots of the experimental and simulated data of oleic acid and xylose consumption in the synthesis of xylose oleate in MEK catalyzed by L435.....107
Figure 5.4. Xylose and oleic acid conversion profiles in the synthesis of xylose oleate catalyzed by L435 in a vortex flow reactor.....108
Figure 5.5. L435 operational stability.....109

SUPPLEMENTARY MATERIAL

Figure S4. Lineweaver-Burk plot for the initial reaction rates of xylose consumption for L435 at 1.4 mM of oleic acid 200
Figure S5. Lineweaver-Burk plot for the initial reaction rates of oleic acid consumption for L435 at 7 mM of xylose 201
Figure S6. Mass spectrum of xylose monooleate with m/z = 413.2 202
Figure S7. Mass spectrum of xylose dioleate with m/z = 677.5 203
Figure S8. Mass spectrum of xylose trioleate with m/z = 941.7.....204

STABILIZATION AND SELECTIVITY ALTERATION OF LIPOZYME 435 BY ITS COATING WITH POLYETHYLENEIMINE: COMPARISON OF THE BIOCATALYST PERFORMANCE IN THE SYNTHESIS OF XYLOSE FATTY ESTERS

Figure 5.6. Inactivation courses of different Lipozyme 435 preparations in methyl ethyl ketone.....132
Figure 5.7. Residual activities of different Lipozyme 435 preparations after incubation for 12 h at 60 ºC in aqueous solutions, pHs 5, 7, and 9 133
Figure 5.8. (a) Xylose and lauric acid conversion profiles in the synthesis of xylose laurate; (b) Xylose and palmitic acid conversion profiles in the synthesis of xylose palmitate.....135
Figure 5.9. (a) Xylose and lauric acid conversion profiles in the synthesis of xylose laurate; (b) Xylose and palmitic acid conversion profiles in the synthesis of xylose palmitate.....136
Figure 5.10. Operational stability of uncoated and PEI-coated (2 KDa) Lipozyme 435 biocatalysts.....139

SUPPLEMENTARY MATERIAL

Figure S9. Mass spectrum of xylose monolaurate with m/z = 331.1 (xylose: lauric acid molar ratio of 1:5) 205
Figure S10. Mass spectrum of xylose dilaurate with m/z = 513.3 (xylose: lauric acid molar ratio of 1:5) 206
Figure S11. Mass spectrum of xylose trilaurate with m/z = 695.3 (xylose: lauric acid molar ratio of 1:5) 207
Figure S12. Mass spectrum of xylose monolaurate with m/z = 331.1 (xylose: lauric acid molar ratio of 5:1) 208
Figure S13. Mass spectrum of xylose dilaurate with m/z = 513.3 (xylose: lauric acid molar ratio of 5:1) 209
Figure S14. Mass spectrum of xylose trilaurate with m/z = 695.4 (xylose: lauric acid molar ratio of 5:1) 210
Figure S15. Mass spectrum of xylose monopalmitate with m/z = 387.2 (xylose: palmitic acid molar ratio of 1:5) 211
Figure S16. Mass spectrum of xylose dipalmitate with m/z = 627.4 (xylose: palmitic acid molar ratio of 1:5) 212
Figure S17. Mass spectrum of xylose tripalmitate with m/z = 865.5 (xylose: palmitic acid molar ratio of 1:5) 213
Figure S18. Mass spectrum of xylose monopalmitate with m/z = 387.1 (xylose: palmitic acid molar ratio of 5:1) 214
Figure S19. Mass spectrum of xylose dipalmitate with m/z = 627.3 (xylose: palmitic acid molar ratio of 5:1) 215
Figure S20. Mass spectrum of xylose tripalmitate with m/z = 865.4 (xylose: palmitic acid molar ratio of 5:1) 216

PRODUCTION OF SUGARS FROM MIXED HARDWOODS FOR USE IN THE SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY IMMOBILIZED-STABILIZED DERIVATIVES OF *CANDIDA ANTARCTICA* LIPASE B

Figure 5.11. Physical aspects of the hydrolysate extract after PF, UF, NF, and SD stages.....159
Figure 5.12. Comparison of xylose and glucose conversions with spray dried lignocellulosic sugars (SDLS) and commercial sugars (CS).....161
Figure 5.13. Effects of Novozyme 435 PEI-coating on glucose and xylose conversions to SFAEs.....163
Figure 5.14. Effect of enzyme loading on glucose [a] and xylose [b] conversions to sugar fatty acid esters.....164
Figure 5.15. Operational stability of uncoated and PEI-coated Novozyme 435 biocatalysts in the synthesis of SFAEs.....166

SUPPLEMENTARY MATERIAL

Figure S21. Mass spectrum of xylose monooleate with m/z = 413.29.....217
Figure S22. Mass spectrum of xylose dioleate with m/z = 677.53.....218
Figure S23. Mass spectrum of glucose monooleate with m/z = 443.30.....219
Figure S24. Mass spectrum of glucose dioleate with m/z = 707.54.....220

LIST OF TABLES

REVIEWING RESEARCH ON THE SYNTHESIS OF CALB-CATALYZED SUGAR ESTERS INCORPORATING SYSTEMATIC MAPPING PRINCIPLES

Table 3.1. Evidence synthesis and related review terminology 27
Table 3.2. Percentage of occurrences of methodologies applied in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.....37

SUPPLEMENTARY MATERIAL

Table S1. Search methodology for locating articles addressing the synthesis of sugar fatty acid esters 180
Table S2. Records eliminated after title/abstract screening 180
Table S3. Interpretation of Kappa statistic.....180
Table S4. Components and conditions of the reaction system for synthesis of carbohydrate esters and SEs conversions 181

A REVIEW ON THE PRODUCTION AND RECOVERY OF SUGARS FROM LIGNOCELLULOSICS FOR USE IN THE SYNTHESIS OF BIOPRODUCTS

Table 3.3. Pros and cons of some pretreatment methods for processing lignocellulosic materials.....65
Table 3.4. Components of the system for obtaining sugars from lignocellulosics and sugar-derived products among studies that promoted process scale-up 78

SUPPLEMENTARY MATERIAL

Table S5. Search methodology for locating articles addressing the production of sugars from lignocellulosics 189
Table S6. Interpretation of Kappa statistic.....189
Table S7. Components of the system for the obtaining of sugars from lignocellulosics and sugar-derived products.....190

LIPOZYME 435-MEDIATED SYNTHESIS OF XYLOSE OLEATE IN METHYL ETHYL KETONE

Table 5.1. Components and conditions of the reaction system in the synthesis of xylose fatty esters and corresponding conversions.....101
Table 5.2. Kinetic constants of the Ping-Pong Bi-Bi model (Eq. 1) fitted to the enzymatic esterification data (fatty acid and sugar consumption).....106

SUPPLEMENTARY MATERIAL

Table S8. Kinetic constants of the esterification reaction catalyzed by L435 201

STABILIZATION AND SELECTIVITY ALTERATION OF LIPOZYME 435 BY ITS COATING WITH POLYETHYLENEIMINE: COMPARISON OF THE BIOCATALYST PERFORMANCE IN THE SYNTHESIS OF XYLOSE FATTY ESTERS

Table 5.3. Recovered hydrolytic activities (A_H) of the L435 after its surface coating with PEI 129
Table 5.4. Residual activities of different L435 preparations after incubation for 12 h at 60 ºC in methanol/phosphate buffer (80/20 v/v), pH 7.....133

PRODUCTION OF SUGARS FROM MIXED HARDWOODS FOR USE IN THE SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY

IMMOBILIZED-STABILIZED DERIVATIVES OF *CANDIDA ANTARCTICA* LIPASE B

Table 5.5. Chemical composition of the hardwood after steam-explosion pretreatment and enzymatic hydrolysis.....159
Table 5.6. Composition range after enzymatic hydrolysis and purification/concentration of the CHE in two steps: (1) UF (after PF); (2) NF 159
Table 5.7. Powder composition range after spray drying 159

ABBREVIATIONS AND ACRONYMS

SFAEs	Sugar fatty acid esters
CALB	*Candida antarctica* lipase B
N435	Novozyme 435
L435	Lipozyme 435
MEK	Methyl Ethyl Ketone
PEI	Polyethyleneimine
SRs	Systematic reviews
SMs	Systematic maps
CEE	Collaboration for environmental evidence
SEs	Sugar esters
FFAs	Free fatty acids
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
T-but	Ter-butyl alcohol
2M2B	2-Methyl-2-butanol
GRAS	Food grade additive recognized as safe
ILs	Ionic liquids
THF	Tetrahydrofuran
DES	Deep euthetic solvents
MBGs	Microemulsion based organogels
EC	Emulsion capacity
A_H	Hydrolytic activity
HPLC	High performance liquid chromatography
GC	Gas chromatography
MS	Molecular sieves
KDa	Kilodalton
w/v	Mass/volume
w/w	Mass/mass
v/v	Volume/volume
MW	Molecular weight
rpm	rotations per minute
mM	millimolar
TBU	Tributyrin hydrolysis activity
RID	Refractive index detector

FID	Flame ionization detector
OC	Octyl-agarose
DS	Dextran sulfate
EH	Enzymatic hydrolysis
SCI-E	Science citation index expanded
ESCI	Emerging sources citation index
EAs	Enzymatic activities
DOI	Digital object identifier
LHW	Liquid hot water
SE	Steam explosion
AFEX	Ammonia fiber expansion
HMF	Hydroxymethylfurfural
GI	Gamma irradiation
UHP	Ultra-high pressure
PPP	Pentose phosphate pathway
XOS	Xylooligosaccharides
FAMEs	Fatty acid methyl esters
CS	Corn stover
SSF	Simultaneous saccharification and fermentation
OPEFB	Oil palm empty fruit bunches
LB	Lignocellulosic biomass
HSEH	High solids enzymatic hydrolysis
SDLS	Spray dried lignocellulosic sugars
CS	Commercial sugars
MWCOs	Targeted molecular weight cut-offs
wt%	Percentage by weight
Å	Ångstron
g	g-force or RCF (relative centrifugal force)
LAP	Laboratory analytical procedure
CHH	Crude hydrolysate hardwood
CHE	Crude hydrolysate extract
PF	Particulate filtration
UF	Ultrafiltration
NF	Nanofiltration
SD	Spray drying

HTec3	Cellic® HTec3 (hemicellulase complex)
CTec3	Cellic® CTec3 (cellulase and hemicellulase complex)
iCalB	Lipase acrylic resin from *Candida antarctica*, Sigma-Aldrich
RML	Lipase from *Rhizomucor miehei*
PES	Polyethersulfone

SUMMARY

1. GENERAL INTRODUCTION 19
2. OBJECTIVES 24
2.1. SPECIFIC OBJECTIVES 24
3. LITERATURE REVIEW 24
3.1. REVIEWING RESEARCH ON THE SYNTHESIS OF CALB-CATALYZED SUGAR ESTERS INCORPORATING SYSTEMATIC MAPPING PRINCIPLES 24
3.1.1. Introduction 24
3.1.2. Methodology 28
3.1.2.1. Literature searching and database building 28
3.1.2.2. Data coding and data synthesis 30
3.1.2.3. Data handling 30
3.1.3. Results and discussion 31
3.1.3.1. Components of the esterification/transesterification reaction 31
3.1.3.1.1. Reactants 31
3.1.3.1.2. Solvents 33
3.1.3.1.3. Experimental methodologies 36
3.1.3.2. Network analysis 39
3.1.3.3. Limitations and perspectives 42
3.1.3.4. Practical implications 42
3.1.4. Conclusions 44
References 45
Supplementary material 177
3.2. A REVIEW ON THE PRODUCTION AND RECOVERY OF SUGARS FROM LIGNOCELLULOSICS FOR USE IN THE SYNTHESIS OF BIOPRODUCTS 54
3.2.1. Introduction 54
3.2.2. Methodology 56
3.2.2.1. Literature searching and database building 56
3.2.2.2. Data analysis and presentation 58
3.2.3. Results and discussion 60
3.2.3.1. Pretreatment and enzymatic hydrolysis of the lignocellulosic biomass 60
3.2.3.2. Use of the xylose stream as feedstock to synthesize other products 68
3.2.3.3. Co-occurrence analysis of keywords 72
3.2.3.4. Scale-up of the process 77
3.2.3.5. Limitations 79
3.2.4. Conclusions 79
References 81
Supplementary material 189
4. METHODOLOGY 91
5. RESULTS AND DISCUSSION 91
5.1. LIPOZYME 435-MEDIATED SYNTHESIS OF XYLOSE OLEATE IN METHYL ETHYL KETONE 91
5.1.1. Introduction 91
5.1.2. Materials and Methods 95
5.1.2.1. Materials 95
5.1.2.2. Enzymatic synthesis of xylose oleate 95
5.1.2.2.1. Xylose solubility in MEK 95
5.1.2.2.2. Determination of the effect of xylose: oleic acid molar ratio on the reaction performance 95
5.1.2.2.3. Effect of the temperature on the L435 activity profile 96

5.1.2.2.4. Determination of esterification rates for L435....96
a) Determination of initial rates 96
b) Determination of long-term rates....96
5.1.2.2.5. Determination of the L435 operational stability....97
5.1.2.3. Purification and characterization of xylose oleate....97
5.1.2.4. Emulsion capacity assays....98
5.1.2.5. Standard enzymatic activities....98
5.1.2.5.1. L435 hydrolysis activity....98
5.1.2.5.2. L435 esterification activity....98
5.1.2.6. Chromatographic analyses....99
5.1.2.6.1. Xylose determination....99
5.1.2.6.2. Oleic acid determination....99
5.1.2.6.3. Furfural determination....99
5.1.3. Results and discussion....100
5.1.3.1. Enzymatic synthesis of xylose oleate....100
5.1.3.1.1. Effect of xylose: oleic acid molar ratio on the reaction performance....100
5.1.3.1.2. Effect of the temperature on the L435 activity profile....104
5.1.3.1.3. Esterification rates for L435....105
5.1.3.1.4. Conversion profiles in the synthesis of xylose oleate....108
5.1.3.1.5. L435 operational stability....109
5.1.3.2. Purification and characterization of xylose oleate....111
5.1.3.3. Emulsion capacity assays....112
5.1.4. Conclusions....112
References....113
Supplementary material....200
5.2. STABILIZATION AND SELECTIVITY ALTERATION OF LIPOZYME 435 BY ITS COATING WITH POLYETHYLENEIMINE: COMPARISON OF THE BIOCATALYST PERFORMANCE IN THE SYNTHESIS OF XYLOSE FATTY ESTERS....121
5.2.1. Introduction....121
5.2.2. Materials and methods....125
5.2.2.1. Materials....125
5.2.2.2. Coating of the L435 surface....125
5.2.2.3. L435 thermostability....125
5.2.2.4. Free fatty acids enzyme specificity in the synthesis of xylose fatty acid esters....125
5.2.2.5. Purification and characterization of xylose fatty acid esters....126
5.2.2.6. Emulsion capacity assays....126
5.2.2.7. L435 operational stability....127
5.2.2.8. Standard enzymatic activities....127
5.2.2.8.1. Hydrolytic activity....127
5.2.2.8.2. Esterification activity....127
5.2.2.9. Chromatographic analyses....128
5.2.2.9.1. Xylose determination....128
5.2.2.9.2. Free fatty acids determination....128
5.2.2.10. Protein determination....128
5.2.3. Results and discussion....129
5.2.3.1. Coating of the L435 surface....129
5.2.3.2. L435 thermostability....131
5.2.3.3. Free fatty acids enzyme specificity in the synthesis of xylose fatty acid esters....133
5.2.3.4. Partial purification and characterization of xylose fatty acid esters....137
5.2.3.5. Emulsion capacity assays....138
5.2.3.6. L435 operational stability....139

5.2.4. Conclusions 140
References 141
Supplementary material 205
5.3. PRODUCTION OF SUGARS FROM MIXED HARDWOODS FOR USE IN THE SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY IMMOBILIZED-STABILIZED DERIVATIVES OF CANDIDA ANTARCTICA LIPASE B 150
5.3.1. Introduction 150
5.3.2. Materials and Methods 153
5.3.2.1. Materials 153
5.3.2.2. Steam-explosion pretreatment of hardwood 153
5.3.2.3. Enzymatic hydrolysis of steam-exploded hardwood 154
5.3.2.4. Reactor configuration 154
5.3.2.5. Purification and concentration of the crude enzymatic hydrolysis extract 155
5.3.2.6. Enzymatic synthesis of sugar fatty acid esters 155
5.3.2.7. N435 PEI-coating 156
5.3.2.8. N435 operational stability in the synthesis of sugar fatty acid esters 156
5.3.2.9. Characterizations 156
5.3.2.9.1. Liquid fraction – acid hydrolysis 156
5.3.2.9.2. Solid fraction 156
5.3.2.9.3. Chromatographic analysis 157
5.3.2.9.4. Mass spectrometry analysis 157
5.3.2.9.5. Emulsion capacity assays 158
5.3.2.10. N435 hydrolytic activity measurements 158
5.3.3. Results and discussion 158
5.3.3.1. Enzymatic hydrolysis of steam-exploded hardwood and purification/concentration of the hydrolysate extract 158
5.3.3.2. Enzymatic synthesis of sugar fatty acid esters 161
5.3.3.2.1. Comparison of spray dried lignocellulosic sugars versus commercial sugars for esterification 161
5.3.3.2.2. Comparison of uncoated N435 versus PEI-coated N435 as biocatalysts 162
5.3.3.3. Effect of enzyme loading on sugar conversions to sugar fatty acid esters 163
5.3.3.4. N435 operational stability in the synthesis of sugar fatty acid esters 165
5.3.3.5. Characterizations – sugar fatty acid esters 167
5.3.3.5.1. Mass spectrometry analysis 167
5.3.3.5.2. Emulsion capacity assays 168
5.3.4. Conclusions 169
References 170
Supplementary material 217
6. GENERAL REMARKS 175
7. SUGGESTIONS FOR FUTURE RESEARCH 176
APPENDIX 221

1. GENERAL INTRODUCTION

Sugar fatty acid esters (SFAEs) are non-ionic, low toxic, and biodegradable surfactants with applications in detergents, oral care products, food, cosmetics, and the pharmaceutical industry (BIDJOU-HAIOUR; KLAI, 2013; CHANG; ZHANG; TANG, 2018; OGAWA et al., 2019; SHIN et al., 2019). These esters hold a market value that is at least two orders of magnitude greater than ethanol (CEPEA, 2022; GONÇALVES *et al.*, 2022; H & Z INDUSTRY CO., 2022). Particularly, xylose fatty acid esters are used in the cosmetic and pharmaceutical sectors, acting to improve skin hydration (BIDJOU-HAIOUR; KLAI, 2013). Xylose is a pentose that can be obtained from the hydrolysis of hemicelluloses, an important component of the lignocellulosic biomass, and is characterized as a renewable, low-cost, and abundant raw material (ABDULMALEK; HAMIDON; RAHMAN, 2016; MÉLINE et al., 2018). This sugar may represent a feasible option as a substrate used to produce SFAEs, since after the hydrolysis of lignocellulosic biomasses the hemicellulose fraction is generally underused (VESCOVI; DOS SANTOS; TARDIOLI, 2017). Nonetheless, xylose usage to obtain SFAEs has been little reported in the scientific literature so far (ABDULMALEK; HAMIDON; RAHMAN, 2016; BIDJOU-HAIOUR; KLAI, 2013; CHANG; SHAW, 2009; GIORGI *et al.*, 2022; MÉLINE et al., 2018; NETA; TEIXEIRA; RODRIGUES, 2015; SHI; LI; CHU, 2011; SIEBENHALLER et al., 2017, 2018; VESCOVI; DOS SANTOS; TARDIOLI, 2017). To date, SFAEs have mainly been produced using glucose (AN *et al.*, 2019; ANDLER *et al.*, 2017; ARCENS *et al.*, 2018; BASYARUDDIN; RAHMAN; ARUMUGAM, 2012; CHAIWUT *et al.*, 2022; CHANG; ZHANG; TANG, 2018; FINDRIK *et al.*, 2016; HA *et al.*, 2010; HOLLENBACH *et al.*, 2022; LIANG *et al.*, 2018; LIN *et al.*, 2016; SIEBENHALLER *et al.*, 2017, 2018; VUILLEMIN *et al.*, 2022; and so on); the use of xylose thus represents a novel use of this sugar.

SFAEs synthesis occurs through an esterification reaction in the presence of chemical catalysts or enzymes, where the carbohydrate acts as the acceptor for an activated acyl group, while fatty acids are the donors of this acyl group (DE LIMA et al., 2018; LIANG et al., 2018). The enzymatic route requires milder operating conditions, facilitates the separation of products, and achieves higher yields than the chemical pathway (ABDULMALEK; HAMIDON; RAHMAN, 2016; SIEBENHALLER et al.,

2018). However, it is first necessary to reduce the cost of enzymes and eliminate expensive chemical processing steps to make this route feasible.

Moreover, the use of enzymes as catalysts in their "free form" is generally accompanied by some downsides related to the instability of their structures when isolated from their natural environment and the difficulty of recovery at the end of the process. To overcome some of these drawbacks, enzymes have been immobilized using different techniques, such as interfacial adsorption on hydrophobic supports, covalent bonds, and ionic interactions (GONÇALVES *et al*., 2019). In addition to immobilization, strategies involving the chemical modification of the surface of enzymes have also been studied to improve the biocatalyst stability against different denaturing agents and maximize its activity (SILVEIRA *et al*., 2019; GUIMARÃES *et al*., 2018), specificity, and selectivity (CABRERA *et al*., 2009). Coating immobilized enzymes with polyethyleneimine (PEI), for example, can promote their stabilization and reduce leaching from the support, maintaining the reversibility of immobilization (FERNANDEZ-LOPEZ *et al*., 2017; HIRATA *et al*., 2016; PEIRCE *et al*., 2016; VIRGEN-ORTÍZ *et al*., 2017) and escalating the specificity and regioselectivity of derivatives (PALOMO *et al*., 2007).

Based on the considerations raised, this research proposes to study the synthesis of sugar fatty acid esters (mostly xylose esters) using immobilized-stabilized derivatives of *Candida antarctica* lipase B as catalysts of the reaction. In general terms, this research did aim at (1) selecting appropriate esterification conditions in an 80 mL-reactor and scaling-up to a 4L-reactor; (2) allowing the biocatalyst recovering from the product chain and its reuse with no significant reductions in the product yield through the combination of immobilization and chemical modification of the enzyme surface; and (3) using both commercial and lignocellulosic sugars as substrates in the synthesis of these biosurfactants.

The literature review about the abovementioned topic of study was presented below in the form of 2 scientific articles published on the journals "Critical Reviews in Biotechnology" (https://doi.org/10.1080/07388551.2021.1888071) and "Industrial Crops and Products" (https://doi.org/10.1016/j.indcrop.2022.115213). Results achieved throughout this doctorate were presented and discussed in the form of more three scientific articles. Two of them have already been published on the journals "Molecules" (https://doi.org/10.3390/molecules26113317) and "International Journal of Biological Macromolecules" (https://doi.org/10.1016/j.ijbiomac.2021.10.052). The other article has

been submitted for publication on the journal “Biofuels, Bioproducts & Biorefining” and is currently under review.

REFERENCES

ABDULMALEK, E.; HAMIDON, N. F.; RAHMAN, M. B. A. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents. **Journal of Molecular Catalysis B: Enzymatic**, vol. 132, p. 1–4, 2016. DOI https://doi.org/10.1016/j.molcatb.2016.06.010.

AN, D.; ZHANG, X.; LIANG, F.; XIAN, M.; FENG, D.; YE, Z. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A**, vol. 577, p. 257–264, 2019. DOI https://doi.org/10.1016/j.colsurfa.2019.05.079.

ANDLER, S. M.; WANG, L.; ROTELLO, V. M.; GODDARD, J. M. Influence of hierarchical interfacial assembly on lipase stability and performance in deep eutectic solvent. **Journal of Agricultural and Food Chemistry**, vol. 65, no. 9, p. 1907–1914, 2017. DOI https://doi.org/10.1021/acs.jafc.6b05372.

ARCENS, D.; GRAU, E.; GRELIER, S.; CRAMAIL, H.; PERUCH, F. 6-O-glucose palmitate synthesis with lipase: Investigation of some key parameters. **Molecular Catalysis**, vol. 460, p. 63–68, 2018. DOI https://doi.org/10.1016/j.mcat.2018.09.013.

BASYARUDDIN, M.; RAHMAN, A.; ARUMUGAM, M. Microwave assisted enzymatic synthesis of fatty acid sugar ester in ionic liquid-tert-butanol biphasic solvent system. **Asian Journal of Chemistry**, vol. 24, no. 11, p. 5058–5062, 2012.

BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. https://doi.org/10.14233/ajchem.2013.13973.

CABRERA, Z.; FERNANDEZ-LORENTE, G.; FERNANDEZ-LAFUENTE, R.; PALOMO, J. M.; GUISAN, J. M. Enhancement of Novozym-435 catalytic properties by physical or chemical modification. **Process Biochemistry**, vol. 44, no. 2, p. 226–231, 2009. DOI https://doi.org/10.1016/j.procbio.2008.10.005.

CEPEA - CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA. **Ethanol**. Piracicaba, São Paulo, Brazil: 2022. Available at: https://www.cepea.esalq.usp.br/br/indicador/etanol.aspx.

CHAIWUT, P.; JIRARAT, A.; TIENSRI, N.; SANGTHONG, S. Green synthesis optimization of glucose palm oleate and its potential use as natural surfactant in cosmetic emulsion. **Cosmetics**, vol. 9, no. 4, p. 76, 2022. DOI https://doi.org/10.3390/cosmetics9040076.

CHANG, P.; ZHANG, Z.; TANG, S. Lipase-catalyzed synthesis of sugar ester in mixed biphasic system of ionic liquids and supercritical carbon dioxide. **Journal of the Chinese Chemical Society**, vol. 65, no. 4, p. 452–458, 2018. DOI https://doi.org/10.1002/jccs.201700278.

CHANG, S. W.; SHAW, J. F. Biocatalysis for the production of carbohydrate esters. **New Biotechnology**, vol. 26, no. 3–4, p. 109–116, 2009. DOI https://doi.org/10.1016/j.nbt.2009.07.003.

DE LIMA, L. N.; MENDES, A. A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W.; GIORDANO, R. L. C. Performance of different immobilized lipases in the syntheses of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, 2018. DOI https://doi.org/10.3390/molecules23040766.

FERNANDEZ-LOPEZ, L.; PEDRERO, S. G.; LOPEZ-CARROBLES, N.; VIRGEN-ORTÍZ, J. J.; GORINES, B. C.; OTERO, C.; FERNANDEZ-LAFUENTE, R. Physical

crosslinking of lipase from *Rhizomucor miehei* immobilized on octyl agarose via coating with ionic polymers: Avoiding enzyme release from the support. **Process Biochemistry**, vol. 54, p. 81–88, 2017. DOI https://doi.org/10.1016/j.procbio.2016.12.018.

FINDRIK, Z.; MEGYERI, G.; GUBICZA, L.; BÉLAFI-BAKÓ, K.; NEMESTÓTHY, N.; SUDAR, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. **Journal of Cleaner Production**, vol. 112, p. 1106–1111, 2016. DOI https://doi.org/10.1016/j.jclepro.2015.07.098.

GIORGI, V.; BOTTO, E.; FONTANA, C.; MEA, L. D.; VAZ JR., S.; MENÉNDEZ, P.; RODRÍGUEZ, P. Enzymatic production of lauroyl and stearoyl monoesters of D-xylose, L-arabinose, and D-glucose as potential lignocellulosic-derived products, and their evaluation as antimicrobial agents. **Catalysts**, vol. 12, no. 6, p. 610, 2022. DOI https://doi.org/10.3390/catal12060610.

GONÇALVES, M. C. P.; KIECKBUSCH, T.G.; PERNA, R.F.; FUJIMOTO, J.T.; MORALES, S. A. V.; ROMANELLI, J. P. Trends on enzyme immobilization researches based on bibliometric analysis. **Process Biochemistry**, vol. 76, p. 95–110, 2019. DOI https://doi.org/10.1016/j.procbio.2018.09.016.

GONÇALVES, M. C. P.; ROMANELLI, J. P.; CANSIAN, A. B. M.; PUCCI, E. F. Q.; GUIMARÃES, J. R.; TARDIOLI, P. W.; SAVILLE, B. A. A review on the production and recovery of sugars from lignocellulosics for use in the synthesis of bioproducts. **Industrial Crops and Products**, vol. 186, p. 115213, 2022. DOI https://doi.org/10.1016/j.indcrop.2022.115213.

GUIMARÃES, J. R.; GIORDANO, R. L. C.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W. Evaluation of strategies to produce highly porous cross-linked aggregates of porcine pancreas lipase with magnetic properties. **Molecules**, vol. 23, no. 11, p. 13–16, 2018. DOI https://doi.org/10.3390/molecules23112993.

H & Z INDUSTRY CO. **Certification of Analysis**. Jinan, Shandong, China: 2022.

HA, S. H.; HIEP, N. M.; LEE, S. H.; KOO, Y. M. Optimization of lipase-catalyzed glucose ester synthesis in ionic liquids. **Bioprocess and Biosystems Engineering**, vol. 33, p. 63–70, 2010. DOI https://doi.org/10.1007/s00449-009-0363-4.

HIRATA, D. B.; ALBUQUERQUE, T. L.; RUEDA, N.; SÁNCHEZ-MONTERO, J. M.; GARCIA-VERDUGO, E.; PORCAR, R.; FERNANDEZ-LAFUENTE, R. Advantages of heterofunctional octyl supports: Production of 1,2-dibutyrin by specific and selective hydrolysis of tributyrin catalyzed by immobilized lipases. **ChemistrySelect**, vol. 1, no. 12, p. 3259–3270, 2016. DOI https://doi.org/10.1002/slct.201600274.

HOLLENBACH, R.; DELAVAULT, A.; GEBHARDT, L.; SOERGEL, H.; MUHLE-GOLL, C.; OCHSENREITHER, K.; SYLDATK, C. Lipase-mediated mechanoenzymatic synthesis of sugar esters in dissolved unconventional and neat reaction systems. **Sustainable Chemistry & Engineering**, vol. 10, p. 10192–10202, 2022. DOI https://doi.org/10.1021/acssuschemeng.2c01727.

LIANG, M. Y.; CHEN, Y.; BANWELL, M. G.; WANG, Y.; LAN, P. Enzymatic preparation of a homologous series of long-chain 6-O-acylglucose esters and their evaluation as emulsifiers. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 15, p. 3949–3956, 2018. DOI https://doi.org/10.1021/acs.jafc.8b00913.

LIN, X. S.; ZHAO, K. H.; ZHOU, Q. L.; XIE, K. Q.; HALLING, P. J.; YANG, Z. Synthesis of glucose laurate with excellent productivity. **Bioresources and Bioprocessing**, vol. 3, no. 2, p. 1–7, 2016. DOI https://doi.org/10.1186/s40643-015-0080-6.

MÉLINE, T.; MUZARD, M.; DELEU, M.; RAKOTOARIVONINA, H.; PLANTIER-ROYON, R.; RÉMOND, C. D-xylose and L-arabinose laurate esters: Enzymatic synthesis, characterization and physico-chemical properties. **Enzyme and Microbial**

Technology, vol. 112, p. 14–21, 2018. DOI https://doi.org/10.1016/j.enzmictec.2018.01.008.

NETA, N. A. S.; TEIXEIRA, J. A.; RODRIGUES, L. R. Sugar ester surfactants: Enzymatic synthesis and applications in food industry. **Critical Reviews in Food Science and Nutrition**, vol. 55, no. 5, p. 595–610, 2015. DOI https://doi.org/10.1080/10408398.2012.667461.

OGAWA, S.; ENDO, A.; KITAHARA, N.; YAMAGISHI, T.; AOYAGI, S.; HARA, S. Factors determining the reaction temperature of the solvent-free enzymatic synthesis of trehalose esters. **Carbohydrate Research**, vol. 482, p. 107739, 2019. DOI https://doi.org/10.1016/j.carres.2019.06.018.

PALOMO, J. M.; FILICE, M.; FERNANDEZ-LAFUENTE, R.; TERRENI, M.; GUISAN, J. M. Regioselective hydrolysis of different peracetylated β-monosaccharides by immobilized lipases from different sources. Key role of the immobilization. **Advanced Synthesis and Catalysis**, vol. 349, no. 11–12, p. 1969–1976, 2007. DOI https://doi.org/10.1002/adsc.200700055.

PEIRCE, S.; TACIAS-PASCACIO, V. G.; RUSSO, M. E.; MARZOCCHELLA, A.; VIRGEN-ORTÍZ, J. J.; FERNANDEZ-LAFUENTE, R. Stabilization of *Candida antarctica* lipase B (CALB) immobilized on octyl agarose by treatment with polyethyleneimine (PEI). **Molecules**, vol. 21, no. 6, 2016. DOI https://doi.org/10.3390/molecules21060751.

SHI, Y. G.; LI, J. R.; CHU, Y. H. Enzyme-catalyzed regioselective synthesis of sucrose-based esters. **Journal of Chemical Technology and Biotechnology**, vol. 86, no. 12, p. 1457–1468, 2011. DOI https://doi.org/10.1002/jctb.2711.

SHIN, D. W.; MAI, N. L.; BAE, S. W.; KOO, Y. M. Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. **Enzyme and Microbial Technology**, vol. 126, p. 18–23, 2019. DOI https://doi.org/10.1016/j.enzmictec.2019.03.004.

SIEBENHALLER, S.; HAJEK, T.; MUHLE-GOLL, C.; HIMMELSBACH, M.; LUY, B.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.; HAHN, T.; ZIBEK, S.; SYLDATK, C. Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. **Bioresources and Bioprocessing**, vol. 4, no. 25, 2017. DOI https://doi.org/10.1186/s40643-017-0155-7.

SIEBENHALLER, S.; KIRCHHOFF, J.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.; MUHLE-GOLL, C.; LUY, B.; HAITZ, F.; HAHN, T.; ZIBEK, S.; SYLDATK, C.; OCHSENREITHER, K. Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. **Frontiers in Chemistry**, vol. 6, no. 421, 2018. DOI https://doi.org/10.3389/fchem.2018.00421.

SILVEIRA, E. A.; MORENO-PEREZ, S.; BASSO, A.; SERBAN, S.; PESTANA-MAMEDE, R.; TARDIOLI, P. W.; SANCHEZ-FARINAS, C.; CASTEJON, N.; FERNANDEZ-LORENTE, G.; ROCHA-MARTIN, J.; GUISAN, J. M. Biocatalyst engineering of *Thermomyces Lanuginosus* lipase adsorbed on hydrophobic supports: Modulation of enzyme properties for ethanolysis of oil in solvent-free systems. **Journal of Biotechnology**, vol. 289, p. 126–134, 2019. DOI https://doi.org/10.1016/j.jbiotec.2018.11.014.

VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, Á.; BARBOSA, O.; RODRIGUES, R. C.; FERNANDEZ-LAFUENTE, R.

Polyethylenimine: A very useful ionic polymer in the design of immobilized enzyme biocatalysts. **Journal of Materials Chemistry B**, vol. 5, no. 36, p. 7461–7490, 2017. DOI https://doi.org/10.1039/c7tb01639e.
VUILLEMIN, M. E.; HUSSON, E.; LACLEF, S.; JAMALI, A.; LAMBERTYN, V.; PILARD, S.; CAILLEU, D.; SARAZIN, C. Improving the environmental compatibility of enzymatic synthesis of sugar-based surfactants using green reaction media. **Process Biochemistry**, vol. 117, p. 30–41, 2022. DOI https://doi.org/10.1016/j.procbio.2022.03.015.

2. OBJECTIVE

Enzymatically synthesize sugar fatty acid esters by esterification of sugars (mainly xylose; commercial or obtained from lignocellulosic biomass) with free fatty acids in methyl ethyl ketone (MEK) medium.

2.1. SPECIFIC OBJECTIVES

- ✓ Evaluate the acylation of commercial xylose with free fatty acids catalyzed by commercial immobilized lipases (Novozyme 435®, Lipozyme 435®).
- ✓ Study the kinetics of the esterification reaction and the enzyme specifity regarding the acyl donor chain.
- ✓ Improve the operational stability of the commercial immobilized lipases by coating their surface with a layer of polyethylenimine (PEI).
- ✓ Determine the optimal lignocellulosic biomass treatment conditions (pretreatment, enzymatic hydrolysis, purification, and concentration steps) to obtain a xylose-rich extract.
- ✓ Evaluate the uncoated and PEI-coated lipases in the synthesis of sugar fatty acid esters from the xylose/glucose extract from lignocellulosic biomass.
- ✓ Evaluate the reaction scale-up from an 80 mL to a 4 L-reactor.
- ✓ Purify and characterize the sugar esters produced according to their structures and emulsifying properties.

3. LITERATURE REVIEW

3.1. REVIEWING RESEARCH ON THE SYNTHESIS OF CALB-CATALYZED SUGAR ESTERS INCORPORATING SYSTEMATIC MAPPING PRINCIPLES

3.1.1. Introduction

The quality of evidence reviews has been a concern in several research fields (e.g., LEARY *et al.*, 2016; SIMONS; REIMER, 2009; TRANFIELD; DENYER; SMART, 2003) from the medical sector to environmental sciences (MOHER *et al.*, 2009; ROMANELLI *et al.*, 2020; WOODCOCK; PULLIN; KAISER, 2014). In light of this,

rigorous evidence review methodologies, such as systematic reviews and maps, were first developed in the health sector as a gold standard for collecting and synthesizing primary studies (COOK; MULROW; HAYNES, 1997). These approaches consist of a series of guidelines expressly designed to ensure high levels of objectivity and transparency in literature reviews, providing an appropriate basis for conducting reliable evidence syntheses (BURDA *et al.*, 2011; COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2013; HIGGINS, 2011; MOHER *et al.*, 2009; POPOVICH *et al.*, 2012; PULLIN; STEWART, 2006; SHEA *et al.*, 2007; ZUMSTEG; COOPER; NOON, 2012).

Systematic review (SR) methods include several steps that ensure the synthesis is reliable, such as comprehensive searches across multiple bibliographic sources, transparent screening of retrieved articles, critical appraisal of study quality, review for possible biases, well-documented extraction and reporting of data, and appropriate quantitative or qualitative data synthesis (DWAN *et al.*, 2013; PETERSEN; VAKKALANKA; KUZNIARZ, 2015; ROMANELLI *et al.*, 2020). Systematic maps (SMs) are methodologically similar to SR, but they are often used to catalogue evidence, identifying knowledge gaps, trends, and research clusters (JAMES; RANDALL; HADDAWAY, 2016).

In order to standardize systematic reviews methods, formal coordinating review bodies have been established across various disciplines, including Cochrane in healthcare, the Campbell Collaboration in social welfare, and the Collaboration for Environmental Evidence (CEE) in conservation and environmental management (HADDAWAY *et al.*, 2019).

In the realm of chemical engineering there is no specialized organization devoted to guiding evidence synthesis conduct. Consequently, the majority of reviews published in this research field could be classified as non-systematic by those standards, since they did not follow guidelines specifically designed to avoid errors and bias into the review process (e.g., COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018; HIGGINS, 2011). Furthermore, if those reviews failed to survey a comprehensive evidence base or effectively document all review steps (which is a common pitfall with some traditional reviews (e.g, ROBERTS; STEWART; PULLIN, 2006), then there is much room for the implementation of unsound actions to inform research, given they can mislead the review conclusions (STEWART, 2016; WHITTAKER, 2010; WOODCOCK; PULLIN; KAISESR, 2014).

Given this context, in an effort to advance with rigorous methods for conducting evidence reviews in chemical engineering, we incorporated principles of systematic mapping (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018; HIGGINS, 2011) to evaluate the research involving the synthesis of sugar fatty acid esters (SFAEs), or simply sugar esters (SEs). This research subject has presented an average annual growth of nearly 15% in its publication rate over the last decade (Figure S1, supplementary material), which follows the increasing demand of consumers for healthier products in recent years, as well as the emergence of environmental regulations during this period (CHANG; SHAW, 2009; VESCOVI; DOS SANTOS; TARDIOLI, 2017).

SEs represent a class of surfactants based on a sugar moiety and a fatty acid tail. They are non-toxic and biodegradable amphiphilic molecules, which have diverse applications as emulsifiers in food, cosmetics, and pharmaceutical products, in addition to being used as antitumor agents and plant growth inhibitors (GRÜNINGER; DELAVAULT; OCHSESNREITHER, 2019; TAN; DOU, 2020). The synthesis of these compounds can be performed by chemical processes as well as by microbial or enzymatic conversions, through an esterification/transesterification reaction between a sugar and a fatty acid or vinyl ester of fatty acid, where the carbohydrate acts as the acceptor for an activated acyl group (FOLEY, 2019; KERTHY *et al.*, 2019; VESCOVI; DOS SANTOS; TARDIOLI, 2017).

Obtaining SFAEs through the enzymatic pathway may represent a better alternative, as it requires smooth operating conditions, facilitates product separation, achieves high yields, and presents high specificity and selectivity (CHANG; SHAW, 2009; SIEBENHALLER *et al.*, 2018; VESCOVI; DOS SANTOS; TARDIOLI, 2017). Among the biocatalysts which can be used in these reactions, the most common ones are lipases (ABBAS; COMEAU, 2003; ABDULMALEK *et al.*, 2012; ABDULMALEK; HAMIDON; RAHMAN, 2016; CHAMOULEAU *et al.*, 2001; CHANG; SHAW, 2009; COULON *et al.*, 1996; DEGN; ZIMMERMANN, 2001; FERRER *et al.*, 2005; KHAN; RATHOD, 2015; NETA; TEIXEIRA; RODRIGUES, 2015; TARAHOMJOO; ALEMZADEH, 2003; VESCOVI *et al.*, 2017, 2016; VESCOVI; DOS SANTOS; TARDIOLI, 2017). These enzymes, especially *Candida antarctica* lipases B (CALB), have been frequently employed in the synthesis of esters in nonaqueous mediums (ADLERCREUTZ, 2013; BIDJOU-HAIOUR; KLAI, 2013; FERNANDEZ-LAFUENTE, 2010; GUMEL *et al.*, 2011; KHAN; RATHOD, 2015; KOBAYASHI, 2011). CALB is a highly enantioselective and efficient catalyst, which leads to reactions

with high yields under moderate conditions (HASEGAWA; AZUMA; TAKAHASHI, 2008; KUNDYS *et al.*, 2018; RAVELO *et al.*, 2015). Thus, researchers have sought to make the production of sugar fatty acid esters feasible through the biochemical route, by reducing the cost of the enzyme and eliminating expensive processing steps (ABDULMALEK; HAMIDON; RAHMAN, 2016; GUMEL *et al.*, 2011; KAPOOR; GUPTA, 2012).

In this review, our evaluation is restricted to a specific subset of scientific articles retrieved from five bibliographic sources: Web of Science (Core Collection: SCI-E and ESCI), Scopus, PubMed (Central; PMC), CAB Direct, and SciELO. For clarity, we used review terminologies presented in Table 3.1. The objectives of this study are two-fold: (1) to present trends and gaps across the literature on the CALB-catalyzed synthesis of sugar esters to subsidize future studies on this topic; (2) to introduce principles of systematic mapping to the field of chemical engineering.

Table 3.1. Evidence synthesis and related review terminology.

Term	**Definition**	**References**
Evidence review	An overarching term for articles that collate and summarizes multiple primary studies related to a specific, policy-relevant question.	Collaboration for environmental evidence, 2013
Evidence synthesis	"A distinct element in the review process" that combines results from primary studies to derive findings from all available evidence. This "occurs once the evidence base has been accumulated and the data of interest extracted".	Pope; Mays, 2007
Grey literature	Evidence not published in commercial publications, i.e., thesis and dissertations, research and committee reports, government reports, conference papers, ongoing research.	Paez, 2017
Primary study	Research that is collected firsthand rather than found in a book, database, or journal. It is often based on principles of the scientific method, where researchers develop research questions or hypotheses and collect data on events, objects, or people that is measurable, observable, and	Driscoll, 2011

	replicable. The ultimate goal in conducting primary research is to learn about something new that can be confirmed by others and to eliminate our own bias in the process.	
Systematic review	"A review of a clearly formulated question that use systematic and explicit methods to identify, select and critically appraise relevant research, and to collect and analyze data from the studies that are included within the review. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies".	Collaboration for environmental evidence, 2013
Systematic map	Systematic mapping does not attempt to answer a specific question as do systematic reviews, but instead collates, describes, and catalogs available evidence relating to a topic or question of interest. The included studies can be used to identify evidence for policy-relevant questions, knowledge gaps (to help direct future primary research), and knowledge clusters (subsets of evidence that may be suitable for Secondary research, e.g., systematic review).	Berger-Tal et al., 2018; James; Randall; Haddaway, 2016

3.1.2. Methodology

3.1.2.1. Literature searching and database building

We established the design of this review by following the criteria issued by the Collaboration for Environmental Evidence (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018). We determined the scope and focus of this study in close cooperation with environmental synthesists and experts in the subject addressed, primarily in Brazil.

When searching for relevant literature, we used online bibliographic sources, applying the following search string to retrieve titles, abstracts, and keywords of related publications: "sugar* ester*" OR "sugar* fatty acid* ester*" OR "fatty acid* sugar* ester*" OR "carbohydrate* ester" OR "carbohydrate* esters" OR "carbohydrate* fatty acid* ester*" OR "fatty acid* carbohydrate* ester*". Searches were restricted to articles published between 2010 and 2019 to obtain a sample of recent publications. We last

updated the database on May 2, 2020 (see the complete searching procedures in Table S1 and Figure S2 – Supplementary Material).

Web-searches first returned a total of 731 articles. After an analysis of coverage and overlapping to remove replicates, the database was reduced to 457 records. To eliminate unrelated publications unwittingly included in the reference list after this survey, the screening process was conducted by first analyzing titles and abstracts, considering the following inclusion criteria: i) articles should be a primary study about the synthesis of sugar esters (i.e. review articles were not eligible for inclusion); ii) studies should contemplate the synthesis of SES through the biochemical route; iii) the enzyme applied in the synthesis should be CALB. A total of 166 records were then subjected to a full-text analysis. As a result, a population of 57 primary studies were selected to be included in this study. A summary of the methodology employed is presented in Figure 3.1 and a detailing of the papers eliminated after title/abstract screening and full-text analysis are shown in Table S2 and Figure S3 (supplementary material), respectively.

Recognizing the potential for subjective decisions at the inclusion/exclusion stage (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018), a sample of 50 articles were analyzed by a second assessor, with decisions being compared using the Kappa test of agreement to ensure the repeatability of the process (COHEN, 1960). A Kappa score of 0.852 (95% Lower/Upper Confidence Limit) was obtained, which indicates almost perfect agreement between reviewers and that decisions were sufficiently repeatable (Table S3 – Supplementary Material). Furthermore, in order to promote transparency to the application of systematic mapping principles, a list containing all articles excluded after the full-text analysis is provided along with reasons for exclusion (supplementary material).

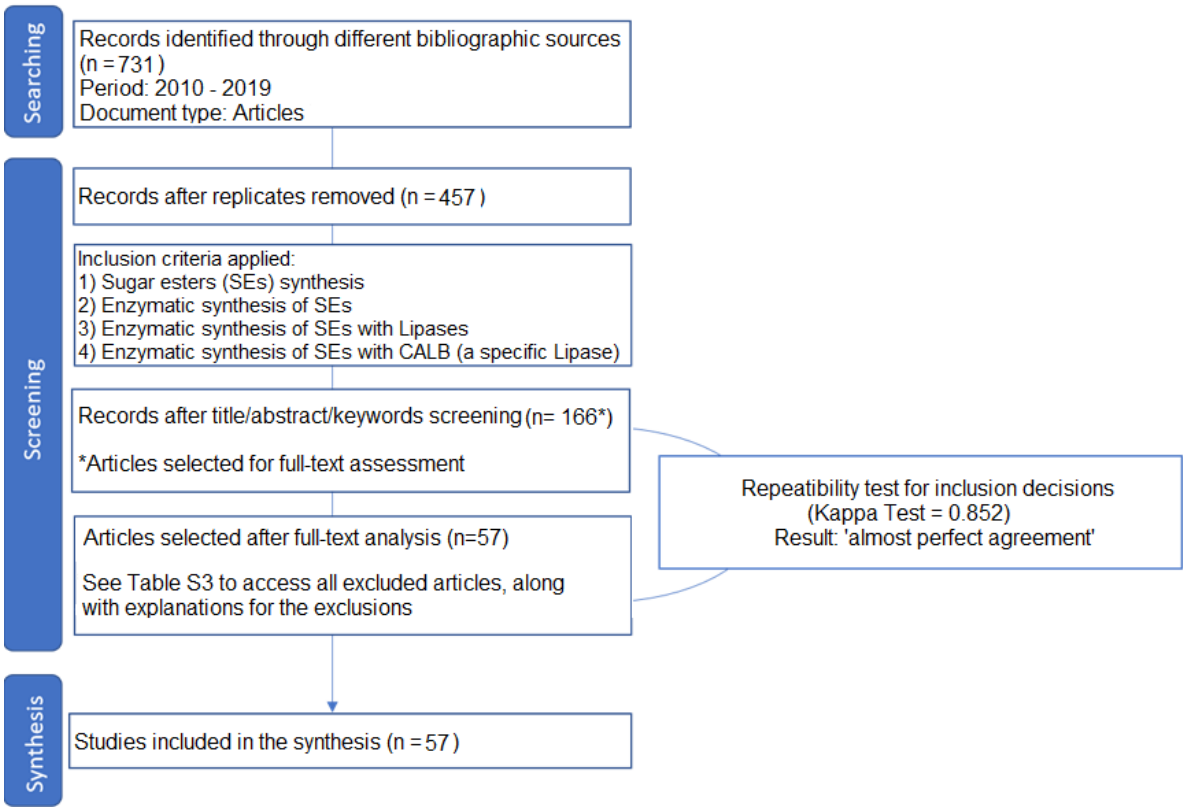


Figure 3.1. Flow Diagram for the selection of studies. (Adapted from ROSES Flow Diagram for Systematic Maps. Version 1.0).

3.1.2.2. Data coding and data synthesis

Articles meeting our inclusion criteria (N=57) were subjected to data coding and a synthesis of results. Despite the extensive literature that exists on CALB mediated synthesis of sugar fatty acid esters, important aspects of the components of this reaction remain to be clarified (GUMEL *et al.*, 2011). Considering this, here we elucidated some of these aspects, including the use of certain sugars (acyl acceptors), fatty acids/vinyl esters (acyl donors), solvents, and methodologies for the reaction, as well as their effects on SES productivities. The conditions of each reaction in our dataset are available in the supplementary material (Table S4).

3.1.2.3. Data handling

We used MS Excel (v. 2016) to perform calculations and OriginPro software (version 8.5) to develop graphs. The software R was used to accomplish the screening process – 'revtools package' (R CORE, 2019). Additionally, we used the terms involving acyl acceptors, acyl donors, solvents, and methodologies of our database to explore the hot topics of the CALB-catalyzed SES synthesis with the assistance of network maps drawn by VOSviewer software (version 1.16.15). We used the text mining functions to

construct and visualize the co-occurrences of these “keywords”, and then present trends across the included literature. VOSviewer is a software tool expressly designed for the analysis of bibliometric data (GONÇALVES *et al*., 2019; VAN ECK; WALTMAN, 2010).

3.1.3. Results and discussion

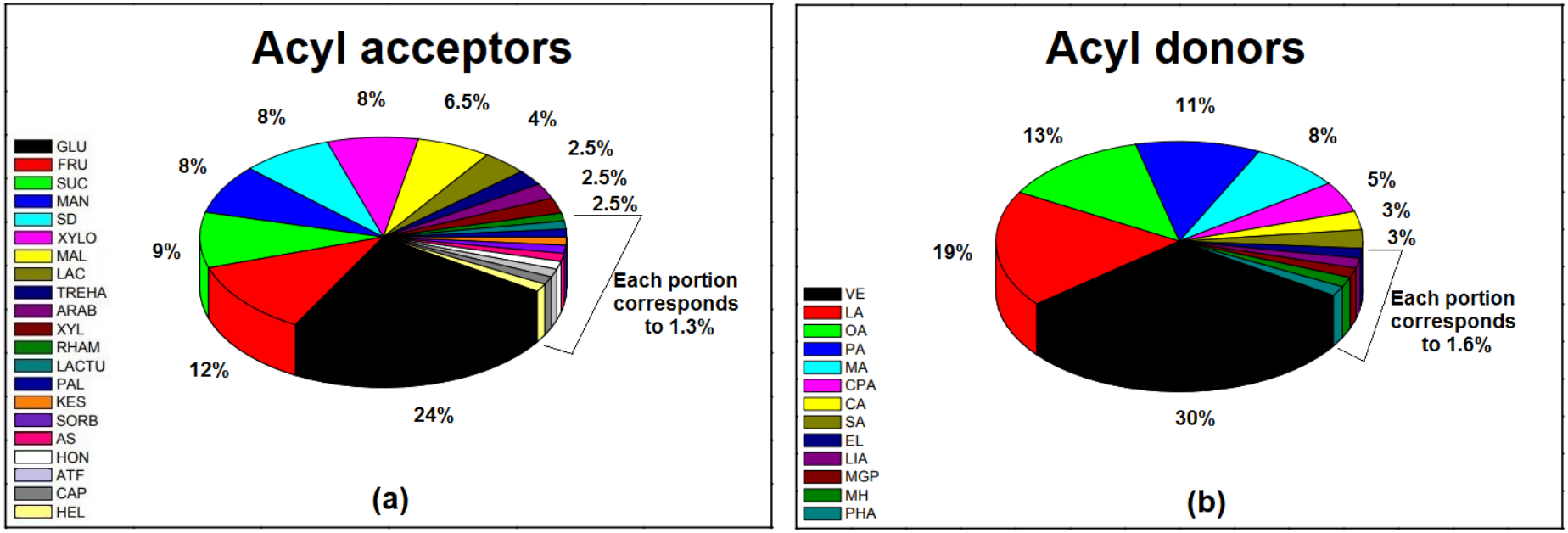
3.1.3.1. Components of the esterification/transesterification reaction

3.1.3.1.1. Reactants

The lipase catalyzed SFAEs synthesis allows for a reduction in the number of reaction steps, prevents the occurrence of racemization, and presents minimal protection due to regiospecificity. Many important factors are involved in this synthesis, such as the reactants (acyl acceptors, acyl donors), the solvent system, experimental techniques applied, the temperature, among other factors (CHANG; SHAW, 2009).

Substrates used to synthesize sugar fatty acid esters, namely sugars and fatty acids, greatly influence the efficiency of a lipase catalyzing a given esterification process (NETA; TEIXEIRA; RODRIGUES, 2015). We found that the sugars most used as acyl acceptors in the carbohydrate esters synthesis (Figure 3.2-a) were simple monosaccharides, such as glucose, fructose, and mannose, although some disaccharides were also used (sucrose, maltose, lactose) among studies we evaluated. However, the use of these carbohydrates to produce SEs in non-polar organic solvents has been described as a limiting factor for the reaction, due to their low solubility in these mediums (DE LIMA *et al.*, 2018; MAI *et al*., 2014). To overcome this drawback, the use of sugar derivatives has been reported. Nonetheless, the preparation of these derivatives requires additional synthesis steps that make the process even more expensive (DE PAULA; BARBOZA; DE CASTRO, 2005).

In contrast, among sugars scarcely reported in the evaluated literature (~5% of the publications), we identified the direct use of renewable raw materials and frequently available resources, such as the application of lignocellulosic biomass or agave syrup and honey. Boyère *et al.* (2011) evaluated the use of agave syrup and honey as substrates and solvents, simultaneously, in the synthesis of sugar esters. Despite the high-water content, authors obtained promising results, which may open the door for other natural substrates like maple syrup, rice syrup or corn syrup. Additionally, Puat, Taib, and Suhaimi (2010) used lignocellulosic biomass to synthesize SFAEs with better emulsifying properties than commercial surfactants.



Acyl acceptors: GLU: Glucose; SUC: Sucrose; FRU: Fructose; MAN: MannoSEs; SD: Sugar derivatives; MAL: MaltoSEs; XYLO: Xylose; LAC: LactoSEs; THEHA: TrehaloSEs; ARAB: ArabinoSEs; XYL: Xylitol; RHAM: RhamnoSEs; LACTU: LactuloSEs; PAL: PalatinoSEs; KES: KestoSEs; SORB: Sorbitol; AS: Agave syrup; HON: Honey; ATF: Agave Fructans; CAP: Caprolactone; HEL: Helicid.
Acyl donos: VE: Vinyl esters; LA: Lauric acid; PA: Palmitic acid; OA: Oleic acid; MA: Myristic acid; CA: Capric acid; CPA: Caprylic acid; SA: Stearic acid; EL: Ethyl laurate; LIA: Linoleic acid; MGP: Methyl-D-glucopyranoside; MH: Methyl hexanoate; PHA: Bacterial polyhydroxyalkanoates.

Figure 3.2. Proportion of acyl acceptors (a) and donors (b) used as reagents in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.

Furthermore, the main component of hemicelluloses, xylose, was identified as another low-cost and renewable raw material that was reported infrequently in our dataset, representing approximately 8% of the occurrences. Considering this scenario, from an industrial point of view, we believe that this lack of studies employing xylose as an acyl acceptor may create opportunities for the development of studies in the realm of chemical engineering, given that after the hydrolysis of the lignocellulosic biomass, the hemicellulose fraction is generally underused and, consequently, could present itself as a viable option. As a result, the use of all these renewable materials may lead in future to a further link between biochemistry and the food, pharmaceutical or cosmetics industry in the production of alternative sugar esters.

Regarding acyl donors (Figure 3.2-b), vinyl esters (~30% of the occurrences) provided, in general, synthesis of sugar esters with high yields. On the other hand, the use of free fatty acids (FFAs) represents 63% of the occurrences, with lauric, palmitic, and oleic acids accounting for roughly 43% of them. FFAs can successfully be used for the synthesis of sugar esters, in lieu of more activated acyl donors, such as vinyl esters, which are more expensive and generate toxic products with a potential inhibitory effect for lipases (BERGER; FABER, 1991). By using FFAs in esterification reactions, the only byproduct formed is water, which is safe and can be easily removed from the reaction mixture by using water-adsorbent reagents (CASAS-GODOY *et al.*, 2016). However, in some cases, vinyl esters have been used because they are more reactive than FFAs (CRUCES *et al.*, 2001; LIANG *et al.*, 2018; SUGIHARA, 1953). Therefore, the use of FFAs still leads to the possibility of the emergence of new studies, in an attempt to overcome this issue.

3.1.3.1.2. Solvents

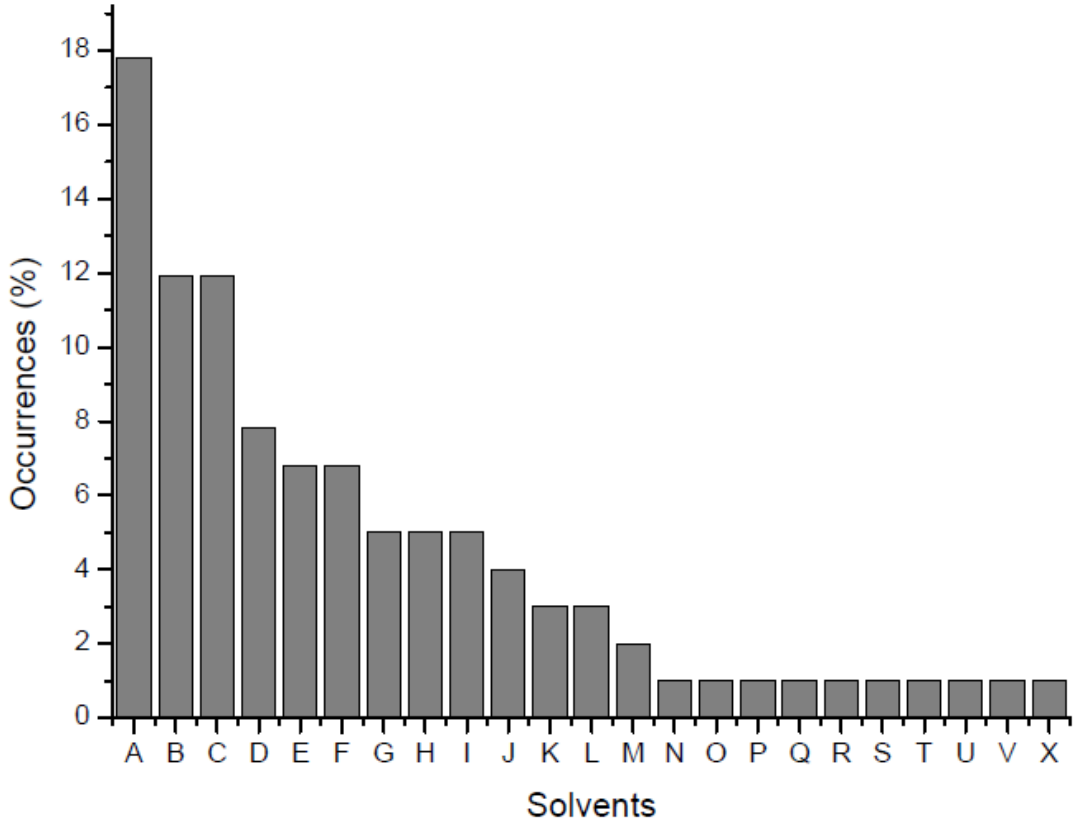
A nonaqueous solvent is essential for lipase catalyzed synthesis of SFAEs. The solubility of sugars and fatty acids in organic solvents is generally markedly different and this complicates the esterification reaction, as a high concentration of both the reactants within a single phase is difficult to achieve (GUMEL *et al.*, 2011). Some of the commonly used organic solvents, such as dimethylformamide (DMF), pyridine or dimethylsulfoxide (DMSO), can dissolve saccharides. However, environmental and health risks of these substances have driven the search for other suitable reaction solvents (GUMEL *et al.*, 2011). Besides, they usually have very negative effects for enzymes and are not

compatible with many applications of carbohydrate-derived products (DE LIMA *et al.*, 2018).

Hence, the solvent must reconcile carbohydrate solubility and low polarity to reduce damage on the enzyme. Additionally, the solvent must not present reactive hydroxyl groups able to compete with the sugars, carboxylic groups or esters bonds, which could be substrates of the enzyme and reduce the product yield and/or generate by-products. In general, tertiary alcohols meet these requirements, (e.g. ter-butyl alcohol (T-but) and 2-Methyl-2-butanol (2M2B)), as lipases do not recognize them as substrates (CASTRO *et al.*, 2004; FALLAVENA *et al.*, 2014; KUMARI *et al.*, 2009).

Among the solvents we assessed (Figure 3.3), T-but presented the highest percentage of occurrences (18). This compound was successfully applied in the lipase-catalyzed synthesis of sugar esters, leading to high yields (KOBAYASHI; EHARA, 2010; PERIN; FELISBERTI, 2018), good operational stability for biocatalysts (CHÁVEZ-FLORES *et al.*, 2017), and higher solubility of carbohydrates in the reaction media (PERIN; FELISBERTI, 2018). In addition, according to Degn and Zimmermann (2001), Adachi and Kobayashi (2005), and Kobayashi (2011), this solvent is suitable for the synthesis of sugar esters due to the fact that it is sufficiently hydrophobic (logP of 0.80) to prevent lipases inactivation, but hydrophilic enough to dissolve the carbohydrates. However, T-but cannot be used in food additives (BIDJOU-HAIOUR; KLAI, 2013).

Another important solvent highlighted in Figure 3.3 was 2M2B (12% of the occurrences), which has been applied to synthesize sugar esters with high conversions, productivities, selectivity, and initial reaction rates (FAVRELLE *et al.*, 2011; LIN *et al.*, 2016; PÖHNLEIN *et al*., 2014). In contrast, Li *et al.* (2015) showed that fructose laurate synthesis was preferred by CALB in methyl-ethyl-ketone (MEK) rather than 2M2B, which can be justified by the fact that MEK could help CALB fold into a conformation favoring mono-ester to di-ester conversion, which is responsible for the different selectivity of CALB in 2M2B and MEK. Moreover, MEK is considered a food grade additive recognized as safe (GRAS). Nonetheless, in our database, there are few occurrences (N=2) that address the use of this solvent for producing SEs, opening room for the development of more studies about this topic.



Solvents: A: Tert-Butyl alcohol; B: 2-Methyl-2-butanol (2M2B); C: Ionic liquid; D: Tetrahydrofuran (THF); E: Acetone; F: Hexane; G: Dimethyl sulfoxide (DMSO); H: Deep eutetic solvents (DES); I: Dimethylformamide (DMF); J: Pyridine; K: AcetonitrILse; L: Solvent free; M: Methyl Ethyl Ketone (MEK); N: Heptane; O: Toluene; P: Ethyl acetate; Q: Isooctane; R: Formamide; S: Benzene; T: Agave syrup; U: Honey; V: Chloroform; X: Ethanol.

Figure 3.3. Percentage of solvent occurrences used in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipase B.

Another example of solvents that might be used in esterification/transesterification reactions are ionic liquids (MAI *et al.*, 2014; REETZ, 2002; SHI; LI; CHU, 2011). Our results indicated that ionic liquids (ILSs) (12% of the occurrences) have been highly reported as a good solvent option for SFAEs synthesis (ANDLER *et al.*, 2017; FISCHER *et al.*, 2013; SIEBENHALLER *et al.*, 2018). The conversions obtained in ILSs are, in general, closely correlated with their properties, depending on the cation and anion of the ionic liquid. However, the use of these solvents is very challenging, especially due to their high cost, long reaction time, low yield, enzyme denaturation, difficult recoveries, and reuse of the ILs and the enzyme (ANDLER *et al.*, 2017). To minimize some of these issues, most ionic liquids are employed in a bisolvent system. Ji *et al.* (2015) and Zhao *et al.* (2016) showed that the use of a solvent mixture composed of ILs and 2M2B has been demonstrated to be a favorable option for sugar ester synthesis. Siebenhaller *et al*. (2018) used the combination of supercritical CO_2

and ILs, which was not only able to improve the reaction rate and yield, but also made the product separation and enzyme/ILs recycling easier. The use of other solvent mixtures, including T-but, acetone, DMF, DMSO, THF, and acetonitrile has also improved the reaction rate and maximum yield (FISCHER *et al.*, 2013; HA; HIEP; KOO, 2010; KOBAYASHI; EHARA, 2010; YANG; ZHAO; LIU, 2013).

On the other hand, we found that deep euthetic solvents (DES) are among those requiring further exploration for sugar ester synthesis, representing only 5% of the occurrences in our dataset. By the application of a DES, sugars are easily available in the reaction solvent. This is an elegant way to avoid the negative effect of the low solubility of sugars in water-free solvents (SIEBENHALLER *et al.*, 2017). For the enzymatic synthesis of sugar esters it was not only possible to replace the use of organic solvents with DES, it was also shown that the alternative use of DES provides further benefits for this synthesis, e.g., the ability of one DES to act as both substrate and solvent at the same time (ZHANG *et al.*, 2013).

Solvent-free systems are another example of reaction medium that was minimally addressed in the literature assessed, accounting for only 3% of the occurrences. This approach encourages the design of processes and products that reduce the use and generation of hazardous chemicals (OGAWA *et al.*, 2019). According to Galonde *et al*. (2013a), sugar esters synthesized under solvent-free conditions lowered the surface tension, produced stable oils-in-water emulsions to a degree similar to a commercially available surfactant, and did not require downstream purification. Therefore, this is an overarching approach that we considered could be more applicable to the synthesis of SFAEs in the search for new eco-friendly alternatives.

3.1.3.1.3. *Experimental methodologies*

To assure the enzymatic obtaining of sugar esters with similar characteristics to those conferred by commercial surfactants, it is necessary to apply some experimental techniques in the course of the synthesis, which allow from a simple monitoring of the reaction to determine the optimized operational parameters. These methods have been tested worldwide with the objective to overcome the main issues in the enzymatic synthesis of sugar esters, such as the high cost of the biocatalyst, the low solubility of sugars in non-polar organic media (which makes this process highly complex), and the insufficient SEs yields, among other factors. So, we designed an appraisal of these indicators according to their occurrences in our database (Table 3.2).

Table 3.2. Percentage of occurrences of methodologies applied in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipases B.

Methodologies	**Occurrences (%)**
1) Application of factorial designs	4.0
2) Optimization of the reaction conditions (not factorial designs)	4.0
3) Influence of fatty acids with different chain length as acyl donors	5.0
4) Influence of the solvents on operational parameters	11.1
5) Influence of molecular sieves on sugar ester synthesis	2.5
6) Evaluation of surface tension and emulsifying properties	8.2
7) Analysis of enzyme thermal, operational, and pH stability	8.2
8) Product purification, characterization, and quantification	9.6
9) Evaluation of enzyme kinetics, sugar ester yields, and mass transfer limitations	5.0
10) Influence of experimental conditions on conversions	7.6
11) Evaluation of CALB selectivity	3.0
12) CALB immobilization or the use of pre-immobilized CALB	27.8
13) Enzymatic reaction under ultrasound irradiation	1.0
14) Use of fluidized bed reactors	0.5
15) Use of microwave reactors	0.5
16) Use of supersaturated sugar solutions	2.0

Regarding the main strategies by which authors of research articles could increase sugar esters productivities (Table 3.2), we highlight CALB immobilization, the evaluation of the influence of solvents on some operational parameters, and the appraisal of reaction conditions.

CALB immobilization has been the most widely used technique in SFAEs synthesis (95% of the publications) over the last decade. The immobilization of this biocatalyst promotes stabilization compared to the soluble formulation; it also increases volumetric activity, retains or improves chemo-, enantio- and regioselectivity, and allows for easier product purification and recovery (CASAS-GODOY *et al.*, 2016; DULĘBA *et al.*, 2019; HIRATA *et al.*, 2016; RODRIGUES *et al.*, 2019).

Diverse immobilization techniques were reported in our dataset, which include immobilization by interfacial adsorption on hydrophobic supports, covalent bonding, and ionic interactions. The methods listed included CALB immobilization on different pre-

treated supports, such as chitosan, celite, silica magnetic microparticles, octyl-silica (CHÁVEZ-FLORES *et al.*, 2017; DE LIMA *et al.*, 2016; NETA *et al.*, 2012; VESCOVI *et al.*, 2016), and microemulsion based organogels (MBGs) (RAHMAN *et al.*, 2012; SUTILI *et al.*, 2015). We also verified that the use of commercial pre-immobilized CALB (Novozym 435, N435) occurred in about 82% of the publications, and achieved the highest sugar ester yields (ABDULMALEK; HAMIDON; RAHMAN, 2016; FINDRIK *et al.*, 2016; MAI *et al.*, 2014; MÉLINE *et al.*, 2018; NETA *et al.*, 2012; NETA *et al.*, 2011; PAPPALARDO *et al.*, 2017). N435 is based on CALB immobilization via interfacial adsorption on a macroporous resin (Lewatit VP OC 1600) formed by poly (methyl methacrylate) crosslinked with divinylbenzene (ORTIZ *et al.*, 2019).

Another method that showed significant relevance in obtaining carbohydrate esters (Table 3.2) evaluates the influence of several solvents on sugar solubility, enzyme activity and stability, and sugar ester yields, and presented a coverage of approximately 40% of the studies. Moreover, roughly 30% of the articles addressed: the appraisal of the reaction conditions (enzymatic load, fatty acid/sugar molar ratio, reaction time, and temperature); the evaluation of surface tensions and emulsifying properties; the analysis of enzyme thermal, operational, and pH stability; and the use of product purification, characterization, and quantification strategies.

Conversely, some of the analyzed approaches represented only a minor percentage of the occurrences. Nonetheless, they showed a great influence on the quality of the products, and we think they could be further explored in future studies on CALB-catalyzed SEs synthesis. These methodologies include: the influence of molecular sieves on SEs synthesis (2.5% of the occurrences), which allows for enhancement of the product yield, by displacing the reaction equilibrium towards synthesis rather than hydrolysis (NOTT; BROGNAUX, 2012) through the continuous removal of water as the reaction produces it (GUMEL *et al.*, 2011); the use of experimental designs (4.0% of the occurrences), which represent a useful tool when it comes to optimizing and developing a better understanding of enzymatic synthesis of glycosylated compounds (GALONDE *et al.*, 2013b); the application of supersaturated sugar solutions (2.0% of the occurrences), which enhance enzyme activity and stability (ADNANI *et al.*, 2010), and can also be employed to overcome the problem of the restricted sugar solubility in the reaction (ADNANI *et al.*, 2010; SHIN *et al.*, 2019), in addition to improving sugar esters productivities (SHIN *et al.*, 2019).

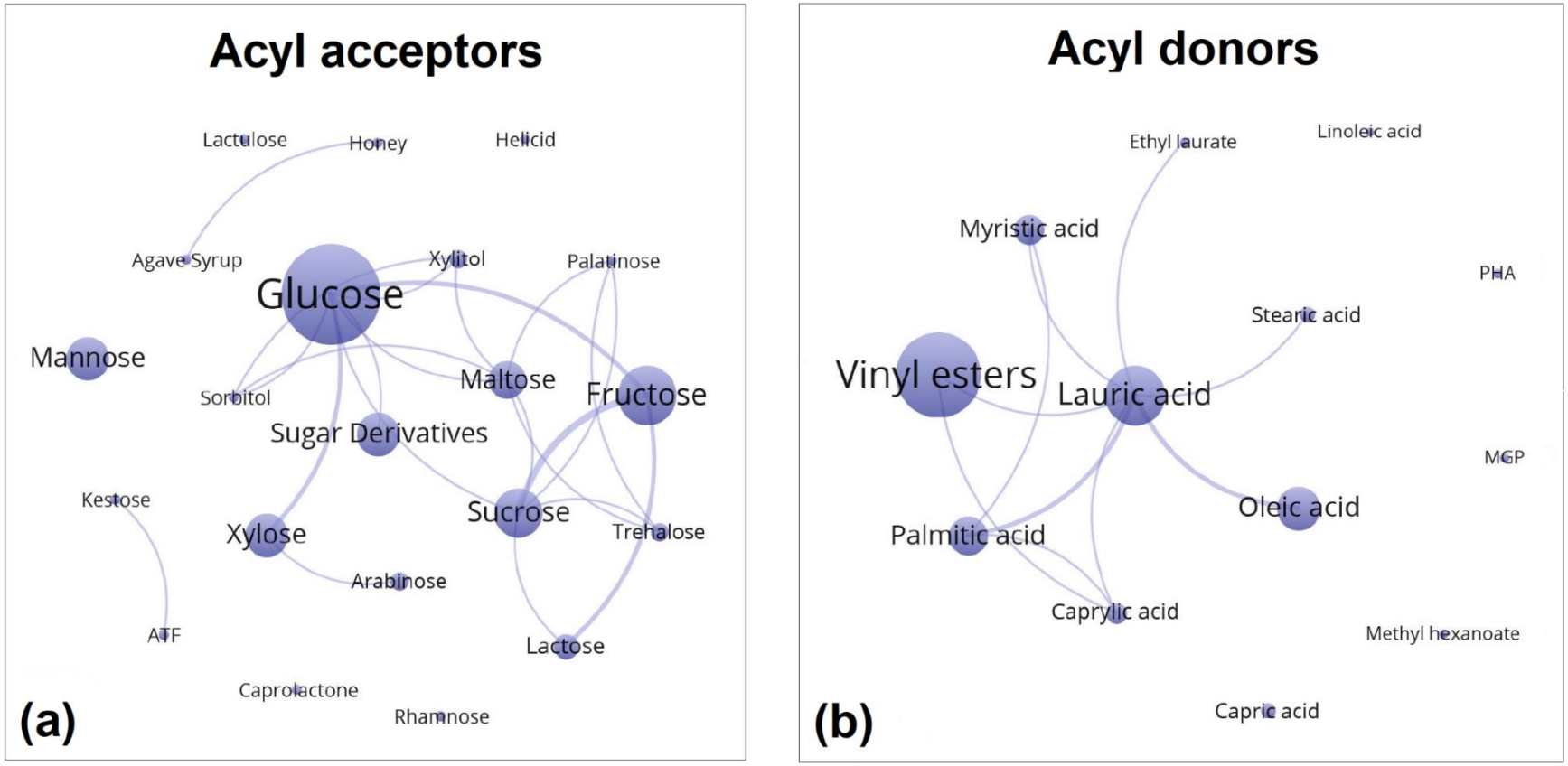
On top of these gaps, the use of ultrasound homogenization accounts for only 1.0% of occurrences and, as already stated by Ha *et al*. (2010) and Ye *et al*. (2014), could be better employed as an economically competitive reaction system on an industrial scale. Finally, microwave and fluidized bed reactors represented only 0.5% of the occurrences (each one of them), but proved to be efficient reactor designs by providing significant improvements for sugar esters conversions and productivities (JIA *et al.*, 2017; YANG; ZHAO; LIU, 2013).

3.1.3.2. Network analysis

Network maps allowed us to elucidate the structure, network distribution, and frequency of the co-occurrence of related terms, so as to clarify the research hotspots in the field of enzymatic SEs synthesis (GAO; HUANG; ZHANG, 2019). Our analysis revealed that 21 acyl acceptors and 13 acyl donors were cited by the studies we investigated (Figure 3.4). The highest number of occurrences was shown by the terms glucose (~24%), fructose (~12%), and sucrose (~9%) (acyl acceptors) (a); and by free fatty acids (~63%) – with lauric, oleic, and palmitic acids accounting for ~43% of the occurrences - and vinyl esters (~30%) (acyl donors) (b).

The arrangement of Figure 3.4-a reveals that the stablished network depicts the use of more than one source of sugar in only about 23% of the publications, which may indicate that most studies are not evaluating the effects of varying this substrate on SFAEs synthesis. The investigation of the effects of the use of several sugars as acyl acceptors is essential to obtain optimal reaction conditions, which represent, among other factors, a compromise between enzyme activity and the substrate choice.

In Figure 3.4-b, lauric acid (C-12:0) presented the highest number of co-occurrences (N=7), followed by palmitic (C-16:0), and caprylic (C-8:0) acids (each one with 3 co-occurrences). The increased co-occurrences of these substrates in the literature we gathered may have been caused by favoring the catalysis for the esterification process, given the high selectivity of the CALB for these long and medium chain fatty acids (GUMEL *et al.*, 2011). However, the effect of fatty acid (the acyl donor) chain length on lipase activity is difficult to generalize.



Acyl acceptors: ATF: Agave TequILsana Fructans. Acyl donors: PHA: Bacterial polyhydroxyalkanoates; MGP: Methyl-D-glucopyranoside.

Figure 3.4. Network analysis of acyl acceptors (a) and acyl donors (b) addressed by our dataset as reagents in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipase B. The size of the node is proportional to the number of occurrences, and the thickness of the edges represents co-occurrences between items.

The commonly used CALB Novozym 435, for example, has a higher specificity towards the unbranched long chain fatty acids (CAO; BORNSCHEUER; SCHMID, 1999; JUHL *et al.*, 2010; SOULTANI; ENGASSESR; GHOUL, 2001).

Nonetheless, even with the large utilization of free fatty acids (FFAs) in these esterification reactions, a co-occurrence network among FFAs occurs in only 14% of the publications. This may reflect that the evaluation of different chain length fatty acids as acyl donors does not serve as a priority methodology in obtaining SFAEs. Through the application of this method, SEs productivities could be greatly influenced by the enhanced specificity of the enzyme in certain cases. Table 3.2 also justified this affirmation, by showing that this approach represents only 5.0% of the occurrences.

We identified the use of 23 solvents and 16 methodologies in our data set (Figure 3.5). Among solvents (a), the most recurrent terms were T-but, 2M2B, and ionic liquids, as previously stated, which accounted for roughly 18, 12, and 12% of the occurrences, respectively. The largest co-occurrence networks include the use of T-but, 2M2B, THF, and DMF.

In general, the strong interaction between all items of the mapping could be justified by the fact that ~40% of the publications addressed the synthesis of SFAEs by testing more than one solvent in the reaction medium. This may reflect that these studies evaluated the impact of the solvent choice on the product quality, since a hydrophobic solvent is not necessarily a good choice for achieving optimal rates and degrees of conversion, depending on the solubility of the hydrophilic substrate (DEGN; ZIMMERMANN, 2001). Therefore, the investigation of the effect of different solvents or solvent mixtures is essential for enhancing SEs productivities. Moreover, we verified that these solvents are generally used in the form of a bisolvent system (25% of the publications), in an attempt to ensure greater solubilization of sugars in the reaction medium.

Among the methods applied in the production of SEs (Fig. 5-b), ~63% (N=10) occurred over eight times, indicating the predominance of some methodological techniques such as: CALB immobilization; influencing of the solvent on operational parameters; the purification, characterization, and quantification of the products. The wide utilization of these methods may be justified by the growing market demand for environmentally friendly and economically feasible products. Moreover, the assessment of these approaches showed, as expected, significant co-occurrences among all techniques. The highest interactions occurred between the methodology indicated by 12

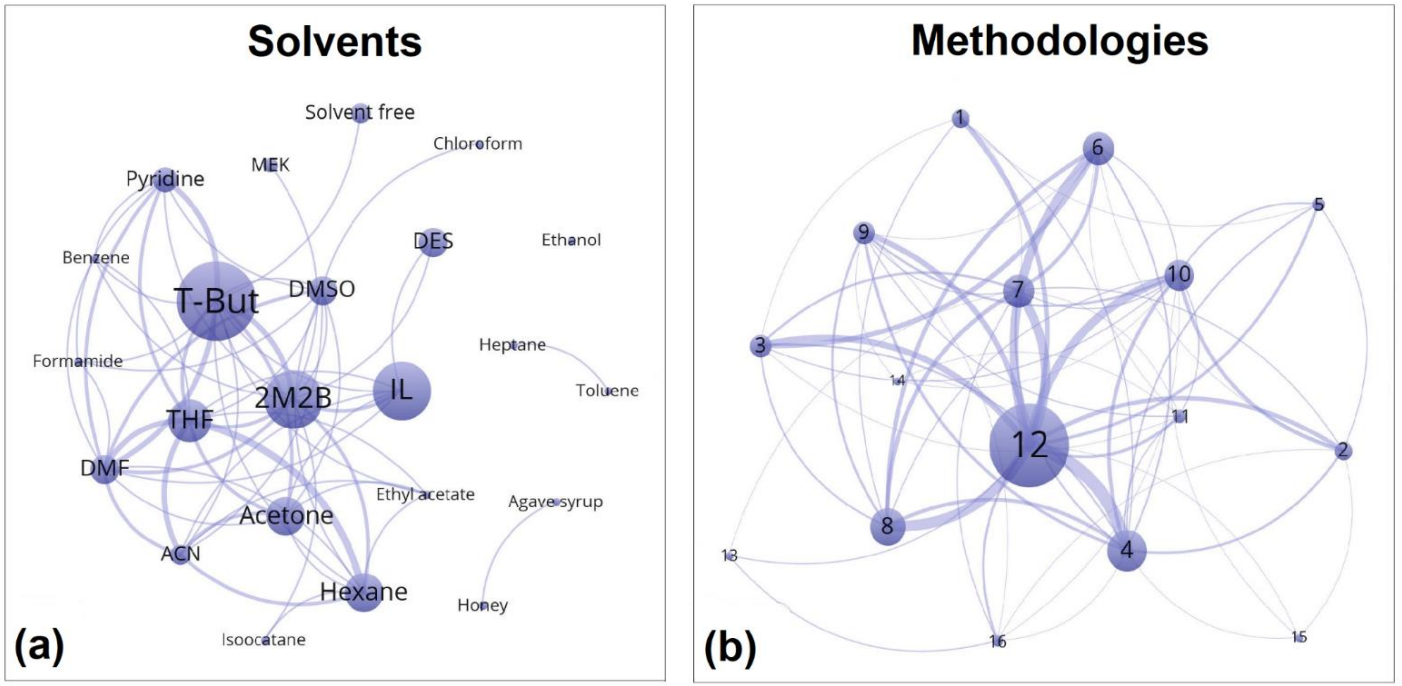
(CALB immobilization or the use of pre-immobilized CALB) and the strategies represented by 4, 6, 7, and 8. The purpose of combining these approaches is generally to reduce the cost of the process by presenting the possibility of recovering the biocatalyst from the product stream and reusing it for several cycles.

3.1.3.3. Limitations and perspectives for future studies

Reliable evidence reviews must have, as one of the main principles of their approaches, comprehensive search strategies, through which they may capture as much of the relevant scientific information for the synthesis as possible (ABDULLA *et al.*, 2015). In the case of this particular study, we only retrieved publications in the period from 2010 to 2019. Therefore, we acknowledge that the total number of all available articles in the theme could have been underestimated, taking into account that the timespan before 2010 may also be relevant to discuss trends over time. Nonetheless, we consider this study an important baseline to introduce the recent research direction into this field as well as the principles of more rigorous review methods to the chemical engineering field.

3.1.3.4. Practical implications

Systematic review methods are admittedly time- and resource-intensive, due to their requirements of coordinate multiple reviewers, process large numbers of retrieved documents, and the involvement of a team of expert advisors (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018). Nonetheless, when researchers or organizations operate under extremely strict time requirements, conducting a full systematic approach may not represent a viable option. Thus, as an attempt to reconcile tight time frames and a desire to increase the reliability of reviews, it is worthwhile to adopt, as much as possible, the principles of systematic review methods to improve the transparency, reproducibility, and objectivity of reviews. Doing so will facilitate their access and judgment by independent researchers, the appraisal of the reliability of their conclusions, and the evaluation of how the work was carried out, in addition to avoiding controversial reviews (BERGER-TAL *et al.*, 2018).



Solvents: T-But: Tert-Butyl alcohol; 2M2B: 2-Methyl-2-butanol; ILS: Ionic liquid; THF: Tetrahydrofuran; DMSO: Dimethyl sulfoxide; DES: Deep eutetic solvents; DMF: Dimethylformamide; ACN: AcetonitrILse; MEK: Methyl Ethyl Ketone. Techniques: 1: Application of factorial designs; 2: Optimization of the reaction conditions (not factorial designs); 3: Influence of fatty acids with different chain length as acyl donors; 4: Influence of the solvents on the sugar solubility , enzyme activity, lipases stability, and sugar esters yields; 5: Influence of molecular sieves on sugar esters synthesis; 6: Evaluation of surface tension and emulsifying properties; 7: Analysis of thermal, operational, and pH stability; 8: Purification, characterization, and quantification of the products; 9: Evaluation of enzyme kinetics, sugar esters yields, and mass transfer limitations; 10: Influence of experimental conditions on conversion; 11: Selective enzymatic synthesis of sugar esters; 12: CALB immobilization or the use of pre-immobilized CALB; 13: Enzymatic reaction under ultrasound irradiation for sugar esters synthesis; 14: Use of fluidized bed reactors for sugar esters synthesis; 15: Use of microwave reactors for sugar esters synthesis; 16: Use of supersaturated sugar solutions.

Figure 3.5. Network analysis of solvents (a) and methodologies (b) addressed by our dataset in the enzymatic synthesis of sugar esters with *Candida antarctica* Lipase B. The size of the node is proportional to the number of occurrences, and the thickness of the edges represents co-occurrences between items.

3.1.4. Conclusions

In this review, we incorporated some principles of systematic mapping into the research addressing the synthesis of CALB-catalyzed sugar esters over the last ten years in order to identify perspectives and research opportunities for specific topics inside this realm of chemical engineering. As suggested here, while reviews in this area are not always of high methodological rigorous standards, this paper can be influential in developing the evidence base and increasing the current knowledge on this topic.

The following findings can be drawn:

• The simple monosaccharides were the most cited acyl acceptors among studies we investigated, despite their low solubility in non-polar organic solvents. Sugar derivatives were used to minimize this issue, but their preparation makes the process even more expensive;

• Renewable raw materials could be further explored in our data set, by considering their outstanding results on the enzymatic synthesis of sugar esters;

• Due to the high selectivity of CALB for long and medium chain fatty acids, they presented the highest number of co-occurrences in our population of articles;

• There is still room for improvement of studies with free fatty acids, since in some cases vinyl esters are still preferentially used as acyl donors due to their higher reactivity when compared to FFAs;

• The solvents most used to synthesize SEs (T-but and 2M2B) reconcile carbohydrate solubility and low polarity to reduce the damage on the enzyme;

• The use of eco-friendly and food-grade solvents has received little attention in the literature we assessed. Solvent-free systems and methyl ethyl ketone can provide new opportunities to fill these gaps.

• CALB immobilization, the evaluation of the influence of solvents on operational properties, and the appraisal of the reaction conditions were the main methodologies applied by articles we investigated, following the growing market demand for environmentally friendly and economically feasible products;

• The use of molecular sieves, experimental designs, supersaturated sugar solutions, ultrasound homogenization, and microwave and fluidized bed reactors showed a great influence on the quality of SEs synthesized, although they represented a minor percentage of the occurrences;

• The co-occurence analysis of keywords involving solvent terms showed that most of the papers evaluated different solvents as reaction media (mostly in the form of a bisolvent system) and also the impact of their choice on SEs productivities;
• The significant co-occurrences among all methods we assessed generally occurred in an attempt to reduce the cost of the process with the possibility of recovering the biocatalyst from the product stream and reusing it;
• The comprehensiveness of SEs synthesis could rely, among other factors, on the evaluation of the effects of varying acyl acceptors and acyl donors in the enzymatic reaction; however, research articles we investigated have not frequently addressed this issue.

Funding

This work was supported by: Fundacão de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [grant numbers 2019/23908-4, 2019/08533-4, and 2016/10636-8]; Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil (CNPq) [grant number 308212/2017-7]; Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) [Finance Code 001].

References

ABBAS, H.; COMEAU, L. Aroma synthesis by immobilized lipase from *Mucor sp*. **Enzyme and Microbial Technology**, vol. 32, no. 5, p. 589–595, 2003. DOI https://doi.org/10.1016/S0141-0229(03)00022-X.

ABDULLA, H.; SMITH, K.; ATHERTON, P. J.; IDRIS, I. Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown : a systematic review and meta-analysis. **Diabetologia**, vol. 59, no. 1, p. 44-55, 2015. DOI https://doi.org/10.1007/s00125-015-3751-0.

ABDULMALEK, E.; HAMIDON, N. F.; ABDUL RAHMAN, M. B. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents. **Journal of Molecular Catalysis B: Enzymatic**, vol. 132, p. 1–4, 2016. DOI https://doi.org/10.1016/j.molcatb.2016.06.010.

ABDULMALEK, E.; SALAMI, H.; SAUPI, M.; TEJO, B. A.; BASRI, M.; BAKAR, A.; NOOR, R.; RAJA, Z.; RAHMAN, A.; BASYARUDDIN, M.; RAHMAN, A. Improved enzymatic galactose oleate ester synthesis in ionic liquids. **Journal of Molecular Catalysis. B: Enzymatic**, vol. 76, p. 37–43, 2012. DOI https://doi.org/10.1016/j.molcatb.2011.12.004.

ADACHI, S.; KOBAYASHI, T. Synthesis of esters by immobilized-lipase-catalyzed condensation reaction of sugars and fatty acids in water-miscible organic solvent. **Journal of Bioscience and Bioengineering**, vol. 99, no. 2, p. 87–94, 2005. DOI https://doi.org/10.1263/jbb.99.87.

ADLERCREUTZ, P. Immobilisation and application of lipases in organic media. **Chemical Society Reviews**, vol. 42, no. 15, p. 6406–6436, 2013. DOI

https://doi.org/10.1039/c3cs35446f.
ADNANI, A.; BASRI, M.; ABDUL, E.; BAKAR, A.; BASYARUDDIN, M.; RAHMAN, A.; CHAIBAKHSH, N.; NOOR, R.; RAJA, Z.; RAHMAN, A. Optimization of lipase-catalyzed synthesis of xylitol ester by Taguchi robust design method. **Industrial Crops and Products**, vol. 31, p. 350–356, 2010. DOI https://doi.org/10.1016/j.indcrop.2009.12.001.
ANDLER, S. M.; WANG, L.; ROTELLO, V. M.; GODDARD, J. M. Influence of hierarchical interfacial assembly on lipase stability and performance in deep eutectic solvent. **Journal of Agricultural and Food Chemistry**, vol. 65, no. 9, p. 1907–1914, 2017. DOI https://doi.org/10.1021/acs.jafc.6b05372.
BERGER-TAL, O.; GREGGOR, A. L.; MACURA, B.; ADAMS, A.; BLUMENTHAL, A.; BOUSKILA, A.; CANDOLIN, U.; DORAN, C. Systematic reviews and maps as tools for applying behavioral ecology to management and policy. **Behavioral Ecology**, p. 1–8, 2018. DOI https://doi.org/10.1093/beheco/ary130.
BERGER, B.; FABER, K. “Immunization” of lipase against acetaldehyde emerging in acyl transfer reactions from vinyl acetate. **Journal of the Chemical Society**, no. 17, p. 1198–1200, 1991. DOI https://doi.org/10.1039/C39910001198.
BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. DOI https://doi.org/10.14233/ajchem.2013.13973.
BOYÈRE, C.; FAVRELLE, A.; BROZE, G.; LAURENT, P.; NOTT, K.; PAQUOT, M.; BLECKER, C.; JÉRÔME, C.; DEBUIGNE, A. Lipase catalysis and thiol-Michael addition: a relevant association for the synthesis of new surface-active carbohydrate esters. **Carbohydrate Research**, vol. 346, no. 14, p. 2121–2125, 2011. DOI https://doi.org/10.1016/j.carres.2011.07.011.
BURDA, B. U.; NORRIS, S. L.; HOLMER, H. K.; OGDEN, L. A.; SMITH, M. E. B. Quality varies across clinical practice guidelines for mammography screening in women aged 40 e 49 years as assessed by AGREE and AMSTAR instruments. **Journal of Clinical Epidemiology**, vol. 64, no. 9, p. 968–976, 2011. DOI http://dx.doi.org/10.1016/j.jclinepi.2010.12.005.
CAO, L.; BORNSCHEUER, U. T.; SCHMID, R. D. Lipase-catalyzed solid-phase synthesis of sugar esters. Influence of immobilization on productivity and stability of the enzyme. **Journal of Molecular Catalysis B: Enzymatic**, vol. 6, no. 3, p. 279–285, 1999. DOI https://doi.org/10.1016/S1381-1177(98)00083-6.
CASAS-GODOY, L.; ARRIZON, J.; ARRIETA-BAEZ, D.; PLOU, F. J.; SANDOVAL, G. Synthesis and emulsifying properties of carbohydrate fatty acid esters produced from Agave tequilana fructans by enzymatic acylation. **Food Chemistry**, vol. 204, p. 437–443, 2016. DOI http://dx.doi.org/10.1016/j.foodchem.2016.02.153.
CASTRO, H. F.; MENDES, A. A.; SANTOS, J. C.; AGUIAR, C. L. Modificação de óleos e gorduras por biotransformação. **Química Nova**, vol. 27, no. 1, p. 146–156, 2004. DOI https://doi.org/10.1590/s0100-40422004000100025.
CHAMOULEAU, F.; COULON, D.; GIRARDIN, M.; GHOUL, M. Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media. **Journal of Molecular Catalysis B: Enzymatic**, vol. 11, no. 4–6, p. 949–954, 2001. DOI https://doi.org/10.1016/S1381-1177(00)00166-1.
CHANG, S. W.; SHAW, J. F. Biocatalysis for the production of carbohydrate esters. **New Biotechnology**, vol. 26, no. 3–4, p. 109–116, 2009. DOI https://doi.org/10.1016/j.nbt.2009.07.003.
CHÁVEZ-FLORES, L. F.; BELTRAN, H. I.; ARRIETA-BAEZ, D.; REYES-DUARTE, D. Regioselective synthesis of lactulose esters by *Candida antarctica* and *Thermomyces*

lanuginosus lipases. **Catalysts**, vol. 7, no. 9, p. 263, 2017. DOI https://doi.org/10.3390/catal7090263.

COHEN, J. A coefficient of agreement for nominal scales. **Educational and Psychological Measurement**, vol. XX, no. 1, p. 37–46, 1960. DOI https://doi.org/https://doi.org/10.1177/001316446002000104.

COLLABORATION FOR ENVIRONMENTAL EVIDENCE. **Guidelines and standards for evidence synthesis in environmental management**. Version 5, 2018. Available at: www.environmentalevidence.org/information-for-authors.

COLLABORATION FOR ENVIRONMENTAL EVIDENCE. **Guidelines for systematic review and evidence synthesis in environmental management**. Version 4, 2013. Available at: https://www.environmentalevidence.org/Documents/Guidelines/Guidelines4.2.pdf.

COOK, D. J.; MULROW, C. D.; HAYNES, R. B. Systematic Reviews: synthesis of best evidence for clinical decisions. **Annals of Internal Medicine**, vol. 126, no. 5, p. 389–391, 1997. DOI https://doi.org/doi:10.7326/0003-4819-126-5-199703010-00006.

COULON, D.; ISMAIL, A.; GIRARDIN, M.; ROVEL, B.; GHOUL, M. Effect of different biochemical parameters on the enzymatic synthesis of fructose oleate. **Journal of Biotechnology**, vol. 51, p. 115-121, 1996. DOI https://doi.org/10.1016/0168-1656(96)01588-x.

CRUCES, M. A.; PLOU, F. J.; FERRER, M.; BERNABÉ, M.; BALLESTEROS, A. Improved synthesis of sucrose fatty acid monoesters. **Journal of the American Oil Chemists' Society**, vol. 78, no. 5, p. 541–546, 2001. DOI https://doi.org/10.1007/s11746-001-0300-5.

DE LIMA, L. N.; MENDES, A. A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W.; GIORDANO, R. L. C. Performance of different immobilized lipases in the syntheses of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, 2018. DOI https://doi.org/10.3390/molecules23040766.

DE LIMA, L. N.; VIEIRA, G. N. A.; KOPP, W.; TARDIOLI, P. W.; GIORDANO, R. L. C. Mono- and heterofunctionalized silica magnetic microparticles (SMMPs) as new carriers for immobilization of lipases. **Journal of Molecular Catalysis B: Enzymatic**, vol. 133, p. 491–499, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2017.04.002.

DE PAULA, A. V.; BARBOZA, J. C. S.; DE CASTRO, H. F. Estudo da influência do solvente, carboidrato e ácido graxo na síntese enzimática de ésteres de açúcares. **Quimica Nova**, vol. 28, no. 5, p. 792–796, 2005. DOI https://doi.org/10.1590/s0100-40422005000500011.

DEGN, P.; ZIMMERMANN, W. Optimization of carbohydrate fatty acid ester synthesis in organic media by a lipase from *Candida antarctica*. **Biotechnology and Bioengineering**, vol. 74, no. 6, p. 483–491, 2001. DOI https://doi.org/10.1002/bit.1139.

DRISCOLL, L. **Introduction to primary research: observations, surveys, and interviews**. Writing spaces: Readings on writing, vol. 2, p. 153-174, 2011.

DULĘBA, J.; CZIRSON, K.; SIÓDMIAK, T.; MARSZAŁŁ, M. P. Lipase B from *Candida antarctica* — the wide applicable biocatalyst in obtaining pharmaceutical compounds. **Medical Research Journal**, vol. 4, no. 3, p. 174–177, 2019. DOI https://doi.org/10.5603/mrj.a2019.0030.

DWAN, K.; GAMBLE, C.; WILLIAMSON, P. R.; KIRKHAM, J. J.; BIAS, R. Systematic review of the empirical evidence of study publication bias and outcome reporting bias — an updated review. **Plos One**, vol. 8, no. 7, 2013. DOI https://doi.org/10.1371/journal.pone.0066844.

FALLAVENA, L. P.; ANTUNES, F. H. F.; ALVES, J. S.; PALUDO, N.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Ultrasound technology and

molecular sieves improve the thermodynamically controlled esterification of butyric acid mediated by immobilized lipase from *Rhizomucor miehei*. **RSC Advances**, vol. 4, no. 17, p. 8675–8681, 2014. DOI https://doi.org/10.1039/c3ra47315e.

FAVRELLE, A.; BOYÈRE, C.; LAURENT, P.; BROZE, G.; BLECKER, C.; PAQUOT, M.; JÉRÔME, C.; DEBUIGNE, A. Enzymatic synthesis and surface active properties of novel hemifluorinated mannose esters. **Carbohydrate Research**, vol. 346, no. 9, p. 1161–1164, 2011. DOI http://dx.doi.org/10.1016/j.carres.2011.04.004.

FERNANDEZ-LAFUENTE, R. Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. **Journal of Molecular Catalysis B: Enzymatic**, vol. 62, no. 3–4, p. 197–212, 2010. DOI https://doi.org/10.1016/j.molcatb.2009.11.010.

FERRER, M.; SOLIVERI, J.; PLOU, F. J.; NIEVES, L.; REYES-DUARTE, D.; CHRISTENSEN, M. Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B , and their antimicrobial properties. **Enzyme and Microbial Technology**, vol. 36, p. 391–398, 2005. DOI https://doi.org/10.1016/j.enzmictec.2004.02.009.

FINDRIK, Z.; MEGYERI, G.; GUBICZA, L.; BÉLAFI-BAKÓ, K.; NEMESTÓTHY, N.; SUDAR, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. **Journal of Cleaner Production**, vol. 112, p. 1106–1111, 2016. DOI http://dx.doi.org/10.1016/j.jclepro.2015.07.098.

FISCHER, F.; HAPPE, M.; EMERY, J.; FORNAGE, A.; SCHÜTZ, R. Enzymatic synthesis of 6- and 6'-O-linoleyl-α-D-maltose: From solvent-free to binary ionic liquid reaction media. **Journal of Molecular Catalysis B: Enzymatic**, vol. 90, p. 98–106, 2013. DOI http://dx.doi.org/10.1016/j.molcatb.2013.01.019.

FOLEY, A. M.; MAGUIRE, A. R. The impact of recent developments in technologies which enable the increased use of biocatalysts. **European Journal of organic chemistry**, vol. 2019, no. 23, p. 3713–3734, 2019. DOI https://doi.org/10.1002/ejoc.201900208.

GALONDE, N.; NOTT, K.; RICHARD, G.; DEBUIGNE, A.; NICKS, F.; JEROME, C.; FAUCONNIER, M. Study of the influence of pure ionic liquids on the lipase-catalyzed (trans)esterification of mannose based on their anion and cation nature. **Current Organic Chemistry**, vol. 17, no. 7, p. 763-770, 2013a. DOI https://doi.org/10.2174/1385272811317070010.

GALONDE, N.; BROSTAUX, Y.; RICHARD, G.; NOTT, K.; JERÔME, C.; FAUCONNIER, M. Use of response surface methodology for the optimization of the lipase-catalyzed synthesis of mannosyl myristate in pure ionic liquid. **Process Biochemistry**, vol. 48, no. 12, p. 1914–1920, 2013b. DOI http://dx.doi.org/10.1016/j.procbio.2013.08.023.

GAO, J.; HUANG, X.; ZHANG, L. Comparative analysis between international research hotspots and national-level policy keywords on artificial intelligence in China from 2009 to 2018. **Sustainability**, vol. 11, no. 23, p. 6574, 2019. DOI http://dx.doi.org/10.3390/su11236574.

GONÇALVES, M. C. P.; KIECKBUSCH, T. G.; PERNA, R. F.; FUJIMOTO, J. T.; MORALES, S. A. V.; ROMANELLI, J. Trends on enzyme immobilization researches based on bibliometric analysis. **Process Biochemistry**, vol. 76, p. 95–110, 2019. DOI https://doi.org/10.1016/j.procbio.2018.09.016.

GRÜNINGER, J.; DELAVAULT, A.; OCHSENREITHER, K. Enzymatic glycolipid surfactant synthesis from renewables. **Process Biochemistry**, 2019. DOI https://doi.org/10.1016/j.procbio.2019.09.023.

GUMEL, A. M.; ANNUAR, M. S. M.; HEIDELBERG, T.; CHISTI, Y. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, vol. 46, no. 11, p. 2079–2090, 2011. DOI http://dx.doi.org/10.1016/j.procbio.2011.07.021.

HA, S. H.; HIEP, N. M.; KOO, Y. Enhanced production of fructose palmitate by lipase-catalyzed esterification in ionic liquids. **Biotechnology and Bioprocess Engineering**, vol. 15, p. 126–130, 2010. DOI https://doi.org/10.1007/s12257-009-3091-3.

HA, S. H.; HIEP, N. M.; LEE, S. H.; KOO, Y.-M. Optimization of lipase-catalyzed glucose ester synthesis in ionic liquids. **Bioprocess and Biosystems Engineering**, vol. 33, p. 63–70, 2010. DOI https://doi.org/10.1007/s00449-009-0363-4.

HADDAWAY, N. R.; FEIERMAN, A.; GRAINGER, M. J.; GRAY, C. T.; AYDER, E. T.; DHAUBANJAR, S.; WESTGATE, M. J. EviAtlas: a tool for visualising evidence synthesis databases. **Environmental Evidence**, vol. 8, no. 1, p. 22, 2019. DOI https://doi.org/10.1186/s13750-019-0167-1.

HASEGAWA, S.; AZUMA, M.; TAKAHASHI, K. Enzymatic esterification of lactic acid, utilizing the basicity of particular polar organic solvents to suppress the acidity of lactic acid. **Journal of Chemical Technology & Biotechnology**, vol. 83, p. 1503–1510, 2008. DOI https://doi.org/10.1002/jctb.1935.

HIGGINS, J. P. T. (Ed.). **Handbook for systematic reviews of interventions**. The Cochrane Collaboration, 2011.

HIRATA, D. B.; ALBUQUERQUE, T. L.; RUEDA, N.; SÁNCHEZ-MONTERO, J. M.; GARCIA-VERDUGO, E.; PORCAR, R.; FERNANDEZ-LAFUENTE, R. Advantages of heterofunctional octyl supports: Production of 1,2-dibutyrin by specific and selective hydrolysis of tributyrin catalyzed by immobilized lipases. **ChemistrySelect**, vol. 1, no. 12, p. 3259–3270, 2016. DOI https://doi.org/10.1002/slct.201600274.

JAMES, K. L.; RANDALL, N. P.; HADDAWAY, N. R. A methodology for systematic mapping in environmental sciences. **Environmental Evidence**, vol. 5, no. 1 , p. 7, 2016. DOI https://doi.org/10.1186/s13750-016-0059-6.

JI, F.; WANG, J.; JIANG, B.; LI, Y.; JI, F.; WANG, J.; JIANG, B.; LI, Y. Efficient mono-acylation of fructose by lipase-catalyzed esterification in ionic liquid co-solvents. **Carbohydrate Research**, vol. 416, p. 51-58, 2015. DOI https://doi.org/10.1016/j.carres.2015.08.009.

JIA, C.; WANG, H.; ZHANG, W.; ZHANG, X.; FENG, B. Efficient enzyme-selective synthesis of monolauryl mannose in a circulating fluidized bed reactor. **Process Biochemistry**, vol. 66, p. 28-32, 2017. DOI https://doi.org/10.1016/j.procbio.2017.12.002.

JUHL, P. B.; DODERER, K.; HOLLMANN, F.; THUM, O.; PLEISS, J. Engineering of *Candida antarctica* lipase B for hydrolysis of bulky carboxylic acid esters. **Journal of Biotechnology**, vol. 150, no. 4, p. 474–480, 2010. DOI http://dx.doi.org/10.1016/j.jbiotec.2010.09.951.

KAPOOR, M.; GUPTA, M. N. Lipase promiscuity and its biochemical applications. **Process Biochemistry**, vol. 47, no. 4, p. 555–569, 2012. DOI http://dx.doi.org/10.1016/j.procbio.2012.01.011.

KERTHY, S.; IBINGA, K.; FABRE, J.; BIKANGA, R.; LOUIS, B. Atypical reaction media and organized systems for the synthesis of low-substitution sugar esters. **Frontiers in Chemistry**, vol. 7, p. 587, 2019. DOI https://doi.org/10.3389/fchem.2019.00587.

KHAN, N. R.; RATHOD, V. K. Enzyme catalyzed synthesis of cosmetic esters and its intensification: A review. **Process Biochemistry**, vol. 50, no. 11, p. 1793–1806, 2015a. DOI http://dx.doi.org/10.1016/j.procbio.2015.07.014.

KOBAYASHI, T. Lipase-catalyzed syntheses of sugar esters in non-aqueous media. **Biotechnology Letters**, vol. 33, no. 10, p. 1911–1919, 2011. DOI https://doi.org/10.1007/s10529-011-0663-z.

KOBAYASHI, T.; EHARA, T. Efficient synthesis of 6-O-palmitoyl-1,2-O-isopropylidene-a-D-glucofuranose in an organic solvent system by lipase-catalyzed

esterification. **Biotechnology Letters**, vol. 32, no. 11, p. 1679–1684, 2010. DOI https://doi.org/10.1007/s10529-010-0334-5.
KUMARI, A.; MAHAPATRA, P.; GARLAPATI, V. K.; BANERJEE, R. Enzymatic transesterification of Jatropha oil. **Biotechnology for Biofuels**, vol. 2, p. 1, 2009. DOI https://doi.org/10.1186/1754-6834-2-1.
KUNDYS, A.; BIAŁECKA-FLORJAŃCZYK, E.; FABISZEWSKA, A.; MAŁAJOWICZ, J. *Candida antarctica* lipase B as catalyst for cyclic esters synthesis, their polymerization and degradation of aliphatic polyesters. **Journal of Polymers and the Environment**, vol. 26, no. 1, p. 396–407, 2018. DOI https://doi.org/10.1007/s10924-017-0945-1.
LEARY, B. C. O.; KVIST, K.; BAYLISS, H. R.; DERROIRE, G.; HEALEY, J. R.; HUGHES, K.; KLEINSCHROTH, F.; SCIBERRAS, M.; WOODCOCK, P.; PULLIN, A. S. The reliability of evidence review methodology in environmental science and conservation. **Environmental Science and Policy**, vol. 64, p. 75–82, 2016. DOI http://dx.doi.org/10.1016/j.envsci.2016.06.012.
LI, L.; JI, F.; WANG, J.; LI, Y.; BAO, Y. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. **Enzyme and Microbial Technology**, vol. 69, p. 46–53, 2015. DOI https://doi.org/10.1016/j.enzmictec.2014.12.003.
LIANG, M. Y.; CHEN, Y.; BANWELL, M. G.; WANG, Y.; LAN, P. Enzymatic preparation of a homologous series of long-chain 6-O-acylglucose esters and their evaluation as emulsifiers. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 15, p. 3949–3956, 2018. DOI https://doi.org/10.1021/acs.jafc.8b00913.
LIN, X. S.; ZHAO, K. H.; ZHOU, Q. L.; XIE, K. Q.; HALLING, P. J.; YANG, Z. synthesis of glucose laurate with excellent productivity. **Bioresources and Bioprocessing**, vol. 3, no. 2, p. 1–7, 2016. DOI https://doi.org/10.1186/s40643-015-0080-6.
MAI, N. L.; AHN, K.; BAE, S. W.; SHIN, D. W.; MORYA, V. K.; KOO, Y. M. Ionic liquids as novel solvents for the synthesis of sugar fatty acid ester. **Biotechnology Journal**, vol. 9, no. 12, p. 1565–1572, 2014. DOI https://doi.org/10.1002/biot.201400099.
MÉLINE, T.; MUZARD, M.; DELEU, M.; RAKOTOARIVONINA, H.; PLANTIER-ROYON, R.; RÉMOND, C. D-Xylose and L-arabinose laurate esters: Enzymatic synthesis, characterization and physico-chemical properties. **Enzyme and Microbial Technology**, vol. 112, p. 14–21, 2018. DOI https://doi.org/10.1016/j.enzmictec.2018.01.008.
MOHER, D.; LIBERATI, A.; TETZLAFF, J.; ALTMAN, D. G.; GROUP, T. P. Preferred reporting items for systematic reviews and meta-analyses: the prisma statement. **Plos Medicine**, vol. 6, no. 7, 2009. DOI https://doi.org/10.1371/journal.pmed.1000097.
NETA, N. A. S.; DOS SANTOS, J. C. S.; SANCHO, S. O.; RODRIGUES, S.; GONÇALVES, L. R. B.; RODRIGUES, L. R.; TEIXEIRA, J. A. Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions. **Food Hydrocolloids**, vol. 27, no. 2, p. 324–331, 2012. DOI http://dx.doi.org/10.1016/j.foodhyd.2011.10.009.
NETA, N. A. S.; TEIXEIRA, J. A.; RODRIGUES, L. R. Sugar ester surfactants: Enzymatic synthesis and applications in food industry. **Critical Reviews in Food Science and Nutrition**, vol. 55, no. 5, p. 595–610, 2015. DOI https://doi.org/10.1080/10408398.2012.667461.
NETA, N. A. S.; PERES, A. M.; TEIXEIRA, J. A.; RODRIGUES, L. R. Maximization of fructose esters synthesis by response surface methodology. **New Biotechnology**, vol. 28, no. 4, p. 349–355, 2011. DOI https://doi.org/10.1016/j.nbt.2011.02.007.

NOTT, K.; BROGNAUX, A. (Trans)esterification of mannose catalyzed by lipase B from *Candida antarctica* in an improved reaction medium using co-solvents and molecular sieve. **Preparative Biochemistry and Biotechnology**, vol. 42, no. 4, p. 348–363, 2012. DOI http://dx.doi.org/10.1080/10826068.2011.622330.

OGAWA, S.; ENDO, A.; KITAHARA, N.; YAMAGISHI, T.; AOYAGI, S.; HARA, S. Factors determining the reaction temperature of the solvent-free enzymatic synthesis of trehalose esters. **Carbohydrate Research**, vol. 482, p. 107739, 2019. DOI https://doi.org/10.1016/j.carres.2019.06.018.

ORTIZ, C.; FERREIRA, M. L.; BARBOSA, O.; DOS SANTOS, J. C. S.; RODRIGUES, R. C.; BERENGUER-MURCIA, Á.; BRIAND, L. E.; FERNANDEZ-LAFUENTE, R. Novozym 435: the "perfect" lipase immobilized biocatalyst? **Catalysis Science & Technology**, vol. 9, no. 10, p. 2380–2420, 2019. DOI https://doi.org/10.1039/C9CY00415G.

PAEZ, A. Gray literature: An important resource in systematic reviews. **Journal of Evidenced-Based Medicine**, vol. 10, no. 3, p. 233-240, 2017. DOI https://doi.org/10.1111/jebm.12266.

PAPPALARDO, V. M.; BOERIU, C. G.; ZACCHERIA, F.; RAVASIO, N. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, vol. 433, p. 383–390, 2017. DOI http://dx.doi.org/10.1016/j.mcat.2017.02.029.

PERIN, G. B.; FELISBERTI, M. I. Enzymatic synthesis and structural characterization of methacryloyl-D-fructose- and methacryloyl-D-glucose-based monomers and poly(methacryloyl-D-fructose)-based hydrogels. **Journal of Chemical Technology and Biotechnology**, vol. 93, no. 6, p. 1694-1704, 2018. DOI https://doi.org/10.1002/jctb.5542.

PETERSEN, K.; VAKKALANKA, S.; KUZNIARZ, L. Guidelines for conducting systematic mapping studies in software engineering: An update. **Information and Software Technology**, vol. 64, p. 1–18, 2015. DOI http://dx.doi.org/10.1016/j.infsof.2015.03.007.

PÖHNLEIN, M.; SLOMKA, C.; KUKHARENKO, O.; GÄRTNER, T.; WIEMANN, L. O.; SIEBER, V.; SYLDATK, C.; HAUSMANN, R. Enzymatic synthesis of amino sugar fatty acid esters. **European Journal of Lipid Science and Technology**, vol. 116, p. 423–428, 2014. DOI http://dx.doi.org/10.1002/ejlt.201300380.

POPE, C.; MAYS, N.; POPAY, J. **Synthesizing qualitative and quantitative health evidence. a guide to methods.** London, United Kingdom: Buckingham: Open University Press, 2007.

POPOVICH, I.; WINDSOR, B.; JORDAN, V.; SHOWELL, M.; SHEA, B.; FARQUHAR, C. M. Methodological quality of systematic reviews in subfertility: a comparison of two different approaches. **Plos One**, vol. 7, no. 12, p. 50403, 2012. DOI https://doi.org/10.1371/journal.pone.0050403.

PUAT, S.B.A.; TAIB, M.; SUHAIMI, H. The catalytic properties of htab-alkanol-hydrocarbon-water microemulsion system for esterification. **Journal of Sustainability Science and Management**, vol. 5, no. 1, p. 87–93, 2010.

PULLIN, A. S.; STEWART, G. B. Guidelines for systematic review in conservation and environmental management. **Conservation Biology**, vol. 20, no. 6, p. 1647–1656, 2006. DOI https://doi.org/10.1111/j.1523-1739.2006.00485.x.

R CORE. R: A language and environment for statistical computing. Vienna, 2019.

RAHMAN, M.; ARUMUGAN, M.; KHAIRUDDIN, N. S. K.; ABDULMALEK, E.; BASRI, M.; SALLEH, A. Microwave assisted enzymatic synthesis of fatty acid sugar ester in ionic liquid-tert-butanol biphasic solvent system. **Asian Journal of Chemistry**,

vol. 24, p. 5058–5062, 2012.
RAVELO, M.; FUENTE, E.; BLANCO, Á.; LADERO, M.; GARCÍA-OCHOA, F. Esterification of glycerol and ibuprofen in solventless media catalyzed by free CALB: Kinetic modelling. **Biochemical Engineering Journal**, vol. 101, p. 228–236, 2015. DOI http://dx.doi.org/10.1016/j.bej.2015.06.002.
REETZ, M. T. Lipases as practical biocatalysts. **Current Opinion in Chemical Biology**, vol. 6, no. 2, p. 145–150, 2002. DOI https://doi.org/10.1016/S1367-5931(02)00297-1.
ROBERTS, P. D.; STEWART, G. B.; PULLIN, A. S. Are review articles a reliable source of evidence to support conservation and environmental management ? A comparison with medicine. **Biological Conservation**, vol. 132, p. 409-423, 2006. DOI https://doi.org/10.1016/j.biocon.2006.04.034.
RODRIGUES, R. C.; VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, Á.; ALCANTARA, A. R.; BARBOSA, O.; ORTIZ, C.; FERNANDEZ-LAFUENTE, R. Immobilization of lipases on hydrophobic supports: immobilization mechanism, advantages, problems, and solutions. **Biotechnology Advances**, vol. 37, no. 5, p. 746–770, 2019. DOI https://doi.org/10.1016/j.biotechadv.2019.04.003.
ROMANELLI, J. P.; MELI, P.; NAVES, R. P.; ALVES, M. C.; RODRIGUES, R. R. Reliability of evidence review methods in restoration ecology. **Conservation Biology**, vol. 35, no. 1, p. 142-154, 2020. DOI https://doi.org/10.1111/cobi.13661.
SHEA, B. J.; BOUTER, L. M.; PETERSON, J.; BOERS, M.; ANDERSSON, N.; ORTIZ, Z.; RAMSAY, T.; BAI, A.; SHUKLA, V. K. External validation of a measurement tool to assess systematic reviews (AMSTAR). **Plos One**, vol. 2, no. 12, 2007. DOI https://doi.org/10.1371/journal.pone.0001350.
SHI, Y. G.; LI, J. R.; CHU, Y. H. Enzyme-catalyzed regioselective synthesis of sucrose-based esters. **Journal of Chemical Technology and Biotechnology**, vol. 86, no. 12, p. 1457–1468, 2011. DOI https://doi.org/10.1002/jctb.2711.
SHIN, D. W.; MAI, N. L.; BAE, S. W.; KOO, Y. M. Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. **Enzyme and Microbial Technology**, vol. 126, p. 18–23, 2019. DOI https://doi.org/10.1016/j.enzmictec.2019.03.004.
SIEBENHALLER, S.; HAJEK, T.; MUHLE-GOLL, C.; HIMMELSBACH, M.; LUY, B.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.; HAHN, T.; ZIBEK, S.; SYLDATK, C. Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. **Bioresources and Bioprocessing**, vol. 4, no. 25, 2017. DOI https://doi.org/10.1186/s40643-017-0155-7.
SIEBENHALLER, S.; KIRCHHOFF, J.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.; MUHLE-GOLL, C.; LUY, B.; HAITZ, F.; HAHN, T.; ZIBEK, S.; SYLDATK, C.; OCHSENREITHER, K. Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. **Frontiers in Chemistry**, vol. 6, no. 421, 2018. DOI https://doi.org/10.3389/fchem.2018.00421.
SIMONS, A.; BROCKE, J. V.; NIEHAVES, B.; NIEHAVES, B.; REIMER, K.; PLATTFAUT, R.; CLEVEN, A. **Reconstructing the giant: on the importance of rigour in documenting the literature search process**. 161: ECIS 2009 Proceedings, 2009.
SIÓDMIAK, T.; MANGELINGS, D.; VANDER HEYDEN, Y.; ZIEGLER-BOROWSKA, M.; MARSZAŁŁ, M. P. High enantioselective Novozym 435-catalyzed esterification of (R,S)-flurbiprofen monitored with a chiral stationary phase. **Applied Biochemistry and Biotechnology**, vol. 175, no. 5, p. 2769–2785, 2015. DOI https://doi.org/10.1007/s12010-014-1455-4.
SOULTANI, S.; ENGASSER, J. M.; GHOUL, M. Effect of acyl donor chain length and

sugar/acyl donor molar ratio on enzymatic synthesis of fatty acid fructose esters. **Journal of Molecular Catalysis B: Enzymatic**, vol. 11, no. 4–6, p. 725–731, 2001. DOI https://doi.org/10.1016/S1381-1177(00)00162-4.

STEWART, G. Meta-analysis in applied ecology. **Biology Letters**, vol. 6, no. 1, p. 78-81, 2016. DOI https://doi.org/10.1098/rsbl.2009.0546.

SUGIHARA, J. M. Relative reactivities of hydroxyl groups of carbohydrates. **Advances in Carbohydrate Chemistry**, vol. 8, p. 1–44, 1953. DOI https://doi.org/10.1016/S0096-5332(08)60097-1.

SUTILI, F. K.; NOGUEIRA, D. D. O.; LEITE, S. G. F.; MIRANDA, L. S. M.; DE SOUZA, R. O. M. A. Lipase immobilized in microemulsion based organogels (MBGs) as an efficient catalyst for continuous-flow esterification of protected fructose. **RSC Advances**, vol. 5, no. 47, p. 37287–37291, 2015. DOI http://dx.doi.org/10.1039/C5RA04686F.

TAN, J.; DOU, Y. Deep eutectic solvents for biocatalytic transformations: focused lipase-catalyzed organic reactions. **Applied Microbiology and Biotechnology**, 2020. DOI https://doi.org/10.1007/s00253-019-10342-y.

TARAHOMJOO, S.; ALEMZADEH, I. Surfactant production by an enzymatic method. **Enzyme and Microbial Technology**, vol. 33, no. 1, p. 33–37, 2003. DOI https://doi.org/10.1016/S0141-0229(03)00085-1.

TRANFIELD, D.; DENYER, D.; SMART, P. Towards a methodology for developing evidence-informed management knowledge by means of systematic review. **British Journal of Management**, vol. 14, no. 3, p. 207–222, 2003. DOI https://doi.org/10.1111/1467-8551.00375.

VAN ECK, N. J.; WALTMAN, L. Software survey: VOSviewer , a computer program for bibliometric mapping. **Scientometrics**, vol. 84, no. 2, p. 523–538, 2010. DOI https://doi.org/10.1007/s11192-009-0146-3.

VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

VESCOVI, V.; GIORDANO, R. L. C.; MENDES, A. A.; TARDIOLI, P. W. Immobilized lipases on functionalized silica particles as potential biocatalysts for the synthesis of fructose oleate in an organic solvent/water system. **Molecules**, vol. 22, no. 2, 2017. DOI https://doi.org/10.3390/molecules22020212.

VESCOVI, V.; KOPP, W.; GUISÁN, J. M.; GIORDANO, R. L. C.; MENDES, A. A.; TARDIOLI, P. W. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octyl and multifunctional amino-glutaraldehyde spacer arms. **Process Biochemistry**, vol. 51, no. 12, p. 2055–2066, 2016. DOI http://dx.doi.org/10.1016/j.procbio.2016.09.016.

WHITTAKER, R. J. Meta-analysis and mega-mistakes: calling time on meta-analysis of the species richness-productivity relationship. **Ecology**, vol. 91, no. 9, p. 2522–2533, 2010. https://www.jstor.org/stable/27860825.

WOODCOCK, P.; PULLIN, A. S.; KAISER, M. J. Evaluating and improving the reliability of evidence syntheses in conservation and environmental science: A methodology. **Biological Conservation**, vol. 176, p. 54–62, 2014. DOI http://dx.doi.org/10.1016/j.biocon.2014.04.020.

YANG, R.; ZHAO, X.; LIU, X. Novel and highly efficient regioselective route to helicid esters by Lipozyme TLL. **Plos One**, vol. 8, no. 11, p. 11–16, 2013. DOI https://doi.org/10.1371/journal.pone.0080715.

YE, R.; HAYES, D. G.; BURTON, R. Effects of particle size of sucrose suspensions and

pre-incubation of enzymes on lipase-catalyzed synthesis of sucrose oleic acid esters. **Journal of the American Oil Chemists' Society**, vol. 91, p. 1891–1901, 2014. DOI https://doi.org/10.1007/s11746-014-2537-8.
ZHANG, X.; NIE, K.; WANG, M.; LIU, L.; LI, K.; WANG, F.; TAN, T.; DENG, L. Site-specific xylitol dicaprate ester synthesized by lipase from *Candida sp*. 99-125 with solvent-free system. **Journal of Molecular Catalysis B: Enzymatic**, vol. 89, p. 61–66, 2013. DOI http://dx.doi.org/10.1016/j.molcatb.2012.12.006.
ZHAO, K.; CAI, Y.; LIN, X.; XIONG, J.; HALLING, P. J. Enzymatic synthesis of glucose-based fatty acid esters in bisolvent systems containing ionic liquids or deep eutectic solvents. **Molecules**, vol. 21, no. 10, p. 1294, 2016. DOI https://doi.org/10.3390/molecules21101294.
ZUMSTEG, J. M.; COOPER, J. S.; NOON, M. S. Systematic review checklist a standardized technique for assessing and reporting reviews of life cycle assessment data. **Journal of Industrial Ecology**, vol. 16, p. 12-21, 2012. DOI https://doi.org/10.1111/j.1530-9290.2012.00476.x.

3.2. A REVIEW ON THE PRODUCTION AND RECOVERY OF SUGARS FROM LIGNOCELLULOSICS FOR USE IN THE SYNTHESIS OF BIOPRODUCTS

3.2.1. Introduction

In the upcoming decades, the replacement of fossil resources demands cost-effective and technically feasible solutions. Renewable carbon sources from lignocellulosic biomasses (LBs) are among the promising options (CANILHA *et al.*, 2012; FARMANBORDAR; AMIRI; KARIMI, 2020; NEUMANN *et al.*, 2016; SANTIBÁÑEZ *et al.*, 2020). LB is inexpensive, environmentally friendly, and one of the most abundant yet underutilized resources in the world (ANWAR; GULFRAZ; IRSHAD, 2014; RAVINDRAN; JAISWAL, 2016; TAYYAB *et al.*, 2018). It mainly consists of cellulose (40–50% on average), hemicellulose (25–30%), lignin (15–20%), and traces of pectin, nitrogen, and inorganic compounds (BORGES *et al.*, 2014; CHEN *et al.*, 2017; FONSECA, 2015; ROJAS *et al.*, 2014). This biomass's high degree of polymerization makes its cell walls stable and difficult to degrade (BEHERA *et al.*, 2014; CHAULA *et al.*, 2014). Thus, methods for converting plant material into monomeric sugars (mostly glucose from cellulose and xylose from xylan) are required for their further use as feedstocks in the production of a range of value-added products (RAVINDRAN; JAISWAL, 2016). These procedures comprise pretreatment and enzymatic hydrolysis (EH) steps (ANGARITA *et al.*, 2015; BATISTA *et al.*, 2019; BURKE *et al.*, 2009a; CANILHA *et al.*, 2012; DELABONA *et al.*, 2012, 2013; FURLAN *et al.*, 2012; RODRÍGUEZ-ZÚÑIGA *et al.*, 2015), which are generally undertaken in conjunction

with downstream sugar-recovery to meet the target product attributes (MILESSI-ESTEVES *et al.*, 2019).

Pretreatment is necessary to provide internal access to biomass by the enzymes that will be used in the hydrolysis stage, i.e., such process promotes enzyme and substrate interaction to stimulate the subsequent EH (CHANDRA; ARANTES; SADDLER, 2015; MICHALSKA *et al.*, 2012). Each pretreatment technology presents a different mechanism of action on the plant structure, inducing either physical or chemical modifications. Several approaches have been used for this process, such as autohydrolysis, acid hydrolysis, ammonia activation, kraft pulping, organic solvent pulping, hot water pretreatment, ammonia percolation, lime pretreatment, caustic solvent pulping, alkaline peroxide pretreatment, among other methods (Burke *et al*., 2009a, 2009b; Pourbafrani *et al*., 2014). The selection of a suitable pretreatment strategy depends on the LB source, economic viability, environmental sustainability of the process (minimizing chemical, heat, and power requirements) (FURLAN *et al.*, 2016; KUMAR *et al.*, 2009; LONGATI *et al.*, 2018; YU *et al.*, 2016, 2015), formation of degradation byproducts, process yield, production scale, as well as configurations employed in the hydrolysis and further processing (ABO *et al.*, 2019; BHUTTO *et al.*, 2017; HENDRIKS; ZEEMAN, 2009; SOLTANIAN *et al.*, 2020).

The first step in the EH process consists of breaking down cellulose and hemicellulose (containing xylan) into soluble oligosaccharides of glucose and xylose, respectively. Then, enzyme complexes are used to convert oligosaccharides to monomeric sugars suitable for fermentation or catalytic transformation to bioproducts (BURKE *et al*., 2009b). This approach is designed to maximize enzyme performance, controlling the effect of inhibitors while optimizing hydrolysis rates and sugar yields (BURKE *et al*., 2009a).

After obtaining monomeric sugars from lignocellulosics through an appropriate pretreatment method and EH, the material is recovered and purified to extract the desired sugar(s) (KUMAR; SHARMA; SINGH, 2017). Sugar recovery methods may include solid/liquid systems, chromatographic and membrane separations, ion exchange or crystallization, among others. These strategies can be applied to (1) separate the extracts from the non-treated biomass (physical separations); (2) recover the glucose and/or the xylose (the main monosaccharides resulting from the hydrolysis of the cellulosic and hemicellulosic fractions of the LB, respectively) (POLETTO *et al.*, 2020; SANTIBÁÑEZ *et al.*, 2020) through purification approaches, aiming for their later use in the synthesis of

a variety of products, including sugars, biofuels, and value-added chemicals. The use of the hexoses is widely known, but usage of pentose sugars is gaining more visibility in this realm as well, because of the opportunity to increase the sugar and process yields from lignocellulosics, since the hemicellulose stream was often underexploited after the LB hydrolysis (GENG *et al.*, 2019; VESCOVI; DOS SANTOS; TARDIOLI, 2017). Nevertheless, like many other commercial processes, the overall development of these processes demands the tailoring of specific attributes of the sugar products required for different applications (HUANG *et al.*, 2010; SAINI; CHANDEL; SHARMA, 2020), considering throughput and purity targets and, when applicable, the scale-up of the process (BANERJEE *et al.*, 2012; CHENG *et al.*, 2020; KOPPRAM *et al.*, 2013; WANG; UNREAN; FRANZÉN, 2016).

Here, we survey the peer-reviewed literature addressing the production of sugars from lignocellulosics, focusing on xylose use (with or without other sugars) as a substrate in the synthesis of biochemical products. Potential knowledge gaps and trends within this subject are also explored.

3.2.2. Methodology

3.2.2.1. Literature searching and database building

We performed a systematic search (ROMANELLI *et al.*, 2021a) of the peer-reviewed literature from the Web of Science platform (bibliographic resources - core collection: Science Citation Index Expanded (SCI-E) and Emerging Sources Citation Index (ESCI)), besides Scopus, CAB Direct, and SciELO platforms. We chose the SCI-E and ESCI database within the Web of Science platform because the first one covers the majority of significant scientific results, as well as other online database that also contain citation information such as Google Scholar and Science Direct, while the ESCI contains complete records of papers indexed by journals not yet covered by SCI-E. Journals indexed in ESCI reach the minimum standards of editorial quality, but as they are relatively new, they are still under evaluation to be indexed in SCI-E. Thus, relevant scientific results that can influence the bibliometric metrics can also be found in this database (ROMANELLI *et al.*, 2021b).

The following search string was used to retrieve titles, abstracts, and keywords of related publications: “lignocellulos*” AND ("sugar*" OR "carbohydrat*" OR "glycid*") AND (“enzym* hydrol*”) AND ("pre treat*" OR "pre-treat*"). The database was last updated on September 1, 2021. The full search history is available (Table S5 -

Supplementary Material). Web-searches first returned a total of 973 articles. After an analysis of coverage and overlapping to remove replicates, the database was reduced to 827 publications, which were first screened on their title and abstract to remove non-related articles. Only primary research (i.e., original data specific to a particular research study) that comprised the two following eligibility criteria were included: i) studies should address the production of sugars from lignocellulosics using pre-treatment and/or enzymatic hydrolysis steps; ii) studies should include methods to recover the sugars produced (physical separation of the extracts and/or purification techniques) aiming to use the xylose fraction (with or without other sugars) as feedstock to synthesize other products. Moreover, papers meeting these inclusion criteria that also contemplate the scale-up of the process (the entire operation or just a part of it) were identified and further discussed in this review.

After this first screening step, 137 papers were excluded (i.e., these records did not match any of our inclusion criteria). To ensure transparency at this stage, we listed the excluded studies along with the reasons for exclusion (papers initially excluded - supplementary material). After that, we performed the full-text assessment of the 690 remaining studies. Among them, only 58 papers used the xylose fraction (alone or in combination with other sugars) to synthesize other products. The other 632 papers obtained sugars from lignocellulosics after pretreatment and/or enzymatic hydrolysis but did not further process these carbohydrates into products. Considering that this literature review is focused on the use of monomers obtained after LB conversion, the final database used to inform the analysis and conclusions in this review comprised 58 primary studies. They were selected after the screening process summarized in Figure 3.6, which was designed following the criteria issued by the Collaboration for Environmental Evidence (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018). We note that there is no specialized organization devoted to guide evidence synthesis conducted in the realm of chemical engineering.

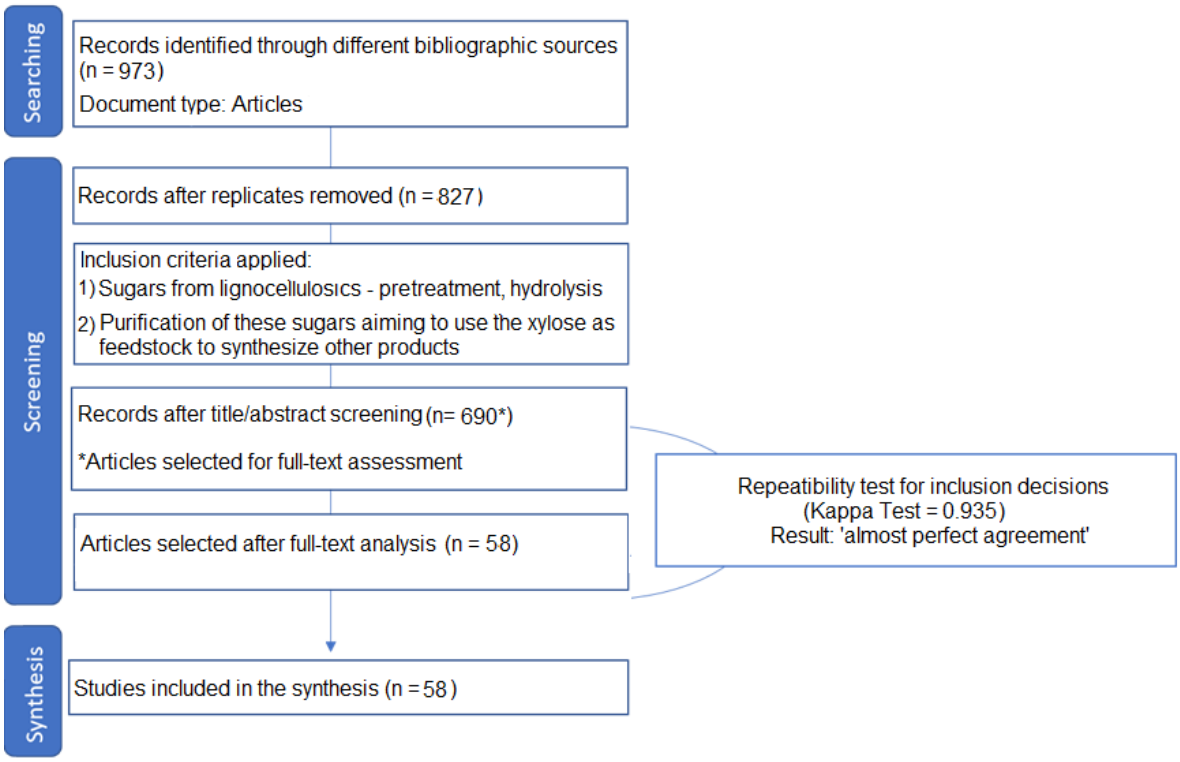


Figure 3.6. Flow Diagram for the selection of studies (Adapted from ROSES, Flow Diagram for Systematic Maps. Version 1.0).

Recognizing the potential for subjective decisions in the screening stage (inclusion-exclusions) (COLLABORATION FOR ENVIRONMENTAL EVIDENCE, 2018), a sample of retrieved papers (n = 40) was also analyzed by a second assessor. To achieve a high level of repeatability, assessors' decisions were compared using Cohen's kappa test of agreement. Scoring decisions (between assessors 1 and 2) were analyzed considering the percentage of agreement between assessors. Kappa values may range from 0 to 1, and higher values indicate greater agreement. Scores lower than 0.6 indicate inconsistencies among assessors so that the inclusion criteria must be redefined (COHEN, 1960). Further information on the kappa test is provided in the supplementary material (Table S6). We obtained a Kappa score of 0.935, which indicates excellent agreement between assessors, and that the decisions in the screening stage were satisfactorily repeatable.

3.2.2.2. Data analysis and presentation

Included articles (n=58) were subjected to data synthesis to elucidate relevant aspects of the LB bioconversion, including pretreatment techniques, enzymatic activities (EAs) (enzymatic hydrolysis stage), sugar-recovery methods, and sugar-derived compounds.

We used MS Excel (v. 2016) to perform calculations and OriginPro software (version 8.5) to plot graphs. The software R was used to conduct the analysis of coverage and overlapping (revtools package) (R CORE, 2019). This analysis is required after retrieving a list of references from different academic bibliographic sources (the fundamental stage of the systematic mapping). The traditional approach to review the retrieved evidence is to then sort the information, locating and removing duplicate entries by viewing titles and DOI (Digital Object Identifier) numbers. 'Revtools' provides the interface for this task.

We also conducted a co-occurrence analysis of keywords with the main methodologies and products present in our database through the construction of bibliometric maps (VAN ECK; WALTMAN, 2010). The co-occurrence analysis can be understood as the counting of paired data within a 'collection unit'. For example, considering the term 'pretreatment method' as a whole group of methodologies for treating the LB, within this group we may have 'liquid hot water (LHW)' and 'steam explosion (SE)' as an example of co-occurrence if they are both cited in the same study. Here the data are the 'LHW' and the 'SE', and the 'collection unit' is the group 'pretreatment method'. In this example, the paired data are {LHW and SE} and they occur once. Of course, more pretreatment methods can be cited at the same time in a primary study, so the pairings become more numerous as each item (e.g., LHW) is paired with each other item. For instance, if in addition to the two pretreatment methods, a third is also cited, say, ammonia fiber expansion (AFEX), then there are three pairings ({LHW and SE}, {LHW and AFEX}, {SE and AFEX}), each with a count of one. The 'collection unit' and the data therein can be varied (e.g., a questionnaire with the responses to the questions as the elements or a paper's bibliography with each cited author as the focus of the analysis). The combination of all the paired data within a "collection unit" forms the support for the analysis. In our study, we analyzed data from four different 'collection units': pretreatment methods, enzymatic activities, sugar-recovery methods, and sugar-derived products. The excel spreadsheet with the categorization of our data into 'collection units' is provided as a supplementary material to this review (co-occurrence analysis).

The analysis of co-occurrence aims to investigate the level of association among items of a 'collection unit'. If a pairing is done only once in the support, the association is tenuous or spurious. If the pairing is done many times, then the association is made stronger with each additional pairing. In the previous example, both pretreatment methods

{LHW and SE} were cited within the same study. If only one or two papers performed this scope of research and cited both methods, we would have little association. On the other hand, following the previously described example, if many studies cited the combination {LHW and AFEX} to perform their analysis, then we would have an extremely strong relationship between these two components for that certain scope of study.

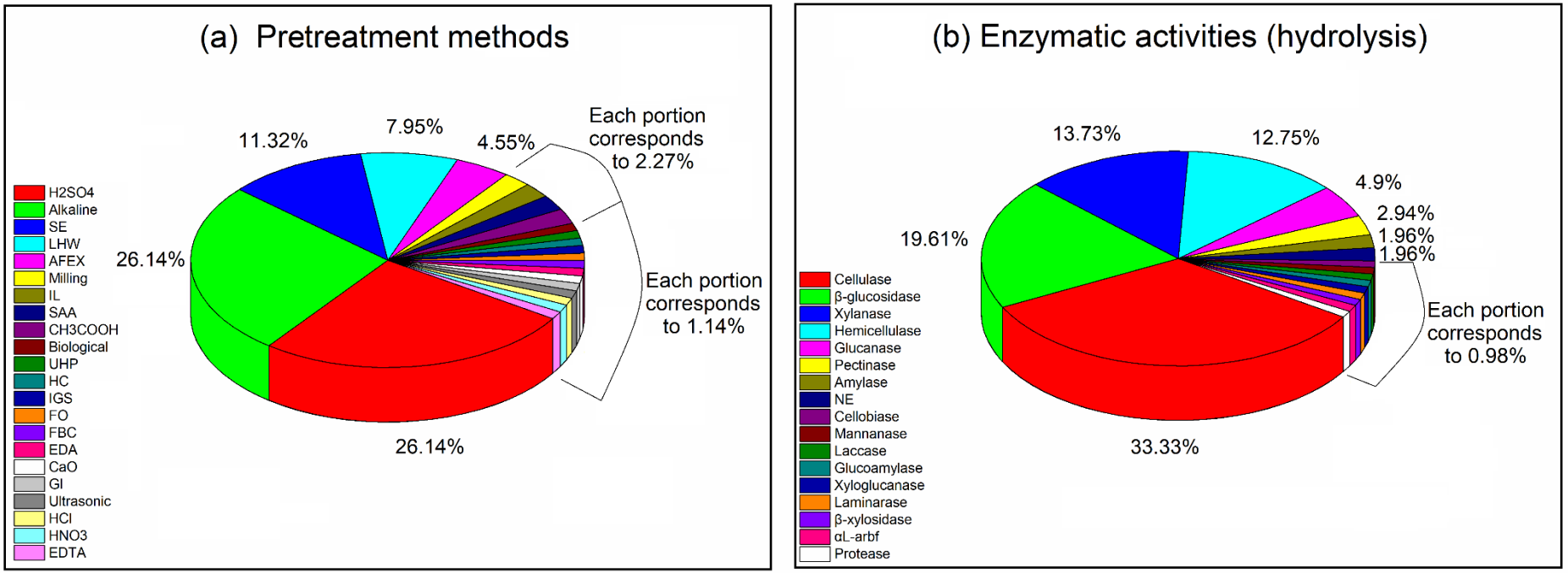
3.2.3. Results and discussion

The components of the system for obtaining sugars from lignocellulosics and the resulting sugar-derived products that are present in our database are available in Table S7 (Supplementary Material).

3.2.3.1. Pretreatment and enzymatic hydrolysis of the lignocellulosic biomass

We found that several pretreatment methodologies have been applied to improve enzyme accessibility and digestibility in the subsequent EH step. They include chemical, physical, physico-chemical, and biological methods, as shown in Figure 3.7 (a).

The H_2SO_4 acidolysis and the alkaline pretreatments (chemical methods) represented 52% of the occurrences. The first approach has the objective of solubilizing the hemicellulose, and by this, making the cellulose better accessible. The solubilized hemicelluloses (oligomers) can be subjected to hydrolytic reactions producing monomers, but also furfural, hydroxymethylfurfural (HMF), solubilized lignin, among other volatile degradation products (YANG; WYMAN, 2008). The degradation compounds that are produced during pretreatment may also be inhibitors that can have a significant influence on enzyme activity during the enzymatic hydrolysis stage. They can reduce enzyme activity, thus preventing some cellulose and hemicellulose from being liberated (FENG *et al.*, 2012). Additionally, the acid pretreatment with H_2SO_4 can be conducted with dilute acid (in concentrations of 0.2–2.5% w/w) or with concentrated acid. Most of the papers within our collection of relevant literature used the dilute H_2SO_4 method to perform the pretreatment. This method represents a more attractive option for the pretreatment of lignocellulosics because its inhibitor production, solubilization of hemicellulose, and precipitation of solubilized lignin are less pronounced than those of the strong acid method (BHUTTO *et al.*, 2017; HENDRIKS; ZEEMAN, 2009).



H2SO4: Sulfuric acid acidolysis; SE: Steam explosion; LHW: Liquid hot water; AFEX: Ammonia fibre expansion; IL: Ionic liquid; AHP: Alkaline hydrogen peroxide; SWH: Subcritical water hydrolysis; SAA: Soaking in aqueous ammonia; CH3COOH: Acetic acid acidolysis; SO2: Sulfur dioxide-impregnated steam explosion; UHP: Ultra-high pressure; HC: Hydrodynamic cavitation; IGS: Imadazole green solvent; FO: Fenton oxidation; FBC: Fenton-bipyridine catalyzed; EDA: Ethylenediamine; CaO: Calcium oxide; GI: Gamma irradiation; SPORL: Sulfite pretreatment to overcome recalcitrance of lignocelluloses; HCl: hydrochloric acid acidolysis; HNO3: Nitric acid acidolysis; EDTA: Ethylenediaminetetra acetic acid; NE: Not specified; αL-arbf: αL-arabinofuranosidase.

Figure 3.7. Pretreatment methods (a) and enzymatic activities (hydrolysis step) (b) used in the obtaining of xylose (and other sugars) from lignocellulosics (number of occurrences - %).

Conversely, other acid pretreatments (with HCl, HNO_3, and CH_3COOH) constituted only 4% of the occurrences in our dataset. Hydrochloric acid (HCl) is more volatile, easier to recover, and attacks biomass better than H_2SO_4; similarly, nitric acid (HNO_3) possesses good cellulose to-sugar conversion rates (BENSAH; MENSAH, 2013; TUTT; KIKAS; OLT, 2012). However, both acids are expensive compared to sulphuric acid. Moreover, the use of nitric acid would be undesirable due to the hazards associated with the production of nitrocellulose. The acetic acid (CH_3COOH) pretreatment is least effective at xylan solubilization, but it can dissolve more lignin than other acid pretreatments (CHEN *et al.*, 2020; ZHANG; PEI; WANG, 2016). We note the acetic acid can be a key factor in autohydrolysis (steam explosion and liquid hot water) processes, whereby structural acetate is released and catalyzes the hydrolysis of hemicellulose and cellulose. The use of acidic pretreatments can represent a better (or inferior) choice depending on the target final product. For example, acid pretreatment is more attractive for methane production than for ethanol production, because methanogens can handle compounds like furfural and HMF (degradation compounds produced during acid pretreatments) to a certain concentration and with an acclimatization period. For both ethanol and methane production, the chance on soluble lignin production is a risk, because this compound is often inhibiting for both processes. Methanogens are however, capable of adapting to such inhibitors (XIAO; CLARKSON, 1997).

During alkaline pretreatment, the first reactions taking place are solvation and saponification. These processes cause a swollen state of the biomass and make it more accessible for enzymes. This methodology is very efficient at solubilizing some types of lignin (the lignin solubilization rate varies with hard or softwoods and with straw, grasses, etc.) and part of the hemicellulose, and at reducing cellulose crystallinity. Nonetheless, it can also produce components that inhibit the later processing of these sugars (WOICIECHOWSKI *et al.*, 2020). Within our dataset, most papers (~77%) that performed the alkaline pretreatment used sodium hydroxide (NaOH) as the chemical agent of the process. To a minor extent, some other publications used calcium hydroxide ($Ca(OH)_2$) and sodium sulfite (Na_2SO_3), each one representing only ~9% of the studies. The use of NaOH was much more common in the literature we assessed because it usually led to a greater increase in the production of sugars (or sugar-derived products) over controls (i.e., without alkaline pretreatment) than the other alkaline pretreatment chemicals.

By way of comparison between alkaline and acid pretreatments (regarding the hydrolysis of hemicellulose), acids will clearly break down hemicellulose in the LB, but they also catalyze the formation of inhibitors, so, while you may get very good hemicellulose conversion, the xylose yield may not be as good because of the formation of these degradation products. Still, it is possible to balance temperature, reaction time, and pH to achieve a reasonable xylose yield. It is most effective to use mild conditions coupled with longer residence times to partially solubilize the xylan and use enzymes to break down the soluble oligosaccharides into monomers. The alkaline treatment, in turn, can be effective for partial lignin solubilization, and by breaking the lignin-hemicellulose-glucan bonds, some hemicellulose can be solubilized. However, the fraction solubilized tends to be less than that observed with acid-catalyzed pretreatment, and you still need xylanases to break down the solubilized fraction into xylose. On the other hand, one advantage of the alkaline pretreatment is that the xylose is preserved with little or no production of furfural or other inhibitors. Therefore, among these chemical pretreatment methods, it seems that there is no clear-cut "best method" to produce xylose. Furthermore, roughly 16% of the occurrences used both pretreatments combined (dilute acid + alkaline). With the aggressive conditions adopted in these classical pretreatments, they led to simple and fast processing with a high energetic yield, and generation of hazardous waste, despite giving low cellulosic/hemicellulosic sugar yields.

Given this context, some other technologies have gained commercial interest in an attempt to minimize some of these issues. LHW, SE, and AFEX pretreatments (physico-chemical methods) contributed to 24% of the occurrences in our database. In the LHW process, the acetyl groups attached to hemicelluloses are separated from the plant structure hydrolytically, using hot water at high temperatures and pressures, while also releasing organic acids (typically acetate). The release of acids decrease the pH of the reaction mixture, creating conditions for a mild acid hydrolysis (BURKE *et al*., 2009a). Two types of systems can be differentiated: subcritical operations (below the critical point of the water) and supercritical processes beyond this point (RUIZ *et al.*, 2020). Depending upon the severity of the reaction (it is usually performed at 150–230 °C for 10–50 min and pressures from 4.9–20 bar), degradation products may still be produced, such as furfural, HMF, formic acid, levulinic acid, and other organic compounds (BURKE *et al*., 2009a; RUIZ *et al*., 2020). Also, in this method, it is possible to separately recover the cellulose and hemicellulose sugar streams, but the hemicellulose sugar stream is usually of very low concentration and hence needs additional intensive

evaporation to remove water and yield appropriate sugar concentrations for further processing. This tends to increase capital and operating costs, though this drawback can be reduced through solvent recycle during extraction (RUIZ *et al.*, 2020).

In the SE pretreatment, biomass is exposed to high pressure and high-temperature steam for a designated period, after which a sudden depressurization leads to the rapid lease of vapor from within the biomass, dramatically reducing its size. During the depressurization process, the "exploded" material is ejected from the reactor and recovered in the explosion tank. The efficiency of this operation is generally influenced by two factors: the retention time and the temperature/pressure *(*BURKE *et al*., 2009a; JACQUET *et al*., 2015; SMICHI *et al*., 2020). As with the LHW pretreatment, the high temperatures of the SE method can promote the condensation and precipitation of soluble lignin, which, if not promptly removed, can block access to (hemi)cellulose and reduce biomass hydrolysis efficiency (GURAGAIN; VADLANI, 2021; LI; GELLERSTEDT, 2008). Depending upon the type of biomass and operating conditions, SE may lead to the formation of inhibitory compounds (AGBOR *et al.*, 2011; TEIXEIRA *et al.*, 2014). Compared with other pretreatment methods, SE represent a significantly lower environmental impact and capital investment for the process. Liquid hot water processes, for instance, increase the hydraulic load in the system and thus increase overall energy consumption while leading to the generation of many non-fermentable molecules (ABO *et al.*, 2019; SMICHI *et al.*, 2020). By comparison, the steam from the SE pretreatment is recovered and used downstream for product recovery/purification (e.g., distillation), and thus has a relatively lower energy footprint among lignocellulosic conversion processes.

AFEX, in turn, is a thermochemical methodology that use gaseous ammonia (with steam) as the main reactant for biomass pretreatment (CHUNDAWAT *et al.*, 2020; TEYMOURI *et al.*, 2005). During this process, biomass and gaseous ammonia are in contact in the presence of different steam/ammonia loadings. Next, the biomass experiences a rapid depressurization during the release of ammonia/steam from the reactor (ZHAO; SHAO; CHUNDAWAT, 2020). AFEX is a dry-to-dry process that cause no significant change in the biomass composition and produces a low amount of inhibitory degradation compounds (BHUTTO *et al.*, 2017). However, ammonia is hazardous, malodorous, and has corrosive properties (BRODEUR *et al.*, 2011). This method also requires high capital and utility costs, mainly due to the requirement of an

ammonia recovery system (BHUTTO *et al.*, 2017) and ammonia containment at all vessels and transfer units such as pumps where ammonia is present.

Among pretreatment strategies infrequently reported in our dataset, ultrasonic, gamma irradiation (GI), and ultra-high pressure (UHP) methods each represented only 1% of the occurrences. Such technologies could be more explored within our research theme since they have the potential to generate less waste than the traditional approaches and reduce the production of impurities and inhibitors (JOE *et al.*, 2015; SEESURIYACHAN; KAWEE-AI; CHAIYASO, 2017; YANG *et al.*, 2011). Although these approaches are not always feasible, their effectiveness can be improved through the use of a combined pretreatment method. For example, Joe et al. (2015) combined GI and a low concentration of aqueous alkali solution to enhance the enzymatic digestibility of rice straw for the production of lipids. GI also increased the conversion of glucose-induced by the alkaline pretreatment. Nevertheless, these pretreatments have not been used at a pilot or commercial scale, and are not easily scalable, which presents a major barrier. Thus, in the biorefinery sector, a more holistic view of pretreatment strategies is required to secure the optimal use of the whole biomass. In general, the biomass pretreatment step should be cost-effective (avoiding expensive construction materials, catalysts, reagents, or neutralization steps), with low energy demands, and easily included in the process integration (BHUTTO *et al.*, 2017; RUIZ *et al.*, 2020). It is difficult to satisfy all of these objectives, and thus, strategic trade-offs are required, considering technical and financial metrics for the overall process. In Table 3.3, we summarize the main advantages and disadvantages of some of the pretreatment methods cited in this paper.

Table 3.3. Pros and cons of some pretreatment methods for processing lignocellulosic materials.

Pretreatment	Advantages	Disadvantages	References
H_2SO_4 acidolysis	Hydrolyzes hemicellulose and lignin; shorter retention time and smaller process vessels compared to other pretreatments	High costs; aggressive conditions; formation of inhibitors; low cellulosic/hemicellulosic sugar yields; generation of hazardous waste; high energetic demand; may promote the condensation and precipitation of soluble lignin	NANDA *et al*., 2014 REZANIA *et al.*, 2017
Alkaline	Hydrolyzes hemicellulose and lignin; increase accessible surface area	High costs; aggressive conditions; formation of inhibitors; low cellulosic/hemicellulosic sugar yields; generation of hazardous waste; high energetic demand; long residence times; irrecoverable salts	WYMAN *et al*., 2005 MOSIER *et al*., 2005

		formed and incorporated into biomass; low digestibility of softwoods	
Steam explosion	Hydrolyzes hemicellulose and lignin; cost-effective and low environmental impact process; low amount of inhibitors produced	High temperatures may catalyze inhibitor formation from sugars; may promote the solubilization and subsequent condensation and precipitation of soluble lignin	JACQUET *et al.*, 2015 SMICHI *et al.*, 2020
LHW	Elevated recovery rates for pentoses; cost-effective and low environmental impact process; low amount of inhibitors produced	High-energy consumption; high hydraulic load; longer retention times increase capital costs and may contribute to degradation of monosaccharides	RUIZ *et al.*, 2020 TOMÁS-PEJÓ *et al.*, 2008
AFEX	Hydrolyzes hemicellulose and lignin; cause no significant change in the biomass composition; low amount of inhibitors produced; increase accessible surface area and saccharification rate	High costs; ammonia is hazardous, malodorous, and has corrosive properties; not efficient for biomass with high lignin content	BHUTTO *et al.*, 2017 ZHAO *et al.*, 2020
Ultrasonic	Faster process; less waste generation than the traditional approaches; reduces the production of impurities and inhibitors	High capital and operating costs; high-energy consumption; lack of studies using this method at pilot or commercial scale	YANG *et al.*, 2011
GI	Mutagenic effect that increases cellulase activity; less waste generation than the traditional approaches; reduces the production of impurities and inhibitors	High capital and operating costs; lack of studies using this method at pilot or commercial scale	JOE *et al.*, 2015
UHP	less waste generation than the traditional approaches; reduces the production of impurities and inhibitors	High capital and operating costs; high-energy consumption; lack of studies using this method at pilot or commercial scale	SEESURIYACHAN *et al.*, 2017

Enzymatic hydrolysis is used to transform pretreated cellulose and hemicellulose into oligosaccharides and monomeric sugars (ABO *et al.*, 2019). The EH step operates with milder conditions and is more selective than chemical (acid) hydrolysis, reducing

inhibitor production. The high cost of enzymes remains a challenge for industrial-scale implementation. Accordingly, research efforts have currently focused on reducing enzyme costs by improving their activity, developing new cellulolytic cocktails (ABO *et al.*, 2019; JATOI *et al.*, 2021), and adapting pretreatment conditions to improve enzyme accessibility and biomass reactivity. For example, Wu *et al*. (2020) adapted pretreatment conditions by incorporating acid groups onto the lignin and Ling *et al*. (2020) conducted a novel pretreatment comprising levulinic acid based deep eutectic solvents.

Figure 3.7 (b) shows that 33% of the records we assessed used cellulase activities to obtain monomeric sugars, and nearly 85% of them were used in conjunction with other EAs. 46% of the occurrences used β-glucosidase and hemicellulase activities (each one appearing in 20 and 27% of the studies, respectively). The main use for these enzymes and their functions are further discussed below (Item 3.3). Also, within the literature we investigated, 13% of the occurrences used crude enzyme cocktails, and 82% of the studies employed commercial enzymes (enzyme complexes containing various EAs), which are more feasible and widely used for large-scale processes.

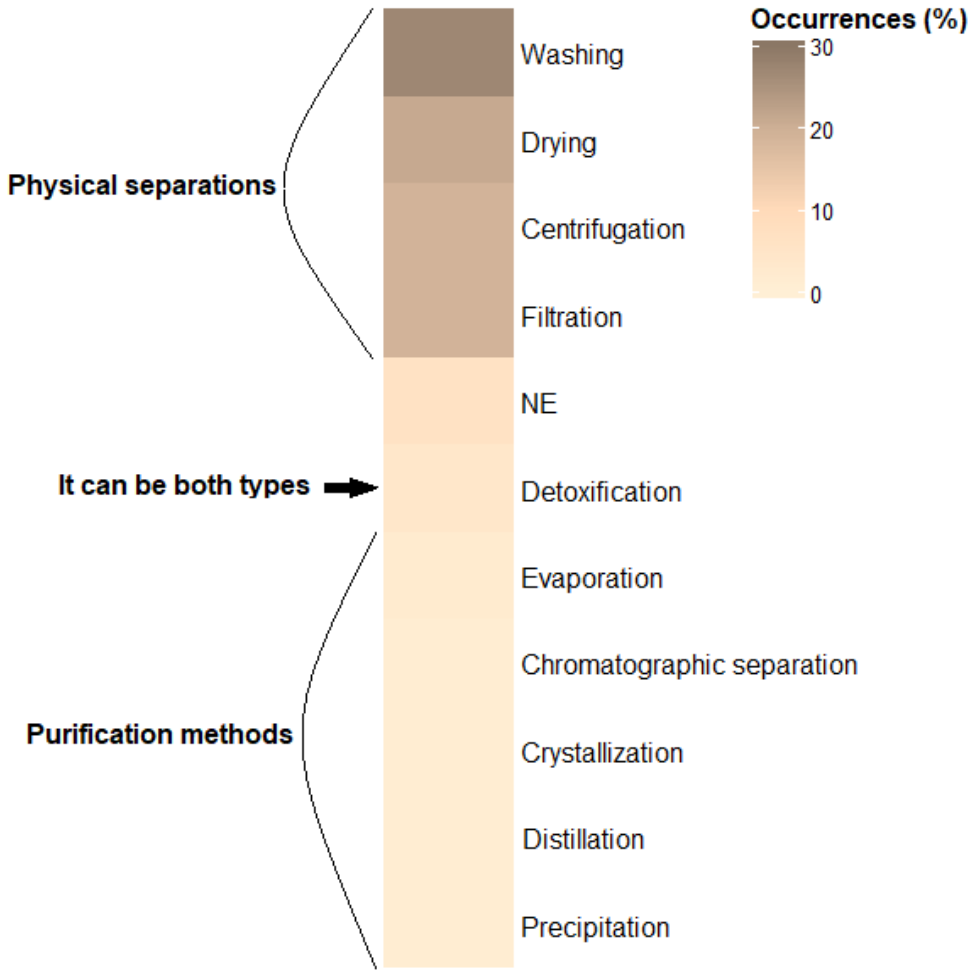
5% of the studies did not specify the EA used during the hydrolysis. Some other EAs (e.g., laccase, glucoamylase, xyloglucanase, laminarinase, β-xylosidase, αL-arabinofuranosidase, and protease) were occasionally reported in our database; each one corresponding to only ~1% of the occurrences. In terms of functionality, xyloglucanases catalyze the hydrolysis of xyloglucan in hemicellulose (BENKO *et al.*, 2008), β-xylosidases break down the nonreducing ends of xylo-oligosaccharides into xylose (HOWARD; JONES; PURDOM, 1959), and αL-arabinofuranosidases hydrolyze some terminal glycosidic linkages to arabinofuranosides of arabinan, arabinoxylan, and arabinogalactan (MARGOLLES; REYES-GAVILÁN, 2003). Glucoamylases and laminarinases promote the hydrolysis of starch/dextrin (SLIVINSKI *et al.*, 2011) and laminarim (SALYERS; PALMER; WILKINS, 1977), respectively, into glucose. Laccase play a role in the degradation of lignin (COHEN; PERSKY; HADAR, 2002) and proteases have been suggested to be essential for β-1,4-endo-glucanase activation (MIAO *et al.*, 2019). Despite the limited reporting of these enzymes in our database, they may still be relevant to the hydrolysis of lignocellulosic materials, particularly as a minor but important component of a multi-enzyme cocktail/blend.

Apart from the EAs used in this stage of the lignocellulosics bioconversion, the hydrolysis yield and rate can be affected by several other factors, which include: (i) type, source, efficiency, and loading of enzymes; (ii) nature of the pretreatment and

composition of the LB; (iii) accessible surface area and pore volume of the substrate; (iv) hydrolysis time; (v) cell wall thickness/coarseness (limits liquid penetration) (JATOI *et al.*, 2021; VASIĆ; KNEZ; LEITGEB, 2021). Physical factors such as solids loading, mixing, reactor design, and impeller configuration also play a role (GAONA; LAWRYSHYN; SAVILLE, 2021, 2019, 2015). The EH efficiency can also be influenced by the addition of non-ionic surfactants or polymers containing poly (ethylene glycol) to the hydrolysis medium or by the use of cost-effective additives such as soybean protein, tryptone, peptone, and maize zein, since they can reduce the enzyme loading required for effective hydrolysis and/or increase sugar yields (BRONDI *et al.*, 2019, 2020; CHENG *et al.*, 2020; SAHA *et al.*, 2017). The integration and optimization of all these process parameters may result in the commercial development of a pathway to produce high-value and commodity products.

3.2.3.2. Use of the xylose stream as feedstock to synthesize other products

The monomeric sugars obtained after the pretreatment and EH of lignocellulosics are the target molecules to be separated and/or purified to recover xylose (and other desired sugars) for use as feedstock in the synthesis of other compounds. Here, we assessed sugar recovery methods (physical separations and purification techniques) reported by our dataset. Among physical separation approaches (Figure 3.8), washing, drying, centrifugation, and (macro) filtration represented 86% of the occurrences (27, 21, 19, and 19%, respectively). These solid/liquid separation systems were simply used to recover the crude sugar extracts (containing a variety of monosaccharides, including xylose) for later use. Albeit they occurred between different stages of the overall process. The washing step, for example, was mainly employed after pretreatment (before hydrolysis) to remove inhibitors that would affect enzyme hydrolysis and fermentation. This process was usually followed by a drying step in an oven (lab scale) to concentrate extracts to be used during enzymatic hydrolysis, which would not be feasible at a larger scale, where other concentration approaches might be used (e.g., with a press, reverse osmosis, evaporation, etc.). Other methods (e.g., centrifugation and filtration) were typically employed after enzyme hydrolysis to recover crude hydrolysates.



NE: Not specified.

Figure 3.8. Assortment of sugar recovery methods (physical separations to collect crude extracts and purification methods) (number of occurrences - %).

Purification strategies (evaporation, distillation, precipitation, crystallization, and chromatographic separation) represented only 6% of the occurrences. This shows that most of the papers that we assessed further processed the crude extracts directly since these purification methodologies require more sophisticated equipment to recover a specific sugar stream from the hydrolysates, which may explain the less frequent use of these methods.

In 4% of studies, a detoxification method was used to detoxify crude extracts or enzymatic hydrolysates by targeting inhibitor(s) produced during lignocellulosics pretreatment. This technique could fit into either category (physical separation or purification). For example, we can remove inhibitors by washing, but also by chromatographic separation or evaporation. However, the detoxification process can be expensive and represent a large portion of the operational costs depending on the product, which is why the selection of the right method is of major importance (HUANG *et al.*,

2008). But even with high costs, these expenses can sometimes be justified by the value of the final product.

After the production and recovery of xylose and other sugars, they are usually processed catalytically, enzymatically, or by fermentation to obtain value-added chemicals. The xylose-derived products present in our dataset (not necessarily exclusively from xylose) are shown in Figure 3.9. Most publications (62%) used this C5 fraction to produce ethanol. Currently, the majority of the ethanol production comes from C-6 sugars, but poor or non-utilization of the C-5 portion is a serious impediment to the economic production of lignocellulosic ethanol (KAMOLDEEN *et al.*, 2017). The fermentation of xylose, however, is a challenging task (GIORDANO *et al.*, 2014; MILESSI-ESTEVES *et al.*, 2019). This is because the traditional microorganism used for bioethanol production, *Saccharomyces cerevisiae*, is not naturally able to metabolize xylose. Several strategies have been therefore developed to allow this yeast to generate metabolic intermediates that can be funneled into the endogenous metabolism through the pentose phosphate pathway (PPP) (KUHAD *et al.*, 2011; YOUNG; LEE; ALPER, 2010). Through a cascade of reactions in the PPP, the glycolytic intermediates are finally fermented to ethanol (BRAT; BOLES; WIEDEMANN, 2009).

Despite all the research effort to improve pentose processing so far, the fermentation rate of C-5 fractions is still remarkably slower than that of glucose. Major limitations include the lack of specific and efficient pentose transporters, the consumption of reaction intermediates by competing enzymatic reactions and pathways, and the fact that the acetyl content is normally higher in pentose-rich materials. So, the stress caused by acetic acid is more pronounced in xylose-rich hydrolysates (ALMEIDA *et al.*, 2011; OREB *et al.*, 2012). Some other microorganisms, such as *Candida shehatae*, *Scheffersomyces stipitis*, and *Pachysolen tannophilus* (MUSSATTO *et al.*, 2012; ROBAK; BALCEREK, 2020), have been used to promote pentose fermentation, but they exhibited slow cell growth during C-5 fermentation, leading to a decline in cell activity (LIGTHELM *et al.*, 1988) and the maintenance of high ethanol yields only for a short period (WIRAWAN *et al.*, 2020). In an attempt to minimize these issues, Wirawan *et al*. (2020) promoted media supplementation with a nutrient solution containing yeast extract, peptone, and xylose under aerobic conditions, which maintained the cellular activity during xylose fermentation. Still, further studies are required to ensure prolonged cell viability and activity.

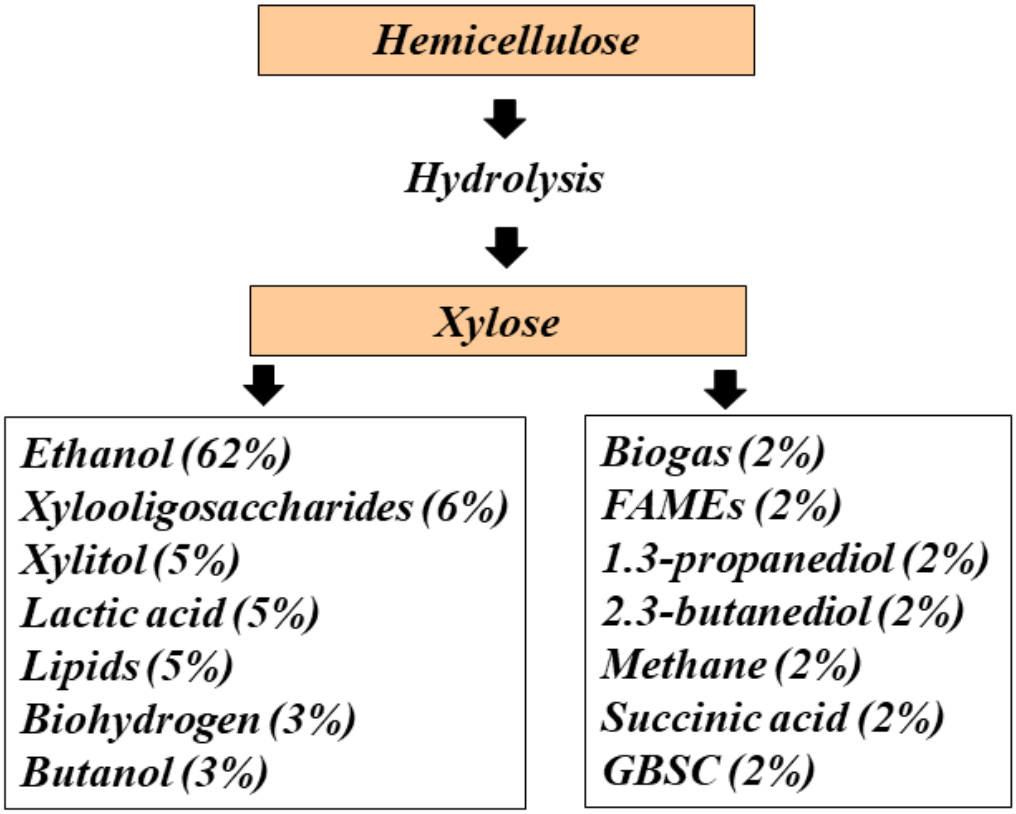


Figure 3.9. Xylose (and other sugars)-derived products (number of occurrences - %). FAMEs: Fatty acid methyl esters; GBSC: Growth of *Bacillus subtilis* cells.

The production of xylooligosaccharides (XOS) is another use for xylose that is gaining commercial interest (representing 6% of the occurrences in the records we evaluated). XOS are short-chain oligomers with prebiotic activity that have applications as ingredients in the nutraceutical industry (MILESSI *et al.*, 2016; NASCIMENTO *et al.*, 2016; SANTIBÁÑEZ *et al.*, 2020). Santibáñez *et al*. (2020) and Poletto *et al*. (2020) affirmed that the most generally recommended XOS production strategy is the two-stage approach through alkaline or acidic pretreatments and EH. However, more innovative strategies (using ultrasonic and UHP pretreatments followed by EH) were reported in our dataset. These approaches allowed the achievement of high XOS yields with a low degree of polymerization (POLETTO *et al.*, 2020).

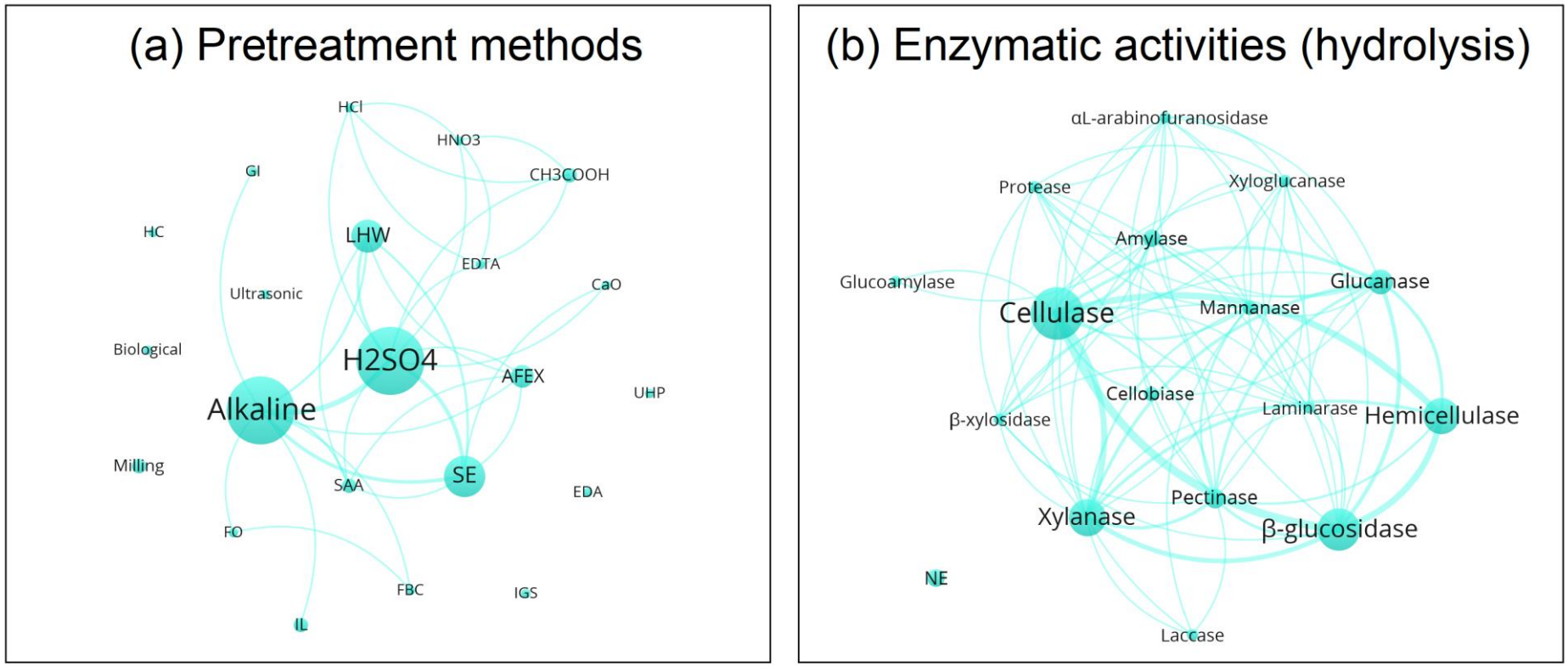
We also highlight the growing production of xylitol, lactic acid, and lipids from xylose, each representing 5% of the occurrences. Conversely, some other products (e.g., biogas, fatty acid methyl esters (FAMEs), diols, methane, succinic acid, and growth of *Bacillus subtilis* cells only constitute a minor fraction of the occurrences (up to 2% each) and, therefore, may represent an opportunity for new studies in these incipient realms.

3.2.3.3. Co-occurrence analysis of keywords

We conducted an analysis of co-occurrence among the main methodologies and products of our database. This assessment allows us to elucidate the structure, network distribution, and frequency of co-occurrence of these terms to clarify the research hotspots and fields that are still little explored, based upon the publications we retrieved.

Bibliometric maps revealed that 22 pretreatment methods and 17 EAs were cited by the studies we investigated (Figure 3.10). The arrangement of Figure 3.10 (a) depicts the use of more than one pretreatment method in ~33% of the papers, which may indicate that most of the studies are not evaluating the effects of varying the type of pretreatment on the characteristics of the target product. H_2SO_4 acidolysis and alkaline pretreatments presented the highest number of co-occurrences (N=9), followed by H_2SO_4 acidolysis and SE (N=5), alkaline and SE (N=4), and H_2SO_4 acidolysis and LHW (N=3). Also, two-step pretreatment protocols are gaining interest and can improve lignin removal versus conventional methods (i.e., simple pretreatments) (BARCELOS *et al.*, 2016; CHANDEL *et al.*, 2014; DA SILVA *et al.*, 2018; JASPREET; TAGGAR; KOCHER, 2019; KAUR; KUHAD, 2019; SHANKAR *et al.*, 2019; WISCHRAL *et al.*, 2019). It is widely known that lignin hinders the hydrolysis of lignocellulosic materials since this polyphenolic macromolecule offers a physical barrier that prevents access of enzymes to native cellulose and/or hemicellulose (WISCHRAL *et al.*, 2019), and may also contribute to non-specific binding of enzymes.

In Figure 3.10 (b), a co-occurrence network among EAs occurs in about 76% of the publications. This outcome suggests that the use of enzyme preparations containing different EAs served as a priority in studies to obtain sugars (including xylose) from lignocellulosics. We verified a high number of co-occurrences among cellulase and β-glucosidase activities (N=25), β-glucosidase and glucanase (N=6), as well as cellulase and glucanase (N=4). The network collaboration established among such EAs was already expected since cellulolytic cocktails comprise cellulases (to convert crystalline cellulose region into glucose, cellobiose, and cellooligosaccharides) (BAJAJ *et al.*, 2009; CHOI; JIAO; LEE, 2020), endoglucanases (which attack regions of low crystallinity, creating free-chain ends), exoglucanases (which remove cellobiose units from the free-chain ends), and β-glucosidases (which hydrolyze cellobiose to produce glucose) (NIGAM, 2013).



H2SO4: Sulfuric acid acidolysis; SE: Steam explosion; LHW: Liquid hot water; AFEX: Ammonia fibre expansion; IL: Ionic liquid; AHP: Alkaline hydrogen peroxide; SWH: Subcritical water hydrolysis; SAA: Soaking in aqueous ammonia; CH3COOH: Acetic acid acidolysis; SO2: Sulfur dioxide-impregnated steam explosion; UHP: Ultra-high pressure; HC: Hydrodynamic cavitation; IGS: Imadazole green solvent; FO: Fenton oxidation; FBC: Fenton-bipyridine catalyzed; EDA: Ethylenediamine; CaO: Calcium oxide; GI: Gamma irradiation; SPORL: Sulfite pretreatment to overcome recalcitrance of lignocelluloses; HCl: hydrochloric acid acidolysis; HNO3: Nitric acid acidolysis; EDTA: Ethylenediaminetetra acetic acid; NE: Not specified.

Figure 3.10. Bibliometric analysis of pretreatment methods (a) and enzymatic activities (hydrolysis step) (b) used in the obtaining of xylose (and other sugars) from lignocellulosics. The size of the node is proportional to the number of occurrences, and the thickness of the edges represents co-occurrences between items.

Hemicellulase systems, in turn, break down glycosidic bonds of the hemicellulose, promoting the degradation of polysaccharides into pentose sugars (e.g., xylose and arabinose) and hexose sugars (e.g., mannose, glucose, galactose, and fructose). These systems generally include endo- and exo-xylanases and xylosidases (TAHA *et al.*, 2016), which are specific EAs required to degrade the most common hemicellulose constituents. Some enzymatic activities contained in hemicellulase cocktails can also hydrolyze ester bonds of acetate or ferulic acid side groups in the plant cell wall structure (BROEKER *et al.*, 2018; LINTON, 2020; SAHA, 2003).

As most studies evaluated the use of xylose + glucose as feedstocks to synthesize different products, a great number of co-occurrences was found between cellulases and hemicellulases (N=16), cellulases and xylanases (N=13), β-glucosidases and hemicellulases (N=15), and β-glucosidases and xylanases (N=8). Accordingly, the bibliometric analysis presented in Figure 3.10 (b) indicated that research efforts have been directed towards the use of commercial enzyme complexes capable of hydrolyzing different polysaccharides during the EH stage. Yet, further research is required to enhance hemicellulose/cellulose to xylose/glucose conversions by finding appropriate LB structures. Also, new suitable pretreatment protocols that can increase the efficiency of EAs must be developed.

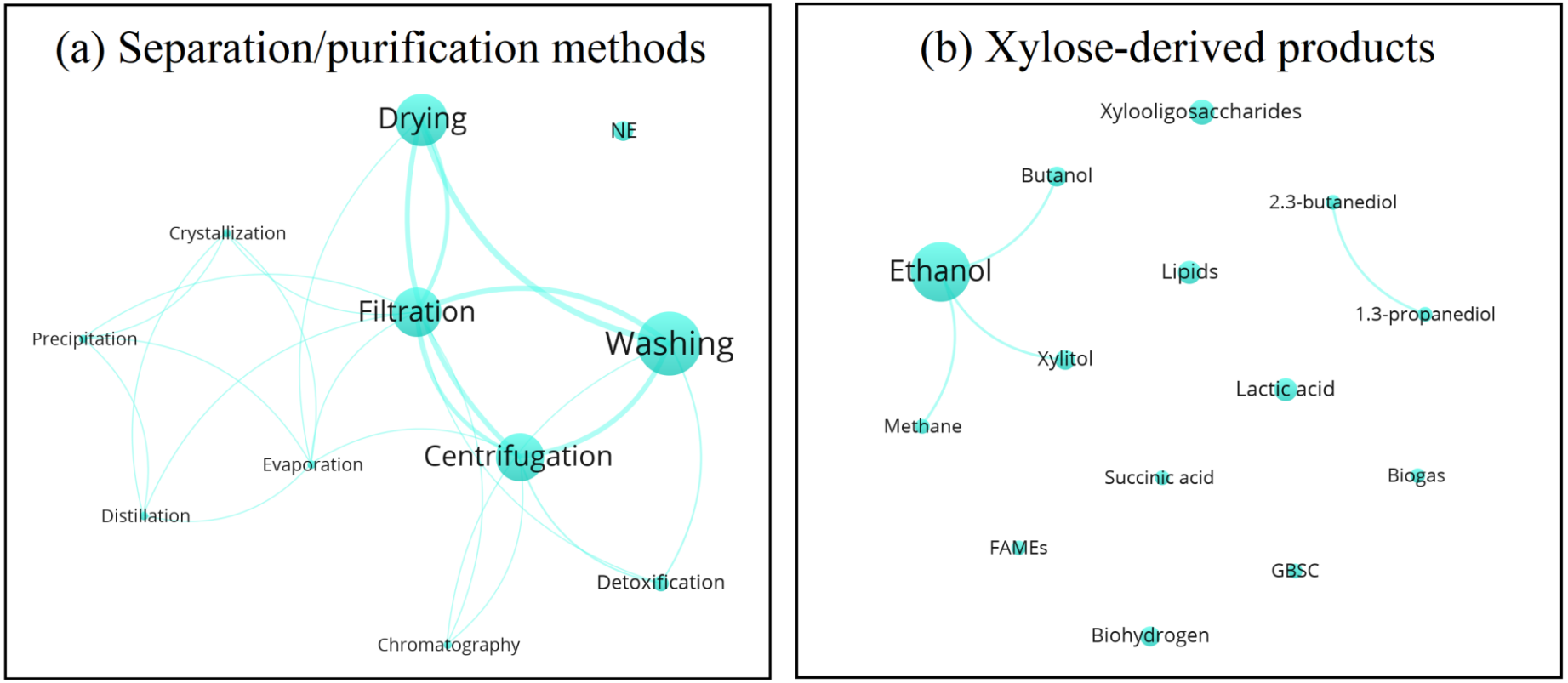
In Figure 3.11, 11 sugar recovery methods and 14 xylose-derived products were listed in the records we evaluated. In section (a), the strong interaction between most items of the mapping may be justified by the fact that ~60% of the publications addressed the recovery of sugars by more than one methodology. Among the remaining studies, 28% used only one technique, and 12% did not specify if any method was employed. The highest number of co-occurrences was identified between the following physical separations: (1) washing and drying (N=22); (2) (macro) filtration and washing (N=17); (3) centrifugation and washing (N=16); (4) centrifugation and drying (N=15); (5) (macro) filtration and drying (N=11); (6) centrifugation and (macro) filtration (N=10). The combination of these methods allowed the recovery of crude extracts (containing xylose and other sugars) for subsequent use to obtain a variety of products.

Some other procedures (purification methods), including distillation, precipitation, crystallization, and chromatographic separation that were less frequent in our dataset (1% of the occurrences each one) presented a high number of co-occurrences with other items of the mapping (mostly physical separations). In these cases, the extracts were first physically separated, xylose was recovered from the enzymatic hydrolysates

through one or more purification methods and then further processed (with or without other monosaccharides). This is distinct from most of the papers that simply used physical separations to get the crude extracts (without any subsequent purification step) for later use.

In Figure 3.11 (b), only ~9% of the papers obtained more than one product from the xylose, which indicates a weak co-occurrence network among xylose-derived goods. The simultaneous synthesis of different products from xylose was reported in five articles of our database (FRANCESCHIN *et al.*, 2011; HONG *et al.*, 2015; KOPPRAM *et al.*, 2013; NANDA; DALAI; KOZINSKI, 2014; ZUO *et al.*, 2012). Nanda, Dalai, and Kozinski (2014) synthesized ethanol and butanol from xylose and glucose after dilute acid pretreatment and EH of three lignocellulosic feedstocks (pinewood, timothy grass, and wheat straw). Franceschin *et al*. (2011) produced ethanol and xylitol from rye straw. A complete process from the feedstock to the end products was designed addressing all major operations: LHW pretreatment, fermentation to ethanol and xylitol, as well as the separation and purification of both products. Koppram *et al*. (2013) also synthesized ethanol and xylitol by evaluating the performance of *Saccharomyces cerevisiae* strains in the fermentation of xylose and glucose in hydrolysates of steam pretreated corncobs. Zuo *et al*. (2012) investigated the production of ethanol and methane from corn stover (CS).

After the development of a novel soaking pretreatment (NaOH and aqueous ammonia), the authors performed the SSF (simultaneous saccharification and fermentation) of pretreated CS for ethanol synthesis. For methane production, the re-soaked CS and residues of SSF were anaerobically digested. Lastly, Hong *et al*. (2015) obtained 1,3-propanediol and 2,3-butanediol from CS after a mild alkaline pretreatment and EH of cellulose and hemicellulose, followed by fermentation to diols. The other ~92% of papers obtained only one xylose-derived product per study. This lack of papers addressing the concomitant production of dissimilar products from xylose could open space for future researchers to develop new approaches with this goal. On the other hand, this might also be a strength, since the selective production of a single compound may make it easier to purify products, and multiple products may imply a difficult, less effective process.



NE: Not specified; FAMEs: Fatty acid methyl esters; GBSC: Growth of Bacillus subtilis cells.

Figure 3.11. Bibliometric analysis of separation/purification methods used to obtain xylose (and other sugars) from lignocellulosics (a) and sugar-derived products (b). The size of the node is proportional to the number of occurrences, and the thickness of the edges represents co-occurrences between items.

3.2.3.4. Scale-up of the process

Based upon optimal results from the production and recovery of sugars from LBs, as well as their further processing to obtain bioproducts, some studies selected appropriate conditions to scale up the process (Table 3.4).

Among these publications, Koppram *et al*. (2013) scaled up the SSF of xylose-rich hydrolysates of steam pretreated corn cobs from laboratory to demo scale. It was possible to successfully reproduce the SSF process at the demonstration scale. Results also suggested that the potential of SSF to synthesize ethanol and xylitol is improved in combination with pre-fermentation and a fed-batch process of substrate and enzymes. Banerjee *et al*. (2012) performed the integrated scale-up of the entire process (alkaline CS pretreatment, EH, pre-fermentation, and fermentation of xylose and glucose to ethanol). The treatment of the enzymatic hydrolysate with activated carbon before the fermentation step had little effect on glucose fermentation, but markedly improved the utilization of xylose, presumably due to the removal of soluble aromatic inhibitors. In addition, results indicated that the alkaline pretreatment is readily scalable and can be integrated with EH and fermentation.

Kamoldeen *et al*. (2017) assessed the potential of ethanol production from oil palm empty fruit bunches (OPEFB) by co-fermentation of its glucose and xylose components. The authors scaled up the bioconversion of mild alkaline-treated hydrolysates of OPEFB in the SSF process (from shake flasks to laboratory bioreactors). They achieved a far higher theoretical ethanol yield in the bioreactor than the one found using shake flasks. This outcome can be probably associated with the steadily controlled fermentation condition of the laboratory-scale bioreactor compared to the shake flask, which is subject to pH changes.

In the study conducted by Wang *et al*. (2016), the scale-up of the high-solids SSF of pretreated wheat straw was implemented through a multi-feed approach (a fed-batch operation with additions of substrates, enzymes, and cells). Following this strategy, reproducible and scalable results were obtained from SSF performed in a bioreactor containing up to 22% w/w insoluble solids. A high insoluble solids content is needed to reach product yields and titers that replicate conditions encountered in large-scale operations.

Table 3.4. Components of the system for obtaining sugars from lignocellulosics and sugar-derived products among studies that promoted process scale-up.

Pretreatment methods	Enzymatic activities (hydrolysis)	Sugar recovery methods	Sugar-derived products	Scales	Microorganisms	Productivities	References
SE	Cellulase, Hemicellulase, β-glucosidase	Centrifugation	Ethanol, Xylitol	From lab to Demo	*S. cerevisiae* KE6-12	~0.15 g/L/h (ethanol) ~0.005 g/L/h (xylitol)	KOPPRAM *et al*., 2013
Alkaline	Glucanase, Hemicellulase, β-glucosidase, Xylanase, Pectinase	Drying, Centrifugation Washing	Ethanol	From lab to Demo	*S. cerevisiae* Y35	~0.11 g/L/h	BANERJEE *et al*., 2012
Alkaline	Cellulase, Hemicellulase	Washing, Drying	Ethanol	From shake flasks to lab	*S. cerevisiae* ATCC 24858	0.21 g/L/h	KAMOLDEEN *et al*., 2017
SE, H_2SO_4	Cellulase, Hemicellulase, β-glucosidase	(Macro) Filtration	Ethanol	From lab to Demo	*S. cerevisiae* KE6-12.A	Up to ~0.4 g/L/h	WANG *et al*., 2016

SE: Steam explosion; H_2SO_4: H_2SO_4 acidolysis.

Some challenges associated with this methodology (e.g., increased mass transfer resistance and inhibitor concentrations) (KOPPRAM *et al.*, 2013) could be managed by effective mixing and reactor design/operation to account for slurry viscosity and enzyme-substrate interactions that affect the overall process performance (GAONA; LAWRYSHYN; SAVILLE, 2021, 2019, 2015). The optimization of these scalable systems allowing high solids loadings through experiments and simulations is key to acquiring industrially relevant data, distinct from the data available from smaller scale systems that must operate at lower solids content and non-representative conditions.

3.2.3.5. Limitations

Our search strategy was designed using two databases from the Web of Science platform (SCI-E and ESCI), besides Scopus, CAB Direct, and SciELO. We chose not to search for grey literature (i.e., evidence not published in commercial publications, such as thesis and dissertations, research and committee reports, government reports, conference papers, and ongoing research (GONÇALVES *et al.*, 2021b), limiting our dataset to primary studies published in scientific journals. We may have thus underestimated the available information and publications on the subject addressed in this study; our conclusions must be evaluated in light of these circumstances. Nonetheless, we consider this review as an important contribution to show the direction of research in this field.

3.2.4. Conclusions

This study revealed that some classical pretreatment methods (e.g., alkaline and acidolysis) are still among the most used methodologies for the initial processing of LB. However, the conditions used in these methods are aggressive, leading to the formation of degradation by-products, reducing cellulosic/hemicellulosic sugar yields, and requiring high levels of energy. Some other technologies, such as LHW and SE, were used to minimize some of these issues, mainly inhibitors and waste generation. Other environmentally friendly approaches (ultrasonic, GI, and UHP pretreatments) were little reported in our dataset but have mainly been confined to small-scale applications thus far, suggesting room for research to develop these techniques. Although high costs are associated with these strategies, their effectiveness can be improved using combined pretreatment methods (e.g., GI and alkaline pretreatments), which can enhance lignin removal compared to that obtained with single pretreatment techniques.

The EH stage was generally conducted using commercial enzyme cocktails (enzymatic complexes containing diverse EAs), which are more feasible and commonly used in large-scale processes than crude preparations. In addition, as most of the publications evaluated the use of xylose + glucose as feedstocks to synthesize different products, a great number of co-occurrences were found among EAs specifically designed for the hydrolysis of polysaccharides containing both sugars.

Most papers directly processed crude extracts (containing xylose and other sugars) from enzymatic hydrolysis slurries after simple physical separations, since purification methodologies require more sophisticated equipment to recover a specific sugar stream from the hydrolysates, which may explain the less frequent use of these methods. Detoxification methods were used post pretreatment or post hydrolysis when inhibitors needed to be removed. This increased process costs, although these costs can be justified for high-value products.

Ethanol was the main sugar-derived product addressed by the studies we assessed, despite (or perhaps because of) the challenges related to the production of ethanol from C-5 sugars. There is potential to address some of these challenges by media supplementation with nutrients, but further studies are still required to best enhance cell viability and activity. The production of XOS, xylitol, lactic acid, and lipids has gained more commercial interest, whereas products including biogas, FAMEs, diols, methane, and succinic acid are less commonly investigated. The lack of studies addressing the simultaneous production of different sugar-derived goods could open space for the development of new strategies in this regard or suggest that the synthesis of multiple products may imply a difficult, less effective process. Lastly, within the studies that selected appropriate conditions to scale-up the LB bioconversion, we noted that the use of systems allowing high solids loadings was key to reach product yields representative of commercial operations.

In general, all the LB treatment steps represent a challenge to the synthesis of bioproducts due to the generation of inhibitory compounds (reducing the efficiency of the production of sugars), the high capital and operating costs of pretreatment processes, the costly enzyme preparations (EH stage), and the complex structure of lignocellulosic materials, among other factors. This review showed that research in this field has established new approaches that address these problems and can facilitate industrial development, even though more large-scale testing is needed.

Funding

This work was supported by “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP) [grant numbers 2016/10636-8, 2019/23908-4, 2019/08533-4, 2021/06525-4, and 2021/07958-1]; in part by “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) [Finance Code 001] and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) [project numbers 315092/2020-3 and 141111/2020-8].

References

ABO, B. O.; GAO, M.; WANG, Y.; WU, C.; MA, H.; WANG, Q. Lignocellulosic biomass for bioethanol: An overview on pretreatment, hydrolysis and fermentation processes. **Reviews on Environmental Health**, vol. 34, no. 1, p. 57–68, 2019. DOI https://doi.org/10.1515/reveh-2018-0054.

AGBOR, V. B.; CICEK, N.; SPARLING, R.; BERLIN, A.; LEVIN, D. B. Biomass pretreatment: Fundamentals toward application. **Biotechnology Advances**, vol. 29, no. 6, p. 675–685, 2011. DOI http://dx.doi.org/10.1016/j.biotechadv.2011.05.005.

ALMEIDA, J. R. M.; RUNQUIST, D.; NOGUÉ, V. S. I.; LIDÉN, G.; GORWA-GRAUSLUND, M. F. Stress-related challenges in pentose fermentation to ethanol by the yeast *Saccharomyces cerevisiae*. **Biotechnology Journal**, vol. 6, p. 286–299, 2011. DOI https://doi.org/10.1002/biot.201000301.

ANGARITA, J. D.; SOUZA, R. B. A.; CRUZ, A. J. G.; BISCAIA, E. C.; SECCHI, A. R. Kinetic modeling for enzymatic hydrolysis of pretreated sugarcane straw. **Biochemical Engineering Journal**, vol. 104, p. 10–19, 2015. DOI http://dx.doi.org/10.1016/j.bej.2015.05.021.

ANWAR, Z.; GULFRAZ, M.; IRSHAD, M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. **Journal of Radiation Research and Applied Sciences**, vol. 7, no. 2, p. 163–173, 2014. DOI http://dx.doi.org/10.1016/j.jrras.2014.02.003.

BAJAJ, B. K.; PANGOTRA, H.; WANI, M. A.; SHARMA, P.; SHARMA, A. Partial purification and characterization of a highly thermostable and pH stable endoglucanase from a newly isolated *Bacillus* strain M-9. **Indian Journal of Chemical Technology**, vol. 16, no. 5, p. 382–387, 2009.

BANERJEE, G.; CAR, S.; LIU, T.; WILLIAMS, D. L.; MEZA, S. L.; WALTON, J. D.; HODGE, D. B. Scale-up and integration of alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis, and ethanolic fermentation. **Biotechnology and Bioengineering**, vol. 109, no. 4, p. 922–931, 2012. DOI https://doi.org/10.1002/bit.24385.

BARCELOS, C. A.; MAEDA, R. N.; SANTA ANNA, L. M. M.; PEREIRA, N. Sweet sorghum as a whole-crop feedstock for ethanol production. **Biomass and Bioenergy**, vol. 94, p. 46–56, 2016. DOI https://doi.org/10.1016/j.biombioe.2016.08.012.

BATISTA, G.; SOUZA, R. B. A.; PRATTO, B.; DOS SANTOS-ROCHA, M. S. R.; CRUZ, A. J. G. Effect of severity factor on the hydrothermal pretreatment of sugarcane straw. **Bioresource Technology**, vol. 275, p. 321–327, 2019. DOI https://doi.org/10.1016/j.biortech.2018.12.073.

BEHERA, S.; ARORA, R.; NANDHAGOPAL, N.; KUMAR, S. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. **Renewable and Sustainable Energy Reviews**, vol. 36, p. 91–106, 2014. DOI

http://dx.doi.org/10.1016/j.rser.2014.04.047.
BENKO, Z.; SIIKA-AHO, M.; VIIKARI, L.; RECZEY, K. Evaluation of the role of xyloglucanase in the enzymatic hydrolysis of lignocellulosic substrates. **Enzyme and Microbial Technology**, vol. 43, p. 109–114, 2008. DOI https://doi.org/10.1016/j.enzmictec.2008.03.005.
BENSAH, E. C.; MENSAH, M. Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. **International Journal of Chemical Engineering**, vol. 2013, 2013. DOI https://doi.org/10.1155/2013/719607.
BHUTTO, A. W.; QURESHI, K.; HARIJAN, K.; ABRO, R.; ABBAS, T.; BAZMI, A. A.; KARIM, S.; YU, G. Insight into progress in pre-treatment of lignocellulosic biomass. **Energy**, vol. 122, p. 724–745, 2017. DOI https://doi.org/10.1016/j.energy.2017.01.005.
BORGES, D. G.; BARALDO JUNIOR, A.; FARINAS, C. S.; GIORDANO, R. L. C.; TARDIOLI, P. W. Enhanced saccharification of sugarcane bagasse using soluble cellulase supplemented with immobilized β-glucosidase. **Bioresource Technology**, vol. 167, p. 206–213, 2014. DOI http://dx.doi.org/10.1016/j.biortech.2014.06.021.
BRAT, D.; BOLES, E.; WIEDEMANN, B. Functional expression of a bacterial xylose isomerase in *Saccharomyces cerevisiae*. **Applied and Environmental Microbiology**, vol. 75, no. 8, p. 2304–2311, 2009. DOI https://doi.org/10.1128/AEM.02522-08.
BRODEUR, G.; YAU, E.; BADAL, K.; COLLIER, J.; RAMACHANDRAN, K. B.; RAMAKRISHNAN, S. Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. **Enzyme Research**, vol. 2011, no. 1, 2011. DOI https://doi.org/10.4061/2011/787532.
BROEKER, J.; MECHELKE, M.; BAUDREXL, M.; MENNERICH, D.; HORNBURG, D.; MANN, M.; SCHWARZ, W. H.; LIEBL, W.; ZVERLOV, V. V. The hemicellulose - degrading enzyme system of the thermophilic bacterium *Clostridium stercorarium*: comparative characterisation and addition of new hemicellulolytic glycoside hydrolases. **Biotechnology for Biofuels**, vol. 11, p. 229, 2018. DOI https://doi.org/10.1186/s13068-018-1228-3.
BRONDI, M. G.; ELIAS, A. M.; FURLAN, F. F.; GIORDANO, R. C.; FARINAS, C. S. Performance targets defined by retro-techno-economic analysis for the use of soybean protein as saccharification additive in an integrated biorefinery. **Scientific Reports**, vol. 10, no. 1, p. 1–13, 2020. DOI https://doi.org/10.1038/s41598-020-64316-6.
BRONDI, M. G.; VASCONCELLOS, V. M.; GIORDANO, R. C.; FARINAS, C. S. Alternative low-cost additives to improve the saccharification of lignocellulosic biomass. **Applied Biochemistry and Biotechnology**, vol. 187, no. 2, p. 461–473, 2019. DOI https://doi.org/10.1007/s12010-018-2834-z.
BURKE, M; SAVILLE, B.; ISHIZAWA, C. **Enzymatic treatment of lignocellulosic materials**. Unitated States: [*2009/0098616*], 2009a.
BURKE, Murray; SAVILLE, B.; ISHIZAWA, C. **Treatment of lignocellulosic materials utilizing disc refining and enzymatic hydrolysis**. Unitated States: [*2009/0098618*], 2009b.
CANILHA, L.; CHANDEL, A. K.; MILESSI, T. S. D. S.; ANTUNES, F. A. F.; FREITAS, W. L. D. C.; FELIPE, M. D. G. A.; DA SILVA, S. S. Bioconversion of sugarcane biomass into ethanol: An overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. **Journal of Biomedicine and Biotechnology**, vol. 2012, p. 1–15, 2012. DOI https://doi.org/10.1155/2012/989572.
CHANDEL, A. K.; ANTUNES, F. A.; ANJOS, V.; BELL, M. J. V; RODRIGUES, L. N.; POLIKARPOV, I.; DE AZEVEDO, E. R.; BERNARDINELLI, O. D.; ROSA, C. A.; PAGNOCCA, F. C.; DA SILVA, S. S. Multi-scale structural and chemical analysis of

sugarcane bagasse in the process of sequential acid-base pretreatment and ethanol production by *Scheffersomyces shehatae* and *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, vol. 7, p. 63, 2014. DOI https://doi.org/https://doi.org/10.1186/1754-6834-7-63.

CHANDRA, R. P.; ARANTES, V.; SADDLER, J. Steam pretreatment of agricultural residues facilitates hemicellulose recovery while enhancing enzyme accessibility to cellulose. **Bioresource Technology**, vol. 185, p. 302–307, 2015. DOI https://doi.org/https://doi.org/10.1016/j.biortech.2015.02.106.

CHAULA, Z.; SAID, M.; JOHN, G.; MANYELE, S.; MHILU, C. Modelling the suitability of pine sawdust for energy production via biomass steam explosion. **Smart Grid and Renewable Energy**, vol. 2014, p. 1–7, 2014. DOI https://doi.org/10.4236/sgre.2014.51001.

CHEN, H.; LIU, J.; CHANG, X.; CHEN, D.; XUE, Y.; LIU, P.; LIN, H.; HAN, S. A review on the pretreatment of lignocellulose for high-value chemicals. **Fuel Processing Technology**, vol. 160, p. 196–206, 2017. DOI http://dx.doi.org/10.1016/j.fuproc.2016.12.007.

CHEN, Y.; YAN, Z.; LIANG, L.; RAN, M.; WU, T.; WANG, B.; ZOU, X.; ZHAO, M.; FANG, G.; SHEN, K. Comparative evaluation of organic acid pretreatment of eucalyptus for kraft dissolving pulp production. **Materials**, vol. 13, no. 2, 2020. DOI https://doi.org/10.3390/ma13020361.

CHENG, M. H.; KADHUM, H. J.; MURTHY, G. S.; DIEN, B. S.; SINGH, V. High solids loading biorefinery for the production of cellulosic sugars from bioenergy sorghum. **Bioresource Technology**, vol. 318, p. 124051, 2020. DOI https://doi.org/10.1016/j.biortech.2020.124051.

CHOI, K. R.; JIAO, S.; LEE, S. Y. Metabolic engineering strategies toward production of biofuels. **Current Opinion in Chemical Biology**, vol. 59, p. 1–14, 2020. DOI https://doi.org/10.1016/j.cbpa.2020.02.009.

CHUNDAWAT, S. P. S.; PAL, R. K.; ZHAO, C.; CAMPBELL, T.; TEYMOURI, F.; VIDETO, J.; NIELSON, C.; WIEFERICH, B.; SOUSA, L.; DALE, B. E.; BALAN, V.; CHIPKAR, S.; AGUADO, J.; BURKE, E.; ONG, R. G. Ammonia fiber expansion (AFEX) pretreatment of lignocellulosic biomass. **Journal of Visualized Experiments**, vol. 2020, no. 158, 2020. DOI https://doi.org/10.3791/57488.

COHEN, J. A coefficient of agreement for nominal scales. **Educational and Psychological Measurement**, vol. XX, no. 1, p. 37–46, 1960. DOI https://doi.org/https://doi.org/10.1177/001316446002000104.

COHEN, R.; PERSKY, L.; HADAR, Y. Biotechnological applications and potential of wood-degrading mushrooms of the genus Pleurotus. **Applied Microbiology and Biotechnology**, vol. 58, p. 582–594, 2002. DOI https://doi.org/10.1007/s00253-002-0930-y.

COLLABORATION FOR ENVIRONMENTAL EVIDENCE. **Guidelines and Standards for Evidence synthesis in Environmental Management**. Version 5., 2018. Available at: www.environmentalevidence.org/information-for-authors.

DA SILVA, F. L.; CAMPOS, A. O.; DOS SANTOS, D. A.; MAGALHÃES, E. R. B.; DE MACEDO, G. R.; DOS SANTOS, E. S. Valorization of an agroextractive residue—Carnauba straw—for the production of bioethanol by simultaneous saccharification and fermentation (SSF). **Renewable Energy**, vol. 127, p. 661–669, 2018. DOI https://doi.org/10.1016/j.renene.2018.05.025.

DELABONA, P. S.; COTA, J.; HOFFMAM, Z. B.; PAIXÃO, D. A. A.; FARINAS, C. S.; CAIRO, J. P. L. F.; LIMA, D. J.; SQUINA, F. M.; RULLER, R.; PRADELLA, J. G. C. Understanding the cellulolytic system of *Trichoderma harzianum* P49P11 and

enhancing saccharification of pretreated sugarcane bagasse by supplementation with pectinase and α-l-arabinofuranosidase. **Bioresource Technology**, vol. 131, p. 500–507, 2013. DOI http://dx.doi.org/10.1016/j.biortech.2012.12.105.

DELABONA, P. S.; FARINAS, C. S.; DA SILVA, M. R.; AZZONI, S. F.; PRADELLA, J. G. C. Use of a new *Trichoderma harzianum* strain isolated from the Amazon rainforest with pretreated sugar cane bagasse for on-site cellulase production. **Bioresource Technology**, vol. 107, p. 517–521, 2012. DOI http://dx.doi.org/10.1016/j.biortech.2011.12.048.

FARMANBORDAR, S.; AMIRI, H.; KARIMI, K. Synergy of municipal solid waste co-processing with lignocellulosic waste for improved biobutanol production. **Waste Management**, vol. 118, p. 45–54, 2020. DOI https://doi.org/10.1016/j.wasman.2020.07.053.

FENG, Y.; QI, X.; JIAN, H. L.; SUN, R. C.; JIANG, J. X. Effect of inhibitors on enzymatic hydrolysis and simultaneous saccharification fermentation for lactic acid production from steam explosion pretreated lespedeza stalks. **BioResources**, vol. 7, no. 3, p. 3755–3766, 2012.

FONSECA, M. A. **Evaluation of xylooligosaccharides production from soy husks**. 2015. 111 f. Federal University of Sao Carlos, 2015. Available at: https://repositorio.ufscar.br/handle/ufscar/7439.

FRANCESCHIN, G.; SUDIRO, M.; INGRAM, T.; SMIRNOVA, I.; BRUNNER, G.; BERTUCCO, A. Conversion of rye straw into fuel and xylitol: A technical and economical assessment based on experimental data. **Chemical Engineering Research and Design**, vol. 89, no. 6, p. 631–640, 2011. DOI https://doi.org/10.1016/j.cherd.2010.11.001.

FURLAN, F. F.; COSTA, C. B. B.; SECCHI, A. R.; WOODLEY, J. M.; GIORDANO, R. C. Retro-Techno-Economic Analysis: Using (Bio)Process Systems Engineering Tools to Attain Process Target Values. **Industrial and Engineering Chemistry Research**, vol. 55, no. 37, p. 9865–9872, 2016. DOI https://doi.org/10.1021/acs.iecr.6b01757.

FURLAN, F. F.; COSTA, C. B. B.; FONSECA, G. C.; SOARES, R. P.; SECCHI, A. R.; CRUZ, A. J. G.; GIORDANO, R. C. Assessing the production of first and second generation bioethanol from sugarcane through the integration of global optimization and process detailed modeling. **Computers and Chemical Engineering**, vol. 43, p. 1–9, 2012. DOI https://doi.org/10.1016/j.compchemeng.2012.04.002.

GAONA, A.; LAWRYSHYN, Y.; SAVILLE, B. Enhancing biomass hydrolysis for biofuel production through hydrodynamic modeling and reactor design. **Energy Science and Engineering**, vol. 7, no. 5, p. 1823–1837, 2019. DOI https://doi.org/10.1002/ese3.394.

GAONA, A.; LAWRYSHYN, Y.; SAVILLE, B. The Effect of fed-batch operation and rotational speed on high-solids enzymatic hydrolysis of hardwood substrates. **Industrial Biotechnology**, vol. 11, no. 5, p. 1–7, 2015. DOI https://doi.org/10.1089/ind.2014.0031.

GAONA, A.; LAWRYSHYN, Y.; SAVILLE, B. A. High-solids enzymatic hydrolysis of biomass: Hydrodynamics and reaction kinetics integration via numerical modeling. **Physics of Fluids**, vol. 33, no. 3, 2021. DOI https://doi.org/10.1063/5.0036138.

GENG, W.; VENDITTI, R. A.; PAWLAK, J. J.; CHANG, H. M. Effect of delignification on hemicellulose extraction from switchgrass, poplar, and pine and its effect on enzymatic convertibility of cellulose-rich residues. **BioResources**, vol. 13, no. 3, p. 4946–4963, 2019. DOI https://doi.org/10.15376/biores.13.3.4946-4963.

GIORDANO, R. L. C.; GIORDANO, R. C.; ZANGIROLAMI, T. C.; TARDIOLI, P. W.; KOPP, W.; MILESSI, T.; SILVA, C. R.; CAVALCANTI-MONTANO, I. D.; GALEANO-SUAREZ, C. A.; ROJAS, M. J.; AL., E. **Catalytic system and process for**

obtaining 2g bioethanol from xylan/xylose oligomers. 2014.
GURAGAIN, Y. N.; VADLANI, P. V. Renewable biomass utilization: A way forward to establish sustainable chemical and processing industries. **Clean Technologies**, vol. 3, no. 1, p. 243–259, 2021. DOI https://doi.org/10.3390/cleantechnol3010014.
HENDRIKS, A. T. W. M.; ZEEMAN, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. **Bioresource Technology**, vol. 100, no. 1, p. 10–18, 2009. DOI https://doi.org/10.1016/j.biortech.2008.05.027.
HONG, E.; KIM, D.; KIM, J.; KIM, J.; YOON, S.; RHIE, S.; HA, S.; RYU, Y. Optimization of alkaline pretreatment on corn stover for enhanced production of 1.3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae* AJ4. **Biomass and Bioenergy**, vol. 77, p. 177–185, 2015. DOI https://doi.org/10.1016/j.biombioe.2015.03.016.
HOWARD, B. Y. B. H.; JONES, G.; PURDOM, M. R. The pentosanases of some rumen B-bacteria. **Biochemical Journal**, vol. 74, no. 1, p. 173–180, 1959. DOI https://doi.org/10.1042/bj0740173.
HUANG, H J; RAMASWAMY, S.; TSCHIRNER, U. W.; RAMARAO, B. V. Separation and purification processes for lignocellulose-to-bioalcohol production. **Bioalcohol production**. New York: Woodhead Publishing Series in Energy, 2010. p. 246–277. DOI https://doi.org/10.1533/9781845699611.3.246.
HUANG, H. J.; RAMASWAMY, S.; TSCHIRNER, U. W.; RAMARAO, B. V. A review of separation technologies in current and future biorefineries. **Separation and Purification Technology**, vol. 62, no. 1, p. 1–21, 2008. DOI https://doi.org/10.1016/j.seppur.2007.12.011.
JACQUET, N.; MANIET, G.; VANDERGHEM, C.; DELVIGNE, F.; RICHEL, A. Application of steam explosion as pretreatment on lignocellulosic material: A review. **Industrial and Engineering Chemistry Research**, vol. 54, no. 10, p. 2593–2598, 2015. DOI https://doi.org/10.1021/ie503151g.
JASPREET, K.; TAGGAR, M. S.; KOCHER, G. S. Fermentation of enzymatically saccharified rice straw hydrolysate for bioethanol production. **Research Journal of Biotechnology**, vol. 14, no. 12, p. 98–103, 2019.
JATOI, A. S.; ABBASI, S. A.; HASHMI, Z.; SHAH, A. K.; ALAM, M. S.; BHATTI, Z. A.; MAITLO, G.; HUSSAIN, S.; KHANDRO, G. A.; USTO, M. A.; IQBAL, A. Recent trends and future perspectives of lignocellulose biomass for biofuel production: a comprehensive review. **Biomass Conversion and Biorefinery**, 2021. DOI https://doi.org/10.1007/s13399-021-01853-8.
JOE, M. H.; KIM, J. Y.; LIM, S.; KIM, D. H.; BAI, S.; PARK, H.; LEE, S. G.; HAN, S. J.; CHOI, J. I. Microalgal lipid production using the hydrolysates of rice straw pretreated with gamma irradiation and alkali solution. **Biotechnology for Biofuels**, vol. 8, no. 1, 2015. DOI https://doi.org/10.1186/s13068-015-0308-x.
KAMOLDEEN, A. A.; LEE, C. K.; WAN ABDULLAH, W. N.; LEH, C. P. Enhanced ethanol production from mild alkali-treated oil-palm empty fruit bunches via co-fermentation of glucose and xylose. **Renewable Energy**, vol. 107, p. 113–123, 2017. DOI https://doi.org/10.1016/j.renene.2017.01.039.
KAUR, A.; KUHAD, R. C. Valorization of rice straw for ethanol production and lignin recovery using combined acid-alkali pre-treatment. **Bioenergy Research**, vol. 12, no. 3, p. 570–582, 2019. DOI https://doi.org/10.1007/s12155-019-09988-3.
KOPPRAM, R.; NIELSEN, F.; ALBERS, E.; LAMBERT, A.; WÄNNSTRÖM, S.; WELIN, L.; ZACCHI, G.; OLSSON, L. Simultaneous saccharification and co-fermentation for bioethanol production using corncobs at lab, PDU and demo scales. **Biotechnology for Biofuels**, vol. 6, no. 1, 2013. DOI https://doi.org/10.1186/1754-6834-6-2.

KUHAD, R. C.; GUPTA, R.; KHASA, Y. P.; SINGH, A.; ZHANG, Y. H. P. Bioethanol production from pentose sugars: Current status and future prospects. **Renewable and Sustainable Energy Reviews**, vol. 15, no. 9, p. 4950–4962, 2011. DOI http://dx.doi.org/10.1016/j.rser.2011.07.058.

KUMAR, P.; BARRETT, D. M.; DELWICHE, M. J.; STROEVE, P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. **Industrial and Engineering Chemistry Research**, vol. 48, no. 8, p. 3713–3729, 2009. DOI https://doi.org/10.1021/ie801542g.

KUMAR, R.; SHARMA, R. K.; SINGH, A. P. Cellulose based grafted biosorbents - Journey from lignocellulose biomass to toxic metal ions sorption applications - A review. **Journal of Molecular Liquids**, vol. 232, p. 62–93, 2017. DOI http://dx.doi.org/10.1016/j.molliq.2017.02.050.

LI, J.; GELLERSTEDT, G. Improved lignin properties and reactivity by modifications in the autohydrolysis process of aspen wood. **Industrial Crops and Products**, vol. 27, no. 2, p. 175–181, 2008. DOI https://doi.org/10.1016/j.indcrop.2007.07.022.

LIGTHELM, M. E.; PRIOR, B. A.; PREEZ, J. C.; BRANDT, V. An investigation of D-{1- 13C} xylose metabolism in *Pichia stipitis* under aerobic and anaerobic conditions. **Applied Microbiology and BiotechnologyBiotechnology**, vol. 1, p. 293–296, 1988. DOI https://doi.org/https://doi.org/10.1007/BF00250458.

LING, Z.; GUO, Z.; HUANG, C.; YAO, L.; XU, F. Deconstruction of oriented crystalline cellulose by novel levulinic acid based deep eutectic solvents pretreatment for improved enzymatic accessibility. **Bioresource Technology**, vol. 305, p. 123025, 2020. DOI https://doi.org/10.1016/j.biortech.2020.123025.

LINTON, S. M. Review: The structure and function of cellulase (endo-β-1,4-glucanase) and hemicellulase (β-1,3-glucanase and endo-β-1,4-mannase) enzymes in invertebrates that consume materials ranging from microbes, algae to leaf litter. **Comparative Biochemistry and Physiology Part - B**, vol. 240, p. 110354, 2020. DOI https://doi.org/10.1016/j.cbpb.2019.110354.

LONGATI, A. A.; LINO, A. R. A.; GIORDANO, R. C.; FURLAN, F. F.; CRUZ, A. J. G. Defining research and development process targets through retro-techno-economic analysis: The sugarcane biorefinery case. **Bioresource Technology**, vol. 263, p. 1–9, 2018. DOI https://doi.org/10.1016/j.biortech.2018.04.102.

MARGOLLES, A.; REYES-GAVILÁN, C. G. D. L. Purification and functional characterization of a novel α-L-arabinofuranosidase from *Bifidobacterium longum* B667. **Applied and Environmental Microbiology**, vol. 69, no. 9, p. 5096–5103, 2003. DOI https://doi.org/10.1128/AEM.69.9.5096.

MIAO, J.; WANG, M.; MA, L.; LI, T.; HUANG, Q.; LIU, D. Effects of amino acids on the lignocellulose degradation by *Aspergillus fumigatus Z5* : insights into performance, transcriptional , and proteomic profiles. **Biotechnology for Biofuels**, vol. 12, no. 4, p. 1–19, 2019. DOI https://doi.org/10.1186/s13068-018-1350-2.

MICHALSKA, K.; MIAZEK, K.; KRZYSTEK, L.; LEDAKOWICZ, S. Influence of pretreatment with Fenton's reagent on biogas production and methane yield from lignocellulosic biomass. **Bioresource Technology**, vol. 119, p. 72–78, 2012. DOI https://doi.org/https://doi.org/10.1016/j.biortech.2012.05.105.

MILESSI-ESTEVES, T. S.; CORRADINI, F. A. S.; KOPP, W.; ZANGIROLAMI, T. C. An innovative biocatalyst for continuous 2g ethanol production from xylo-oligomers by *Saccharomyces cerevisiae* through simultaneous hydrolysis, isomerization, and fermentation (SHIF). **Catalysts**, vol. 9, no. 3, p. 225, 2019. DOI https://doi.org/10.3390/catal9030225.

MILESSI, T. S. S.; KOPP, W.; ROJAS, M. J.; MANRICH, A.; BAPTISTA-NETO, A.;

TARDIOLI, P. W.; GIORDANO, R. C.; FERNANDEZ-LAFUENTE, R.; GUISAN, J. M.; GIORDANO, R. L. C. Immobilization and stabilization of an endoxylanase from Bacillus subtilis (XynA) for xylooligosaccharides (XOs) production. **Catalysis Today**, vol. 259, p. 130–139, 2016. DOI http://dx.doi.org/10.1016/j.cattod.2015.05.032.

MOSIER, N.; WYMAN, C.; DALE, B.; ELANDER, R.; LEE, Y. Y.; HOLTZAPPLE, M.; LADISCH, M. Features of promising technologies for pretreatment of lignocellulosic biomass. **Bioresource Technology**, vol. 96, no. 6, p. 673–686, 2005. DOI https://doi.org/10.1016/j.biortech.2004.06.025.

MUSSATTO, S. I.; MACHADO, E. M. S.; CARNEIRO, L. M.; TEIXEIRA, J. A. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. **Applied Energy**, vol. 92, p. 763–768, 2012. DOI http://dx.doi.org/10.1016/j.apenergy.2011.08.020.

NANDA, S.; DALAI, A. K.; KOZINSKI, J. A. Butanol and ethanol production from lignocellulosic feedstock: Biomass pretreatment and bioconversion. **Energy Science and Engineering**, vol. 2, no. 3, p. 138–148, 2014. DOI https://doi.org/10.1002/ese3.41.

NANDA, S.; MOHAMMAD, J.; REDDY, S. N.; KOZINSKI, J. A.; DALAI, A. K. Pathways of lignocellulosic biomass conversion to renewable fuels. **Biomass Conversion and Biorefinery**, vol. 4, no. 2, p. 157–191, 2014. DOI https://doi.org/10.1007/s13399-013-0097-z.

NASCIMENTO, V. M.; MANRICH, A.; TARDIOLI, P. W.; GIORDANO, R. C.; ROCHA, G. J. M.; GIORDANO, R. L. C. Alkaline pretreatment for practicable production of ethanol and xylooligosaccharides. **Bioethanol**, vol. 2, no. 1, p. 112–125, 2016. DOI https://doi.org/10.1515/bioeth-2016-0008.

NEUMANN, J.; MEYER, J.; OUADI, M.; APFELBACHER, A.; BINDER, S.; HORNUNG, A. The conversion of anaerobic digestion waste into biofuels via a novel thermo-catalytic reforming process. **Waste Management**, vol. 47, p. 141–148, 2016. DOI https://doi.org/10.1016/j.wasman.2015.07.001.

NIGAM, P. S. Microbial enzymes with special characteristics for biotechnological applications. **Biomolecules**, vol. 3, no. 3, p. 597–611, 2013. DOI https://doi.org/10.3390/biom3030597.

OREB, M.; DIETZ, H.; FARWICK, A.; BOLES, E. Novel strategies to improve co-fermentation of pentoses with. **Bioengineered**, vol. 3, no. 6, p. 347–351, 2012.

POLETTO, P.; PEREIRA, G. N.; MONTEIRO, C. R. M.; PEREIRA, M. A. F.; BORDIGNON, S. E.; OLIVEIRA, D. Xylooligosaccharides: Transforming the lignocellulosic biomasses into valuable 5-carbon sugar prebiotics. **Process Biochemistry**, vol. 91, p. 352–363, 2020. DOI https://doi.org/10.1016/j.procbio.2020.01.005.

POURBAFRANI, M.; MCKECHNIE, J.; SHEN, T.; SAVILLE, B. A.; MACLEAN, H. L. Impacts of pre-treatment technologies and co-products on greenhouse gas emissions and energy use of lignocellulosic ethanol production. **Journal of Cleaner Production**, vol. 78, p. 104–111, 2014. DOI http://dx.doi.org/10.1016/j.jclepro.2014.04.050.

R CORE. R: A language and environment for statistical computing. Vienna, 2019.

RAVINDRAN, R.; JAISWAL, A. K. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. **Bioresource Technology**, vol. 199, p. 92–102, 2016. DOI http://dx.doi.org/10.1016/j.biortech.2015.07.106.

REZANIA, S.; DIN, M. F. M.; MOHAMAD, S. E.; SOHAILI, J.; TAIB, S. M.; YUSOF, M. B. M.; KAMYAB, H.; DARAJEH, N.; AHSAN, A. Review on pretreatment methods and ethanol production from cellulosic water hyacinth. **BioResources**, vol. 12, no. 1, p. 2108–2124, 2017. DOI https://doi.org/10.15376/biores.12.1.rezania.

ROBAK, K.; BALCEREK, M. Current state-of-the-art in ethanol production from lignocellulosic feedstocks. **Microbiological Research**, vol. 240, p. 126534, 2020. DOI https://doi.org/10.1016/j.micres.2020.126534.

RODRÍGUEZ-ZÚÑIGA, U. F.; CANNELLA, D.; GIORDANO, R. D. C.; GIORDANO, R. D. L. C.; JØRGENSEN, H.; FELBY, C. Lignocellulose pretreatment technologies affect the level of enzymatic cellulose oxidation by LPMO. **Green Chemistry**, vol. 17, no. 5, p. 2896–2903, 2015. DOI https://doi.org/10.1039/c4gc02179g.

ROJAS, M. J.; SIQUEIRA, P. F.; MIRANDA, L. C.; TARDIOLI, P. W.; GIORDANO, R. L. C. Sequential proteolysis and cellulolytic hydrolysis of soybean hulls for oligopeptides and ethanol production. **Industrial Crops & Products**, vol. 61, p. 202–210, 2014. DOI http://dx.doi.org/10.1016/j.indcrop.2014.07.002.

ROMANELLI, J. P.; SILVA, L. G. M.; GONÇALVES, M. C. P.; NAVES, R. P.; DE ALMEIDA, D. R. A.; DE RESENDE, A. F.; RODRIGUES, R. R. Repeatability of the searching process in reviews of restoration outcomes. **Restoration Ecology**, vol. 29, no. 8, p. 1–10, 2021a. DOI https://doi.org/10.1111/rec.13496.

ROMANELLI, J. P.; GONÇALVES, M. C. P.; PESTANA, L. F. D. A.; SOARES, J. A. H.; BOSCHI, R. S.; ANDRADE, D. F. Four challenges when conducting bibliometric reviews and how to deal with them. **Environmental Science and Pollution Research**, vol. 28, p. 60448–60458, 2021b. DOI https://doi.org/https://doi.org/10.1007/s11356-021-16420-x.

RUIZ, H. A.; CONRAD, M.; SUN, S. N.; SANCHEZ, A.; ROCHA, G. J. M.; ROMANÍ, A.; CASTRO, E.; TORRES, A.; RODRÍGUEZ-JASSO, R. M.; ANDRADE, L. P.; SMIRNOVA, I.; SUN, R. C.; MEYER, A. S. Engineering aspects of hydrothermal pretreatment: From batch to continuous operation, scale-up and pilot reactor under biorefinery concept. **Bioresource Technology**, vol. 299, p. 122685, 2020. DOI https://doi.org/10.1016/j.biortech.2019.122685.

SAHA, B. C. Hemicellulose bioconversion. **Journal of Industrial Microbiology and Biotechnology**, vol. 30, p. 279–291, 2003. DOI https://doi.org/10.1007/s10295-003-0049-x.

SAHA, K.; R, U. M.; SIKDER, J.; CHAKRABORTY, S.; DA SILVA, S. S.; DOS SANTOS, J. C. Membranes as a tool to support biorefineries: Applications in enzymatic hydrolysis, fermentation and dehydration for bioethanol production. **Renewable and Sustainable Energy Reviews**, vol. 74, p. 873–890, 2017. DOI https://doi.org/10.1016/j.rser.2017.03.015.

SAINI, S.; CHANDEL, A. K.; SHARMA, K. K. Past practices and current trends in recovery and purification of first generation ethanol: A learning curve for lignocellulosic ethanol. **Journal of Cleaner Production**, p. 122357, 2020. DOI https://doi.org/10.1016/j.jclepro.2020.122357.

SALYERS, A. A.; PALMER, J. K.; WILKINS, T. D. Laminarinase (β-Glucanase) activity in bacteroides from the human colon. **Applied and Environmental Microbiology**, vol. 33, no. 5, p. 1118–1124, 1977. DOI https://doi.org/10.1128/aem.33.5.1118-1124.1977.

SANTIBÁÑEZ, L.; HENRÍQUEZ, C.; CORRO-TEJEDA, R.; BERNAL, S.; ARMIJO, B.; SALAZAR, O. Xylooligosaccharides from lignocellulosic biomass: A comprehensive review. **Carbohydrate Polymers**, vol. 251, p. 117118, 2020. DOI https://doi.org/10.1016/j.carbpol.2020.117118.

SEESURIYACHAN, P.; KAWEE-AI, A.; CHAIYASO, T. Green and chemical-free process of enzymatic xylooligosaccharide production from corncob: Enhancement of the yields using a strategy of lignocellulosic destructuration by ultra-high pressure pretreatment. **Bioresource Technology**, vol. 241, p. 537–544, 2017. DOI

https://doi.org/10.1016/j.biortech.2017.05.193.
SHANKAR, K.; KULKARNI, N. S.; JAYALAKSHMI, S. K.; SREERAMULU, K. Saccharification of the pretreated husks of corn, peanut and coffee cherry by the lignocellulolytic enzymes secreted by *Sphingobacterium sp*. ksn for the production of bioethanol. **Biomass and Bioenergy**, vol. 127, 2019. DOI https://doi.org/10.1016/j.biombioe.2019.105298.
SLIVINSKI, C. T.; VINICIUS, A.; MACHADO, L.; IULEK, J. Biochemical characterisation of a glucoamylase from *Aspergillus niger* produced by solid-state fermentation. **Brazilian archives of biology and technology**, vol. 54, no. 3, p. 559–568, 2011. DOI https://doi.org/https://doi.org/10.1590/S1516-89132011000300018.
SMICHI, N.; MESSAOUDI, Y.; ALLAF, K.; GARGOURI, M. Steam explosion (SE) and instant controlled pressure drop (DIC) as thermo-hydro-mechanical pretreatment methods for bioethanol production. **Bioprocess and Biosystems Engineering**, vol. 43, no. 6, p. 945–957, 2020. DOI https://doi.org/10.1007/s00449-020-02297-6.
SOLTANIAN, S.; AGHBASHLO, M.; ALMASI, F.; HOSSEINZADEH-BANDBAFHA, H.; NIZAMI, A. S.; OK, Y. S.; LAM, S. S.; TABATABAEI, M. A critical review of the effects of pretreatment methods on the exergetic aspects of lignocellulosic biofuels. **Energy Conversion and Management**, vol. 212, p. 112792, 2020. DOI https://doi.org/10.1016/j.enconman.2020.112792.
TAHA, M.; FODA, M.; SHAHSAVARI, E.; ABURTO-MEDINA, A.; ADETUTU, E.; BALL, A. Commercial feasibility of lignocellulose biodegradation: Possibilities and challenges. **Current Opinion in Biotechnology**, vol. 38, p. 190–197, 2016. DOI http://dx.doi.org/10.1016/j.copbio.2016.02.012.
TAYYAB, M.; NOMAN, A.; ISLAM, W.; WAHEED, S.; ARAFAT, Y.; ALI, F.; ZAYNAB, M.; LIN, S.; ZHANG, H.; LIN, W. Bioethanol production from lignocellulosic biomass by environment-friendly pretreatment methods: A review. **Applied Ecology and Environmental Research**, vol. 16, no. 1, p. 225–249, 2018. DOI https://doi.org/10.15666/aeer/1601_225249.
TEIXEIRA, R. S.; SILVA, A. S.; MOUTTA, R. O.; FERREIRA-LEITÃO, V. S.; BARROS, R. R.; FERRARA, M. A.; BON, E. P. Biomass pretreatment: a critical choice for biomass utilization via biotechnological routes. **BMC Proceedings**, vol. 8, no. 4, p. 8–10, 2014. DOI https://doi.org/10.1186/1753-6561-8-s4-o34.
TEYMOURI, F.; LAUREANO-PEREZ, L.; ALIZADEH, H.; DALE, B. E. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. **Bioresource Technology**, vol. 96, p. 2014–2018, 2005. DOI https://doi.org/10.1016/j.biortech.2005.01.016.
TOMÁS-PEJÓ, E.; OLIVA, J. M.; BALLESTEROS, M. Realistic approach for full-scale bioethanol production from lignocellulose: A review. **Journal of Scientific and Industrial Research**, vol. 67, no. 11, p. 874–884, 2008.
TUTT, M.; KIKAS, T.; OLT, J. Influence of different pretreatment methods on bioethanol production from wheat straw. **Agronomy Research**, vol. 10, no. 1, p. 269–276, 2012.
VAN ECK, N. J.; WALTMAN, L. Software survey : VOSviewer , a computer program for bibliometric mapping. **Scientometrics**, vol. 84, no. 2, p. 523–538, 2010. DOI https://doi.org/10.1007/s11192-009-0146-3.
VASIĆ, K.; KNEZ, Ž.; LEITGEB, M. Bioethanol production by enzymatic hydrolysis from different lignocellulosic sources. **Molecules**, vol. 26, no. 3, 2021. DOI https://doi.org/10.3390/molecules26030753.
VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose

fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

WANG, R.; UNREAN, P.; FRANZÉN, C. J. Model-based optimization and scale-up of multi-feed simultaneous saccharification and co-fermentation of steam pre-treated lignocellulose enables high gravity ethanol production. **Biotechnology for Biofuels**, vol. 9, no. 1, 2016. DOI https://doi.org/10.1186/s13068-016-0500-7.

WIRAWAN, F.; CHENG, C.; LO, Y.; CHEN, C.; CHANG, J. Continuous cellulosic bioethanol co-fermentation by immobilized *Zymomonas mobilis* and suspended *Pichia stipitis* in a two-stage process. **Applied Energy**, vol. 266, p. 114871, 2020. DOI https://doi.org/10.1016/j.apenergy.2020.114871.

WISCHRAL, D.; ARIAS, J. M.; MODESTO, L. F.; PASSOS, D. F.; PEREIRA, N. Lactic acid production from sugarcane bagasse hydrolysates by *Lactobacillus pentosus*: Integrating xylose and glucose fermentation. **Biotechnology Progress**, vol. 35, no. 1, 2019. DOI https://doi.org/10.1002/btpr.2718.

WOICIECHOWSKI, A. L.; DALMAS NETO, C. J.; VANDENBERGHE, L. P. S.; DE CARVALHO NETO, D. P.; SYDNEY, A. C. N.; LETTI, L. A. J.; KARP, S. G.; TORRES, L. A. Z.; SOCCOL, C. R. Lignocellulosic biomass: Acid and alkaline pretreatments and their effects on biomass recalcitrance – Conventional processing and recent advances. **Bioresource Technology**, vol. 304, p. 122848, 2020. DOI https://doi.org/10.1016/j.biortech.2020.122848.

WU, J.; CHANDRA, R. P.; TAKADA, M.; LIU, L. Y.; RENNECKAR, S.; KIM, K. H.; KIM, C. S.; SADDLER, J. N. Enhancing enzyme-mediated cellulose hydrolysis by incorporating acid groups onto the lignin during biomass pretreatment. **Frontiers in Bioengineering and Biotechnology**, vol. 8, p. 1–8, 2020. DOI https://doi.org/10.3389/fbioe.2020.608835.

WYMAN, C. E.; DALE, B. E.; ELANDER, R. T.; HOLTZAPPLE, M.; LADISCH, M. R.; LEE, Y. Y. Coordinated development of leading biomass pretreatment technologies. **Bioresource Technology**, vol. 96, no. 18, p. 1959–1966, 2005. DOI https://doi.org/https://doi.org/10.1016/j.biortech.2005.01.010.

XIAO, W.; CLARKSON, W. W. Acid solubilization of lignin and bioconversion of treated newsprint to methane. **Biodegradation**, vol. 8, p. 61-66, 1997.

YANG, B.; WYMAN, C. E. Pretreatment: the key to unlocking low-cost cellulosic ethanol. **Biofuels bioproducts & Biorefining**, vol. 2, no. 1, p. 26–40, 2008. DOI https://doi.org/doi:10.1002/bbb.49.

YANG, H.; WANG, K.; SONG, X.; XU, F. Production of xylooligosaccharides by xylanase from Pichia stipitis based on xylan preparation from triploid *Populas tomentosa*. **Bioresource Technology**, vol. 102, no. 14, p. 7171–7176, 2011. DOI https://doi.org/10.1016/j.biortech.2011.03.110.

YOUNG, E.; LEE, S.; ALPER, H. Optimizing pentose utilization in yeast: the need for novel tools and approaches. **Biotechnology for Biofuels**, vol. 3, no. 24, p. 1–12, 2010. DOI https://doi.org/10.1186/1754-6834-3-24.

YU, Q.; LIU, J.; ZHUANG, X.; YUAN, Z.; WANG, W.; QI, W.; WANG, Q.; TAN, X.; KONG, X. Liquid hot water pretreatment of energy grasses and its influence of physico-chemical changes on enzymatic digestibility. **Bioresource Technology**, vol. 199, p. 265–270, 2016. DOI https://doi.org/10.1016/j.biortech.2015.07.086.

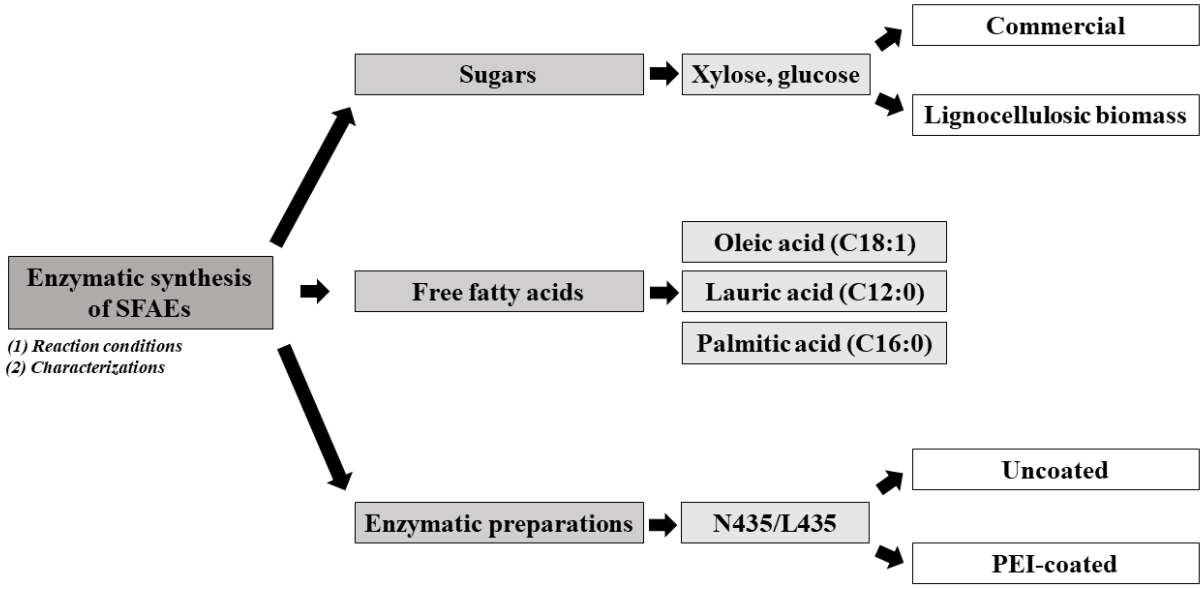
YU, Q.; XU, C.; ZHUANG, X.; YUAN, Z.; HE, M.; ZHOU, G. Xylo-oligosaccharides and ethanol production from liquid hot water hydrolysate of sugarcane bagasse. **Bioresources**, vol. 10, no. 1, p. 30–40, 2015. DOI https://doi.org/10.15376/biores.10.1.30-40.

ZHANG, K.; PEI, Z.; WANG, D. Organic solvent pretreatment of lignocellulosic

biomass for biofuels and biochemicals: A review. **Bioresource Technology**, vol. 199, p. 21–33, 2016. DOI http://dx.doi.org/10.1016/j.biortech.2015.08.102.
ZHAO, C.; SHAO, Q.; CHUNDAWAT, S. P. S. Recent advances on ammonia-based pretreatments of lignocellulosic biomass. **Bioresource Technology**, vol. 298, p. 122446, 2020. DOI https://doi.org/10.1016/j.biortech.2019.122446.
ZUO, Z.; TIAN, S.; CHEN, Z.; LI, J.; YANG, X. Soaking pretreatment of corn stover for bioethanol production followed by anaerobic digestion process. **Applied Biochemistry and Biotechnology**, vol. 167, no. 7, p. 2088–2102, 2012. DOI https://doi.org/10.1007/s12010-012-9751-3.

4. METHODOLOGY

Materials and methods used to perform experiments for this thesis will be presented in the following topic (as part of the structure of the published articles). Here (Scheme 4.1), only a diagram will be presented to summarize the whole methodology used to conduct this research.



Scheme 4.1. Summary of the methodology employed in this study.

5. RESULTS AND DISCUSSION

5.1. LIPOZYME 435-MEDIATED SYNTHESIS OF XYLOSE OLEATE IN METHYL ETHYL KETONE

5.1.1. Introduction

Sugar fatty acid esters (SFAEs) are non-ionic, low toxic, and biodegradable surfactants widely used for food processing, personal care, protein extraction, and as antimicrobial and insecticidal agents (BIDJOU-HAIOUR; KLAI, 2013; CHANG;

ZHANG; TANG, 2018; OGAWA *et al.*, 2019; SHIN *et al.*, 2019). Particularly, xylose fatty esters have been used to improve skin hydration in the cosmetic and pharmaceutical sectors (BIDJOU-HAIOUR; KLAI, 2013). Xylose is a monosaccharide that can be used as a renewable, low-cost, and abundant raw material for SFAEs production. It can be obtained from the hydrolysis of hemicelluloses, one of the main components of the lignocellulosic biomass (ABDULMALEK; HAMIDON; RAHMAN, 2016; MÉLINE *et al.*, 2018). However, the use of xylose in the synthesis of SFAEs has been sparsely reported in the literature (ABDULMALEK; HAMIDON; RAHMAN, 2016; BIDJOU-HAIOUR; KLAI, 2013; CHANG; SHAW, 2009; MÉLINE *et al.*, 2018; NETA; TEIXEIRA; RODRIGUES, 2015; SHI; LI; CHU, 2011; SIEBENHALLER *et al.*, 2017, 2018; VESCOVI; DOS SANTOS; TARDIOLI, 2017), making room for the development of research in this area. Considering this scenario, xylose may represent a feasible substrate in the production of SFAEs, since, after the hydrolysis of lignocellulosic biomasses, the hemicellulose fraction is generally underused (VESCOVI; DOS SANTOS; TARDIOLI, 2017).

The synthesis of xylose fatty esters may occur through either an esterification or a transesterification reaction in the presence of chemical catalysts or enzymes. Xylose acts as the acceptor for the acyl group, while fatty acids/fatty acid esters perform as donors (or activated acyl donors) of this acyl group (DE LIMA *et al.*, 2018; LIANG *et al.*, 2018). In the chemical route, the reaction is carried out at high temperatures with an alkaline or metallic catalyst. This pathway consumes a large amount of energy, presents low selectivity and the purification of the esters formed is complex (BOUZAOUIT; BIDJOU-HAIOUR, 2015; MÉLINE *et al.*, 2018), because of the toxic by-products produced (GUMEL *et al.*, 2011). Conversely, the production of these surfactants through an enzymatic route requires milder operating conditions, facilitates the separation of products, achieves high yields, and presents high specificity and selectivity (ABDULMALEK; HAMIDON; RAHMAN, 2016; GUMEL *et al.*, 2011; KAPOOR; GUPTA, 2012; SIEBENHALLER *et al.*, 2018). Nonetheless, nowadays this route is not the most feasible option for large-scale production. It is still necessary to reduce the cost of biocatalysts and eliminate expensive processing steps.

Lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) are the most used enzymes in the enzymatic synthesis of SFAEs. Several lipases have been evaluated for this purpose, among them lipases from *Candida antarctica*, *Candida rugosa*, *Candida cylindracea*, *Rhizomucor miehei*, *Bacillus subtilis*, *Bacillus licheniformis*, and the porcine pancreatic

lipase (CHANG; SHAW, 2009; GUMEL *et al.*, 2011; NETA; TEIXEIRA; RODRIGUES, 2015; TENG *et al.*, 2020). In particular, the species *Candida antarctica* produces two different lipases: A and B. Fraction B (CALB) is probably the most widely used hydrolases in the field of biocatalysis, conducting reactions with high activities and yields under mild conditions (KUNDYS *et al.*, 2018). CALB is a highly selective and efficient catalyst, applied in numerous organic reactions, with emphasis on the esterification in solvent-free systems and organic solvents (ARCENS *et al.*, 2018; BIDJOU-HAIOUR; KLAI, 2013; MA *et al.*, 2018; SIEBENHALLER *et al.*, 2017).

The use of such enzymes as catalysts in the free form presents some issues related to the instability of their structures and the difficulty of recovery and reuse at the end of the process (GONÇALVES *et al*., 2019). To minimize these drawbacks, CALB immobilization was addressed in about 95% of the publications that encompass the synthesis of SFAEs (in the time span from 2010 to 2019) (GONÇALVES *et al.*, 2021). The interfacial adsorption of lipases on hydrophobic supports (RODRIGUES *et al.*, 2019), covalent bonds, and ionic interactions are among the immobilization techniques reported in this period (ADLERCREUTZ, 2013). These methods included the use of different supports, such as chitosan, celite, silica magnetic microparticles, octyl silica (CHÁVEZ-FLORES *et al.*, 2017; DE LIMA *et al.*, 2016; NETA *et al.*, 2012; VESCOVI *et al.*, 2016), and microemulsion-based organogels (MBGs) (RAHMAN *et al.*, 2012).

Specifically, the use of the commercial CALB physically adsorbed on a macroporous resin (Novozyme 435 and Lipozyme 435) was reported in about 80% of the publications on the synthesis of sugar esters over the last decade (GONÇALVES *et al.*, 2021). Novozyme 435 (N435) and Lipozyme 435 (L435) are CALBs immobilized on Lewatit VP OC 1600, a poly methacrylic acid cross-linked with divinylbenzene. The described loading of CALB onto this support ranges between 8.5 and 20% *w/w* (SIÓDMIAK *et al.*, 2015). CALB immobilized on acrylic resin has outstanding activity and stability in hydrophobic organic media, besides producing high yields of carbohydrate esters when used in these reactions (ABDULMALEK; HAMIDON; RAHMAN, 2016; FINDRIK *et al.*, 2016; MAI *et al.*, 2014; MÉLINE *et al.*, 2018; NETA *et al.*, 2012; NETA *et al.*, 2011; PAPPALARDO *et al.*, 2017). However, due to enzyme displacement or leaching from the support, multiple reuse cycles of these enzymes are often unfeasible (ORTIZ *et al.*, 2019). Even so, its use is a good first approach to analyze the feasibility of the CALB use in a specific process.

The solvent used in the CALB catalyzed synthesis of SFAEs is another variable that should be carefully evaluated. It must reconcile sugar solubility and low polarity to reduce the damage on the enzyme structure and does not present reactive hydroxyl groups, which could be substrates of the enzyme and reduce the product yield and/or generate by-products. In general, tertiary alcohols meet these requirements (CASTRO *et al.*, 2004; FALLAVENA *et al.*, 2014; KUMARI *et al.*, 2009). Nonetheless, many of them are not permitted for the manufacture of food additives and should be replaced by biocompatible solvents. Methyl ethyl ketone (MEK) is an example of these solvents useful for food manipulation and has given promising results in the synthesis of sugar esters, even with low to moderate solubilization of sugars (BIDJOU-HAIOUR; KLAI, 2013; LI *et al.*, 2015).

In this new research effort, we evaluated the performance of the benchmark immobilized CALB (Lipozyme 435) on the synthesis of xylose oleate in MEK to obtain the most suitable esterification conditions. The esterification reaction is a controlled process where the yields are determined by the system's thermodynamics. The catalysts only determine the reaction feasibility, i.e., if the enzyme is inhibited or inactivated, the thermodynamic yield will be not reached (HALLING, 1994; KASCHE, 1986). Water is a by-product of this reaction that can be eliminated by diverse techniques to improve the yields (evaporation, molecular sieves, etc.) (CASTILLO *et al.*, 1997; COLOMBIÉ *et al.*, 1998). However, if the biocatalyst activity is high enough, the accumulation of water in the support particles may become a problem. If a water phase is formed, it can drive to the inhibition or inactivation of the enzyme (DOSSAT; COMBES; MARTY, 1999; MARTY; DOSSAT; CONDORET, 1997). This issue has been solved by some authors using very hydrophobic supports or ultrasounds (ALVES *et al.*, 2014; SÉVERAC *et al.*, 2011; FALLAVENA *et al.*, 2014; GRAEBIN *et al.*, 2012; MARTINS; FRIEDRICH; *et al.*, 2013; MARTINS; SCHEIN; *et al.*, 2013; PALUDO *et al.*, 2015; POPPE *et al.*, 2013). Additionally, we assessed the kinetic parameters of the reaction, the biocatalyst reuse, the purification of the sugar esters synthesized, and their emulsion capacity for further industrial application with a commercial surfactant as a comparison. The successful formation of esters was evaluated by mass spectroscopy.

5.1.2. Materials and Methods

5.1.2.1. Materials

Candida antarctica lipase B immobilized on Lewatit VP OC 1600 (Lipozyme 435, L435) was donated by Novozymes Latin America (Araucária, Brazil). Tributyrin, butyric acid, and n-butanol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Xylose, heptane, and oleic acid were obtained from Synth (São Paulo, Brazil). MEK was purchased from Neon (São Carlos, Brazil). Molecular sieves (3 Å) were obtained from JT Baker (New Jersey, NJ, USA). All other reagents and solvents were of analytical or HPLC/GC grade and were used without any previous treatment.

5.1.2.2. Enzymatic synthesis of xylose oleate

The synthesis of xylose oleate was conducted through the esterification reaction between oleic acid (C18:1) and commercial xylose (as a model compound), using MEK as solvent. The biocatalyst used in this reaction (L435) contains 1432 U of esterification/g of the enzyme. One unit of esterification activity (U) was defined as the initial rate of butyl butyrate synthesis (mmol/L/min) under the conditions described in section 5.1.2.5.2. All experiments were performed in duplicate. Results were expressed as mean ± mean standard deviation (σ).

5.1.2.2.1. Xylose solubility in MEK

The xylose solubility was evaluated in the chosen organic solvent. To this goal, we added 50, 25, 10, and 5 mmol/L of xylose in MEK in closed tubes. After 24 h under continuous stirring (200 rpm) in a Marconi shaker (MA 430/1) (Marconi®, Piracicaba, Brazil) at 60 °C, the solutions or suspensions were centrifuged at 10,000 rpm (12,857 × g) for 2 min (35 °C). The supernatant was then prepared for the quantification of solubilized xylose by liquid chromatography (Section 5.1.2.6.1 - see below).

5.1.2.2.2. Determination of the effect of xylose: oleic acid molar ratio on the reaction performance

The effect of xylose: oleic acid molar ratio in the synthesis of xylose oleate was studied under the following conditions: molar ratios of 1:1, 1:2.5, 1:5, 2.5:1, and 5:1, with the xylose concentration fixed at 7 mmol/L; 0.22 g of molecular sieves/g of water produced; 0.5% w/v of L435; temperature of 60 °C for 24 h under stirring (200 rpm) in a Marconi shaker. Subsequently, the biocatalyst was separated by centrifugation (10,000 rpm, 2 min, 35 °C) and 1 mL of the supernatant was dried at 70 °C for 24 h. After

evaporating the solvent, samples were prepared for the quantification of unconverted xylose and oleic acid by liquid and gas chromatography, see sections 5.1.2.6.1 and 5.1.2.6.2, respectively.

5.1.2.2.3. Effect of the temperature on the L435 activity profile

The esterification reaction was conducted at different temperatures (50, 55, 60, 70, 80 °C) with 0.17, 0.23, and 0.33% w/v of L435 at the following conditions: 200 rpm, xylose: oleic acid molar ratio of 1:5, 0.22 g of molecular sieves/g of water produced. Samples were collected over 5, 10, 15, 20, and 30 min of reaction and the initial xylose consumption rates were determined according to the methodology described in section 5.1.2.6.1 (see below). Enzymatic activities were then obtained in U/g, where 1 Unit represents the consumption of 1 µmol of xylose/minute/gram of L435.

5.1.2.2.4. Determination of esterification rates for L435

a) Determination of initial rates

Esterification reactions were conducted at 60 °C under continuous stirring (200 rpm) for 15 min, with 0.23% w/v of L435 and 0.22 g of molecular sieves/g of water produced. In one set of experiments, the xylose concentration was varied from 0.28 to 7 mmol/L with a fixed quantity of oleic acid (1.4 mmol/L), while in another set of experiments, the amount of oleic acid was varied from 1.4 to 106 mmol/L with a fixed quantity of xylose (7 mmol/L). The biocatalyst was separated by centrifugation at 10,000 rpm for 2 min (35 °C) and the supernatant was then prepared for the quantification of unconverted xylose as described in section 5.1.2.6.1 (see below).

b) Determination of long-term rates

The following conditions were used to conduct esterification reactions in a vortex flow reactor (80 mL): 60 °C; 2,000 rpm; 1% w/v of L435; and xylose: oleic acid molar ratio of 1:5. The reactor specifications were: radius ratio ($\eta = R_{in}/R_{out}$) of 0.24, aspect ratio ($\Gamma = L/d$) of 6.72, and rotation of the inner cylinder (metallic rod coupled to an IKA overhead stirrer, IKA Werke GmbH & Co. KG, Breisgau, Germany) at 2,000 rpm stirring. Tests were performed in the presence of molecular sieves (0.22 g/g of water produced). Samples were collected after 30 min, 1 h, 3, 6, 9, 12, and 24 h of reaction, and quantified according to the methodologies presented in sections 5.1.2.6.1 and 5.1.2.6.2 (see below).

5.1.2.2.5. Determination of the L435 operational stability

To evaluate the operational stability of the biocatalyst, the L435 catalyzed synthesis of xylose oleate was performed in 10 successive 12 h-reaction batches. Reactions were conducted in a vortex flow reactor under the following conditions: 60 °C, continuous stirring (2,000 rpm), xylose: oleic acid molar ratio of 1:5 (7 mmol/L of xylose), 0.22 g of molecular sieves/g of water produced, 10% w/v of L435. After each batch, the biocatalyst and molecular sieves were recovered by centrifugation (35 °C; 10,000 rpm; 2 min) and washed with MEK at room temperature in a commercial sieve, which retained all molecular sieves. L435 was anew washed with MEK and subjected to vacuum filtration to remove the remaining solvent and reused in a new synthesis. Xylose and oleic acid conversions were measured by liquid and gas chromatography, as described in Sections 5.1.2.6.1 and 5.1.2.6.2 (see below), respectively.

5.1.2.3. Purification and characterization of xylose oleate

The purification of the crude sugar ester reaction product followed a protocol adapted from Wagner *et al*. (1991). In a beaker, 20 mg of the esterification reaction product were added to 2 mL of an ethanol-water solution (50:50, v/v), and magnetically stirred. After complete homogenization, the stirring was stopped, and the solution was left standing for 1 hour at room temperature. The precipitate was collected by centrifugation at 10,000 rpm for 15 min and mixed with 2 mL of the ethanol-water solution at room temperature. The ethanol-water solution washed precipitate was collected by centrifugation as described above. This last step was repeated (a total of two washes with alcohol-water solution). The recovered ethanol-water solution washed precipitate was then mixed with 2 mL of MEK at room temperature. The MEK-washed precipitate was recovered by centrifugation as previously described. This step was repeated three times and the recovered organic solvent washed precipitate was dried in a vacuum oven.

The purified xylose oleate was then characterized by mass spectrometry using an electrospray ionization quadrupole time-of-flight MS/MS operated in the negative mode on an Agilent 6545 ESI-QTOF-MS instrument, across the mass range of 50-1200 Da. Samples were dissolved in methanol and analyzed by FIA at a flow rate of 0.35 mL/min and a volume injection of 5.0 µL at 38 °C. The mobile phase consisted of H_2O + 0.1% formic acid and MeCN + 0.1% formic acid 20:80 with an analysis time of 4.0 min. The ESI source conditions were as follows: capillary voltage 2500 V, nozzle voltage 500V,

gas temperature 350 °C, drying gas 13 L/min, nebulizer 35 psi, sheaf gas 320 °C in a flow of 10 L/min, fragmentor 120 V, skimmer 80 V, and collision energy voltage of 20 and 22 V. Data were analyzed with the use of the Qualitative Navigator B.08.000 software.

5.1.2.4. Emulsion capacity assays

Emulsion capacity (EC) assays were performed by dissolving 10 mg of the purified reaction product in 1 mL of water in a test tube and mixing the resultant product with 2 mL of kerosene. The content of the tube was continuously homogenized for 2 min at room temperature (25 °C) and then left standing for 24 hours. Tests were performed in duplicate, and the standard deviations were less than 1%. After this period, the height of the emulsified region and the height of the total column were measured, and the ECs were calculated according to Equation 1 (CAVALCANTI *et al.*, 2017). Sucrose monolaurate was used as a standard for these experiments.

$$EC\ (\%) = \frac{H_e}{H_t}.100 \quad (1)$$

Where:

H_e: height of the emulsified region;

H_t: height of the total column.

5.1.2.5. Standard enzymatic activities

5.1.2.5.1. L435 hydrolysis activity

The protocol to measure the L435 hydrolysis activity in tributyrin is an adaptation of the methodology described by Beisson *et al*. (2000). The method is based on the hydrolysis of a mixture of 1.5 mL of tributyrin, 6 mL of 100 mmol/L phosphate buffer, pH 7.3, and 16.5 mL of distilled water, at 37 °C for 5 min. For these assays, 24 mg of L435 were added to the medium. The released butyric acid was titrated with 0.02 mol/L KOH solution in pH-Stat titrator (Titrando 907, Metrohm, Herisau, Switzerland). A unit of tributyrin hydrolysis activity is defined as the amount of enzyme that releases 1 μmol of butyric acid per minute under the conditions described above.

5.1.2.5.2. L435 esterification activity

The L435 esterification activity was determined through the esterification of butyric acid (0.1 mol/L) with butanol (0.1 mol/L) in heptane (10 mL) at 37 °C. The reaction was conducted in agitated closed flasks (250-300 rpm) and monitored by the consumption of butyric acid by titration with 0.02 mol/L KOH solution, using

phenolphthalein as an indicator (DE LIMA *et al.*, 2018; KOPP *et al.*, 2015; VESCOVI *et al.*, 2016). For this assay, 0.0025, 0.0035, and 0.005 g of L435 were added to the medium.

5.1.2.6. Chromatographic analyses

The reliability of all chromatographic methods applied in this research was confirmed by samples of known concentrations with amounts close to those expected (≥ 90% accuracy).

5.1.2.6.1. Xylose determination

The final reaction medium was centrifuged, and 1 mL of the supernatant was collected. After evaporating the solvent, 1 mL of water was added to the samples and after homogenization, it was filtered using 0.22 μm syringe filters. The xylose concentration was measured using an HPLC Breeze equipped with a refractive index detector (RID) and a Sugar Pak-I column (300 x 6.5 mm x 10 μm) maintained at 80 °C. A solution of EDTA-Ca (50 mg/L) was used as eluent, at 0.5 mL/min. The injection volume was 20 μL, with a run time of 20 min (VESCOVI; DOS SANTOS; TARDIOLI, 2017).

Similar conversions were identified by another method, which anew confirms the reliability of this protocol. The second one performs the washing and homogenization of the final reaction medium with distilled water and removes the aqueous phase for xylose quantification.

5.1.2.6.2. Oleic acid determination

The reaction medium was centrifuged, and 1 mL of the supernatant was collected. The concentration of oleic acid in the samples was measured using an Agilent gas chromatograph 7890A (Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a Restek Rtx-wax column (30 m × 0.25 mm × 0.25 μm; Restek Corporation, Bellefonte, PA, USA) maintained at 200 °C for 1 min, 230 °C for 1 min (10 °C/min), and 250 °C for 5 min (5 °C/min). Helium was used as carrier gas at a flow rate of 1.8 mL/min, with a run time of 14 min (The essential chromatography & spectroscopy catalog, 2011/2012).

5.1.2.6.3. Furfural determination

The presence of the xylose degradation product (furfural) was verified using an HPLC Breeze equipped with a UV–visible detector (274 nm) and a C-18 (Ascentis

Express) column (10 cm x 46 mm x 2.7 μm, Supelco, Bellefonte, USA) maintained at 40 °C. A solution of acetonitrile/water 1:8 v/v with 1% acetic acid was used as eluent, at 0.8 mL/min. The injection volume was 20 μL, with a run time of 15 min (MILESSI *et al.*, 2020).

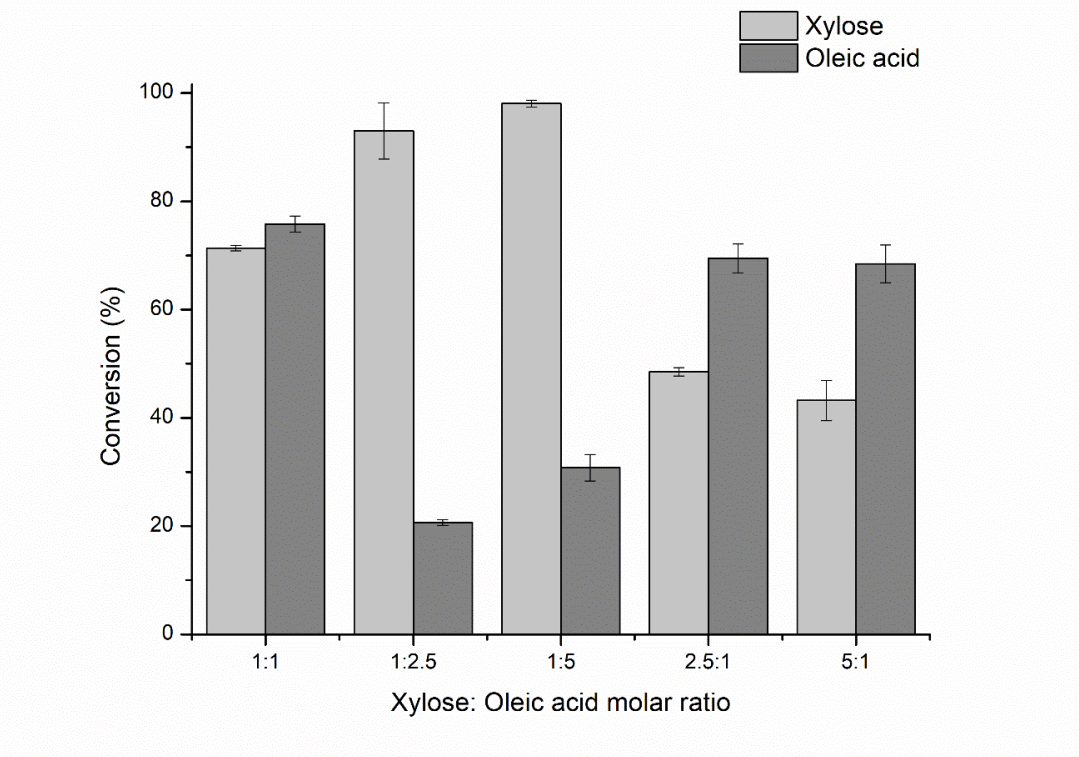
5.1.3. Results and discussion

5.1.3.1. Enzymatic synthesis of xylose oleate

5.1.3.1.1. Effect of the xylose: oleic acid molar ratio on the process performance

After evaluating the xylose solubility in MEK (7.16 ± 0.35 mM), we assessed the xylose/oleic acid molar ratios in the esterification reaction to optimize the production of xylose oleate. From the results shown in Figure 5.1, an excess of oleic acid (1:5 molar ratio), that turns the hydroxyl and acid molar ratio into 1:1.25, had a positive effect on the reaction, leading to a consumption of 98% xylose and 31% oleic acid. This suggested that xylose was modified with one or two molecules of oleic acid, as the ratio is 1.25 molecules of oleic acid per hydroxyl group of each xylose molecule. Very likely the lipase selectivity prevented the incorporation of more fatty acid molecules. Pappalardo *et al.* (2017) reported that in the esterification of palmitic acid with L-(+)-arabinose (1:1 molar ratio) the Novozyme 435 preferentially catalyzed the acylation of the primary hydroxyl group at the C-5 position of the carbohydrate because the secondary hydroxyl groups are less accessible to the active site of the enzyme. In fact, when we used the xylose: oleic acid molar ratio of 1:1, both xylose and oleic acid conversions were very close (around 70 and 75%, respectively, Figure 5.1), indicating that almost only one hydroxyl group of the xylose was substituted.

However, the use of an excess of fatty acid enhances the substrate availability for the enzyme and facilitates the acylation, also shifting the equilibrium in the direction of the synthesis, thus, leading to higher xylose conversions (TARAHOMJOO; ALEMZADEH, 2003). Some studies addressing the lipase-catalyzed synthesis of xylose fatty esters also used an excess of fatty acid to sugar, as shown in Table 5.1.



Reaction conditions: 60 °C, 24 h-reaction, 200 rpm, 7 mM of xylose, 0.5% w/v of L435, with molecular sieves.

Figure 5.1. Optimization of the xylose: oleic acid molar ratio in the synthesis of xylose oleate in methyl ethyl ketone.

Table 5.1. Components and conditions of the reaction system in the synthesis of xylose fatty esters and corresponding conversions.

Acyl donor	Xylose: FA molar ratio	Solvent	Conditions	Biocatalyst	Conversions (%)	References
Capric acid	1:4	DMSO; ACT	60 °C, 24h	N435	64 (FAC)	Abdulmalek; Hamidon; Rahman, 2016
Oleic/Lauric acids	1:5	T-but	46-55 °C, 48h	CALB IM T2-350 CALB-SMMP-O CALB-SMMP-OG	XC: 52/54 (46 °C); 65 (55 °C) 50 (46 °C); 61/66 (55 °C) 57/50 (46 °C); 69/66 (55 °C)	De Lima *et al*., 2016
Vinyl laurate	1:3	2M2B; HEX; THF	50 °C, 4h	N435	74.8 (XC)	Méline *et al*., 2018
Butyric/caprylic/oleic acids	1:5	T-but, T-pent	60 °C, 24h	PPL-OS	70 (XC)	Vescovi; Dos Santos; Tardioli, 2017
Acrylic acid	1:4.2	T-but	55 °C, 48 h	CALB IM NN	4.2 (XC)	Tsukamoto *et al*., 2008
Oleic acid	1:5	MEK	60 °C, 24 h	L435	98 (XC), 31 (FAC)	This study

T-but: Tert-Butyl alcohol; T-pent: Tert-Pentyl alcohol; 2M2B: 2-Methyl-2-butanol; THF: Tetrahydrofuran; DMSO: Dimethyl sulfoxide; ACT: Acetone; HEX: Hexane; CALB-SMMP-O: CALB immobilized on silica magnetic microparticles and functionalized with octyl groups; CALB-SMMP-O-G: CALB immobilized on silica magnetic microparticles and functionalized with octyl and aldehyde groups; PPL-OS: Porcine pancreatic lipase immobilized on octyl-silica; CALB IM N: CALB immobilized on a macroporous acrylic acid resin by Novo Nordisk A/S; FAC: Fatty acid consume; XC: Xylose consume.

Conversely, an excess of sugar had a negative effect on the reaction performance, with lower yields even with respect to the acid. These lower yields after 24 h should be related to a kinetic problem, since thermodynamically the yields regarding the minority substrate should increase. It seems that oleic acid has some positive effect on the reaction kinetics, and its reduction produces some negative effects. Perhaps, the acylation of the enzyme by the acid is the limiting step of the reaction and when decreasing the concentration of the acid, the effect on the reaction rate is rather significant (in fact, as determined in section 5.1.3.1.3, the Km for this compound is high—see below). Moreover, an excess of oleic acid (a mild detergent) may increase the solubility of the xylose in the solvent or prevent its adsorption on some surfaces. Finally, it can produce some positive changes in the enzyme conformation. Although it has been shown that an excess of oleic acid prevents the xylose adsorption to the molecular sieves (as described below), the other effects may also cause a decrease in the yields when the oleic acid concentration decreases.

When the molar ratio of 1:5 was used, if the reaction produced only monoesters, the maximum theoretical oleic acid conversion should be ~20%, regarding the xylose conversion of 98%. Nonetheless, the reaction achieved experimentally around 31% oleic acid conversion, suggesting that not only monoesters were produced (in fact, mass spectrometry later confirmed the presence of mono- and diesters, besides the presence of some minority of triesters — section 5.1.3.2, see below). The formation of this mixture of esters may be due, among other factors, to the solvent used in the reaction, as reported by Li *et al*. (2015), who described significant differences in the diester/monoester molar ratios after the direct esterification of fructose with lauric acid catalyzed by Novozyme 435 with 2-methyl-2-butanol (2M2B) and MEK as solvents. In that paper, when using MEK, the diester/monoester molar ratio was ~3:1, while when using 2M2B, it was 1:1. Furthermore, when the authors directly employed monoester and the fatty acid in the synthesis of diesters, the maximum initial rate of diester synthesis in MEK was 2.2-fold greater than the rate in 2M2B, indicating that CALB conformation in MEK preferred the monoesters as substrate more than in 2M2B. Therefore, the use of MEK as a solvent may partially explain the formation of diesters pointed out here. Although the CALB is reported to produce preferentially sugar monoesters (PAPPALARDO *et al.*, 2017; SHI; LI; CHU, 2011), intramolecular acyl migrations can occur, mainly during long time processing, thus allowing to produce heterogeneous mixtures of esters with different degrees of acylation, a typical outcome observed in chemical processing of sugar esters,

requiring laborious and costly protection and deprotection of hydroxyl groups (FERNANDEZ-LORENTE *et al.*, 2003; FILICE *et al.*, 2009; GUMEL *et al.*, 2011; HERNANDEZ *et al.*, 2011).

To synthesize only monoesters using the molar ratios of 2.5:1 and 5:1, the highest xylose conversions should be theoretically 28% for an oleic acid conversion of ~70% and 14% for an oleic acid conversion of 68.5%, respectively. However, experimentally higher xylose conversions were reached (48.5% for the 2.5:1 molar ratio and 43.2% for the 5:1 molar ratio, as shown in Figure 5.1), which are not plausible considering that the acid was used as a limiting reagent in this set of experiments. The logical explanation for this is that xylose may be somehow decomposing, precipitating, or reacting, and when the yields are calculated, they are overestimated. The presence of the xylose degradation product (JÖNSSON; MARTÍN, 2016) (furfural, section 5.1.2.6.3) was not observed.

After discarding the modification of xylose to furfural, we checked if this sugar can be adsorbed in some of the material employed in the reaction. Thus, we conducted some assays to verify if the xylose depletion only occurs for its consumption during the esterification reaction, and not by long time precipitation in the presence of the reaction product, which could affect the calculation of the conversions based on remaining unmodified xylose. We found that the final precipitate not dissolved in water (from the samples to be quantified by HPLC analysis—section 5.1.2.6.1) did not contain xylose.

We also washed the biocatalyst before and after the reaction (24 h) with water and a high ionic strength buffer and performed the subsequent xylose quantification, to check if xylose could be adsorbed in the support. The same procedure was conducted with the molecular sieves (MS) used in the reaction to capture the released water (GANDHI *et al.*, 2000; MENSAH; CARTA, 1999; VULFSON *et al.*, 2003). Results demonstrated that the sugar was not adsorbed on Lipozyme 435, while 11.23 ± 2.20% of the xylose was adsorbed on MS at the end of the reaction when an excess of xylose was used. The synthesis of xylose oleate catalyzed by the soluble CALB (Lipozyme CALB L) was also conducted under the conditions aforementioned. The xylose adsorption percentage on MS was 14.32 ± 5.37%. We performed this same approach within only 15 min of reaction catalyzed by Lipozyme 435 and verified that 10.87 ± 0.82% of the xylose was adsorbed on MS (again only when using an excess of xylose). This means that, in this case (i.e., excess of sugar), after only 15 min of reaction, MS surface retained a significant percentage of our hydrophilic by-product (residual xylose). This, in our assay, was

confused with a conversion of this reagent. When using an excess of oleic acid (1:2.5 and 1:5), the xylose was not adsorbed on MS at all, being no longer a problem.

Just for academic purposes, we used the mean of those results (11.05 ± 0.25%) as a correction factor for the observed xylose conversions using an excess of xylose (molar ratios of 2.5:1 and 5:1). Figure 5.1 shows the xylose conversions already corrected. With the molar ratios with an excess of fatty acid this correction was not necessary and so, the conversions remained the same. Though, the xylose conversions are still higher than those expected from the oleic acid conversion when the fatty acid was the minority reagent, suggesting that some other factor may be relevantly affecting this reaction when using low oleic acid (as discussed above) and must be deeper investigated in further studies. Nevertheless, we intend to have a full conversion of xylose to esters, therefore conditions using a molar excess of sugar were not interesting for our goal. That way, we have continued with the research using an excess of oleic acid.

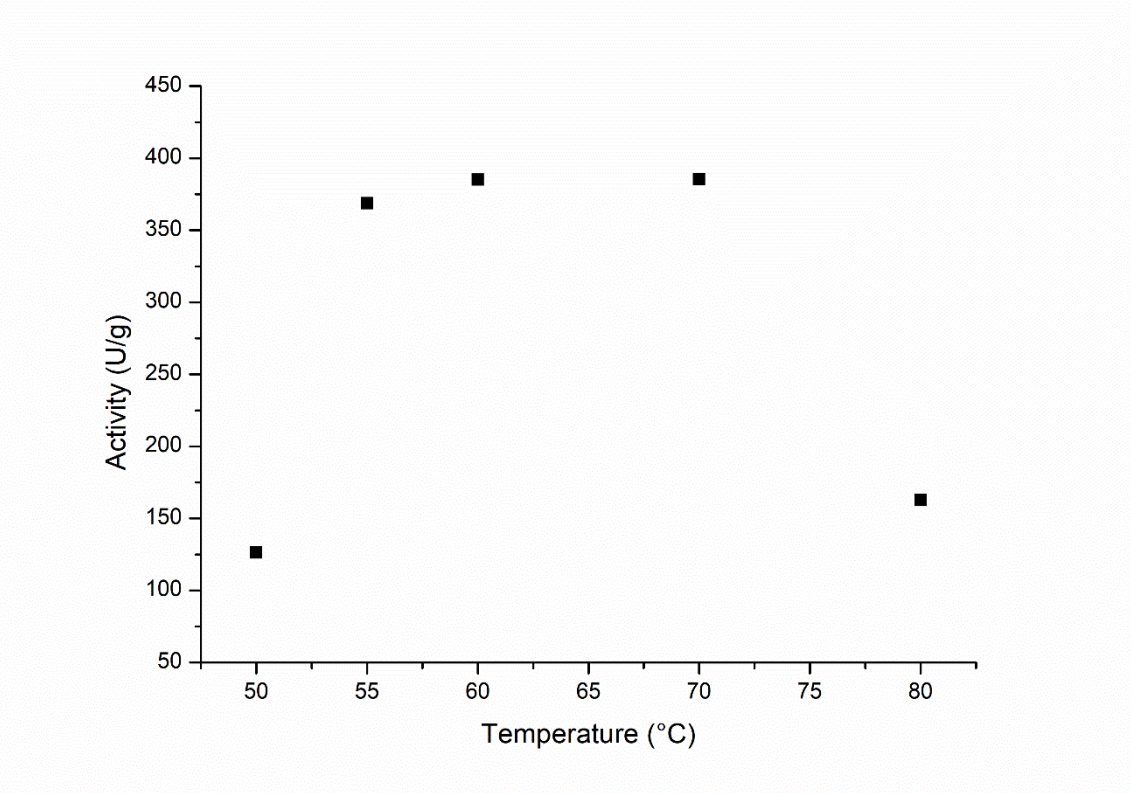
Some studies already evaluated the use of molecular sieves in the enzyme-catalyzed synthesis of xylose fatty esters (DE LIMA *et al.*, 2016; MÉLINE *et al.*, 2018; TSUKAMOTO *et al*., 2008; VESCOVI; DOS SANTOS; TARDIOLI, 2017). Among those authors, Tsukamoto *et al*. (2008) found higher xylose conversions in the presence of MS when compared to the conversions in the absence of them. Nonetheless, they did not assess whether the xylose was adsorbed on the MS surface, which could mask the results. In view of our findings, we selected the xylose: oleic acid molar ratio of 1:5 for subsequent assays of xylose acylation.

5.1.3.1.2. Effect of the temperature on the L435 activity profile

Figure 5.2 shows the Lipozyme 435 activity profile in the esterification reaction for the temperature range from 50 to 80 °C. The highest activities (expressed as a function of the xylose consumption) occurred between 55 and 70 °C. A temperature higher than 70 °C greatly reduced the enzyme activity (>55%, at 80 °C) and therefore the substrate conversion.

Temperature influences both the reaction rate and the inactivation rate of the enzyme. At 50 °C the enzyme activity is about 30% of the maximum activity. Considering that there is no significant enzyme inactivation during the initial rate assays, it shows that the reaction is favored from 60 to 70 °C. The reduction in the enzyme activity observed for temperatures above 70 °C is very likely due to the enzyme thermal inactivation (SCHMIDELL *et al*., 2001). Ljunger, Adlercreutz, and Mattiasson (1994), Lortie (1997),

Oguntimein, Erdmann, and Schmid (1993), and Schlotterbeck *et al*. (1993) also reported a similar behavior with the same biocatalyst in other esterification reactions. In our study, a temperature of 60 °C was chosen to perform the subsequent assays.

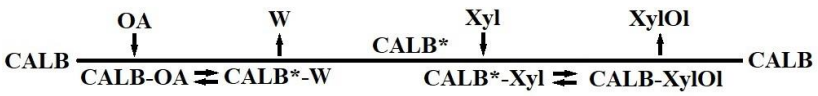


Reaction conditions: xylose: oleic acid molar ratio of 1:5, 200 rpm, enzyme load of 0.16; 0.23 and 0.33% w/v, with molecular sieves, in methyl ethyl ketone.

Figure 5.2. Activity profile as a function of the temperature for L435.

5.1.3.1.3. Esterification rates for L435

The effects of a wide range of concentrations of both substrates (xylose and oleic acid) on the reaction rates were studied (see the reaction conditions in Section 5.1.2.2.4). The Ping-Pong Bi-Bi model (appendix) was selected after a careful analysis of the literature to describe this reaction mechanism, as shown in Scheme 1. According to this mechanism, oleic acid initially binds to the CALB (Lipozyme 435) to form the non-covalent CALB-oleic acid (CALB-OA) that is subsequently transformed to an acyl-CALB intermediate, releasing the first product, water. Then, the acyl-CALB intermediate (CALB*) binds xylose (Xyl), forming the CALB-xylose oleate (CALB-XylOl), which then yields the ester product, xylose oleate (XylOl), and the free CALB (Lipozyme 435).



Scheme 1. Ping-Pong Bi-Bi model of the reaction mechanism. OA is oleic acid, W is water, Xyl is xylose, and XylOl is xylose oleate.

The rate equation for the Ping-Pong Bi-Bi mechanism is as follows:

$$v = \frac{v_{máx}.[OA].[Xyl]}{K_{m_{(OA)}}.[Xyl] + K_{m_{(Xyl)}}.[OA] + [OA].[Xyl]} \quad (2)$$

where v is the initial reaction rate, $[OA]$ and $[Xyl]$ are the respective concentrations of oleic acid and xylose, $v_{máx}$ is the maximum velocity or limiting rate, $K_{m_{(OA)}}$ and $K_{m_{(Xyl)}}$ are the respective Michaelis constants for oleic acid and xylose.

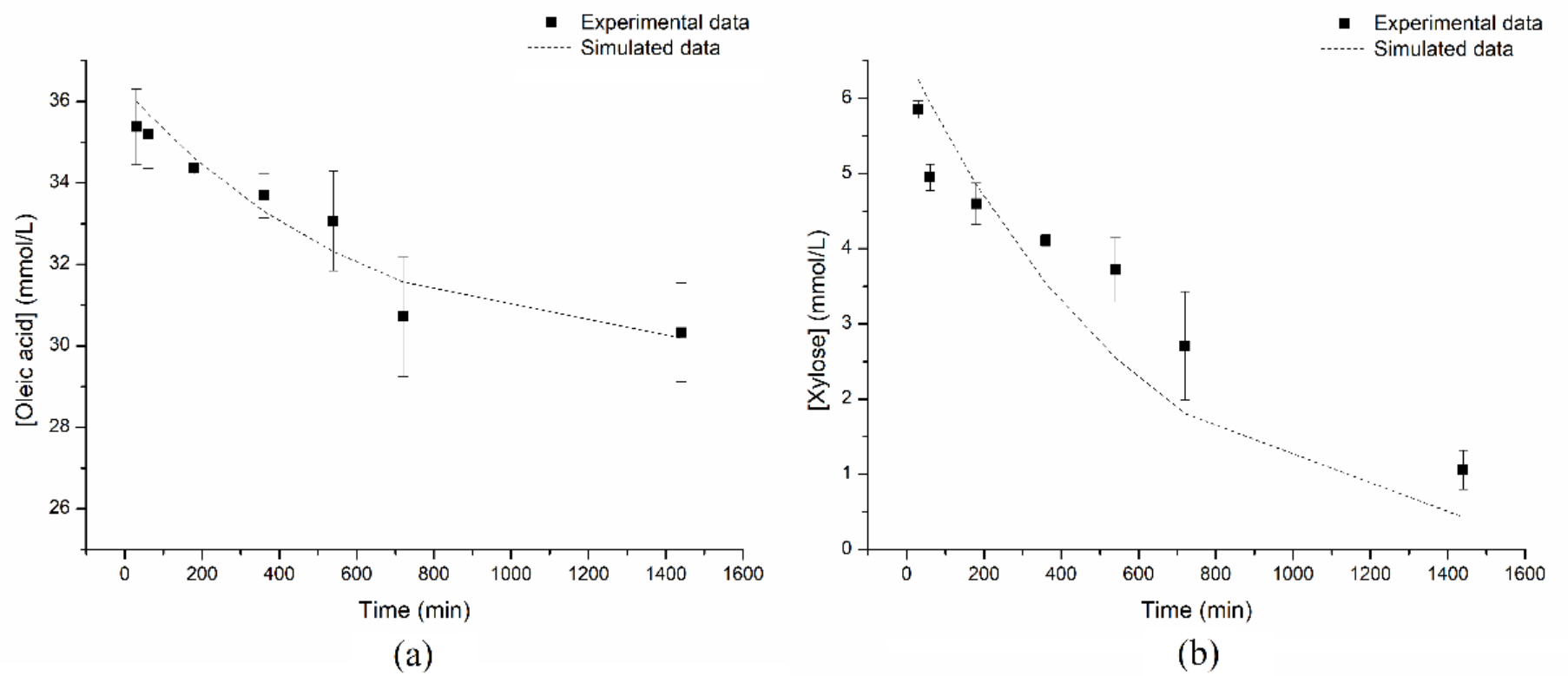
Kinetic constants of initial rate assays were estimated by Lineweaver-Burk plots generated by a linear regression method (OriginPro 8.5 Software) (Figures S4 and S5, Table S8—Supplementary Materials) and used as "initial guesses" in the fitting of the Ping-Pong Bi-Bi model to experimental data of a long-term assay (carried out in Force 2.0 Module). Table 5.2 shows the kinetic parameters for the model (Equation (2)) fitted to the long-term experimental data. They were of the same order of magnitude as those found by Zaidan *et al*. (2012), who synthesized lactose fatty esters from capric acid in the presence of free lipase and lipase immobilized on a new support of mica clay. Moreover, it can be expected from the K_m values that commercial immobilized CALB (Lipozyme 435) has a poor affinity by both xylose and oleic acid, as the values are over 1 M for oleic acid and over 0.75 M for xylose.

Li *et al*. (2015) also found high Km values for lauric acid (11.6 M) and fructose (6.5 M) in the esterification catalyzed by N435 in 2M2B (50 °C, sugar/acid molar ratio of 1:1), using the Ping Pong Bi Bi model without inhibition. Dang, Obiri, and Hayes (2005) reported Km values of 0.4 M for both oleic acid and fructose in T-but, anew employing the non-inhibited Ping Pong Bi Bi model. The esterification was conducted at 65 °C, at a sugar/acid molar ratio of 1:1, and catalyzed by Lipozyme IM, a commercial lipase from *Rhizomucor miehei* immobilized on microporous anionic resin beads. The oleic acid and xylose consumption profiles of experimental and simulated data (from the kinetic constants calculated in this paper) are in Figure 5.3. Results indicated that the model fitted to the experimental data.

Table 5.2. Kinetic constants of the Ping-Pong Bi-Bi model (Eq. 2) fitted to the enzymatic esterification data (fatty acid and sugar consumption).

Parameters	
V_{max} (mmol/L/min)	1.57
$K_{m(OA)}$ (mM)	1120.40
$K_{m(Xyl)}$ (mM)	754.16

Reaction conditions: 60 °C; 2,000 rpm; 1.0% w/v of L435; xylose: oleic acid molar ratio of 1:5; with the addition of molecular sieves. Samples were collected after 30 min, 1 h, 3, 6, 9, 12, and 24 h of reaction.

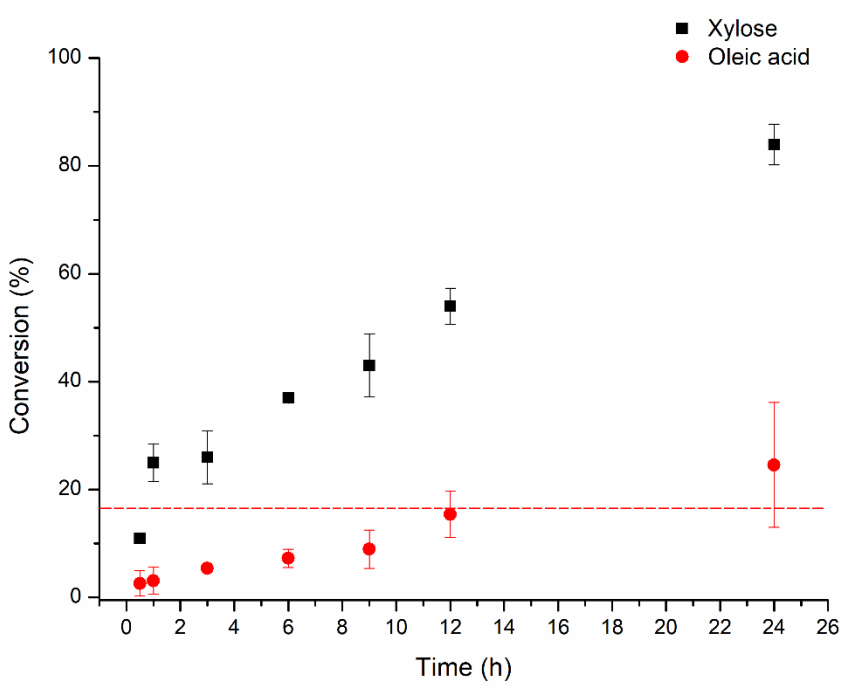


Reaction conditions (experimental data): 24 h-reaction; 60 °C; 2,000 rpm; enzyme load of 1.0% w/v; xylose: oleic acid molar ratio of 1:5; with molecular sieves.

Figure 5.3. Plots of the experimental and simulated data of oleic acid (a) and xylose (b) consumption in the synthesis of xylose oleate in MEK catalyzed by L435.

5.1.3.1.4. Conversion profiles in the synthesis of xylose oleate

Xylose and oleic acid conversion profiles during the esterification reaction are presented in Figure 5.4. Here, we used an excess of fatty acid to xylose, since we already demonstrated that this condition provided higher conversions in the Lipozyme 435 catalyzed esterification reaction. Almost all xylose was consumed after 24 h of reaction (84%) and 24.6% of the oleic acid was depleted after the same period, anew suggesting that more than one hydroxyl group per xylose molecule was modified by the acyl moiety of the oleic acid. De Lima et al. (2016) also reported a high xylose conversion (~65%) in the synthesis of xylose oleate catalyzed by immobilized CALB (CALB IM—T2-350) in tert-butyl alcohol (55 °C, xylose: oleic acid molar ratio of 1:5, 24 h). Vescovi, Dos Santos, and Tardioli (2017) achieved a xylose conversion around 70% (60 °C, 24 h, 1:5 xylose/oleic acid molar ratio in tert-butyl alcohol) in the synthesis of xylose oleate catalyzed by porcine pancreatic lipase immobilized on octyl-silica. Abdulmalek, Hamidon, and Rahman (2016) documented capric acid conversions ranging from 50 to 64% (depending on the solvent system, which could be: acetone, acetone:DMSO, T-but:DMSO, hexane:DMSO) in the synthesis of xylose caproate catalyzed by Novozyme 435 at 60 °C after 24 h-reaction (xylose: capric acid molar ratio of 1:4).



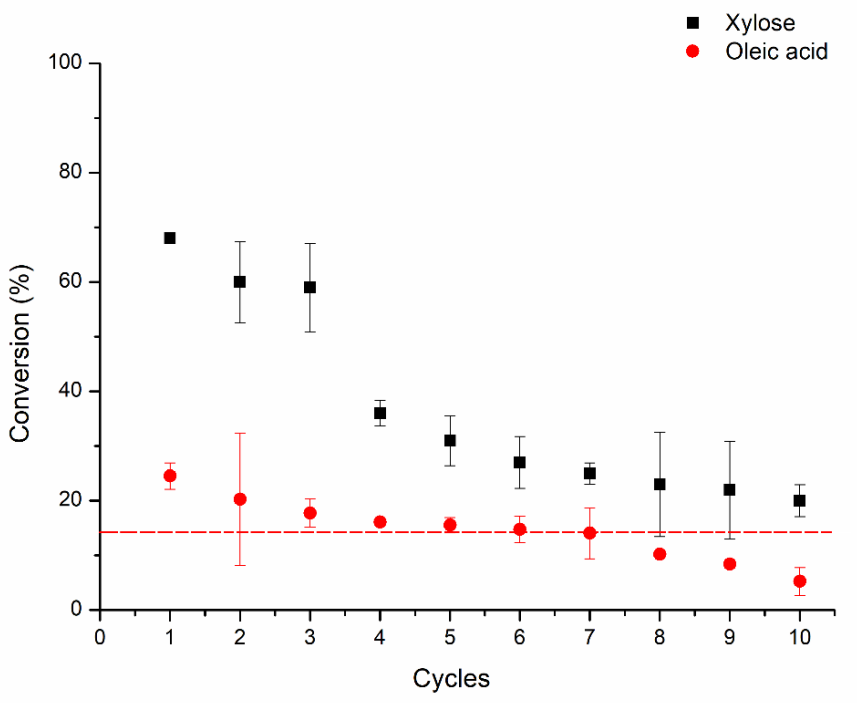
Reaction conditions: 60 °C; 2,000 rpm; enzyme load of 1.0% w/v; xylose: oleic acid molar ratio of 1:5; with molecular sieves.

Figure 5.4. Xylose and oleic acid conversion profiles in the synthesis of xylose oleate catalyzed by L435 in a vortex flow reactor. The dotted line represents the expected maximum conversion of oleic acid regarding the modification of only one hydroxyl group of the xylose.

Bidjou-Haiour and Klai (2013) achieved ~50% lauric acid conversion at 60 °C after 24 h using MEK as a cosolvent in the synthesis of xylose laurate catalyzed by Novozyme 435 (xylose: lauric acid molar ratio of 1:1); so the abovementioned results emphasize the importance of the conversions achieved in this study through the use of the Lipozyme 435 biocatalyst in the synthesis of xylose fatty acid esters.

5.1.3.1.5. L435 operational stability

In industrial applications, recovery and reuse (recycling) of enzymes are often an essential cost-reduction measure. The effect of repeated use of Lipozyme 435 on the substrates conversions was investigated in successive reaction batches (Figure 5.5). Results demonstrated a decrease of 48 and 19% in the xylose and oleic acid conversions, respectively, after ten 12 h-cycles. Vescovi et al. (2016) also reported a conversion decrease of 31% (in the synthesis of fructose oleate) after nine 6 h-batches with the Novozyme 435. Ward, Fang, and Li (1997) described a drop of 55% in the conversions after eight 24 h reaction cycles. They synthesized a sugar ester from 1,2-o-isopropylidene-n-xylofuranose and arachidonic acid. Moreover, the authors estimated that 8–10% of the Novozyme 435 activity was lost per cycle at the end of the reuse. In another study, the conversion of glucose esters from different N-fatty acyl glycines decreased 30% for the 5th 9 h cycle with Novozyme 435 (AN *et al.*, 2019).



Reaction conditions of each batch: 60 °C; 2,000 rpm; enzyme load of 10% w/v; xylose: oleic acid molar ratio of 1:5; 12 h-cycles; with molecular sieves.

Figure 5.5. L435 operational stability.

This decrease in the conversions over the reuse could be partially explained by the CALB desorption from the support while the product is engendered, as was reported by De Lima *et al*. (2016), Neta et al. (2012), and Vescovi *et al*. (2016). Indeed, we verified a reduction of ~50 ± 0.77% in the Lipozyme 435 hydrolytic activity (Section 5.1.2.5.1) after 12 h of incubation in the reaction medium of xylose oleate synthesis.

Since Lipozyme 435 presents the CALB non-covalently linked to the support (Lewatit VP OC 1600), this behavior could be attributed to some main aspects that may greatly facilitate the CALB release from the matrix: (1) a high temperature may weaken the enzyme-support interaction (RUEDA *et al.*, 2015) (although this interaction is very strong, CALB has a very small lid) (MANOEL *et al.*, 2015); (2) high concentrations of substrates-products with surfactant properties (e.g., oleic acid and xylose oleate) could break those interactions and anew cause the lipases release to the medium (VIRGEN-ORTÍZ *et al.*, 2017).

Another fact that could decrease the operational stability of the CALB is the incomplete removal of the compounds adsorbed to its surface between each batch. For instance, water (a side-product of the esterification reaction) is adsorbed on molecular sieves to shift the equilibrium to the direction of the synthesis (COLOMBIÉ *et al.*, 1998; MENSAH; CARTA, 1999). If this water formation is more rapid than the diffusion, and the support pores or matrix are more hydrophilic than the reaction medium, water can be accumulated inside the biocatalyst forming an aqueous phase (MARTINS *et al.*, 2013). This way, acids can also be accumulated in this environment, exposing the enzyme to low pH values and promoting enzyme inactivation by these drastic conditions (MARTINS *et al.*, 2011). Furthermore, water accumulation could make the thermodynamics of the process unfavorable in the enzyme proximity, reducing the enzyme activity *(*ORTIZ *et al*., 2019).

Additionally, a minor reason for these reductions in the conversions could be the known mechanical fragility of Lewatit under stirring (GARCIA-GALAN *et al.*, 2011; SANTOS *et al.*, 2015). This may be an important negative feature when considering the use of Lipozyme 435 in mechanically stirred batch reactors. Nonetheless, this issue was minimized with the use of a stirred reactor configuration less aggressive with the mechanical structure of the beads (a vortex flow reactor) (LIU *et al*., 2013). Further, the support solubility in MEK could represent another cause of the low operational stability of Lipozyme 435 in the synthesis of SFAEs that cannot be discarded (RUEDA *et al.*, 2015).

These drawbacks have been considered as the main problems for the industrial implementation of Lipozyme 435 (IDRIS; BUKHARI, 2012). Thus, in accordance with other authors, we demonstrated the limited repeatability and stability of Lipozyme 435, which are crucial factors for cost reduction in large-scale sugar esters production. This could be solved using a lower temperature, which will require longer reaction times. The final decision will depend on the weight that the different parameters may be given in a specific factory (necessity of the use of a fresh enzyme or enlarge the reaction cycles). Another alternative may be to improve the biocatalyst stability by using a better support or by modifying the Lipozyme 435 surface (BARBOSA *et al.*, 2012; BILAL *et al.*, 2019; RUEDA *et al.*, 2016).

5.1.3.2. Purification and characterization of xylose oleate

The esterification reaction here described results in a crude sugar ester reaction product containing the sugar ester (xylose oleate), the biocatalyst (Lipozyme 435), the anhydrous solvent (MEK), as well as unreacted sugar (xylose), and unreacted fatty acid (oleic acid). So, a method for purifying and separating the sugar ester from the crude reaction product is required.

Samples of the crude sugar ester reaction product from the esterification of xylose with oleic acid were purified according to an adaptation of the methodology described by Wagner *et al*. (1991) (Section 5.1.2.3). Most of the unreacted xylose and fatty acid molecules were removed after this process (≥89.64% and 97.01, respectively).

To confirm the successful enzymatic formation of xylose oleate, the purified samples were then characterized by mass spectrometry (see Figures S6–S8 in the Supplementary Materials). Mass spectra of the $[M\text{-}H]^-$ ion with *m/z* = 413.2 and 677.5 revealed that Lipozyme 435 mainly produced xylose mono- and dioleate (xylose: oleic acid molar ratio of 1:5). To a lesser extent, we also detected the presence of xylose trioleate (*m/z* = 941.7). Therefore, the possible formation of a mixture of esters mentioned above was confirmed here by mass spectrometry. Although immobilized CALB (Novozyme 435) has been reported to be selective to modify only one hydroxyl group at the C5 primary position (PAPPALARDO *et al.*, 2017), it has also been described that the solvent can change this behavior, with MEK preferentially leading to the formation of diester/monoester at an approximate molar ratio of 3 to 1 (LI *et al.*, 2015).

5.1.3.3. Emulsion capacity assays

SFAEs are nonionic surfactants that exhibit emulsifying, stabilizing, and detergency effects, thus finding applications in the food, cosmetic, detergent, and pharmaceutical industry (NETA; TEIXEIRA; RODRIGUES, 2015). The emulsion capacity (EC) of the sugar ester synthesized and purified in this study (xylose oleate in MEK using a xylose: oleic acid molar ratio of 1:5) was of the same order of magnitude of a commercial sugar ester (sucrose monolaurate); 6.25 and 10%, respectively. This may enable the future application of those esters as industrial surfactants, along with other indicators to be assessed. The EC is highly correlated with the medium hydrophobicity (AI; XIAO; JIANG, 2021). Sugar esters are molecules bearing a hydrophilic and a hydrophobic moiety. The hydrophobic one consists of a fatty acid, whereas the hydrophilic one consists of a sugar molecule (EL-LAITHY; SHOUKRY; MAHRAN, 2011). As the proportion of hydrophobic moieties of the molecule increases, the emulsion capacity is significantly reduced (AI; XIAO; JIANG, 2021). Thus, as sugar esters afford mixtures of compounds differing in their degree of esterification and/or the position of acylation, our product may contain more than one fatty acid residue (as confirmed by mass spectrometry) and, consequently, its hydrophobicity is enhanced. This means that esterification reactions that preferentially produce carbohydrate monoesters (which is the case of the commercial surfactant evaluated in this study) instead of a mixture of esters tend to present higher emulsion capacities and so, emulsion stabilities. Thus, the favored formation of sugar monoesters may represent an attractive approach for their commercial application (LIU, 2017; NETA; TEIXEIRA; RODRIGUES, 2015).

5.1.4. Conclusions

The benchmark immobilized CALB (Lipozyme 435) was proven to be efficient for xylose oleate synthesis in methyl ethyl ketone, yielding high substrates conversions. An excess of oleic acid markedly favored the reaction catalyzed by Lipozyme 435. Besides, when an excess of sugar was used, since the first 15 min of reaction, molecular sieves retained nearly 11% of our hydrophilic reagent (xylose), producing an apparent increase in the conversions. Thus, for this case, the use of different means of water removal from the product stream is required, such as salt hydrate pairs, saturated salt solutions, or pervaporation.

The predicted Ping Pong Bi Bi kinetic model presented an adequate fit to the experimental data (oleic acid and xylose consumption profiles) and the kinetic constants

determined for this model revealed that Lipozyme 435 has a poor affinity for both substrates. The emulsion capacity of our purified product was close to that of a commercial product (also a sugar ester). Lipozyme 435, therefore, has considerable potential for use in the production of biosurfactants based on xylose and oleic acid. Nevertheless, its limited repeatability and stability turn the cost reduction in the large-scale xylose oleate production difficult. Moreover, the preferential formation of xylose monoesters may represent a better alternative for their commercial use, and, in this study, we identified the presence of a mixture of xylose mono-, di- and trioleate.

Funding: This research was funded by the "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP, grant numbers 2016/10636-8 and 2019/23908-4), in part by "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES, Finance Code 001), "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, project number 315092/2020-3), "Ministerio de Ciencia e Innovación" (Spanish Government, project number CTQ2017-86170-R), and "Consejo Superior de Investigaciones Científicas" (CSIC, project number AEP045).

Acknowledgments: The authors would like to thank Maria Fátima das Graças Fernandes da Silva for the technical support in the conduction of the mass spectrometry analyzes. The authors also thank Novozymes Latin America (Araucária, Brazil) for the donation the imobilized CALB (Lipozyme 435).

References

ABDULMALEK, E.; HAMIDON, N. F.; RAHMAN, M. B. A. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents. **Journal of Molecular Catalysis B: Enzymatic**, vol. 132, p. 1–4, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2016.06.010.

ADLERCREUTZ, P. Immobilisation and application of lipases in organic media. **Chemical Society Reviews**, vol. 42, no. 15, p. 6406–6436, 2013. DOI https://doi.org/10.1039/c3cs35446f.

AGILENT. **The essential chromatography & spectroscopy catalog**. 2012.

AI, M.; XIAO, N.; JIANG, A. Molecular structural modification of duck egg white protein conjugates with monosaccharides for improving emulsifying capacity. **Food Hydrocolloids**, vol. 111, p. 106271, 2021. DOI https://doi.org/10.1016/j.foodhyd.2020.106271.

ALVES, J. S.; GARCIA-GALAN, C.; SCHEIN, M. F.; SILVA, A. M.; BARBOSA, O.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Combined effects of ultrasound and immobilization protocol on butyl acetate synthesis catalyzed by CALB. **Molecules**, vol. 19, no. 7, p. 9562–9576, 2014. DOI https://doi.org/10.3390/molecules19079562.

AN, D.; ZHANG, X.; LIANG, F.; XIAN, M.; FENG, D.; YE, Z. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A**, vol. 577, p. 257–264, 2019. DOI https://doi.org/10.1016/j.colsurfa.2019.05.079.

ARCENS, D.; GRAU, E.; GRELIER, S.; CRAMAIL, H.; PERUCH, F. 6-O-glucose palmitate synthesis with lipase: Investigation of some key parameters. **Molecular Catalysis**, vol. 460, p. 63–68, 2018. DOI https://doi.org/10.1016/j.mcat.2018.09.013.

BARBOSA, O.; RUIZ, M.; ORTIZ, C.; FERNÁNDEZ, M.; TORRES, R.; FERNANDEZ-LAFUENTE, R. Modulation of the properties of immobilized CALB by chemical modification with 2,3,4-trinitrobenzenesulfonate or ethylendiamine. Advantages of using adsorbed lipases on hydrophobic supports. **Process Biochemistry**, vol. 47, no. 5, p. 867–876, 2012. DOI http://dx.doi.org/10.1016/j.procbio.2012.02.026.

BEISSON, F.; TISS, A.; RIVIÈRE, C.; VERGER, R. Methods for lipase detection and assay: A critical review. **European Journal of Lipid Science and Technology**, vol. 102, no. 2, p. 133–153, 2000. DOI https://doi.org/10.1002/(sici)1438-9312(200002)102:2<133::aid-ejlt133>3.0.co;2-x.

BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. DOI https://doi.org/10.14233/ajchem.2013.13973.

BILAL, M.; ZHAO, Y.; NOREEN, S.; SHAH, S. Z. H.; BHARAGAVA, R. N.; IQBAL, H. M. N. Modifying bio-catalytic properties of enzymes for efficient biocatalysis: a review from immobilization strategies viewpoint. **Biocatalysis and Biotransformation**, vol. 37, no. 3, p. 159–182, 2019. DOI https://doi.org/10.1080/10242422.2018.1564744.

BOUZAOUIT, N.; BIDJOU-HAIOUR, C. Optimization of lipase catalyzed synthesis of fatty acid xylose ester using statistical experimental designs. **Der Pharma Chemica**, vol. 7, p. 261–269, 2015.

C. ORTIZ, M.L. FERREIRA, O. BARBOSA, J.C.S. DOS SANTOS, R.C. RODRIGUES, Á. BERENGUER-MURCIA, L.E. BRIAND, R. F.-L. Novozym 435: the “perfect” lipase immobilized biocatalyst? **Catalysis Science & Technology**, vol. 9, no. 10, p. 2380–2420, 2019. DOI https://doi.org/https://doi.org/10.1039/C9CY00415G.

CASTILLO, E.; DOSSAT, V.; MARTY, A.; STÉPHANE CONDORET, J.; COMBES, D. The role of silica gel in lipase-catalyzed esterification reactions of high-polar substrates. **Journal of the American Oil Chemists’ Society**, vol. 74, no. 2, p. 77–85, 1997. DOI https://doi.org/10.1007/s11746-997-0148-3.

CASTRO, H. F.; MENDES, A. A.; SANTOS, J. C.; AGUIAR, C. L. Modificação de óleos e gorduras por biotransformação. **Química Nova**, vol. 27, no. 1, p. 146–156, 2004. DOI https://doi.org/10.1590/s0100-40422004000100025.

CAVALCANTI, R. M. F.; SILVA, D. P. D.; PAZ, M. C. F.; QUEIROZ, J. C. F. Screening, production and characterization of biosurfactants from caatinga´s filamentous Fungi. **International Journal of Pharmaceutical Science Invention**, vol. 6, no. 5, p. 23–28, 2017.

CHANG, P.; ZHANG, Z.; TANG, S. Lipase-catalyzed synthesis of sugar ester in mixed biphasic system of ionic liquids and supercritical carbon dioxide. **Journal of the Chinese Chemical Society**, vol. 65, no. 4, p. 452–458, 2018. DOI https://doi.org/10.1002/jccs.201700278.

CHANG, S. W.; SHAW, J. F. Biocatalysis for the production of carbohydrate esters. **New Biotechnology**, vol. 26, no. 3–4, p. 109–116, 2009. DOI https://doi.org/10.1016/j.nbt.2009.07.003.

CHÁVEZ-FLORES, L. F.; BELTRAN, H. I.; ARRIETA-BAEZ, D.; REYES-DUARTE, D. Regioselective synthesis of lactulose esters by *Candida antarctica* and *Thermomyces*

lanuginosus lipases. **Catalysts**, vol. 7, no. 9, p. 263, 2017. DOI https://doi.org/10.3390/catal7090263.

COLOMBIÉ, S.; TWEDDELL, R. J.; CONDORET, J. S.; MARTY, A. Water activity control: A way to improve the efficiency of continuous lipase esterification. **Biotechnology and Bioengineering**, vol. 60, no. 3, p. 362–368, 1998. DOI https://doi.org/10.1002/(SICI)1097-0290(19981105)60:3<362::AID-BIT13>3.0.CO;2-O.

DANG, H. T.; OBIRI, O.; HAYES, D. G. Feed batch addition of saccharide during saccharide-fatty acid esterification catalyzed by immobilized lipase: Time course, water activity, and kinetic model. **Journal of the American Oil Chemists' Society**, vol. 82, no. 7, p. 487–493, 2005. DOI https://doi.org/10.1007/s11746-005-1098-x.

DE LIMA, L. N.; MENDES, A. A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W.; GIORDANO, R. L. C. Performance of different immobilized lipases in the syntheses of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, 2018. DOI https://doi.org/10.3390/molecules23040766.

DE LIMA, L. N.; VIEIRA, G. N. A.; KOPP, W.; TARDIOLI, P. W.; GIORDANO, R. L. C. Mono- and heterofunctionalized silica magnetic microparticles (SMMPs) as new carriers for immobilization of lipases. **Journal of Molecular Catalysis B: Enzymatic**, vol. 133, p. 491–499, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2017.04.002.

DOSSAT, V.; COMBES, D.; MARTY, A. Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: Influence of the glycerol production. **Enzyme and Microbial Technology**, vol. 25, no. 3–5, p. 194–200, 1999. DOI https://doi.org/10.1016/S0141-0229(99)00026-5.

EL-LAITHY, H. M.; SHOUKRY, O.; MAHRAN, L. G. Novel sugar esters proniosomes for transdermal delivery of vinpocetine: Preclinical and clinical studies. **European Journal of Pharmaceutics and Biopharmaceutics**, vol. 77, no. 1, p. 43–55, 2011. DOI http://dx.doi.org/10.1016/j.ejpb.2010.10.011.

FALLAVENA, L. P.; ANTUNES, F. H. F.; ALVES, J. S.; PALUDO, N.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Ultrasound technology and molecular sieves improve the thermodynamically controlled esterification of butyric acid mediated by immobilized lipase from *Rhizomucor miehei*. **RSC Advances**, vol. 4, no. 17, p. 8675–8681, 2014. DOI https://doi.org/10.1039/c3ra47315e.

FERNANDEZ-LORENTE, G.; PALOMO, J. M.; COCCA, J.; MATEO, C.; MORO, P.; TERRENI, M.; FERNANDEZ-LAFUENTE, R.; GUISAN, J. M. Regio-selective deprotection of peracetylated sugars via lipase hydrolysis. **Tetrahedron**, vol. 59, no. 30, p. 5705–5711, 2003. DOI https://doi.org/10.1016/S0040-4020(03)00876-7.

FILICE, M.; BAVARO, T.; FERNANDEZ-LAFUENTE, R.; PREGNOLATO, M.; GUISAN, J. M.; PALOMO, J. M.; TERRENI, M. Chemo-biocatalytic regioselective one-pot synthesis of different deprotected monosaccharides. **Catalysis Today**, vol. 140, no. 1–2, p. 11–18, 2009. DOI https://doi.org/10.1016/j.cattod.2008.07.016.

FINDRIK, Z.; MEGYERI, G.; GUBICZA, L.; BÉLAFI-BAKÓ, K.; NEMESTÓTHY, N.; SUDAR, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. **Journal of Cleaner Production**, vol. 112, p. 1106–1111, 2016. DOI http://dx.doi.org/10.1016/j.jclepro.2015.07.098.

GANDHI, N. N.; PATIL, N. S.; SAWANT, S. B.; JOSHI, J. B.; WANGIKAR, P. P.; MUKESH, E. D. Lipase-catalyzed esterification. **Catalysis Reviews: Science and Engineering**, vol. 42, no. 4, p. 439–480, 2000. DOI https://doi.org/https://doi.org/10.1081/CR-100101953.

GARCIA-GALAN, C.; BERENGUER-MURCIA, Á.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Potential of different enzyme immobilization strategies to improve

enzyme performance. **Advanced Synthesis and Catalysis**, vol. 353, no. 16, p. 2885–2904, 2011. DOI https://doi.org/10.1002/adsc.201100534.

GONÇALVES, M. C. P., KIECKBUSCH, T. G., PERNA, R. F., FUJIMOTO, J. T., MORALES, S. A. V., ROMANELLI, J. P. Trends on enzyme immobilization researches based on bibliometric analysis. **Process Biochemistry**, vol. 76, p. 95–110, 2019. DOI https://doi.org/10.1016/j.procbio.2018.09.016.

GONÇALVES, M. C. P.; ROMANELLI, J. P.; GUIMARÃES, J. R.; VIEIRA, A. C.; DE AZEVEDO, B. P.; TARDIOLI, P. W. Reviewing research on the synthesis of CALB-catalyzed sugar esters incorporating systematic mapping principles. **Critical Reviews in Biotechnology**, vol. 41, no. 6, p. 865–878, 2021. DOI https://doi.org/10.1080/07388551.2021.1888071.

GRAEBIN, G.; MARTINS, B.; GARCIA-GALAN, C.; FERNANDEZ-LAFUENTE, R.; AYUB, M. A. Z.; RODRIGUES, R. C. Immobilization of Lipase B from *Candida antarctica* on Porous Styrene – Divinylbenzene Beads Improves Butyl Acetate Synthesis. **Biotechnology Progress**, vol. 28, no. 2, p. 406–412, 2012. DOI https://doi.org/10.1002/btpr.1508.

GUMEL, A. M.; ANNUAR, M. S. M.; HEIDELBERG, T.; CHISTI, Y. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, vol. 46, no. 11, p. 2079–2090, 2011. DOI http://dx.doi.org/10.1016/j.procbio.2011.07.021.

HALLING, P. J. Thermodynamic predictions for biocatalysis in nonconventional media: Theory, tests, and recommendations for experimental design and analysis. **Enzyme and Microbial Technology**, vol. 16, no. 3, p. 178–206, 1994. DOI https://doi.org/10.1016/0141-0229(94)90043-4.

HERNANDEZ, K.; GARCIA-VERDUGO, E.; PORCAR, R.; FERNANDEZ-LAFUENTE, R. Hydrolysis of triacetin catalyzed by immobilized lipases: Effect of the immobilization protocol and experimental conditions on diacetin yield. **Enzyme and Microbial Technology**, vol. 48, no. 6–7, p. 510–517, 2011. DOI http://dx.doi.org/10.1016/j.enzmictec.2011.02.005.

IDRIS, A.; BUKHARI, A. Immobilized *Candida antarctica* lipase B: Hydration, stripping off and application in ring opening polyester synthesis. **Biotechnology Advances**, vol. 30, no. 3, p. 550–563, 2012. DOI http://dx.doi.org/10.1016/j.biotechadv.2011.10.002.

JÖNSSON, L. J.; MARTÍN, C. Bioresource technology pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. **Bioresource Technology**, vol. 199, p. 103–112, 2016. DOI https://doi.org/10.1016/j.biortech.2015.10.009.

KAPOOR, M.; GUPTA, M. N. Lipase promiscuity and its biochemical applications. **Process Biochemistry**, vol. 47, no. 4, p. 555–569, 2012. DOI http://dx.doi.org/10.1016/j.procbio.2012.01.011.

KASCHE, V. Mechanism and yields in enzyme catalysed equilibrium and kinetically controlled synthesis of β-lactam antibiotics, peptides and other condensation products. **Enzyme and Microbial Technology**, vol. 8, no. 1, p. 4–16, 1986. DOI https://doi.org/10.1016/0141-0229(86)90003-7.

KOPP, W.; SILVA, F. A.; LIMA, L. N.; MASUNAGA, S. H.; TARDIOLI, P. W.; GIORDANO, R. C.; ARAÚJO-MOREIRA, F. M.; GIORDANO, R. L. C. Synthesis and characterization of robust magnetic carriers for bioprocess applications. **Materials Science and Engineering B: Solid-State Materials for Advanced Technology**, vol. 193, p. 217–228, 2015. DOI http://dx.doi.org/10.1016/j.mseb.2014.12.002.

KUMARI, A.; MAHAPATRA, P.; GARLAPATI, V. K.; BANERJEE, R. Enzymatic transesterification of Jatropha oil. **Biotechnology for Biofuels**, vol. 2, p. 1, 2009. DOI

https://doi.org/10.1186/1754-6834-2-1.
KUNDYS, A.; BIAŁECKA-FLORJAŃCZYK, E.; FABISZEWSKA, A.; MAŁAJOWICZ, J. *Candida antarctica* lipase B as catalyst for cyclic esters synthesis, their polymerization and degradation of aliphatic polyesters. **Journal of Polymers and the Environment**, vol. 26, no. 1, p. 396–407, 2018. DOI https://doi.org/10.1007/s10924-017-0945-1.
LI, L.; JI, F.; WANG, J.; LI, Y.; BAO, Y. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. **Enzyme and Microbial Technology**, vol. 69, p. 46–53, 2015. DOI https://doi.org/10.1016/j.enzmictec.2014.12.003.
LIANG, M. Y.; CHEN, Y.; BANWELL, M. G.; WANG, Y.; LAN, P. Enzymatic preparation of a homologous series of long-chain 6-O-acylglucose esters and their evaluation as emulsifiers. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 15, p. 3949–3956, 2018. DOI https://doi.org/10.1021/acs.jafc.8b00913.
LIU, W. L.; LO, S. H.; SINGCO, B.; YANG, C. C.; HUANG, H. Y.; LIN, C. H. Novel trypsin–FITC@MOF bioreactor efficiently catalyzes protein digestion. **Journal of Materials Chemistry B**, vol. 1, p. 928–932, 2013. DOI https://doi.org/10.1039/c3tb00257h.
LIU, K. J. Enzymatic synthesis of isomaltotriose palmitate and evaluation of its emulsifying property. **Enzyme and Microbial Technology**, vol. 101, p. 51–56, 2017. DOI http://dx.doi.org/10.1016/j.enzmictec.2017.03.004.
LJUNGER, G.; ADLERCREUTZ, P.; MATTIASSON, B. Enzymatic synthesis of octyl-β-glucoside in octanol at controlled water activity. **Enzyme and Microbial Technology**, vol. 16, no. 9, p. 751–755, 1994. DOI https://doi.org/10.1016/0141-0229(94)90031-0.
LORTIE, R. Enzyme catalyzed esterification. **Biotechnology Advances**, vol. 15, no. 1, p. 1–15, 1997. DOI https://doi.org/10.1016/S0734-9750(96)00046-8.
MA, Y. R.; BANWELL, M. G.; YAN, R.; LAN, P. Comparative study of the emulsifying properties of a homologous series of long-chain 6′-O-acylmaltose esters. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 33, p. 8832–8840, 2018. DOI https://doi.org/10.1021/acs.jafc.8b02391.
MAI, N. L.; AHN, K.; BAE, S. W.; SHIN, D. W.; MORYA, V. K.; KOO, Y. M. Ionic liquids as novel solvents for the synthesis of sugar fatty acid ester. **Biotechnology Journal**, vol. 9, no. 12, p. 1565–1572, 2014. DOI https://doi.org/10.1002/biot.201400099.
MANOEL, E. A.; DOS SANTOS, J. C. S.; FREIRE, D. M. G.; RUEDA, N.; FERNANDEZ-LAFUENTE, R. Immobilization of lipases on hydrophobic supports involves the open form of the enzyme. **Enzyme and Microbial Technology**, vol. 71, p. 53–57, 2015. DOI https://doi.org/10.1016/j.enzmictec.2015.02.001.
MARTINS, A. B.; FRIEDRICH, J. L. R.; CAVALHEIRO, J. C.; GARCIA-GALAN, C.; BARBOSA, O.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Improved production of butyl butyrate with lipase from *Thermomyces lanuginosus* immobilized on styrene-divinylbenzene beads. **Bioresource Technology**, vol. 134, p. 417–422, 2013. DOI https://doi.org/10.1016/j.biortech.2013.02.052.
MARTINS, A. B.; GRAEBIN, N. G.; LORENZONI, A. S. G.; FERNANDEZ-LAFUENTE, R.; AYUB, M. A. Z.; RODRIGUES, R. C. Rapid and high yields of synthesis of butyl acetate catalyzed by Novozym 435: Reaction optimization by response surface methodology. **Process Biochemistry**, vol. 46, no. 12, p. 2311–2316, 2011. DOI https://doi.org/10.1016/j.procbio.2011.09.011.
MARTINS, A. B.; SCHEIN, M. F.; FRIEDRICH, J. L. R.; FERNANDEZ-LAFUENTE, R.; AYUB, M. A. Z.; RODRIGUES, R. C. Ultrasound-assisted butyl acetate synthesis catalyzed by Novozym 435: Enhanced activity and operational stability. **Ultrasonics**

Sonochemistry, vol. 20, no. 5, p. 1155–1160, 2013. DOI https://doi.org/10.1016/j.ultsonch.2013.01.018.

MARTY, A.; DOSSAT, V.; CONDORET, J. S. Continuous operation of lipase-catalyzed reactions in nonaqueous solvents: Influence of the production of hydrophilic compounds. **Biotechnology and Bioengineering**, vol. 56, no. 2, p. 232–237, 1997. DOI https://doi.org/10.1002/(SICI)1097-0290(19971020)56:2<232::AID-BIT12>3.0.CO;2-I.

MÉLINE, T.; MUZARD, M.; DELEU, M.; RAKOTOARIVONINA, H.; PLANTIER-ROYON, R.; RÉMOND, C. D-Xylose and L-arabinose laurate esters: Enzymatic synthesis, characterization and physico-chemical properties. **Enzyme and Microbial Technology**, vol. 112, p. 14–21, 2018. DOI https://doi.org/10.1016/j.enzmictec.2018.01.008.

MENSAH, P.; CARTA, G. Adsorptive control of water in esterification with immobilized enzymes. Continuous operation in a periodic counter-current reactor. **Biotechnology and Bioengineering**, vol. 66, no. 3, p. 137–146, 1999. DOI https://doi.org/10.1002/(SICI)1097-0290(1999)66:3<137::AID-BIT1>3.0.CO;2-R.

MILESSI, T. S.; PEREZ, C. L.; ZANGIROLAMI, T. C.; CORRADINI, F. A. S.; SANDRI, J. P.; MORENO, M. R. F.; GIORDANO, R. C.; THEVELEIN, J. M.; GIORDANO, R. L. C. Biotechnology for biofuels repeated batches as a strategy for high 2G ethanol production from undetoxified hemicellulose hydrolysate using immobilized cells of recombinant *Saccharomyces cerevisiae* in a fixed-bed reactor. **Biotechnology for Biofuels**, vol. 13, no. 85, p. 1–12, 2020. DOI https://doi.org/10.1186/s13068-020-01722-y.

NETA, N. A. S.; DOS SANTOS, J. C. S.; SANCHO, S. O.; RODRIGUES, S.; GONÇALVES, L. R. B.; RODRIGUES, L. R.; TEIXEIRA, J. A. Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions. **Food Hydrocolloids**, vol. 27, no. 2, p. 324–331, 2012. DOI http://dx.doi.org/10.1016/j.foodhyd.2011.10.009.

NETA, N. A. S.; TEIXEIRA, J. A.; RODRIGUES, L. R. Sugar ester surfactants: Enzymatic synthesis and applications in food industry. **Critical Reviews in Food Science and Nutrition**, vol. 55, no. 5, p. 595–610, 2015. DOI https://doi.org/10.1080/10408398.2012.667461.

NETA, N. A. S.; PERES, A. M.; TEIXEIRA, J. A.; RODRIGUES, L. R. Maximization of fructose esters synthesis by response surface methodology. **New Biotechnology**, vol. 28, no. 4, p. 349–355, 2011. DOI https://doi.org/10.1016/j.nbt.2011.02.007.

OGAWA, S.; ENDO, A.; KITAHARA, N.; YAMAGISHI, T.; AOYAGI, S.; HARA, S. Factors determining the reaction temperature of the solvent-free enzymatic synthesis of trehalose esters. **Carbohydrate Research**, vol. 482, p. 107739, 2019. DOI https://doi.org/10.1016/j.carres.2019.06.018.

OGUNTIMEIN, G. B.; ERDMANN, H.; SCHMID, R. D. Lipase catalysed synthesis of sugar ester media. **Biotechnology Letters**, vol. 15, no. 2, p. 175–180, 1993. DOI https://doi.org/10.1007/BF00133019.

PALUDO, N.; ALVES, J. S.; ALTMANN, C.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. The combined use of ultrasound and molecular sieves improves the synthesis of ethyl butyrate catalyzed by immobilized *Thermomyces lanuginosus* lipase. **Ultrasonics Sonochemistry**, vol. 22, p. 89–94, 2015. DOI http://dx.doi.org/10.1016/j.ultsonch.2014.05.004.

PAPPALARDO, V. M.; BOERIU, C. G.; ZACCHERIA, F.; RAVASIO, N. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, vol. 433, p. 383–390, 2017. DOI http://dx.doi.org/10.1016/j.mcat.2017.02.029.

POPPE, J. K.; GARCIA-GALAN, C.; MATTE, C. R.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C.; AYUB, M. A. Z. Optimization of synthesis of fatty acid methyl esters catalyzed by lipase B from *Candida antarctica* immobilized on hydrophobic supports. **Journal of Molecular Catalysis B: Enzymatic**, vol. 94, p. 51–56, 2013. DOI http://dx.doi.org/10.1016/j.molcatb.2013.05.010.

RAHMAN, M., ARUMUGAN, M., KHAIRUDDIN, N. S. K., ABDULMALEK, E., BASRI, M., SALLEH, A. Microwave assisted enzymatic S-synthesis of fatty acid sugar ester in ionic liquid-tert-butanol biphasic solvent system. **Asian Journal of Chemistry**, vol. 24, p. 5058–5062, 2012.

RODRIGUES, R. C.; VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, Á.; ALCANTARA, A. R.; BARBOSA, O.; ORTIZ, C.; FERNANDEZ-LAFUENTE, R. Immobilization of lipases on hydrophobic supports: immobilization mechanism, advantages, problems, and solutions. **Biotechnology Advances**, vol. 37, no. 5, p. 746–770, 2019. DOI https://doi.org/10.1016/j.biotechadv.2019.04.003.

RUEDA, N.; DOS SANTOS, J. C. S.; ORTIZ, C.; TORRES, R.; BARBOSA, O.; RODRIGUES, R. C.; BERENGUER-MURCIA, Á.; FERNANDEZ-LAFUENTE, R. Chemical modification in the design of immobilized enzyme biocatalysts: drawbacks and opportunities. **Chemical Record**, vol. 16, no. 3, p. 1436–1455, 2016. DOI https://doi.org/10.1002/tcr.201600007.

RUEDA, N.; DOS SANTOS, J. C. S.; TORRES, R.; ORTIZ, C.; BARBOSA, O.; FERNANDEZ-LAFUENTE, R. Improved performance of lipases immobilized on heterofunctional octyl-glyoxyl agarose beads. **RSC Advances**, vol. 5, no. 15, p. 11212–11222, 2015. DOI https://doi.org/10.1039/c4ra13338b.

SANTOS, J. C. S. D.; BARBOSA, O.; ORTIZ, C.; BERENGUER-MURCIA, A.; RODRIGUES, R. C.; FERNANDEZ-LAFUENTE, R. Importance of the support properties for immobilization or purification of enzymes. **ChemCatChem**, vol. 7, no. 16, p. 2413–2432, 2015. DOI https://doi.org/10.1002/cctc.201500310.

SCHLOTTERBECK, A.; LANG, S.; WRAY, V.; WAGNER, F. Lipase-catalyzed monoacylation of fructose. **Biotechnology Letters**, vol. 15, no. 1, p. 61–64, 1993. DOI https://doi.org/10.1007/BF00131554.

SCHMIDELL, W.; LIMA, U.A.; AQUARONE, E.; BORZANI, W. **Biotecnologia Industrial**. 1ª.: Ltda., Edgard Blucher, 2001.

SÉVERAC, E.; GALY, O.; TURON, F.; PANTEL, C. A.; CONDORET, J. S.; MONSAN, P.; MARTY, A. Selection of CalB immobilization method to be used in continuous oil transesterification: Analysis of the economical impact. **Enzyme and Microbial Technology**, vol. 48, no. 1, p. 61–70, 2011. DOI https://doi.org/10.1016/j.enzmictec.2010.09.008.

SHI, Y. G.; LI, J. R.; CHU, Y. H. Enzyme-catalyzed regioselective synthesis of sucrose-based esters. **Journal of Chemical Technology and Biotechnology**, vol. 86, no. 12, p. 1457–1468, 2011. DOI https://doi.org/10.1002/jctb.2711.

SHIN, D. W.; MAI, N. L.; BAE, S. W.; KOO, Y. M. Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. **Enzyme and Microbial Technology**, vol. 126, p. 18–23, 2019. DOI https://doi.org/10.1016/j.enzmictec.2019.03.004.

SIEBENHALLER, S.; HAJEK, T.; MUHLE-GOLL, C.; HIMMELSBACH, M.; LUY, B.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.; HAHN, T.; ZIBEK, S.; SYLDATK, C. Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. **Bioresources and Bioprocessing**, vol. 4, no. 25, 2017. DOI https://doi.org/10.1186/s40643-017-0155-7.

SIEBENHALLER, S.; KIRCHHOFF, J.; KIRSCHHÖFER, F.; BRENNER-WEISS, G.;

MUHLE-GOLL, C.; LUY, B.; HAITZ, F.; HAHN, T.; ZIBEK, S.; SYLDATK, C.; OCHSENREITHER, K. Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. **Frontiers in Chemistry**, vol. 6, no. 421, 2018. DOI https://doi.org/10.3389/fchem.2018.00421.

SIÓDMIAK, T.; MANGELINGS, D.; VANDER HEYDEN, Y.; ZIEGLER-BOROWSKA, M.; MARSZAŁŁ, M. P. High enantioselective Novozym 435-catalyzed esterification of (R,S)-flurbiprofen monitored with a chiral stationary phase. **Applied Biochemistry and Biotechnology**, vol. 175, no. 5, p. 2769–2785, 2015. DOI https://doi.org/10.1007/s12010-014-1455-4.

TARAHOMJOO, S.; ALEMZADEH, I. Surfactant production by an enzymatic method. **Enzyme and Microbial Technology**, vol. 33, no. 1, p. 33–37, 2003. DOI https://doi.org/10.1016/S0141-0229(03)00085-1.

TENG, Y.; STEWART, S. G.; HAI, Y. W.; LI, X.; BANWELL, M. G.; LAN, P. Sucrose fatty acid esters: synthesis, emulsifying capacities, biological activities and structure-property profiles. **Critical Reviews in Food Science and Nutrition**, vol. 0, no. 0, p. 1–21, 2020. DOI https://doi.org/10.1080/10408398.2020.1798346.

TSUKAMOTO, J.; HAEBEL, S.; VALEN, G. P.; PETER, M. G.; FRANCO, T. T. Enzymatic direct synthesis of acrylic acid esters of mono- and disaccharides. **Journal of Chemical Technology and Biotechnology**, vol. 83, p. 1486–1492, 2008. DOI https://doi.org/10.1002/jctb.1983.

VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

VESCOVI, V.; KOPP, W.; GUISÁN, J. M.; GIORDANO, R. L. C.; MENDES, A. A.; TARDIOLI, P. W. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octyl and multifunctional amino-glutaraldehyde spacer arms. **Process Biochemistry**, vol. 51, no. 12, p. 2055–2066, 2016. DOI http://dx.doi.org/10.1016/j.procbio.2016.09.016.

VIRGEN-ORTÍZ, J. J.; TACIAS-PASCACIO, V. G.; HIRATA, D. B.; TORRESTIANA-SANCHEZ, B.; ROSALES-QUINTERO, A.; FERNANDEZ-LAFUENTE, R. Relevance of substrates and products on the desorption of lipases physically adsorbed on hydrophobic supports. **Enzyme and Microbial Technology**, vol. 96, p. 30–35, 2017. DOI http://dx.doi.org/10.1016/j.enzmictec.2016.09.010.

VULFSON, E. N.; HALLING, P. J.; HOLLAND, H. L.; RHEE, J. S.; KWON, S. J.; HAN, J. J. Water activity control for lipase-catalyzed reactions in nonaqueous media. **Enzymes in Nonaqueous Solvents**, vol. 15, no. 11, p. 135–150, 2003. DOI https://doi.org/10.1385/1-59259-112-4:135.

WAGNER, F. W.;WALTON, M. A. D.; LINCOLN, R. S. M.; LINCOLN, V. H. S. **Separation and purification of sugar esters**. United States: 1991.

WARD, O. P.; FANG, J.; LI, Z. Lipase-catalyzed synthesis of a sugar ester containing arachidonic acid. **Enzyme and Microbial Technology**, vol. 20, no. 1, p. 52–56, 1997. DOI https://doi.org/10.1016/S0141-0229(96)00081-6.

ZAIDAN, U. H.; ABDUL RAHMAN, M. B.; OTHMAN, S. S.; BASRI, M.; ABDULMALEK, E.; ABDUL RAHMAN, R. N. Z. R.; SALLEH, A. B. Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. **Food Chemistry**, vol. 131, no. 1, p. 199–205, 2012. DOI http://dx.doi.org/10.1016/j.foodchem.2011.08.060.

5.2. STABILIZATION AND SELECTIVITY ALTERATION OF LIPOZYME 435 BY ITS COATING WITH POLYETHYLENEIMINE: COMPARISON OF THE BIOCATALYST PERFORMANCE IN THE SYNTHESIS OF XYLOSE FATTY ESTERS

5.2.1. Introduction

The interfacial adsorption of lipases on hydrophobic supports can promote their stabilization, hyperactivation, and purification in a single step (DOS SANTOS *et al.*, 2015a; 2015b; FERNANDEZ-LAFUENTE *et al.*, 1998; PALOMO *et al.*, 2002). This type of immobilization is quite simple, reduces the costs of the process, and allows support reuse (GARCIA-GALAN *et al.*, 2011; RODRIGUES *et al.*, 2019). However, the adsorbed enzyme may undergo lipase desorption under drastic conditions due to the reversibility of the process (e.g., high concentration solvents, high temperatures, extreme pH values) or even by the action of some substrates/products with detergent properties, resulting in the product contamination by the enzyme or in the biocatalyst inactivation (FERNANDEZ-LORENTE *et al.*, 2011; HIRATA *et al.*, 2016a; 2016b; MORENO-PÉREZ; GUISAN; FERNANDEZ-LORENTE, 2014; PIZARRO *et al.*, 2012; SIÓDMIAK *et al.*, 2015).

These drawbacks have been minimized by the surface coating of those immobilized enzymatic preparations through physical (ionic exchange of polymers) or chemical (aminoethylamidation, succinylation, hydroxyethylamidation, etc.) modifications (FERNANDEZ-LORENTE *et al.*, 2008; RODRIGUES *et al.*, 2014; RODRIGUES *et al.*, 2009; RUEDA; DOS SANTOS; ORTIZ; *et al.*, 2015). Such approaches are intended to protect the enzyme from the external environment, providing enhanced stability and a significantly reduced lipase desorption if the enzyme intermolecular crosslinking is achieved (GUIMARÃES *et al.*, 2018; VIRGEN-ORTÍZ *et al.*, 2017a; ZUCCA; FERNANDEZ-LAFUENTE; SANJUST, 2016). The chemical aminations are based on the reaction of the surface carboxylic groups of the enzyme (NAKAMURA *et al.*, 2006) or the modification of all of the surface primary amino groups with different agents (BIANCHI *et al.*, 1993), and these may be relatively complex processes. Otherwise, physical modifications using polymers having many anionic (e.g., dextran sulfate) or cationic (e.g., polyethyleneimine) groups are relatively simple. This type of coating may modify the interactions and mobility in the enzyme surface and so the shape of the active site (CABRERA *et al.*, 2009), besides allowing a physical

crosslinking that prevents the enzyme release (FERNANDEZ-LOPEZ *et al.*, 2017a; 2017b).

In particular, the treatment of lipases immobilized via physical adsorption with polyethyleneimine (PEI) permits physical intermolecular crosslinking, and that way the prevention of enzyme release (the crosslinked proteins must be released simultaneously from their matrix, which is more difficult) (VIRGEN-ORTÍZ *et al.*, 2017a). This approach can also promote some stabilizing effects in the enzyme surface, which can occur for several reasons, including the elimination of free radicals and the promotion of favorable environments that can partition deleterious hydrophobic substances (VIRGEN-ORTÍZ *et al.*, 2017a). Besides, the open structure of this random coil polymer does not prevent the entry of small substrates and can only cause a partition of hydrophobic substrates from the enzymatic environment (ARANA-PEÑA *et al.*, 2020; ARANA-PEÑA; LOKHA; FERNÁNDEZ-LAFUENTE, 2019; FERNANDEZ-LOPEZ *et al.*, 2017a; GUIMARÃES *et al.*, 2018; HIRATA *et al.*, 2016a; FERNANDEZ-LOPEZ, 2017b; PALOMO *et al.*, 2007; PEIRCE *et al.*, 2016; VIRGEN-ORTÍZ *et al.*, 2017b; ZAAK *et al.*, 2017). In some instances, an increase in the enzymatic activity (DOS SANTOS *et al.*, 2014) and the obtaining of biocatalysts with improved specificity or regioselectivity (PALOMO *et al.*, 2007) may occur after the PEI coating. The reversibility of this interaction also allows the support reuse, releasing both the enzyme and PEI by using ionic surfactants, guanidine, among others (PEIRCE *et al.*, 2016).

The PEI coating of the commercially available immobilized CALB produced by Novozymes (adsorbed via interfacial activation on a Lewatit VP OC 1600 resin, a macroporous support formed by poly(methyl methacrylate) crosslinked with divinylbenzene (ORTIZ *et al.*, 2019; CASAS-GODOY *et al.*, 2016; WANG *et al.*, 2015) may represent an alternative to overcome the enzyme leakage frequently reported when using this lipase (ORTIZ *et al.*, 2019; GONÇALVES *et al.*, 2021a; VESCOVI *et al.*, 2016), besides tailoring the biocatalyst final properties. Examples involving the coating of this immobilized enzyme when it is used in anhydrous systems are scarce, and in many instances, they involve very hydrophobic substrates, reducing the enzyme activity due to the PEI treatment, which can prevent the substrate to access the enzyme (VERDASCO-MARTÍN *et al.*, 2016; VILLALBA *et al.*, 2016).

To assess the performance of this benchmark immobilized CALB (uncoated and after the treatment with PEI) within a regioselective reaction model, the enzymatic preparations will be used as catalysts in the synthesis of sugar fatty acid esters (SFAEs).

SFAEs are non-ionic surfactants with low toxicity and great potential for use in the food, pharmaceutical, and cosmetic industries (CHANG; SHAW, 2009; GUMEL *et al.*, 2011; NETA; TEIXEIRA; RODRIGUES, 2015). Their synthesis is usually performed through an esterification reaction between a sugar and a fatty acid, where the carbohydrate acts as the acceptor for an activated acyl group and the fatty acid acts as the donor of this acyl group (FOLEY, 2019; KERTHY *et al.*, 2019; VESCOVI; DOS SANTOS; TARDIOLI, 2017). Now, the substrate is hydrophilic, and the PEI coating could have a moderate effect on the enzyme activity. In the context of enzyme release, this reaction has a special interest: the product is a detergent, and, therefore, it can force the release of the enzyme from the support, producing its inactivation (VIRGEN-ORTÍZ *et al.*, 2017c). In fact, when we produced these compounds using the commercial enzyme, the reuse of the biocatalyst was not possible and this was attributed to the enzyme release from the support (GONÇALVES *et al.*, 2021a).

This reaction is a thermodynamically controlled process where the catalysts only determine the reaction feasibility, i.e., if the enzyme is inhibited or inactivated, the thermodynamic yield will be not reached (HALLING, 1994; KASCHE, 1986). The enzymatic route may represent a preferable industrial alternative to the chemical pathway, since it requires mild operating conditions, facilitates the separation of products, and achieves high yields (ABDULMALEK; HAMIDON; RAHMAN, 2016). However, as in this case we are modifying a multifunctional poly-ol (xylose), changes in the enzyme structure caused by the modification with PEI may drive to changes in the biocatalyst regioselectivity, altering the final composition of the product. These changes caused by the PEI coating have been reported in some enantiospecific processes, but not in regioselectivity (VIRGEN-ORTÍZ *et al.*, 2017a). The enzyme coating with PEI may have other effects, such as the local accumulation of the free fatty acid in the enzyme environment, and this could have also some effects on the enzyme features (VIRGEN-ORTÍZ *et al.*, 2017a). Finally, the PEI may alter the enzyme pH in the water, with the presence of free fatty acids presenting lower effect on the pH of this phase (VIRGEN-ORTÍZ *et al.*, 2017a). All these modifications can change the enzyme apparent or real regioselectivity (RODRIGUES *et al*., 2013). Moreover, if the enzyme regioselectivity is altered and more sugar groups are modified, the thermodynamic yields may be also altered (as there is more fatty acid consumption).

Different acyl acceptors have been used to synthesize SFAEs via biocatalysis. Among them, xylose (the main component of hemicellulose) is a low-cost and renewable

raw material that was reported in only ~8% of these publications over the last decade (GONÇALVES *et al.*, 2021b). The lack of studies employing this carbohydrate in such reactions, added to the fact that the hemicellulose stream is often underexploited after the hydrolysis of lignocellulosic materials, may create opportunities for the development of research in this realm (DE LIMA *et al.*, 2016; F.A. DOTTORI; COOPER; BENSON, 2016; MATHEWS; PAWLAK; GRUNDEN, 2015; MUZARD; DELEU, 2020). As for acyl donors, the use of free fatty acids (FFAs) represents 63% of the occurrences in the CALB-catalyzed synthesis of sugar esters, with lauric, palmitic, and oleic acids accounting for nearly 43% of them (GONÇALVES *et al.*, 2021b). By using FFAs instead of activated acyl donors (e.g., an ester) the only byproduct formed is water, which is safe and can be easily removed from the reaction mixture by using water-adsorbent reagents (CASAS-GODOY *et al.*, 2016).

The selection of a suitable organic solvent for use in the enzymatic synthesis of SFAEs is an essential parameter to be considered in this reaction. The solvent must reconcile carbohydrate solubility and low polarity to reduce the damage on the lipase. It should also not present reactive hydroxyl groups which could be substrates for the enzyme and reduce product yield and/or generate undesired by-products. Predominantly, tertiary alcohols meet these particularities (CASTRO *et al.*, 2004; FALLAVENA *et al.*, 2014; KUMARI *et al.*, 2009). However, they are usually not compatible with food applications of sugar-derived products. To overcome this issue, methyl ethyl ketone (MEK) was used in this research. This solvent has shown promising results in these esterification reactions, even with low solubilization rates of sugars (BIDJOU-HAIOUR; KLAI, 2013; GONÇALVES *et al.*, 2021b; LI *et al.*, 2015).

Accordingly, this study aims to modify the Lipozyme 435 surface with PEI of different sizes. The effects of these modifications in the catalytic properties of the enzyme (activity and stability) were studied. The biocatalysts were then used in the synthesis of xylose fatty esters from lauric and palmitic acids in an organic medium (MEK) to evaluate the substrates conversion profiles and the enzyme reuse. We also assessed the purification of the sugar esters synthesized and their emulsion capacities with a commercial surfactant as a comparison. The successful formation of esters was evaluated by mass spectrometry.

5.2.2. Materials and Methods

5.2.2.1. Materials

Candida antarctica lipase B immobilized on Lewatit VP OC 1600 (Lipozyme® 435) was donated by Novozymes Latin America (Araucária, Brazil). Tributyrin, butyric acid, and n-butanol were purchased from Sigma-Aldrich Co. (St. Louis, USA). Xylose, heptane, and lauric/palmitic acids were obtained from Synth (São Paulo, Brazil). Methyl ethyl ketone (MEK) was purchased from Neon (São Carlos, Brazil). Molecular sieves (3 Å) were obtained from JT Baker (New Jersey, USA). All other reagents and solvents were of analytical grade or HPLC/GC and were used without any previous treatment.

5.2.2.2. Coating of the L435 surface

The treatment of the L435 surface with PEI was performed by adding 100 µL of PEI solution (100 mg/mL) (Molecular weight (MW): 2; 25; and 750 KDa) to a suspension of CALB prepared in 5 mmol/L sodium phosphate buffer, pH 7.0 (biocatalyst /buffer ratio of 1/10 v/v). The reaction medium was incubated at 25 °C under 150 rpm stirring for 60 min. The immobilized derivative coated with PEI was then recovered by vacuum filtration (WILSON *et al.*, 2006).

5.2.2.3. L435 thermostability

For the thermostability assays, different enzyme preparations (non-treated L435; L435 coated with PEI 2; 25; and 750 KDa) were incubated at 1.0% w/v and 60 °C in different media: (1) MEK (72 h); (2) buffer solutions pHs 5, 7, and 9 (12 h); (3) methanol/phosphate buffer (80/20 v/v), pH 7 (12 h). Samples were withdrawn for the measurement of the hydrolytic activities (Section 5.2.2.8.1).

5.2.2.4. Free fatty acids enzyme specificity in the synthesis of xylose fatty acid esters

The synthesis of xylose fatty esters was conducted through the esterification reaction between lauric acid (C12:0) or palmitic acid (C16:0) and the commercial xylose (as a model molecule), using MEK as solvent. The non-modified L435 and the L435 coated with PEI 2 KDa were used as biocatalysts. The reaction was performed in a vortex flow reactor (80 mL) under the following conditions: 60 °C; 2,000 rpm; 1557.23 U of tributyrin hydrolysis activity (TBU)/g of xylose (Section 5.2.2.8.1); xylose: fatty acid molar ratio of 1:5 (in MEK to a xylose concentration of 7 mmol/L); 0.22 g of molecular sieves/g of water produced. Samples were collected after 30 min, 1 h, 3, 6, 9, 12, and 24 h of reaction, and quantified according to the methodologies presented in sections

5.2.2.9.1 and 5.2.2.9.2. All experiments were performed in duplicate. Results were expressed as mean ± mean standard deviation (σ).

5.2.2.5. Purification and characterization of xylose fatty acid esters

The purification of the crude sugar ester reaction product was conducted according to the protocol described in Gonçalves *et al*. (2021a). Briefly, 20 mg of the esterification reaction product were added to 2 mL of an ethanol/water solution (50:50, v/v) and magnetically stirred. After complete homogenization, the stirring was ceased, and the solution was left standing for 1 h at room temperature. The precipitate was collected by centrifugation at 10,000 rpm for 15 min and mixed with 2 mL of the ethanol/water solution at room temperature. The alcohol-water-washed precipitate was collected by centrifugation as described above. This last step was repeated once for a total of two alcohol-water washes. The recovered alcohol-water washed precipitate was then mixed with 2 mL of MEK at room temperature. The organic solvent-washed precipitate was anew recovered by centrifugation. This step was repeated three times and the recovered organic solvent-washed precipitate was dried in a vacuum oven.

The purified products were then characterized by mass spectrometry using an electrospray ionization quadrupole time-of-flight MS/MS operated in the negative mode in an Agilent 6545 ESI-QTOF-MS instrument, across the mass range of 50-1200 Da. Samples were dissolved in methanol and analyzed by FIA at a flow rate of 0.35 mL/min and a volume injection of 5.0 µL at 38 °C. The mobile phase consisted of H_2O+0.1% formic acid and MeCN+0.1% formic acid 20:80 with an analysis time of 4.0 min. The ESI source conditions were as follows: capillary voltage 2500 V, nozzle voltage 500V, gas temperature 350 ºC, drying gas 13 L/min, nebulizer 35 psi, sheaf gas 320 ºC in a flow of 10 L/min, fragmentor 120 V, skimmer 80 V, and collision energy voltage of 20 and 22 V. Data were analyzed with the use of the Qualitative Navigator B.08.000 software.

5.2.2.6. Emulsion capacity assays

The emulsion capacity (EC) assays were performed according to the methodology described in Gonçalves *et al*. (2021a). Succinctly, 10 mg of the purified reaction product were dissolved in 1 mL of water and mixed with 2 mL of kerosene in a test tube. The content of the tube was continuously homogenized for 2 min at room temperature and then left standing for 24 h. Tests were performed in duplicate, and the standard deviations were <1%. After this period, the height of the emulsified region and the height of the total

column were measured, and the ECs were calculated according to Equation 1. Sucrose monolaurate was used as a commercial standard for these experiments.

5.2.2.7. L435 operational stability

The synthesis of xylose laurate catalyzed by L435 (before and after its coating with PEI 2 KDa) was performed in 5 successive 6 h-batches. Reactions were conducted under the following conditions: 60 °C, continuous stirring (2,000 rpm), xylose: lauric acid molar ratio of 1:5 (7 mmol/L of xylose in MEK), 15,572 TBU/g of xylose. After each batch, the biocatalysts were recovered by centrifugation (35 °C; 10,000 rpm; 2 min), washed with MEK, subjected to vacuum filtration to remove the remaining solvent, and reused in a new synthesis. The xylose conversions were measured by liquid chromatography, as described in section 5.2.2.9.1.

5.2.2.8. Standard enzymatic activities

5.2.2.8.1. *Hydrolytic activity*

The protocol to measure the L435 hydrolytic activity in tributyrin is an adaptation of the methodology described by Beisson *et al*. (2000). The method is based on the hydrolysis of a mixture of 1.5 mL of tributyrin, 6 mL of 100 mmol/L phosphate buffer, pH 7.3, and 16.5 mL of distilled water, at 37 °C for 5 min. For these assays, 24 mg of L435 were added to the medium. The released butyric acid was titrated with 0.02 mol/L KOH solution in a pH-Stat titrator (Titrando 907, Metrohm, Herisau, Switzerland). A unit of tributyrin hydrolysis activity (TBU) is defined as the amount of enzyme that releases 1 μmol of butyric acid per minute under the conditions described above.

5.2.2.8.2. Esterification activity

The L435 esterification activity was determined through the esterification of butyric acid (0.1 mol/L) with butanol (0.1 mol/L) in heptane (10 mL) at 37 ºC. The reaction was conducted in agitated closed flasks (250-300 rpm) and monitored by the consumption of butyric acid by titration with 0.02 mol/L KOH solution, using phenolphthalein as the indicator (DE LIMA *et al.*, 2018; KOPP *et al.*, 2015; VESCOVI *et al.*, 2016). The biocatalyst contains 1432 U of esterification/g of the enzyme. One unit of esterification activity (U) was defined as the initial rate of butyl butyrate synthesis (mmol/L/min) under the conditions described above. For this assay, 0.0025, 0.0035, and 0.005 g of L435 were added to the medium.

5.2.2.9. Chromatographic analyses

The reliability of all chromatographic methods applied in this research was confirmed by samples of known concentrations with amounts close to those expected (≥ 90% accuracy).

5.2.2.9.1. Xylose determination

The final esterification reaction medium was centrifuged (10,000 rpm; 35 ºC; 2 min) and 1 mL of the supernatant was collected. After evaporating the solvent, 1 mL of water was added to the samples and their contents were homogenized and filtered using 0.22 µm syringe filters. The xylose concentration was measured using an HPLC Breeze (Waters Co., Milford, MA, USA) equipped with a refractive index detector (RID) and a Sugar Pak-I column (300 x 6.5 mm x 10 µm, Waters Co.) maintained at 80 ºC. A solution of EDTA-Ca (50 mg/L) was used as the eluent, at 0.5 mL/min. The injection volume was 20 µL, with an injection time of 20 min (VESCOVI; DOS SANTOS; TARDIOLI, 2017).

5.2.2.9.2. Free fatty acids determination

The final esterification reaction medium was centrifuged (10,000 rpm; 35 ºC; 2 min) and 1 mL of the supernatant was collected. The concentration of free fatty acids in the samples was measured using an Agilent gas chromatograph 7890A (Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a Restek Rtx-wax column (30 m × 0.25 mm × 0.25 µm; Restek Corporation, Bellefonte, PA, USA) maintained at 200 °C for 1 min, 230 °C for 1 min (10 °C/min), and 250 °C for 5 min (5 °C/min). Helium was used as carrier gas at a flow rate of 1.8 mL/min, with a run time of 14 min (AGILENT, 2012).

5.2.2.10. *Protein determination*

The protein concentration of the L435 supernatant in the final esterification reaction medium (before and after the PEI coating) was determined by the Bradford method (BRADFORD, 1976), using bovine serum albumin as the standard protein. Briefly, 25 µL of protein sample were added to 1 mL of the Bradford reagent (Sigma-Aldrich, St. Louis, USA) and after 5 min of reaction, its absorbance was measured at 595 nm.

5.2.3. Results and discussion

5.2.3.1. Coating of the L435 surface

The L435 surface was coated with polyethyleneimine of different sizes (MWs: 750; 25; and 2 KDa), according to the methodology described in section 5.2.2.2. Table 5.3 shows the recovered hydrolytic activities (%) of the enzyme after these treatments.

Table 5.3. L435 recovered hydrolytic activities (A_H) after its surface coating with PEI.

Molecular weight (KDa)	Recovered A_H (%)
2	59.70 ± 3.73
25	73.57 ± 6.52
750	57.72 ± 2.98

The L435 treatment with polyethyleneimine led to a reduction of about 41.3 ± 1.4% in its initial A_H (Section 2.8.1), except for the coating with PEI 25 KDa, which presented a decrease of only ~26% in the enzyme activity. This reduction in the A_H may occur due to the promotion of additional diffusion limitations of the substrates caused by the reduction in the L435 pores after the coating with PEI or even due to the partition of the fatty acid by the hydrophilic environment generated around the enzyme (ARANA-PEÑA *et al.*, 2020). The PEI layer over the CALB may also increase the tortuosity of the diffusion path that the substrates must follow to reach the active center of the enzyme (that is located in the support surface) and, consequently, increase the already existing diffusion problems because of the high enzymatic load and volumetric activity (PRONK; LINDAHL; KASSON, 2014; REGAN; LILLY; DUNNILL, 1974; SHEN; CHEN, 2007). Cabrera *et al*. (2009) also described a decrease in the Novozyme 435 hydrolytic activities after its coating with polymers of small and large sizes. In another study, Guimarães *et al*. (2018) verified that the modification of the porcine pancreatic lipase surface with PEI led to a loss of ~30% in the initial A_H. It can be assumed that the "wholes" between adsorbed PEI molecules may be larger using the larger polymers. That way, the coating may be more efficient using the small PEI and this may have a higher negative effect on the enzyme activity.

Nevertheless, even with such reductions in the enzymatic activities, these treatments can still be advantageous by preventing the CALB desorption from the support under certain conditions (RUEDA *et al.*, 2016), among other improvements that will be discussed below. So, to verify if these modifications prevented the CALB leakage, the hydrolytic activities of all L435 preparations were measured after 12 h of incubation in the reaction medium of xylose laurate synthesis, as well as the proteins in the supernatant

of this medium (section 2.10.1). We verified a reduction of 37 ± 4.24% in the A_H of the uncoated L435, besides the presence of proteins in the supernatant of the reaction medium, which were in their inactive form (no A_H was detected in the supernatant). These results confirmed the release of the enzyme from the support in the non-modified L435, very likely by the detergent-like properties of the xylose esters. This enzyme leakage is a common pitfall of using physically adsorbed lipases in the presence of detergent-like compounds (e.g., sugar esters) (VIRGEN-ORTÍZ *et al.*, 2017c). The release of this benchmark immobilized CALB from its matrix was also reported by Gonçalves *et al*. (2021a), Saunders and Brask (2010), Chen *et al*. (2007, 2008), Tambe *et al.* (2015), Hilterhaus, Thum, and Liese (2008), Nicolás, Lassalle, and Ferreira (2015), and Weinberger *et al.* (2018). The authors described a significant lipase detachment from the support, a fact that can become a serious problem in the operational stability of this enzyme. Therefore, solving these problems will have a general positive impact in the use of this biocatalyst in this kind of reaction.

Conversely, when the L435 was coated with PEI we verified the maintenance of all its A_H after 12 h of incubation in the final esterification reaction medium, as well as no proteins in the supernatant of this medium. This means that the L435 treatment with PEI prevented the CALB leaching in our crude reaction product. Physical surface treatments of immobilized enzymes generally prevent enzyme desorption because to unbind the lipase from the support the intermolecular-crosslinked proteins (linked by physical intermolecular adsorption) must be released simultaneously from their matrix, which is much more difficult (FERNANDEZ-LOPEZ *et al.*, 2017a; 2017b; FERNANDEZ-LORENTE *et al.*, 2011; 2012).

The prevention of enzyme leakage from immobilized preparations after their modification with different compounds was previously reported. Fernandez-Lopez *et al*. (2017b) coated the CALB immobilized on octyl-agarose (OC) with PEI and dextran sulfate (DS). They found that the CALB release from the OC support in the presence of detergents was greatly reduced after this treatment (PEI plus DS coating). Barbosa *et al*. (2012a) coated the CALB immobilized on OC via interfacial activation with glutaraldehyde, achieving intermolecular chemical crosslinking. Results showed different resistance to desorption by incubation in detergents or solvents. In another study, Fernandez-Lorente *et al*. (2011) promoted the intermolecular crosslinking of the lipase from *Rhizomucor miehei* immobilized on OC using aldehyde dextran. The authors also verified the prevention of enzyme leakage after this treatment. However, this is the first

time that the release of the enzyme in anhydrous medium in the presence of reaction products with mild detergent is shown, and we confirmed that the PEI can prevent this release.

Moreover, the L435 coating with polymers may present some other positive effects in the biocatalyst performance, which include the partition of inactivation compounds (e.g., organic solvents), the increasing of the enzyme stability (thermal and/or operational), and the enhancement of the CALB activity or selectivity/specificity. Some of these aspects will be discussed in more detail below.

5.2.3.2. L435 thermostability

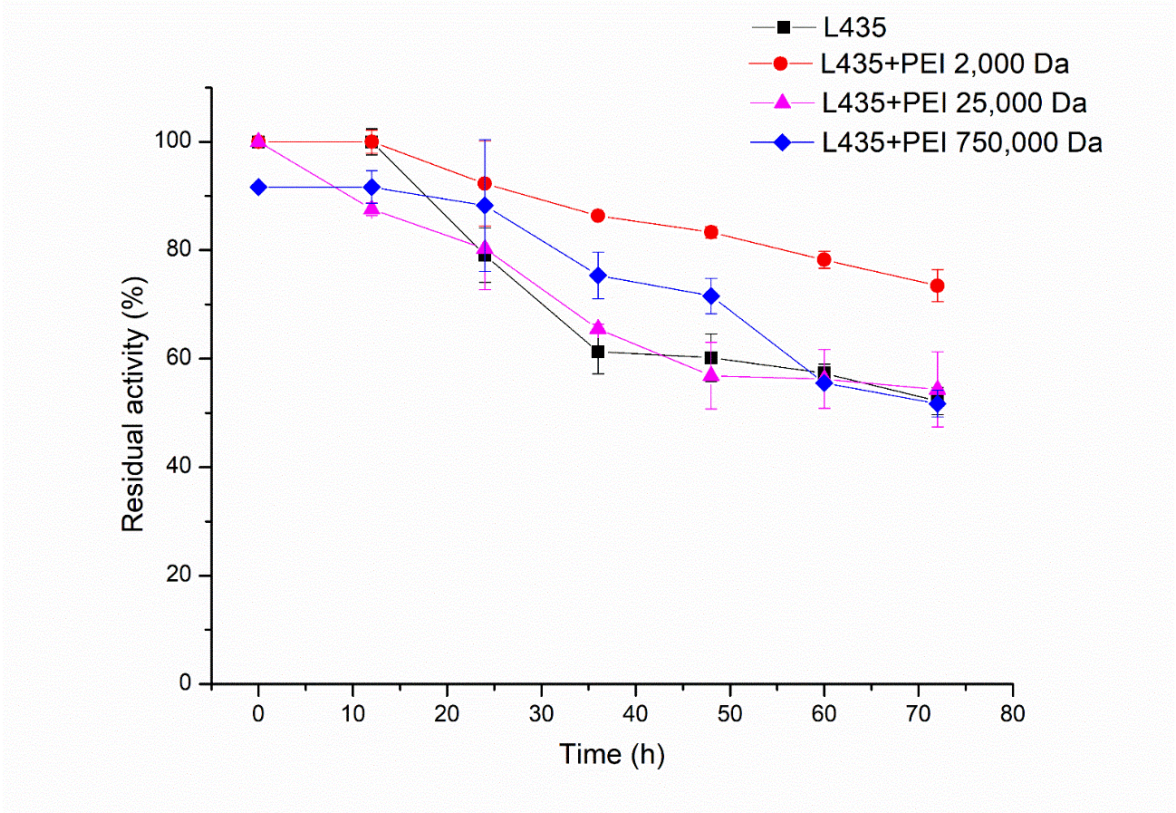
The thermal stabilities of the L435 preparations (uncoated and treated with PEI) were evaluated under different conditions, as shown in Figures 5.6 and 5.7 and Table 5.4. In Figure 5.6 (incubation of the enzymatic preparations in MEK), we found an accentuated reduction (by ~50%) in the recovered activities of the uncoated L435 after 72 h of incubation. This maintenance of half the A_H was similar for the CALB treated with PEI 25 and 750 KDa, after the same period. On the other hand, the L435 modified with PEI 2 KDa (the lowest molecular weight PEI evaluated) presented an approximately linear inactivation profile over time, with retention of ~73% of the initial activity after 72 h, being that way the more stable preparation under these conditions.

The inactivation assays at different pHs (Figure 5.7) showed maintenance of all the L435 initial activity before and after its coating with PEI 2 KDa after 12 h of incubation at pH 5. At pH 7, the non-treated CALB and the enzyme modified with the smallest size PEI retained roughly 54 and 71% of the A_H, respectively, over the same period. Lastly, at pH 9, all preparations were almost fully inactivated. In the incubation of the L435 derivatives in methanol/aqueous phosphate buffer mixtures (Table 5.4), the non-modified L435 and the CALB treated with PEI 2 KDa maintained all their initial activities after 12 h. Curiously, the other preparations were fully inactivated.

These outcomes indicated that the size of the PEI polymer played different effects in the thermal stability of the modified L435, as previously reported by Cabrera *et al*. (2009). The smallest size of the PEI (2 KDa) may have facilitated a full coating of the immobilized enzyme with small “wholes” of it, protecting the enzyme more efficiently (JÄMSÄ *et al.*, 2013; WANG *et al.*, 2018) and increasing its thermostability. Moreover, large polymers cannot access the areas of the protein in close contact with the support due to steric hindrances (CHAUBEY *et al*., 2006; PALOMO *et al*., 2002; ABIAN *et al*.,

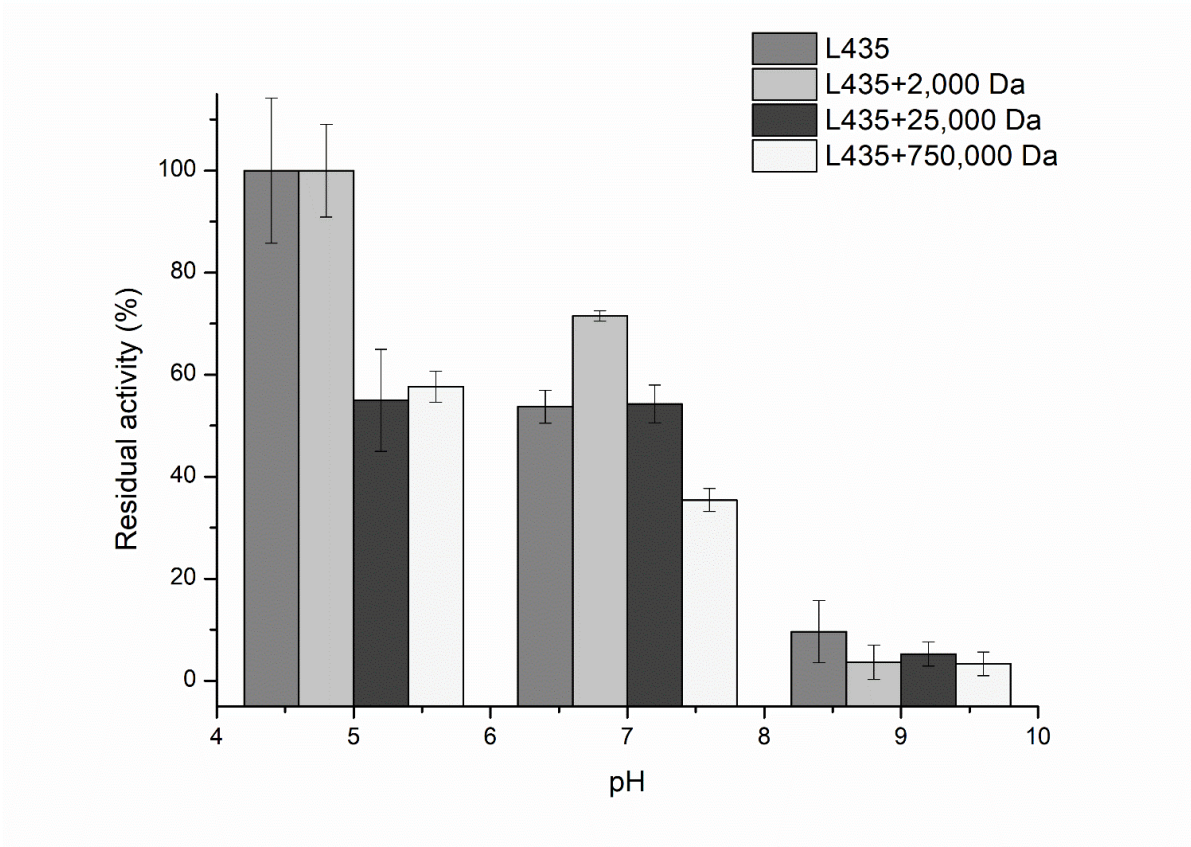
2001), while this may be not a problem for the smaller polymers. This dissimilar modification of the enzyme surface can drive an even destabilization of the enzyme (FERNANDEZ-LAFUENTE *et al*., 1999). Further, if some lipase molecules are unfolded in contact with the PEI, they can also maximize the interaction with the polymer (VIRGEN-ORTÍZ *et al*., 2016).

Guimarães *et al*. (2018), Peirce *et al*. (2016), Fernandez-Lopez *et al*. (2017a; 2017b), Zaak *et al*. (2017), Virgen-Ortíz *et al*. (2017b), Hill, Karboune, and Mateo, (2017), He *et al*. (2015), among other authors, also stated that PEI can be a promising candidate as a thermal stabilizing agent for proteins with diverse applications. Consequently, as the L435 treated with PEI 2 KDa presented: (1) the highest stability in MEK, different aqueous buffer solutions (pHs 5 and 7), and methanol/aqueous mixture; (2) the second-highest recovered activity (regarding the non-modified L435); and (3) does not desorb from the support after incubation in our reaction product; we selected this enzyme preparation, along with the uncoated L435, for subsequent assays of xylose laurate synthesis.



L435: uncoated L435; L435+PEI 750 KDa: L435 coated with PEI 750 KDa; L435+PEI 25 KDa: L435 coated with PEI 25 KDa; L435+PEI 2 KDa: L435 coated with PEI 2 KDa.

Figure 5.6. Inactivation courses of different Lipozyme 435 preparations in methyl ethyl ketone. The experiments were performed at 60 °C. Hydrolytic activities were obtained using tributyrin as the substrate (see section 5.2.2.8.1).



L435: uncoated L435; L435+PEI 750 KDa: L435 coated with PEI 750 KDa; L435+PEI 25 KDa: L435 coated with PEI 25 KDa; L435+PEI 2 KDa: L435 coated with PEI 2 KDa.

Figure 5.7. Residual activities of different Lipozyme 435 preparations after incubation for 12 h at 60 ºC in aqueous solutions, pHs 5, 7, and 9. Hydrolytic activities were obtained using tributyrin as the substrate (see section 5.2.2.8.1).

Table 5.4. Residual activities of different Lipozyme 435 preparations after incubation for 12 h at 60 ºC in methanol/phosphate buffer (80/20 v/v), pH 7. Hydrolytic activities were obtained using tributyrin as the substrate (see section 5.2.2.8.1).

Enzymatic preparation	Residual A_H (%)
L435	100 ± 13.76
L435 + PEI 2 KDa	100 ± 5.95
L435 + PEI 25 KDa	0.49 ± 0.12
L435 + PEI 750 KDa	3.24 ± 0.28

L435: uncoated L435; L435+PEI 750 KDa: L435 coated with PEI 750 KDa; L435+PEI 25 KDa: L435 coated with PEI 25 KDa; L435+PEI 2 KDa: L435 coated with PEI 2 KDa. Experiments were performed at 60 °C.

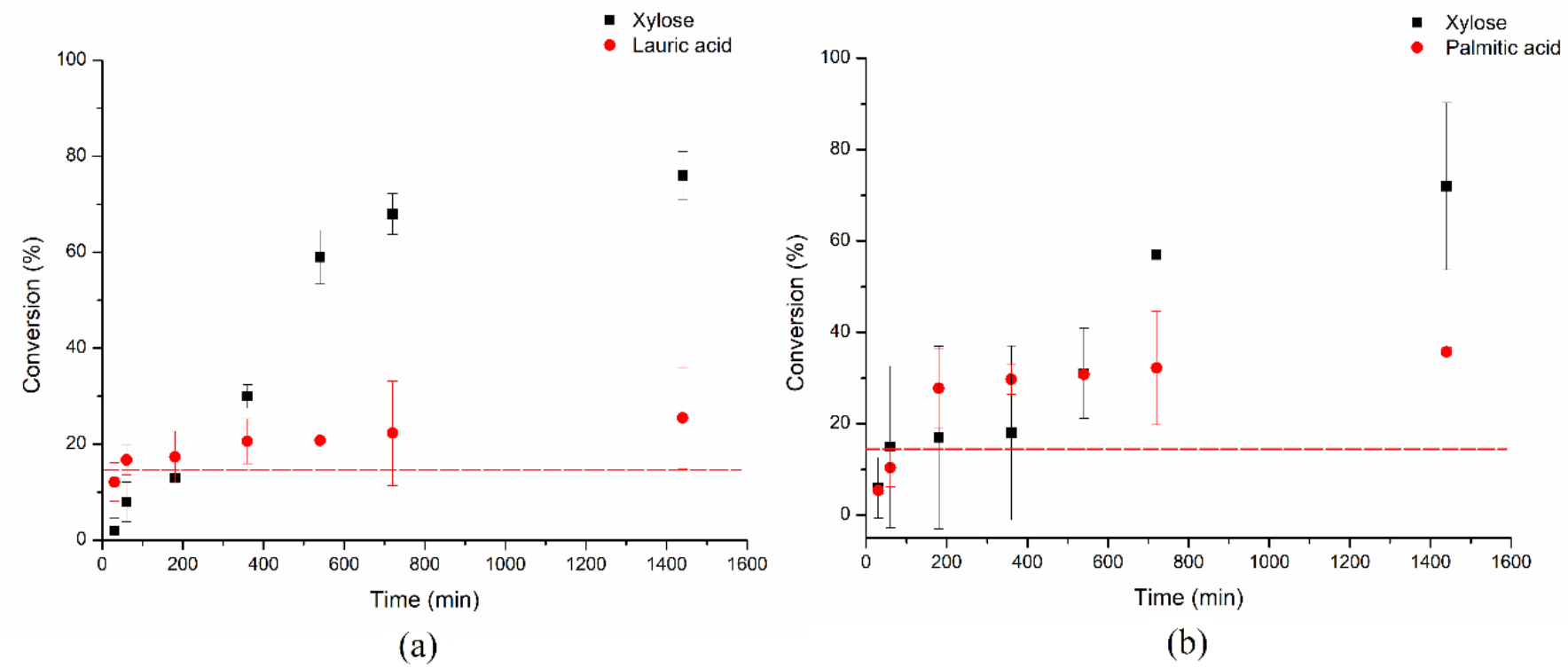
5.2.3.3. Free fatty acids enzyme specificity in the synthesis of xylose fatty acid esters

Given that the literature has demonstrated that in the case of direct esterification the chain length of the fatty acid can affect conversions (RUFINO *et al.*, 2010), we investigated lauric (C12:0), palmitic (C16:0), and oleic (C18:1) acids as acyl donors in the synthesis of xylose fatty esters. Here, we used an excess of fatty acid to xylose, since we already demonstrated that this condition provided higher conversions in the CALB-

catalyzed esterification reaction (GONÇALVES *et al.*, 2021a). Figures 5.8 and 5.9 show the conversion profiles obtained with the uncoated L435 and with the L435 treated with PEI 2 KDa as the biocatalysts of the esterification reaction, respectively.

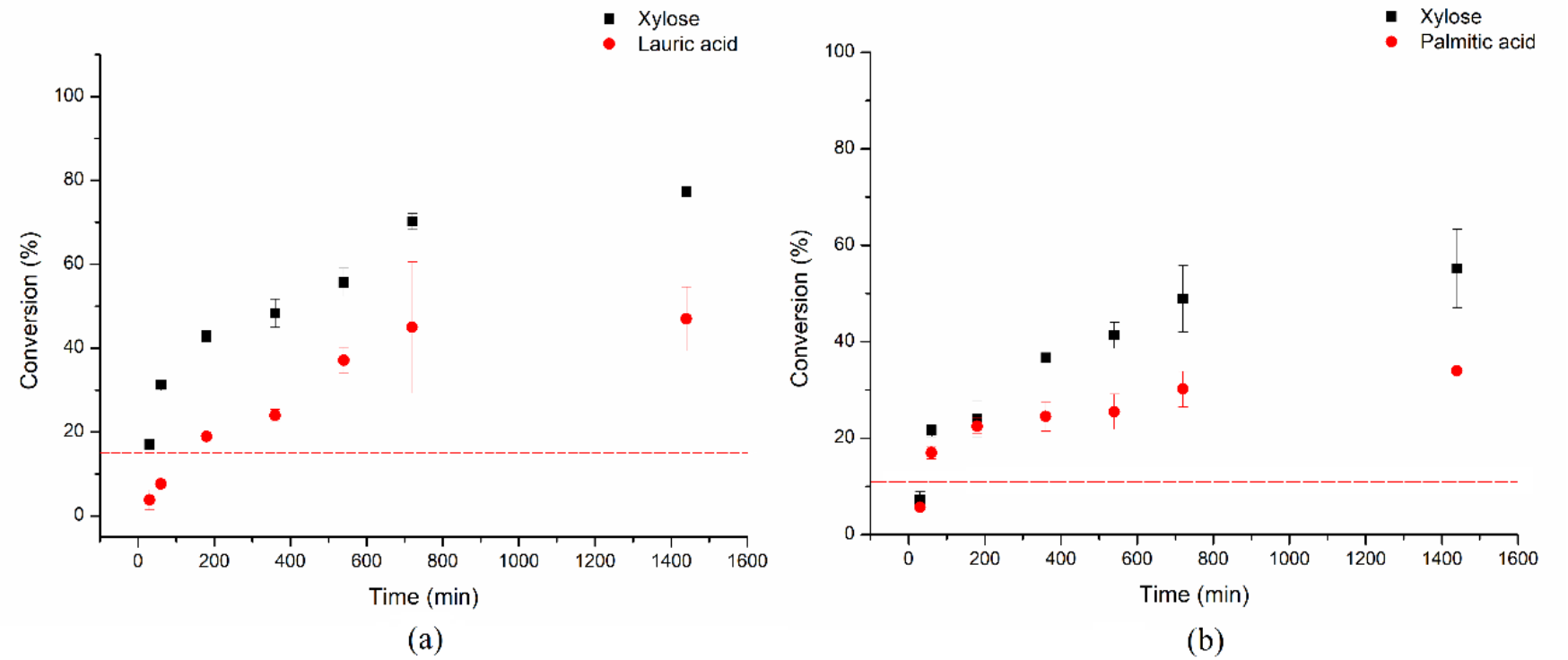
In Figure 5.8, 76% of the xylose and 25% of the lauric acid were depleted after a 24 h-reaction (a), while 72% of the xylose and 36% of the palmitic acid were consumed over the same period (b), implying that a higher modification of the xylose was produced when using the palmitic acid. The maximum lauric/palmitic acid conversions should be ~15% (Figure 5.8) if the reaction produced only monoesters (24 h). However, higher acid consumptions than expected were observed, suggesting that not only monoesters were synthesized, i.e., that more than one hydroxyl group per xylose molecule was substituted by the acyl moiety of the fatty acid. In fact, mass spectrometry later confirmed the presence of a mixture of mono-, di-, and triesters (Section 5.2.3.4, see below), even though the commercial CALB immobilized in a macroporous resin has been described to selectively modify only one hydroxyl group of the carbohydrate at the C5 primary position (PAPPALARDO *et al.*, 2017). In Gonçalves *et al*. (2021a), 84% xylose conversion and 16% oleic acid conversion were achieved in the aforementioned conditions. Thus, the fatty acid utilized as the acyl donor in this esterification reaction influenced the conversions of the limiting reagent, but not in a very drastic way. The best results were obtained when using long and unsaturated oleic acid (GONÇALVES *et al.*, 2021a). Indeed, other researches showed that the L435 has a higher specificity towards long-chain fatty acids (CAO; BORNSCHEUER; SCHMID, 1999; JUHL *et al.*, 2010; SÉVERAC *et al.*, 2011).

In Figure 5.9 (a), we found conversions of 77% (xylose) and 47% (lauric acid) after 24 h-reaction by using the L435 treated with PEI 2 KDa as the biocatalyst. Although the modified xylose is similar, the results showed much higher consumption of the lauric acid than with the non-treated CALB, suggesting that the new biocatalysts produced a higher modification of the xylose molecules. In 4 (b), 55% of the xylose and 34% of the palmitic acid were consumed after the same period. This result showed a reduction in the xylose conversion, while no significant reduction in the palmitic acid consumption was detected, again suggesting a higher degree of modification of the xylose molecules.



Reaction conditions: 60 °C; 2,000 rpm; 1557.23 TBU/g of xylose; xylose: fatty acid molar ratio of 1:5, with molecular sieves.

Figure 5.8. (a) Xylose and lauric acid conversion profiles in the synthesis of xylose laurate; (b) Xylose and palmitic acid conversion profiles in the synthesis of xylose palmitate. Both reactions were catalyzed by the uncoated Lipozyme 435 in a vortex flow reactor. The red dotted lines represent the expected maximum conversion of lauric/palmitic acids regarding the modification of only one hydroxyl group of the xylose.



Reaction conditions: 60 °C; 2,000 rpm; 1557.23 TBU/g of xylose; xylose: fatty acid molar ratio of 1:5, with molecular sieves.

Figure 5.9. (a) Xylose and lauric acid conversion profiles in the synthesis of xylose laurate; (b) Xylose and palmitic acid conversion profiles in the synthesis of xylose palmitate. Both reactions were catalyzed by the Lipozyme 435 coated with PEI 2 KDa in a vortex flow reactor. The red dotted lines represent the expected maximum conversion of lauric/palmitic acids regarding the modification of only one hydroxyl group of the xylose.

This different enzyme specificity may be caused by the concentration of the acid molecules in the enzyme environment (a cationic environment), by alterations of the pH in the water layer around the lipase, or by real conformational changes induced by the PEI modification (VIRGEN-ORTÍZ *et al.*, 2017a). Whatever the reason, different yields were obtained using the modified or the non-modified biocatalyst, and this is a very interesting result.

Similar conversions were found by De Lima *et al*. (2016). They evaluated the performance of the CALB immobilized by hydrophobic adsorption or hydrophobic/covalent linkages in the synthesis of xylose laurate in a tert-butyl alcohol medium, yielding around 60% xylose conversion at 55°C after 48 h under optimized conditions. Bidjou-Haiour and Klai (2013) assessed the synthesis of xylose laurate catalyzed by the Novozyme 435. The authors achieved ~50% lauric acid conversion at 60 ºC after 24 h using MEK as cosolvent. In another study, Ha, Hiep, and Koo (2010) investigated the Novozyme 435-catalyzed esterification of fructose with palmitic acid in ionic liquids mixtures at 60 °C for 14 h, obtaining approximately 75% fructose conversion under optimized reaction conditions. Enayati *et al*. (2018) synthesized lactose fatty esters from lauric, palmitic, and caprylic acids using CALB as the biocatalyst in organic solvents. Results showed conversions up to 77% and 93% for free and immobilized lipases, respectively.

5.2.3.4. Partial purification and characterization of xylose fatty acid esters

Our crude reaction product contains the sugar ester (xylose laurate/palmitate), the biocatalyst (L435), the organic solvent (MEK), as well as unreacted sugar and fatty acid. Thus, to separate the SFAEs from the crude product, samples of the final reaction medium (from the esterification of xylose with lauric or palmitic acids) were purified according to the methodology described in section 5.2.2.5. 97.8% of the lauric acid and ≥89% of the xylose; 86.4% of the palmitic acid and ≥83% of the xylose were removed after this process.

The purified samples were then characterized by mass spectrometry (see Figures S9-S20 in the supplementary material). Mass spectra of the $[M-H]^-$ ion (xylose: fatty acid molar ratio of 1:5) revealed that the L435 mainly produced xylose mono- and diesters (m/z = 331.1 and 513.3 – xylose laurate; m/z = 387.2 and 627.4 – xylose palmitate) (Figures S9, S10, S15 and S16, respectively). To a lesser extent, we also detected the presence of xylose triesters (m/z = 695.3 – xylose laurate; m/z = 865.5 – xylose palmitate)

(Figures S11 and S17, respectively). For the 5:1 molar ratio, xylose mono, di-, and trilaurate (m/z = 331.1, 513.3, and 695.4, respectively) were found in the same order of magnitude (Figures S12-S14). Still using the molar ratio with an excess of sugar, xylose monopalmitate (m/z = 387.1) was identified in higher intensity regarding di- and tripalmitate (m/z = 627.3 and 865.4) (Figures S18-S20). These results showed that an excess of xylose or fatty acid did not affect the CALB selectivity to the point of preventing the formation of di- and triesters. So, the formation of a mixture of sugar esters with different degrees of modification was confirmed here by mass spectrometry.

5.2.3.5. Emulsion capacity assays

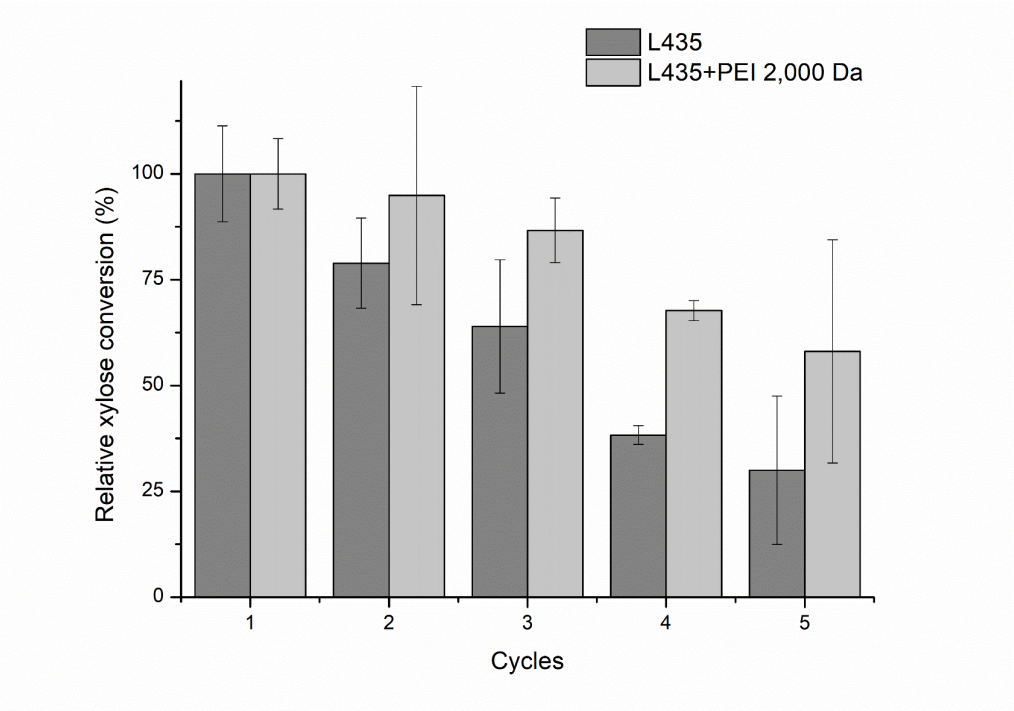
Sugar esters present emulsifying properties as well as a range of other useful physical properties that follow from their amphipathic nature (TENG *et al.*, 2020). The emulsion capacities (ECs) of the purified xylose laurate (12.5%) and xylose palmitate (11.76%) (using a xylose: fatty acid molar ratio of 1:5, in MEK) were of the same order of magnitude of the sucrose monolaurate EC (10%), a commercial sugar ester. This outcome (along with other parameters to be assessed) may allow the future use of the SFAEs synthesized in this study as emulsifiers suitable for deployment in a wide range of practical applications. For instance, laurate and palmitate esters derivatives are often used as detergents, in ice cream, milk beverages, cake batter, and sauces or dressings (YE; HAYES; BURTON, 2014).

Furthermore, the emulsion capacity is a parameter highly correlated with the medium hydrophobicity (AI; XIAO; JIANG, 2021). Sugar esters have hydrophobic characteristics associated with their fatty acyl side chain and hydrophilic hydroxyl groups associated with their carbohydrate moiety. As would be expected, longer acyl chains will increase the hydrophobicity and thereby decrease the EC and so, the emulsion stability (AI; XIAO; JIANG, 2021; TENG *et al.*, 2020). Indeed, the EC of the xylose palmitate was slightly lower than that for the xylose laurate, which has a smaller hydrophobic moiety. Likewise, in Gonçalves *et al*. (2021a), we used a longer-chain fatty acid (oleic acid, C18:1) to synthesize xylose oleate in the same conditions of this study and found an even lower EC (6.25%). Liu (2017) also found lower emulsion capacities for the synthesis of sucrose esters from stearic acid (C18:0) than those obtained from the palmitic acid (C16:0). It is worth noting that such comparisons were made only between sugar esters from the same carbohydrate source.

5.2.3.6. L435 operational stability

We selected the lauric acid as the acyl donor of these esterification reactions to analyze the biocatalysts stabilities due to some reasons: (1) the SFAEs synthesized from this fatty acid presented a higher purification rate than that produced from the palmitic acid (Section 5.2.3.4); (2) the xylose laurate showed an emulsion capacity slightly superior to that of the xylose palmitate (Section 5.2.3.5).

So, reactions were conducted with lauric acid and xylose as substrates and catalyzed by the non-modified L435 or by the L435 coated with PEI 2 KDa. With the non-treated CALB, we verified a decrease of 70% in the relative xylose conversions (i.e., a drop of 37% in the absolute conversions) after five 6 h-cycles (Figure 5.10). In Gonçalves *et al*. (2021a) (using oleic acid as the acyl donor of this reaction), we also identified a significant decrease in the xylose conversions under the same reaction conditions, after ten 12 h-cycles. These reductions in the conversions over the biocatalyst reuse were partially explained by the CALB desorption from the support (a drop of 37 and 50% in the A_H after 12 h-incubation in the reaction medium of xylose laurate and xylose oleate synthesis, respectively). This behavior could be attributed to several causes that may greatly facilitate the CALB release from the matrix of the commercial Lipozyme 435, as discussed in Gonçalves *et al*. (2021a).



Reaction conditions of each batch: 60 °C; 2,000 rpm; 15,572 TBU/g of xylose; xylose: lauric acid molar ratio of 1:5 (in MEK to a xylose concentration of 7 mM); 6 h-cycles.

Figure 5.10. Operational stability of uncoated and PEI-coated (2 KDa) Lipozyme 435 biocatalysts.

In contrast, when the L435 coated with PEI 2 KDa was used as the biocatalyst of this reaction, we identified a decrease of only 42% in the relative xylose conversions (i.e., a drop of 10% in the absolute conversions), after the same five 6 h-cycles (Figure 5.10). This strategy reduces the enzyme desorption problem (VILLALBA *et al.*, 2016) that has been reported using this biocatalyst (GONÇALVES *et al*., 2021b; TENG *et al*., 2020; YE; HAYES; BURTON, 2014), improving enzyme reuse.

Villalba *et al*. (2016) also evaluated the operational stability of the L435 coated with PEI. The authors verified that the biocatalyst remained fully active after 14 consecutive 24 h-reaction cycles of alcoholysis of camelina oil. Fernandez-Lopez *et al*. (2017b) reported the reuse of the CALB immobilized on octyl-agarose and coated with PEI and dextran sulfate. This enzyme catalyzed the hydrolysis of triacetin for 6 cycles (of 2 h each) without any decay in the reaction rates or yields of diacetin.

Besides, we determined the hydrolytic activities of the L435 samples (section 5.2.2.8.1) to compare these two enzymatic preparations before and after the reuse assays. After five 6 h-cycles, we verified a reduction 2.18 ± 0.53-fold greater in the A_H of the non-modified L435 regarding the derivative coated with PEI. This outcome confirms that the addition of the PEI layer to the L435 surface reduced the enzyme leakage and so, raised the L435 operational stability.

Accordingly, in compliance with other authors, we demonstrated here that the limited repeatability of the commercial immobilized CALB (considered as the main problem for the industrial implementation of this enzyme (AN *et al.*, 2019; ORTIZ *et al.*, 2019; GONÇALVES *et al.*, 2021a; IDRIS; BUKHARI, 2012)) can be enhanced by improving the L435 stability through the modification of the biocatalyst surface with PEI. Hence, this approach can be crucial to endorse the large-scale production of sugar esters via the enzymatic route (BARBOSA *et al.*, 2012b; BILAL *et al.*, 2019; RUEDA *et al.*, 2016).

5.2.4. Conclusions

The coating of the immobilized preparation of CALB supplied by Novozymes (Lipozyme 435) with PEI prevented the enzyme desorption from the support in the presence of the crude sugar ester synthesized in this study. The size of the PEI polymer played an important effect in the thermostability of the modified L435. The enzyme treated with the lowest PEI size (2 KDa) presented the highest thermal stability in MEK, buffer solutions (pHs 5 and 7), and methanol/phosphate buffer (pH 7). The L435 (before

and after its coating with low size PEI) yielded high xylose conversions in the synthesis of xylose laurate/palmitate in methyl ethyl ketone. The PEI treatment increased the modification degree of the xylose molecules, suggesting a change in the apparent enzyme selectivity. The L435 treatment with PEI 2 KDa also enhanced the operational stability of this commercial immobilized lipase, allowing its reuse for 5 cycles with only 10% decay in the absolute conversions. Due to the simplicity and short time-consuming of this modification (performed on an enzyme previously immobilized), this strategy seems to be an effective and easy-to-perform tool to tailor the biocatalyst properties, possibly enabling the use of this CALB preparation in large-scale processes. Further, the formation of a mixture of mono-, di-, and tri- xylose esters is still convenient for detergents simple preparation, and the emulsion capacities of our purified products were close to that of a commercial sugar ester.

Funding: This work was supported by the "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP) [grant numbers 2016/10636-8, 2019/23908-4]; in part by "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) [Finance Code 001]; "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) [grant number 315092/2020-3); "Ministerio de Ciencia e Innovación" (Spanish Government) [grant number CTQ2017-86170-R]; and "Consejo Superior de Investigaciones Científicas" (CSIC) [grant number AEP045].

Acknowledgments: The authors would like to thank Maria Fátima das Graças Fernandes da Silva for the technical support in the conduction of the mass spectrometry analysis. The authors also thank Novozymes Latin America (Araucária, Brazil) for the donation of the immobilized CALB (Lipozyme 435).

References

ABBAS, H.; COMEAU, L. Aroma synthesis by immobilized lipase from *Mucor sp*. **Enzyme and Microbial Technology**, vol. 32, no. 5, p. 589–595, 2003. DOI https://doi.org/10.1016/S0141-0229(03)00022-X.

ABDULMALEK, E.; HAMIDON, N. F.; RAHMAN, M. B. A. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents. **Journal of Molecular Catalysis B: Enzymatic**, vol. 132, p. 1–4, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2016.06.010.

ABIAN, O.; MATEO, C.; FERNÁNDEZ-LORENTE, G.; PALOMO, J. M.; FERNÁNDEZ-LAFUENTE, R.; GUISAN, J. M. Stabilization of immobilized enzymes against organic solvents: generation of hyper-hydrophilic micro-environments fully surrounding enzyme molecules. **Biocatalysis and Biotransformation**, vol. 19, p. 489–

503, 2001. DOI https://doi.org/https://doi.org/10.3109/10242420108992032.
AGILENT. **The essential chromatography & spectroscopy catalog**. 2012.
AI, M.; XIAO, N.; JIANG, A. Molecular structural modification of duck egg white protein conjugates with monosaccharides for improving emulsifying capacity. **Food Hydrocolloids**, vol. 111, p. 106271, 2021. DOI https://doi.org/10.1016/j.foodhyd.2020.106271.
AN, D.; ZHANG, X.; LIANG, F.; XIAN, M.; FENG, D.; YE, Z. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A**, vol. 577, p. 257–264, 2019. DOI https://doi.org/10.1016/j.colsurfa.2019.05.079.
ARANA-PEÑA, S.; LOKHA, Y.; FERNÁNDEZ-LAFUENTE, R. Immobilization on octyl-agarose beads and some catalytic features of commercial preparations of lipase A from *Candida antarctica* (Novocor ADL): Comparison with immobilized lipase B from *Candida antarctica*. **Biotechnology Progress**, vol. 35, no. 1, p. 2735, 2019. DOI https://doi.org/10.1002/btpr.2735.
ARANA-PEÑA, S.; RIOS, N. S.; MENDEZ-SANCHEZ, C.; LOKHA, Y.; GONÇALVES, L. R. B.; FERNÁNDEZ-LAFUENTE, R. Use of polyethylenimine to produce immobilized lipase multilayers biocatalysts with very high volumetric activity using octyl-agarose beads: Avoiding enzyme release during multilayer production. **Enzyme and Microbial Technology**, vol. 137, p. 109535, 2020. DOI https://doi.org/10.1016/j.enzmictec.2020.109535.
BARBOSA, O.; TORRES, R.; ORTIZ, C.; FERNANDEZ-LAFUENTE, R. The slow-down of the CALB immobilization rate permits to control the inter and intra molecular modification produced by glutaraldehyde. **Process Biochemistry**, vol. 47, no. 5, p. 766–774, 2012a. DOI http://dx.doi.org/10.1016/j.procbio.2012.02.009.
BARBOSA, O.; RUIZ, M.; ORTIZ, C.; FERNÁNDEZ, M.; TORRES, R.; FERNANDEZ-LAFUENTE, R. Modulation of the properties of immobilized CALB by chemical modification with 2,3,4-trinitrobenzenesulfonate or ethylendiamine. Advantages of using adsorbed lipases on hydrophobic supports. **Process Biochemistry**, vol. 47, no. 5, p. 867–876, 2012b. DOI http://dx.doi.org/10.1016/j.procbio.2012.02.026.
BEISSON, F.; TISS, A.; RIVIÈRE, C.; VERGER, R. Methods for lipase detection and assay: A critical review. **European Journal of Lipid Science and Technology**, vol. 102, no. 2, p. 133–153, 2000. DOI https://doi.org/10.1002/(sici)1438-9312(200002)102:2<133::aid-ejlt133>3.0.co;2-x.
BIANCHI, D.; BATTISTEP, E.; BOSETTI, A.; CESTIA, P.; FELCETEB, Z. Effects of chemical modification on stereoselectivity of *Pseudomonas cepacia* lipase. **Tehkdron: Asymmetry**, vol. 4, no. 5, p. 1–6, 1993. DOI https://doi.org/https://doi.org/10.1016/S0957-4166(00)80115-9.
BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. DOI https://doi.org/10.14233/ajchem.2013.13973.
BILAL, M.; ZHAO, Y.; NOREEN, S.; SHAH, S. Z. H.; BHARAGAVA, R. N.; IQBAL, H. M. N. Modifying bio-catalytic properties of enzymes for efficient biocatalysis: a review from immobilization strategies viewpoint. **Biocatalysis and Biotransformation**, vol. 37, no. 3, p. 159–182, 2019. DOI https://doi.org/10.1080/10242422.2018.1564744.
BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, vol. 72, p. 248–254, 1976. DOI https://doi.org/https://doi.org/10.1006/abio.1976.9999.
CABRERA, Z.; FERNANDEZ-LORENTE, G.; FERNANDEZ-LAFUENTE, R.;

PALOMO, J. M.; GUISAN, J. M. Enhancement of Novozym-435 catalytic properties by physical or chemical modification. **Process Biochemistry**, vol. 44, no. 2, p. 226–231, 2009. DOI https://doi.org/10.1016/j.procbio.2008.10.005.

CAO, L.; BORNSCHEUER, U. T.; SCHMID, R. D. Lipase-catalyzed solid-phase synthesis of sugar esters. Influence of immobilization on productivity and stability of the enzyme. **Journal of Molecular Catalysis B: Enzymatic**, vol. 6, no. 3, p. 279–285, 1999. DOI https://doi.org/10.1016/S1381-1177(98)00083-6.

CASAS-GODOY, L.; ARRIZON, J.; ARRIETA-BAEZ, D.; PLOU, F. J.; SANDOVAL, G. Synthesis and emulsifying properties of carbohydrate fatty acid esters produced from Agave tequilana fructans by enzymatic acylation. **Food Chemistry**, vol. 204, p. 437–443, 2016. DOI http://dx.doi.org/10.1016/j.foodchem.2016.02.153.

CASTRO, H. F.; MENDES, A. A.; SANTOS, J. C.; AGUIAR, C. L. Modification of oils and fats by biotransformation. **Química Nova**, vol. 27, no. 1, p. 146–156, 2004. DOI https://doi.org/10.1590/s0100-40422004000100025.

CHANG, S. W.; SHAW, J. F. Biocatalysis for the production of carbohydrate esters. **New Biotechnology**, vol. 26, no. 3–4, p. 109–116, 2009. DOI https://doi.org/10.1016/j.nbt.2009.07.003.

CHAUBEY, A.; PARSHAD, R.; KOUL, S.; TANEJA, S. C.; QAZI, G. N. Enantioselectivity modulation through immobilization of *Arthrobacter sp*. lipase: kinetic resolution of fluoxetine intermediate. **Journal of Molecular Catalysis B: Enzymatic**, vol. 42, p. 39–44, 2006. DOI https://doi.org/https://doi.org/10.1016/j.molcatb.2006.06.011.

CHEN, B.; HU, J.; MILLER, E. M.; XIE, W.; CAI, M.; GROSS, R. A. *Candida antarctica* lipase B chemically immobilized on epoxy-activated micro- and nanobeads: Catalysts for polyester synthesis. **Biomacromolecules**, vol. 9, p. 463–471, 2008. DOI https://doi.org/10.1021/bm700949x.

CHEN, B.; MILLER, E. M.; MILLER, L.; MAIKNER, J. J.; GROSS, R. A.; YORK, N.; CO, H.; BOX, P. O.; HOUSE, S.; PENNSYL, V.; SYNCHROTRON, N.; SOURCE, L.; BROOKHA, V.; YORK, N. Effects of macroporous resin size on *Candida antarctica* lipase B adsorption, fraction of active molecules, and catalytic activity for polyester synthesis. **Langmuir**, vol. 23, p. 1381–1387, 2007. DOI https://doi.org/https://doi.org/10.1021/la062258u.

DE LIMA, L. N.; MENDES, A. A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W.; GIORDANO, R. L. C. Performance of different immobilized lipases in the syntheses of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, 2018. DOI https://doi.org/10.3390/molecules23040766.

DE LIMA, L. N.; VIEIRA, G. N. A.; KOPP, W.; TARDIOLI, P. W.; GIORDANO, R. L. C. Mono- and heterofunctionalized silica magnetic microparticles (SMMPs) as new carriers for immobilization of lipases. **Journal of Molecular Catalysis B: Enzymatic**, vol. 133, p. 491–499, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2017.04.002.

DOS SANTOS, J. C. S.; GARCIA-GALAN, C.; RODRIGUES, R. C.; DE SANT' ANA, H. B.; GONÇALVES, L. R. B.; FERNANDEZ-LAFUENTE, R. Improving the catalytic properties of immobilized Lecitase via physical coating with ionic polymers. **Enzyme and Microbial Technology**, vol. 60, p. 1–8, 2014. DOI https://doi.org/10.1016/j.enzmictec.2014.03.001.

DOS SANTOS, J. C. S.; RUEDA, N.; SANCHEZ, A.; VILLALONGA, R.; GONÇALVES, L. R. B.; FERNANDEZ-LAFUENTE, R. Versatility of divinylsulfone supports permits the tuning of CALB properties during its immobilization. **RSC Advances**, vol. 5, no. 45, p. 35801–35810, 2015a. DOI http://dx.doi.org/10.1039/C5RA03798K.

DOS SANTOS, J. C. S.; RUEDA, N.; TORRES, R.; BARBOSA, O.; GONÇALVES, L. R. B.; FERNANDEZ-LAFUENTE, R. Evaluation of divinylsulfone activated agarose to immobilize lipases and to tune their catalytic properties. **Process Biochemistry**, vol. 50, no. 6, p. 918–927, 2015b. DOI https://doi.org/10.1016/j.procbio.2015.03.018.

ENAYATI, M.; GONG, Y.; GODDARD, J. M.; ABBASPOURRAD, A. Synthesis and characterization of lactose fatty acid ester biosurfactants using free and immobilized lipases in organic solvents. **Food Chemistry**, vol. 266, p. 508–513, 2018. DOI https://doi.org/10.1016/j.foodchem.2018.06.051.

DOTTORI, F. A.; COOPER, R. A.; BENSON, N. B.; BENECH, R. O. **Fractionation of lignocellulosic biomass for cellulosic ethanol and chemical production**. Unitated States: [2016/0251682], 2016.

FALLAVENA, L. P.; ANTUNES, F. H. F.; ALVES, J. S.; PALUDO, N.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Ultrasound technology and molecular sieves improve the thermodynamically controlled esterification of butyric acid mediated by immobilized lipase from *Rhizomucor miehei*. **RSC Advances**, vol. 4, no. 17, p. 8675–8681, 2014. DOI https://doi.org/10.1039/c3ra47315e.

FERNANDEZ-LAFUENTE, R.; ARMISÉN, P.; SABUQUILLO, P.; FERNÁNDEZ-LORENTE, G.; GUISÁN, J. M. Immobilization of lipases by selective adsorption on hydrophobic supports. **Chemistry and Physics of Lipids**, vol. 93, no. 1–2, p. 185–197, 1998. DOI https://doi.org/10.1016/S0009-3084(98)00042-5.

FERNANDEZ-LAFUENTE, R.; ROSELL, C. M.; CAANAN-HADEN, L.; RODES, L.; GUISAN, J. M. Facile synthesis of artificial enzyme chemistry of immobilized derivatives: Dramatic stabilization of penicillin acylase versus organic solvents. **Enzyme and Microbial Technology**, vol. 24, no. 1–2, p. 96–103, 1999. DOI https://doi.org/https://doi.org/10.1016/S0141-0229(98)00102-1.

FERNANDEZ-LOPEZ, L.; PEDRERO, S. G.; LOPEZ-CARROBLES, N.; VIRGEN-ORTÍZ, J. J.; GORINES, B. C.; OTERO, C.; FERNANDEZ-LAFUENTE, R. Physical crosslinking of lipase from *Rhizomucor miehei* immobilized on octyl agarose via coating with ionic polymers: Avoiding enzyme release from the support. **Process Biochemistry**, vol. 54, p. 81–88, 2017a. DOI http://dx.doi.org/10.1016/j.procbio.2016.12.018.

FERNANDEZ-LOPEZ, L.; VIRGEN-ORTÍZ, J. J.; PEDRERO, S. G.; LOPEZ-CARROBLES, N.; GORINES, B. C.; OTERO, C.; FERNANDEZ-LAFUENTE, R. Optimization of the coating of octyl-CALB with ionic polymers to improve stability and decrease enzyme leakage. **Biocatalysis and Biotransformation**, vol. 36, p. 47–56, 2017b. DOI https://doi.org/https://doi.org/10.1080/10242422.2016.1278212.

FERNANDEZ-LORENTE, G.; FILICE, M.; LOPEZ-VELA, D.; PIZARRO, C.; WILSON, L.; BETANCOR, L.; AVILA, Y.; GUISAN, J. M. Cross-linking of lipases adsorbed on hydrophobic supports: Highly selective hydrolysis of fish oil catalyzed by RML. **Journal of the American Oil Chemists' Society**, vol. 88, no. 6, p. 801–807, 2011. DOI https://doi.org/10.1007/s11746-010-1727-2.

FERNANDEZ-LORENTE, G.; GODOY, C. A.; MENDES, A. A.; LOPEZ-GALLEGO, F.; GRAZU, V.; RIVAS, B.; PALOMO, J. M.; HERMOSO, J.; FERNANDEZ-LAFUENTE, R.; GUISAN, J. M. Solid-phase chemical amination of a lipase from *Bacillus thermocatenulatus* to improve its stabilization via covalent immobilization on highly activated glyoxyl-agarose. **Biomacromolecules**, vol. 9, no. 9, p. 2553–2561, 2008. DOI https://doi.org/10.1021/bm800609g.

FERNANDEZ-LORENTE, G.; BETANCOR, L.; CARRASCOSA, A. V.; PALOMO, J. M.; GUISAN, J. M. Modulation of the selectivity of immobilized lipases by chemical and physical modifications: Release of omega-3 fatty acids from fish oil. **Journal of the American Oil Chemists' Society**, vol. 89, p. 97–102, 2012. DOI

https://doi.org/https://doi.org/10.1007/s11746-011-1885-x.
FOLEY, A. M.; MAGUIRE, A. R. The impact of recent developments in technologies which enable the increased use of biocatalysts. **European Journal of organic chemistry**, vol. 2019, no. 23, p. 3713–3734, 2019. DOI https://doi.org/10.1002/ejoc.201900208.
GARCIA-GALAN, C.; BERENGUER-MURCIA, Á.; FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C. Potential of different enzyme immobilization strategies to improve enzyme performance. **Advanced Synthesis and Catalysis**, vol. 353, no. 16, p. 2885–2904, 2011. DOI https://doi.org/10.1002/adsc.201100534.
GONÇALVES, M. C. P.; AMARAL, J. C.; FERNANDEZ-LAFUENTE, R.; JUNIOR, R. D. S.; TARDIOLI, P. W. Lipozyme 435-mediated synthesis of xylose oleate in methyl ethyl ketone. **Molecules**, vol. 26, p. 3317, 2021a. DOI https://doi.org/https://doi.org/10.3390/molecules26113317.
GONÇALVES, M. C. P.; ROMANELLI, J. P.; GUIMARÃES, J. R.; VIEIRA, A. C.; DE AZEVEDO, B. P.; TARDIOLI, P. W. Reviewing research on the synthesis of CALB-catalyzed sugar esters incorporating systematic mapping principles. **Critical Reviews in Biotechnology**, vol. 41, no. 6, p. 865–878, 2021b. DOI https://doi.org/10.1080/07388551.2021.1888071.
GUIMARÃES, J. R.; DE LIMA CAMARGO GIORDANO, R.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W. Evaluation of strategies to produce highly porous cross-linked aggregates of porcine pancreas lipase with magnetic properties. **Molecules**, vol. 23, no. 11, p. 13–16, 2018. DOI https://doi.org/10.3390/molecules23112993.
GUMEL, A. M.; ANNUAR, M. S. M.; HEIDELBERG, T.; CHISTI, Y. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, vol. 46, no. 11, p. 2079–2090, 2011. DOI http://dx.doi.org/10.1016/j.procbio.2011.07.021.
HE, H.; LUO, H.; CHANG, Y.; LI, X.; WANG, X.; LIANG, C.; WEI, Y.; YU, H.; SHEN, Z. Immobilization and stabilization of cephalosporin C acylase on aminated support by crosslinking with glutaraldehyde and further modifying with aminated macromolecules. **Biotechnology Progress**, vol. 31, p. 387–395, 2015. DOI https://doi.org/10.1002/btpr.
HA, S. H.; HIEP, N. M.; KOO, Y. Enhanced production of fructose palmitate by lipase-catalyzed esterification in ionic liquids. **Biotechnology and Bioprocess Engineering**, vol. 15, p. 126–130, 2010. DOI https://doi.org/10.1007/s12257-009-3091-3.
HALLING, P. J. Thermodynamic predictions for biocatalysis in nonconventional media: Theory, tests, and recommendations for experimental design and analysis. **Enzyme and Microbial Technology**, vol. 16, no. 3, p. 178–206, 1994. DOI https://doi.org/10.1016/0141-0229(94)90043-4.
HILL, A. A.; KARBOUNE, S.; MATEO, C. Investigating and optimizing the immobilization of levansucrase for improved retention of transfructosylation activity and thermal stability. **Process Biochemistry**, vol. 61, p. 63–72, 2017. DOI http://dx.doi.org/10.1016/j.procbio.2017.06.011.
HILTERHAUS, L.; THUM, O.; LIESE, A. Reactor concept for lipase-catalyzed solvent-free conversion of highly viscous reactants forming two-phase systems. **Organic Process Research & Development**, vol. 12, p. 618–625, 2008. DOI https://doi.org/https://doi.org/10.1021/op800070q.
HIRATA, D. B.; ALBUQUERQUE, T. L.; RUEDA, N.; SÁNCHEZ-MONTERO, J. M.; GARCIA-VERDUGO, E.; PORCAR, R.; FERNANDEZ-LAFUENTE, R. Advantages of heterofunctional octyl supports: Production of 1,2-dibutyrin by specific and selective hydrolysis of tributyrin catalyzed by immobilized lipases. **ChemistrySelect**, vol. 1, no. 12, p. 3259–3270, 2016a. DOI https://doi.org/10.1002/slct.201600274.
HIRATA, D. B.; ALBUQUERQUE, T. L.; RUEDA, N.; VIRGEN-ORTÍZ, J. J.; TACIAS-PASCACIO, V. G.; FERNANDEZ-LAFUENTE, R. Evaluation of different

immobilized lipases in transesterification reactions using tributyrin: Advantages of the heterofunctional octyl agarose beads. **Journal of Molecular Catalysis B: Enzymatic**, vol. 133, p. 117–123, 2016b. DOI http://dx.doi.org/10.1016/j.molcatb.2016.08.008.

IDRIS, A.; BUKHARI, A. Immobilized *Candida antarctica* lipase B: Hydration, stripping off and application in ring opening polyester synthesis. **Biotechnology Advances**, vol. 30, no. 3, p. 550–563, 2012. DOI http://dx.doi.org/10.1016/j.biotechadv.2011.10.002.

JÄMSÄ, S.; MAHLBERG, R.; HOLOPAINEN, U.; ROPPONEN, J.; SAVOLAINEN, A.; RITSCHKOFF, A. C. Slow release of a biocidal agent from polymeric microcapsules for preventing biodeterioration. **Progress in Organic Coatings**, vol. 76, no. 1, p. 269–276, 2013. DOI http://dx.doi.org/10.1016/j.porgcoat.2012.09.018.

JUHL, P. B.; DODERER, K.; HOLLMANN, F.; THUM, O.; PLEISS, J. Engineering of *Candida antarctica* lipase B for hydrolysis of bulky carboxylic acid esters. **Journal of Biotechnology**, vol. 150, no. 4, p. 474–480, 2010. DOI http://dx.doi.org/10.1016/j.jbiotec.2010.09.951.

KASCHE, V. Mechanism and yields in enzyme catalysed equilibrium and kinetically controlled synthesis of β-lactam antibiotics, peptides and other condensation products. **Enzyme and Microbial Technology**, vol. 8, no. 1, p. 4–16, 1986. DOI https://doi.org/10.1016/0141-0229(86)90003-7.

KERTHY, S.; IBINGA, K.; FABRE, J.; BIKANGA, R.; LOUIS, B. Atypical reaction media and organized systems for the synthesis of low-substitution sugar esters. **Frontiers in Chemistry**, vol. 7, p. 587, 2019. DOI https://doi.org/10.3389/fchem.2019.00587.

KOPP, W.; SILVA, F. A.; LIMA, L. N.; MASUNAGA, S. H.; TARDIOLI, P. W.; GIORDANO, R. C.; ARAÚJO-MOREIRA, F. M.; GIORDANO, R. L. C. Synthesis and characterization of robust magnetic carriers for bioprocess applications. **Materials Science and Engineering B: Solid-State Materials for Advanced Technology**, vol. 193, p. 217–228, 2015. DOI http://dx.doi.org/10.1016/j.mseb.2014.12.002.

KUMARI, A.; MAHAPATRA, P.; GARLAPATI, V. K.; BANERJEE, R. Enzymatic transesterification of Jatropha oil. **Biotechnology for Biofuels**, vol. 2, p. 1, 2009. DOI https://doi.org/10.1186/1754-6834-2-1.

LI, L.; JI, F.; WANG, J.; LI, Y.; BAO, Y. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. **Enzyme and Microbial Technology**, vol. 69, p. 46–53, 2015. DOI https://doi.org/10.1016/j.enzmictec.2014.12.003.

LIU, K. J. Enzymatic synthesis of isomaltotriose palmitate and evaluation of its emulsifying property. **Enzyme and Microbial Technology**, vol. 101, p. 51–56, 2017. DOI http://dx.doi.org/10.1016/j.enzmictec.2017.03.004.

MATHEWS, S. L.; PAWLAK, J.; GRUNDEN, A. M. Bacterial biodegradation and bioconversion of industrial lignocellulosic streams. **Applied Microbiology and Biotechnology**, vol. 99, p. 2939–2954, 2015. DOI https://doi.org/10.1007/s00253-015-6471-y.

MORENO-PÉREZ, S.; GUISAN, J. M.; FERNANDEZ-LORENTE, G. Selective ethanolysis of fish oil catalyzed by immobilized lipases. **Journal of the American Oil Chemists' Society**, vol. 91, no. 1, p. 63–69, 2014. DOI https://doi.org/10.1007/s11746-013-2348-3.

MUZARD, M.; DELEU, M. Enzymatically-synthesized xylo-oligosaccharides laurate esters as surfactants of interest. **Carbohydrate Research**, vol. 495, p. 1–6, 2020. DOI https://doi.org/10.1016/j.carres.2020.108090.

NAKAMURA, Y.; MATSUMOTO, T.; NOMOTO, F.; UEDA, M.; FUKUDA, H.; KONDO, A. Enhancement of activity of lipase-displaying yeast cells and their application to optical resolution of (R,S)-1-benzyloxy-3-chloro-2-propyl monosuccinate.

Biotechnology Progress, vol. 22, no. 4, p. 998–1002, 2006. DOI https://doi.org/10.1021/bp060136m.

NETA, N. A. S.; TEIXEIRA, J. A.; RODRIGUES, L. R. Sugar ester surfactants: Enzymatic synthesis and applications in food industry. **Critical Reviews in Food Science and Nutrition**, vol. 55, no. 5, p. 595–610, 2015. DOI https://doi.org/10.1080/10408398.2012.667461.

NICOLÁS, P.; LASSALLE, V. L.; FERREIRA, M. L. About the role of typical spacer/crosslinker on the design of efficient magnetic biocatalysts based on nanosized magnetite. **Journal of Molecular Catalysis B: Enzymatic**, vol. 122, p. 296–304, 2015. DOI http://dx.doi.org/10.1016/j.molcatb.2015.09.013.

ORTIZ, C.; FERREIRA, M. L.; BARBOSA, O.; DOS SANTOS, J. C. S.; RODRIGUES, R. C.; BERENGUER-MURCIA, Á.; BRIAND, L. E.; FERNANDEZ-LAFUENTE, R. Novozym 435: the "perfect" lipase immobilized biocatalyst? **Catalysis Science & Technology**, vol. 9, no. 10, p. 2380–2420, 2019. DOI https://doi.org/https://doi.org/10.1039/C9CY00415G.

PALOMO, J. M.; FERNANDEZ-LORENTE, G.; MATEO, C.; FUENTES, M.; FERNANDEZ-LAFUENTE, R.; GUISAN, J. M. Modulation of the enantioselectivity of *Candida antarctica* lipase B via conformational engineering: kinetic resolution of (±)-α-hydroxy-phenylacetic acid derivatives. **Tetrahedron Asymmetry**, vol. 13, p. 1337–1345, 2002. DOI https://doi.org/https://doi.org/10.1016/S0957-4166(02)00325-7.

PALOMO, J. M.; FILICE, M.; FERNANDEZ-LAFUENTE, R.; TERRENI, M.; GUISAN, J. M. Regioselective hydrolysis of different peracetylated β-monosaccharides by immobilized lipases from different sources. Key role of the immobilization. **Advanced Synthesis and Catalysis**, vol. 349, no. 11–12, p. 1969–1976, 2007. DOI https://doi.org/10.1002/adsc.200700055.

PALOMO, J. M.; MUOZ, G.; FERNÁNDEZ-LORENTE, G.; MATEO, C.; FERNÁNDEZ-LAFUENTE, R.; GUISÁN, J. M. Interfacial adsorption of lipases on very hydrophobic support (octadecyl-Sepabeads): Immobilization, hyperactivation and stabilization of the open form of lipases. **Journal of Molecular Catalysis B: Enzymatic**, vol. 19, no. 20, p. 279–286, 2002. DOI https://doi.org/10.1016/S1381-1177(02)00178-9.

PAPPALARDO, V. M.; BOERIU, C. G.; ZACCHERIA, F.; RAVASIO, N. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, vol. 433, p. 383–390, 2017. DOI http://dx.doi.org/10.1016/j.mcat.2017.02.029.

PEIRCE, S.; TACIAS-PASCACIO, V. G.; RUSSO, M. E.; MARZOCCHELLA, A.; VIRGEN-ORTÍZ, J. J.; FERNANDEZ-LAFUENTE, R. Stabilization of *Candida antarctica* Lipase B (CALB) immobilized on octyl agarose by treatment with polyethyleneimine (PEI). **Molecules**, vol. 21, no. 6, 2016. DOI https://doi.org/10.3390/molecules21060751.

PIZARRO, C.; BRAÑES, M. C.; MARKOVITS, A.; FERNÁNDEZ-LORENTE, G.; GUISÁN, J. M.; CHAMY, R.; WILSON, L. Influence of different immobilization techniques for *Candida cylindracea* lipase on its stability and fish oil hydrolysis. **Journal of Molecular Catalysis B: Enzymatic**, vol. 78, p. 111–118, 2012. DOI https://doi.org/10.1016/j.molcatb.2012.03.012.

PRONK, S.; LINDAHL, E.; KASSON, P. M. Dynamic heterogeneity controls diffusion and viscosity near biological interfaces. **Nature Communications**, vol. 5, 2014. DOI https://doi.org/10.1038/ncomms4034.

REGAN, D. L.; LILLY, M. D.; DUNNILL, P. Influence of intraparticle diffuisional limitation on the observed kinetics of immobilized enzymes and on catalyst design. **Biotechnology and Bioengineering**, vol. 16, no. 8, p. 1081–1093, 1974. DOI

https://doi.org/10.1002/bit.260160808.
RODRIGUES, R. C.; BARBOSA, O.; ORTIZ, C.; BERENGUER-MURCIA, Á.; TORRES, R.; FERNANDEZ-LAFUENTE, R. Amination of enzymes to improve biocatalyst performance: coupling genetic modification and physicochemical tools. **RSC Advances**, vol. 4, no. 72, p. 38350–38374, 2014. DOI https://doi.org/10.1039/c4ra04625k.
RODRIGUES, R. C.; GODOY, C. A.; VOLPATO, G.; AYUB, M. A. Z.; FERNANDEZ-LAFUENTE, R.; GUISAN, J. M. Immobilization–stabilization of the lipase from *Thermomyces lanuginosus*: Critical role of chemical amination. **Process Biochemistry**, vol. 44, p. 963–968, 2009. DOI https://doi.org/10.1016/j.procbio.2009.04.015.
RODRIGUES, R. C.; ORTIZ, C.; BERENGUER-MURCIA, A.; TORRES, R.; FERNANDEZ-LAFUENTE, R. Modifying enzyme activity and selectivity by immobilization. **Chemical Society Reviews**, vol. 42, no. 15, p. 6290–6307, 2013. DOI https://doi.org/10.1039/c2cs35231a.
RODRIGUES, R. C.; VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, A.; ALCANTARA, A. R.; BARBOSA, O.; ORTIZ, C.; FERNANDEZ-LAFUENTE, R. Immobilization of lipases on hydrophobic supports: immobilization mechanism, advantages, problems, and solutions. **Biotechnology Advances**, vol. 37, no. 5, p. 746–770, 2019. DOI https://doi.org/10.1016/j.biotechadv.2019.04.003.
RUEDA, N.; DOS SANTOS, J. C. S.; ORTIZ, C.; BARBOSA, O.; FERNANDEZ-LAFUENTE, R.; TORRES, R. Chemical amination of lipases improves their immobilization on octyl-glyoxyl agarose beads. **Catalysis Today**, vol. 259, p. 107–118, 2015. DOI http://dx.doi.org/10.1016/j.cattod.2015.05.027.
RUEDA, N.; DOS SANTOS, J. C. S.; ORTIZ, C.; TORRES, R.; BARBOSA, O.; RODRIGUES, R. C.; BERENGUER-MURCIA, Á.; FERNANDEZ-LAFUENTE, R. Chemical modification in the design of immobilized enzyme biocatalysts: drawbacks and opportunities. **Chemical Record**, vol. 16, no. 3, p. 1436–1455, 2016. DOI https://doi.org/10.1002/tcr.201600007.
RUFINO, A. R.; BIAGGIO, F. C.; SANTOS, J. C.; CASTRO, H. F. Screening of lipases for the synthesis of xylitol monoesters by chemoenzymatic esterification and the potential of microwave and ultrasound irradiations to enhance the reaction rate. **International Journal of Biological Macromolecules**, vol. 47, no. 1, p. 5–9, 2010. DOI http://dx.doi.org/10.1016/j.ijbiomac.2010.04.009.
SAUNDERS, P.; BRASK, J. Improved immobilization supports for *Candida antarctica* lipase B. **Biocatalysis in Polymer Chemistry**, p. 65–82, 2010. DOI https://doi.org/10.1002/9783527632534.ch3.
SÉVERAC, E.; GALY, O.; TURON, F.; PANTEL, C. A.; CONDORET, J. S.; MONSAN, P; MARTY, A. Selection of CalB immobilization method to be used in continuous oil transesterification: Analysis of the economical impact. **Enzyme and Microbial Technology**, vol. 48, p. 61–70, 2011. DOI https://doi.org/10.1016/j.molcatb.2006.06.011.
SHEN, L.; CHEN, Z. Critical review of the impact of tortuosity on diffusion. **Chemical Engineering Science**, vol. 62, no. 14, p. 3748–3755, 2007. DOI https://doi.org/10.1016/j.ces.2007.03.041.
SIÓDMIAK, T.; MANGELINGS, D.; VANDER HEYDEN, Y.; ZIEGLER-BOROWSKA, M.; MARSZAŁŁ, M. P. High enantioselective Novozym 435-catalyzed esterification of (R,S)-flurbiprofen monitored with a chiral stationary phase. **Applied Biochemistry and Biotechnology**, vol. 175, no. 5, p. 2769–2785, 2015. DOI https://doi.org/10.1007/s12010-014-1455-4.
TAMBE, A.; VYASARAYANI, R.; DATLA, A.; PONRATHNAM, S.; DEMNEROVA,

K. Macroporous poly (vinyl acetate-co-divinyl benzene) copolymer beads as adsorptive support for the direct immobilization of *Candida antarctica* lipase B. **Enzyme Engineering**, vol. 4, no. 2, 2015. DOI https://doi.org/10.4172/2329-6674.1000130.

TENG, Y.; STEWART, S. G.; HAI, Y. W.; LI, X.; BANWELL, M. G.; LAN, P. Sucrose fatty acid esters: synthesis, emulsifying capacities, biological activities and structure-property profiles. **Critical Reviews in Food Science and Nutrition**, vol. 0, no. 0, p. 1–21, 2020. DOI https://doi.org/10.1080/10408398.2020.1798346.

VERDASCO-MARTÍN, C. M.; VILLALBA, M.; SANTOS, J. C. S.; FERNANDEZ-LAFUENTE, R.; OTERO, C. Effect of chemical modification of Novozym 435 on its performance in the alcoholysis of camelina oil. **Biochemical Engineering Journal**, vol. 111, p. 75–86, 2016. DOI http://dx.doi.org/10.1016/j.bej.2016.03.004.

VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

VESCOVI, V.; KOPP, W.; GUISÁN, J. M.; GIORDANO, R. L. C.; MENDES, A. A.; TARDIOLI, P. W. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octyl and multifunctional amino-glutaraldehyde spacer arms. **Process Biochemistry**, vol. 51, no. 12, p. 2055–2066, 2016. DOI http://dx.doi.org/10.1016/j.procbio.2016.09.016.

VILLALBA, M.; VERDASCO-MARTÍN, C. M.; DOS SANTOS, J. C. S.; FERNANDEZ-LAFUENTE, R.; OTERO, C. Operational stabilities of different chemical derivatives of Novozym 435 in an alcoholysis reaction. **Enzyme and Microbial Technology**, vol. 90, p. 35–44, 2016. DOI http://dx.doi.org/10.1016/j.enzmictec.2016.04.007.

VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, Á.; BARBOSA, O.; RODRIGUES, R. C.; FERNANDEZ-LAFUENTE, R. Polyethylenimine: A very useful ionic polymer in the design of immobilized enzyme biocatalysts. **Journal of Materials Chemistry B**, vol. 5, no. 36, p. 7461–7490, 2017a. DOI https://doi.org/10.1039/c7tb01639e.

VIRGEN-ORTÍZ, J. J.; PEDRERO, S. G.; FERNANDEZ-LOPEZ, L.; LOPEZ-CARROBLES, N.; GORINES, B. C.; OTERO, C.; FERNANDEZ-LAFUENTE, R. Desorption of lipases immobilized on octyl-agarose beads and coated with ionic polymers after thermal inactivation. Stronger adsorption of polymers/unfolded protein composites. **Molecules**, vol. 22, p. 91, 2017b. DOI https://doi.org/10.3390/molecules22010091.

VIRGEN-ORTÍZ, J. J.; TACIAS-PASCACIO, V. G.; HIRATA, D. B.; TORRESTIANA-SANCHEZ, B.; ROSALES-QUINTERO, A.; FERNANDEZ-LAFUENTE, R. Relevance of substrates and products on the desorption of lipases physically adsorbed on hydrophobic supports. **Enzyme and Microbial Technology**, vol. 96, p. 30–35, 2017c. DOI http://dx.doi.org/10.1016/j.enzmictec.2016.09.010.

VIRGEN-ORTÍZ, J. J.; PEIRCE, S.; TACIAS-PASCACIO, V. G.; CORTES-CORBERAN, V.; MARZOCCHELLA, A.; RUSSO, M. E.; FERNANDEZ-LAFUENTE, R. Reuse of anion exchangers as supports for enzyme immobilization: Reinforcement of the enzyme-support multiinteraction after enzyme inactivation. **Process Biochemistry**, vol. 51, p. 1391–1396, 2016. DOI https://doi.org/https://doi.org/10.1016/j.procbio.2016.06.020.

WANG, F.; MO, J.; HUANG, A.; ZHANG, M.; MA, L. Effects of interaction with gene carrier polyethyleneimines on conformation and enzymatic activity of pig heart lactate dehydrogenase. **Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy**, vol. 204, p. 217–224, 2018. DOI

https://doi.org/10.1016/j.saa.2018.06.048.
WANG, X.; CHEN, Y.; MA, Y.; JIN, Q.; WANG, X. Lipozyme 435-catalyzed synthesis of eicosapentaenoyl ethanolamide in a solvent-free system. **Journal of Molecular Catalysis B: Enzymatic**, vol. 122, p. 233–239, 2015. DOI http://dx.doi.org/10.1016/j.molcatb.2015.09.016.
WEINBERGER, S.; PELLIS, A.; COMERFORD, J. W.; FARMER, T. J.; GUEBITZ, G. M. Efficient physisorption of *Candida antarctica* lipase B on polypropylene beads and application for polyester synthesis. **Catalysts**, vol. 8, p. 369, 2018. DOI https://doi.org/10.3390/catal8090369.
WILSON, L.; FERNÁNDEZ-LORENTE, G.; FERNÁNDEZ-LAFUENTE, R.; ILLANES, A.; GUISÁN, J. M.; PALOMO, J. M. CLEAs of lipases and poly-ionic polymers: A simple way of preparing stable biocatalysts with improved properties. **Enzyme and Microbial Technology**, vol. 39, no. 4, p. 750–755, 2006. DOI https://doi.org/10.1016/j.enzmictec.2005.12.011.
YE, R.; HAYES, D. G.; BURTON, R. Effects of particle size of sucrose suspensions and pre-incubation of enzymes on lipase-catalyzed synthesis of sucrose oleic acid esters. **Journal of the American Oil Chemists' Society**, vol. 91, p. 1891–1901, 2014. DOI https://doi.org/10.1007/s11746-014-2537-8.
ZAAK, H.; FERNANDEZ-LOPEZ, L.; OTERO, C.; SASSI, M.; FERNANDEZ-LAFUENTE, R. Improved stability of immobilized lipases via modification with polyethylenimine and glutaraldehyde. **Enzyme and Microbial Technology**, vol. 106, p. 67–74, 2017. DOI http://dx.doi.org/10.1016/j.enzmictec.2017.07.001.
ZUCCA, P.; FERNANDEZ-LAFUENTE, R.; SANJUST, E. Agarose and its derivatives as supports for enzyme immobilization. **Molecules**, vol. 21, no. 11, p. 1–25, 2016. DOI https://doi.org/10.3390/molecules21111577.

5.3. PRODUCTION OF SUGARS FROM MIXED HARDWOODS FOR USE IN THE SYNTHESIS OF SUGAR FATTY ACID ESTERS CATALYZED BY IMMOBILIZED-STABILIZED DERIVATIVES OF *CANDIDA ANTARCTICA* LIPASE B

5.3.1. Introduction

Lignocellulosic substrates represent an alternative renewable source of carbohydrates for the synthesis of several biofuels and bioproducts (GAONA; LAWRYSHYN; SAVILLE, 2015; TAHERZADEH; KARIMI, 2007). The successful commercialization of these products strongly depends on a conversion technology that can efficiently transform biomass into monomeric sugars. This procedure may comprise lignocellulosic biomass (LB) pretreatment followed by high-solids enzymatic hydrolysis (HSEH), which stands as a promising strategy that can generate high sugar yields, operate under mild conditions, and reduce water usage, translating into smaller reactors and lower operating costs (GAONA; LAWRYSHYN; SAVILLE, 2019; PINO *et al*., 2018). Such approaches are generally undertaken in conjunction with downstream purification and

concentration steps to meet the target product attributes (MILESSI-ESTEVES *et al*., 2019).

In some instances, the HSEH process can be designed to maximize the production of one sugar over the others, i.e., this process can be adapted to selectively produce a larger amount of a specific monomer (PURI; HEAVEN; BANKS, 2013). For this, it is necessary to use a higher dose of specific enzymes that promote the hydrolysis of a certain LB component (e.g., hemicellulose) compared to the enzyme loading for the decomposition of another biomass constituent (e.g., cellulose). Preferentially producing more of a target product during hydrolysis can simplify the downstream purification methods that would otherwise contribute to high operational costs. Moreover, this strategy may even eliminate the necessity for purification of hydrolysates, which supports the conclusions drawn by Gonçalves *et al*. (2022), who investigated the production and recovery of sugars from lignocellulosics for use in the synthesis of bioproducts. The authors showed that the majority of studies directly processed crude extracts without purification of the hydrolysates. However, in some cases, enzymatic hydrolysates are purified to remove high molecular weight and/or low molecular weight impurities using, for example, ion exchange, chromatographic separation, or ultrafiltration/nanofiltration membranes with targeted molecular weight cut-offs (MWCOs). Operating pressures, temperatures, and the use of diafiltration may all be adapted to selectively recover sugars while removing key contaminants. Purified extracts can be further concentrated as needed, using, e.g., reverse osmosis, freeze or spray drying, and evaporative methods such as multiple-effect evaporators.

After the LB conversion into monomeric sugars and purification/concentration of extracts (when needed), the resulting sugars are typically used to synthesize other products. The most common goods obtained from a particular monomeric sugar, xylose, but not necessarily exclusively from this sugar, include ethanol, xylooligosaccharides, xylitol, lactic acid, lipids, biohydrogen, and butanol (GONÇALVES *et al*., 2022). In this study, we evaluated the enzymatic synthesis of high value biosurfactants, so-called sugar fatty acid esters (SFAEs). SFAEs have a market value that is at least two orders of magnitude greater than ethanol (CEPEA, 2022; GONÇALVES *et al*., 2022; H & Z INDUSTRY CO., 2022). To date, SFAEs have mainly been produced using glucose (AN *et al*., 2019; ANDLER *et al*., 2017; ARCENS *et al*., 2018; BASYARUDDIN; RAHMAN; ARUMUGAM, 2012; CHAIWUT *et al*., 2022; CHANG; ZHANG; TANG, 2018; FINDRIK *et al*., 2016; HA *et al*., 2010; HOLLENBACH *et al*., 2022; LIANG *et al*., 2018;

LIN *et al*., 2016; SIEBENHALLER *et al*., 2017, 2018; VUILLEMIN *et al*., 2022; etc.); the use of xylose thus represents a novel use of this sugar.

SFAEs are non-ionic surfactants (CHANG; SHAW, 2009; GUMEL *et al*., 2011; NETA; TEIXEIRA; RODRIGUES, 2015) synthesized through an esterification reaction between a sugar moiety and a fatty acid (GRÜNINGER; DELAVAULT; OCHSENREITHER, 2019), where the sugar acts as the acceptor for an acyl group, while the fatty acid is the donor of the acyl group (DE LIMA *et al*., 2018; LIANG *et al*., 2018). The reaction usually takes place in an organic solvent, producing esters with very good surfactant and emulsifier characteristics (GRÜNINGER; DELAVAULT; OCHSENREITHER, 2019). SFAEs are odorless, tasteless, and non-irritating surfactants, which are very interesting features for applications in food or cosmetic formulations (RECKE *et al*., 2013). In addition, they are biodegradable and non-toxic to the environment.

Chemical or enzymatic catalysis may be used to produce SFAEs. The enzymatic route may represent a more sustainable alternative, as it uses lower operating temperatures/pressures, achieves high yields, and provides high specificity and selectivity to target products, facilitating product separation (CHANG; SHAW, 2009; SIEBENHALLER *et al*. 2018; VESCOVI; DOS SANTOS; TARDIOLI, 2017). Lipases are commonly used as biocatalysts for the synthesis of SFAEs. In particular, microbial lipases from *Candida antartica*, *Candida rugosa*, *Rhizomucor miehei*, *Burkholderia sp*. or *Pseudomonas sp*. are commonly used (GRÜNINGER; DELAVAULT; OCHSENREITHER, 2019). *Candida antarctica* produces two different lipases: A and B. Fraction B (CALB) is probably the most widely used hydrolase in the field of biocatalysis, due to its high activity and yields under mild conditions (KUNDYS *et al*., 2018).

The commercial CALB Novozyme® 435 (N435) was reported in ~80% of the scientific publications investigating the synthesis of SFAEs from 2010 to 2019 (GONÇALVES *et al*., 2021a). N435 is a CALB immobilized by non-covalent linkage and interfacial activation on Lewatit VP OC 1600, a poly-methacrylic acid crosslinked with divinylbenzene (SIÓDMIAK *et al*., 2015). In spite of the extensive use of this enzyme preparation, it is difficult to use it multiple times because of enzyme leaching from the support in the presence of substrates/products with surfactant properties (GONÇALVES *et al*., 2021b). The non-covalent bonds between lipase and the support are disrupted by the presence of fatty acids and/or SFAEs in the reaction medium, leading to lipase loss to the medium (VIRGEN-ORTÍZ *et al*., 2017a). As an alternative, coating

of the N435 surface with polyethyleneimine (PEI) can promote physical intermolecular crosslinking, with the potential to prevent enzyme release while tailoring some of the biocatalyst properties (e.g., enzyme specificity) (FERNANDEZ-LAFUENTE *et al*., 2019; GONÇALVES *et al*., 2021c; VESCOVI *et al*., 2016; VIRGEN-ORTÍZ *et al*., 2017b).

Accordingly, in this research, our main goal is to synthesize SFAEs from renewable resources by using the N435 as catalyst of the esterification reaction. For this, first monomeric sugars (mostly xylose) were produced by HSEH of steam-pretreated hardwood. These carbohydrates were then purified and concentrated to be used as substrates in the synthesis of xylose and glucose oleate. The esterification was catalyzed by the enzyme N435 with and without coating with PEI, in methyl ethyl ketone (MEK) medium. Sugar conversion profiles during the esterification were evaluated, comparing lignocellulosic sugars versus commercial sugars. The effects of using uncoated versus PEI-coated N435 and the effects of N435 loading were also investigated. The operational stability of the enzyme preparations was assessed, along with the emulsion capacities of the synthesized SFAEs, in comparison to a commercial surfactant. The formation of esters was evaluated by mass spectrometry.

5.3.2. Materials and Methods

5.3.2.1. Materials

Candida antarctica lipase B immobilized on Lewatit VP OC 1600 (Novozyme® 435 – N435), Cellic® CTec3 (cellulase and hemicellulase complex), and Cellic® HTec3 (hemicellulase complex) were supplied by Novozymes. Xylose, glucose, oleic acid, methyl ethyl ketone (MEK), molecular sieves (3 Å), polyethyleneimine (PEI) solution (50 wt.% in H_2O), and tributyrin were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). Steam-exploded hardwood was produced by Mascoma Canada (Brampton, ON) at the company's pilot plant in Waterdown, Ontario. All other reagents and solvents were of analytical or HPLC grade and were used without any previous treatment or modification.

5.3.2.2. Steam-explosion pretreatment of hardwood

Mixed hardwoods were pretreated in a system that includes a customized feeder designed to continuously feed wood chips into a high-pressure hydrolyzer, a discharge screw conveyor, and a blow valve plus cyclone to collect and isolate the pretreated

product. Wood chips with length of 3–5 cm were fed to the system using a conveyor at a rate of 1-3 tons/h. Pretreatment conditions were set at 200–208 °C (15-18 bar saturated steam) for 5–7 min. Previous investigations demonstrated that these conditions had less sugar degradation compared to the operation at temperatures >210 ºC, thus reducing the formation of degradation products with potential inhibitory effects on fermentation organisms and hemicellulolytic/cellulolytic enzymes. The steam-exploded wood fiber was used as it was (without washing) and stored in sealed plastic drums until the enzymatic hydrolysis was conducted.

5.3.2.3. Enzymatic hydrolysis of steam-exploded hardwood

The enzymatic hydrolysis was conducted at 50 ºC for 4 h in a 4 L-reactor at 15 wt% total solids, with continuous agitation at 60 rpm. The reactor was prefilled with water and steam-exploded hardwood, which were mixed and preheated to 50 ºC for approximately 30 min prior to the addition of the enzymes. The amounts of water and biomass added were calculated based on the dry matter of the pretreated hardwood and the target dry matter (solids) content of the mixture. The reaction was initiated by adding 1.75 wt% Cellic® HTec3 and 1.0 wt% Cellic® CTec3 (enzyme doses are relative to the dry weight of the biomass) to the reactor.

Over the course of the hydrolysis, the pH was adjusted to 5.5 by adding 4 mol/L NaOH solution. Samples were taken hourly. On completion of the enzymatic hydrolysis, the reactor contents were rapidly cooled to room temperature. The liquid and solid portions were recovered by filtration and refrigerated prior to further acid hydrolysis (to characterize oligomers of xylose and glucose; section 5.3.2.9.1) and sugar analysis using high-performance liquid chromatography (HPLC) (to characterize monomers – soluble xylose and glucose; section 5.3.2.9.3).

5.3.2.4. Reactor configuration

The stainless-steel 4 L-reactor is equipped with a DC motor, and the motor rotation was set to clockwise. An external controller (Mixer Clone – Oracle VM Virtual Box, SunOpta Bioprocess Inc.) was used to control the direction and rotational speed of the motor. Radial-flow impellers (4 inches in outside diameter) with four rectangular blades (90º angle) were mounted on a stainless-steel shaft inside the reactor. The reactor was connected to a recirculating water bath (VWR model 1130A, VWR Scientific Products, McGaw Park, IL) to control the temperature.

5.3.2.5. Purification and concentration of the crude enzymatic hydrolysis extract

The enzymatic hydrolysate (liquid fraction) containing xylose and glucose was first purified using a particulate filter (5 µm) to remove any of the suspended solids. The filtered extract was further purified to remove soluble lignin-derived compounds using a polyethersulfone (PES) 3 KDa ultrafiltration membrane (Synder filtration, VT-2B-6338). The membrane system (Mini Tangent Membranes Inc.) was operated at 150 psi, between 10-25 °C. The retentate from the ultrafiltration was considered waste and the permeate was the feed material of the subsequent concentration stage using a reverse osmosis membrane (Trisep Turboclean RO-8038-ACM2-46; maximum operating pressure of 600 psi).

The concentration of the extract was initiated at the lowest temperature possible (10-20°C), with the retentate recirculated to the feed tank. The process continued until the feed to the system was exhausted. The permeate of this process was considered waste, while the retentate was used in the following drying stage. A spray dryer (Buchi Mini B-290) was used with two fluid nozzles to spray feed solution into the nitrogen carrier gas (45 mmHg). The equipment was set to an inlet temperature of 180 ºC and a feed rate of 1 mL/min. 20%wt of maltodextrin was added to the feed material to reduce the stickiness of the spray dried product. The resulting material was then cooled to room temperature and prepared for further acid hydrolysis and sugar analysis (sections 5.3.2.9.1 and 5.3.2.9.3).

5.3.2.6. Enzymatic synthesis of sugar fatty acid esters

The spray dried product was used as acyl acceptor in the synthesis of SFAEs (mainly xylose oleate, but also glucose oleate). The esterification reaction was conducted between oleic acid (C18:1) and xylose/glucose (in a mass ratio of 3 to 1), using MEK as solvent and N435 as the biocatalyst (before and after coating with PEI 2 KDa– section 5.3.2.7). Commercial xylose and glucose were also used as model compounds to compare esterification performance.

The reaction was conducted in the same reactor configuration described in section 5.3.2.4 under the following conditions: 60 °C; 350 rpm; 1%, 1.5%, and 1.75% w/v of uncoated Novozyme 435 (10000 ± 589 TBU/g), adjusting to the same provided activity when using PEI-coated Novozyme 435 (6042 ± 295 TBU/g) (Section 5.3.2.10). Samples were collected after 1, 3, 6, and 24 h of reaction, and analyzed according to the

methodology presented in section 5.3.2.9.3. Molecular sieves (3 Å) with an adsorption capacity of 22%wt were used for water removal during the reaction.

5.3.2.7. N435 PEI-coating

N435 coating with PEI was performed by adding 100 µL of PEI solution (100 mg/mL) (2 KDa) to a suspension of N435 prepared in 5 mmol/L sodium phosphate buffer, pH 7.0 (biocatalyst/buffer ratio of 1/10 v/v). The reaction medium was incubated at 25°C under 150 rpm stirring for 60 min. PEI-coated N435 was then recovered by vacuum filtration (WILSON *et al*., 2006).

5.3.2.8. N435 operational stability in the synthesis of sugar fatty acid esters

The synthesis of SFAEs catalyzed by N435 (before and after its coating with PEI) was performed in 6 successive 24 h-batches. Reactions were conducted under the following conditions: 60 ºC; continuous stirring (350 rpm); xylose: glucose mass ratio of 3; 1.5% w/v N435 (10000 ± 589 TBU/g) (adjusting to the same provided activity when using the PEI-coated biocatalyst). After each batch, the biocatalyst and molecular sieves were recovered, washed with ethanol and MEK, and subjected to vacuum filtration followed by drying in an oven at 40 ºC to remove the remaining solvent. Molecular sieves were removed using a sieve shaker (Retsch, AS 200; 40-amplitude) with sieves of 20 and 100 mesh to retain the molecular sieves and the enzyme, respectively. Biocatalysts were then reused in a new esterification reaction. Xylose and glucose conversions were calculated based on HPLC measurements, as described in section 5.3.2.9.3.

5.3.2.9. Characterizations

5.3.2.9.1. Liquid fraction – acid hydrolysis

Liquid samples collected over the course of the enzymatic hydrolysis were centrifuged at 14,000 g for 10 min (Galaxy 14D Centrifuge 37001-599; VWR, Radnor, PA). 10 mL of the supernatant of each sample and 348 µL of 72% sulfuric acid were added to press tubes. The contents of the tubes were homogenized and put in the autoclave (Napco 9,000-D) for 1 hour. After cooling the samples, they were prepared for measurements of the sugar content according to section 5.3.2.9.3 below.

5.3.2.9.2. Solid fraction

Solids obtained after (1) steam-explosion pretreatment and (2) enzymatic hydrolysis of pretreated hardwood were analyzed in terms of carbohydrate and insoluble

lignin content according to the NREL Laboratory Analytical Procedure (LAP) #42618 (SLUITER *et al*., 2008). The soluble lignin content was measured by a UV-Vis spectrophotometer (Genesys 10-s, Thermo-Scientific). The ash content was determined according to the NREL LAP #42622 (SLUITER *et al*., 2005) and the dry matter content (% DM) was assessed according to the NREL LAP #42620 (HAMES *et al*., 2008).

5.3.2.9.3. Chromatographic analysis

Samples from the enzymatic hydrolysis of pretreated hardwood were neutralized with calcium carbonate, diluted with deionized water, and filtered through 0.22 µm syringe filters (Millex Syringe filters; Merck Millipore, Tullagreen, Cork, Ireland) into 2 mL HPLC vials (Agilent Technologies, Santa Clara, California, USA). For samples from the esterification reactions, 2 mL of the supernatant were evaporated, 0.5 mL of water was added to each sample, they were homogenized, and syringe filtered into 2 mL HPLC vials. Samples and calibration standards were analyzed using an Agilent HPLC system (Model series 1200, Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) equipped with a refractive index detector (RID) and a Bio-Rad HPX-87P column (1,300 x 7.8 mm x 9 µm, Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) maintained at 80 ºC. Deionized water was used as the eluent at 0.6 mL/min. The injection volume was 20 µL, with a run time of 30 min. HPLC results were tabulated using ChemStation software and the concentrations of xylose and glucose were calculated based on the peak heights (accounting for prior dilutions).

5.3.2.9.4. Mass spectrometry analysis

The purification of the crude esterification reaction product was conducted according to the protocol described in Gonçalves *et al*. (2021c). The purified product was then characterized by mass spectrometry using a Thermo Scientific Q Exactive Orbitrap MS/MS coupled to a nano-electrospray ionization source operated in negative ionization mode, across the mass range of 400–1200 m/z. Samples were dissolved in methanol, loaded into a borosilicate nano emitter (Thermo Scientific), and analyzed by direct infusion onto the mass spectrometer. The nano ESI source conditions were as follows: spray voltage 3000 V, capillary temperature 250 ºC, s-lens RF level 55. Targeted MS data were collected with a normalized collision energy voltage of 35eV. Data were analyzed in the Xcalibur Qual Browser 4.5.445.18 software.

5.3.2.9.5. Emulsion capacity assays

10 mg of the purified esterification reaction product (see Gonçalves *et al*. (2021c) for details about the purification methodology) was dissolved in 1 mL of water and mixed with 2 mL of kerosene in a test tube. The content of the tube was continuously homogenized for 2 min at room temperature and then left standing for 24 h. After this period, the height of the emulsified region and the height of the total column were measured, and the ECs were calculated according to Equation 1 (CAVALCANTI *et al*., 2017). Sucrose monolaurate was used as a commercial standard in this experiment. Tests were performed in duplicate, and intrasample variations were <1%.

5.3.2.10. N435 hydrolytic activity measurements

The protocol to measure the N435 hydrolytic activity in tributyrin is an adaptation of the methodology described by Beisson *et al*. (2000). The method is based on the hydrolysis of a mixture of 1.5 mL of tributyrin, 6 mL of 100 mmol/L phosphate buffer, pH 7.3, and 16.5 mL of distilled water at 37 °C for 5 min. For these assays, 24 mg of L435 were added to the medium. The released butyric acid was titrated with 0.02 mol/L KOH solution. A unit of tributyrin hydrolysis activity (TBU) is defined as the amount of enzyme that releases 1 μmol of butyric acid per minute under the conditions described above.

5.3.3. Results and discussion

5.3.3.1. Enzymatic hydrolysis of steam-exploded hardwood and purification/concentration of the hydrolysate extract

Table 5.5 presents the chemical composition of the hardwood (solid fraction) after (1) steam-explosion pretreatment (steam-exploded hardwood - SEH) and (2) enzymatic hydrolysis (post hydrolysis hardwood - PHH). Table 5.6 summarizes the composition of the crude hydrolysate extract – CHE (liquid fraction) after 4 h of enzymatic hydrolysis and the extract composition after (1) particulate filtration (PF), (2) ultrafiltration (UF), and (3) reverse osmosis (RO). Table 5.7 shows the powder composition after spray drying (SD) of the RO retentate. Images of the material obtained after each of these steps are shown in Figure 5.11.

Table 5.5. Chemical composition of the hardwood (solid fraction) after steam-explosion pretreatment and enzymatic hydrolysis.

	Lignin (%)	Ash (%)	Glucan (%)	Xylan (%)	Galactan (%)	Mannan (%)	Arabinan (%)
SEH	30.3	0.27	47.7	16.3	2.9	4.1	0.0
PHH	34.9	1.05	53.4	9.4	2.8	1.7	0.0

SEH: steam-exploded hardwood. PHH: post hydrolysis hardwood. Enzymatic hydrolysis conditions: 15% w/v solids, 1.75% HTec3, 1.0% CTec3, 4 h, with pH adjustments to 5.5.

Table 5.6. Composition range after enzymatic hydrolysis and purification/concentration of the CHE (liquid fraction) in two steps: (1) UF (after PF); (2) RO.

		Xylose (g)	Xylan (g)	Glucose (g)	Glucan (g)	DM (%)
CHE		55.24-56.21	4.85-9.42	16.62-17.52	0.95-4.36	1.9
UF	Retentate (waste)	13.73-14.56	4.32-5.44	4.50-4.78	1.99-2.36	7.1
	Permeate (RO feed)	32.87-33.06	3.43-3.95	8.59-8.64	1.10-1.29	2.4
RO	Retentate (SD feed)	29.53-31.82	1.78-3.42	7.75-7.93	0.94-1.11	18.0
	Permeate (waste)	0	0.22	0	0.06	0.2

DM: dry matter content. All experiments were performed in duplicate. Results were expressed as intervals between samples.

Table 5.7. Powder composition range after spray drying.

	Xylose (mg/g of powder)	Xylan (mg/g of powder)	Glucose (mg/g of powder)	Glucan (mg/g of powder)	DM (%)
SD	601-605	48-52	216-217	27-33	97

DM: dry matter content. All experiments were performed in duplicate. Results were expressed as intervals between samples.

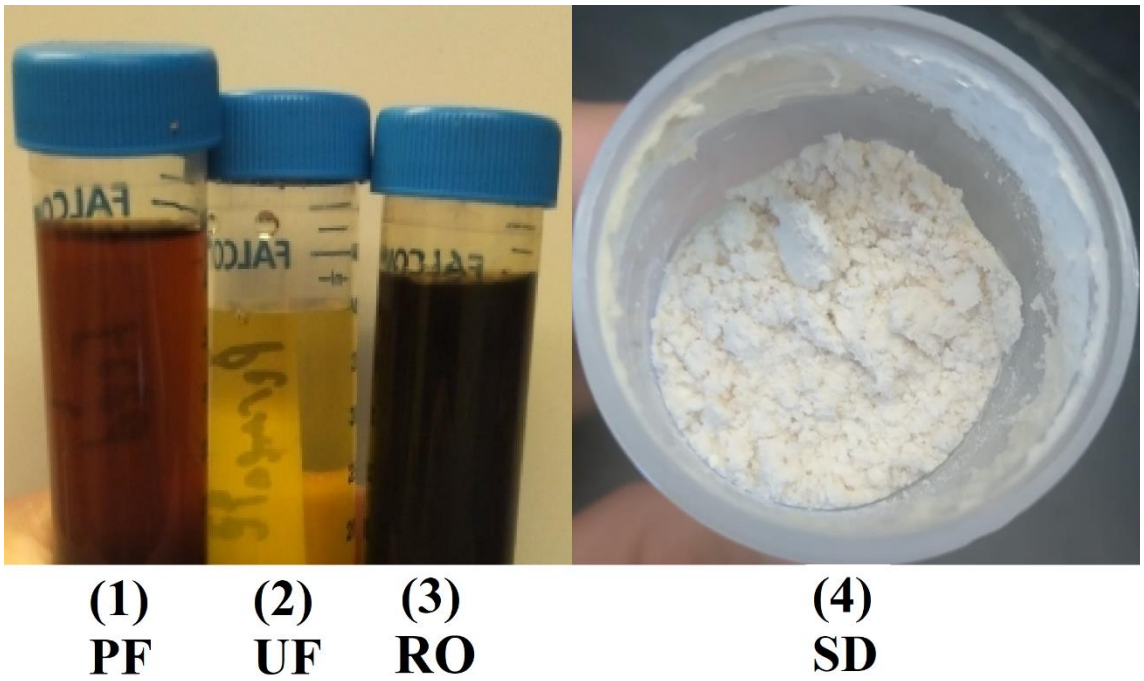


Figure 5.11. Physical aspects of the hydrolysate extract after PF, UF, RO, and SD stages.

A primary goal was to obtain a higher concentration of xylose than glucose by using a higher dose of HTec3 than CTec3 in the enzymatic hydrolysis. Although we aimed to produce mainly xylose, CTec3, a cellulase and hemicellulase complex, was also used to increase xylan solubilization, which increased the xylose yield, albeit with the production of a small amount of glucose. Using this enzyme complex, it was possible to get a xylose: glucose mass ratio of ~3 after spray drying. Thus, this method enhanced xylose production while relatively low levels of glucose production were ensured by adjusting the enzyme dose and the hydrolysis time.

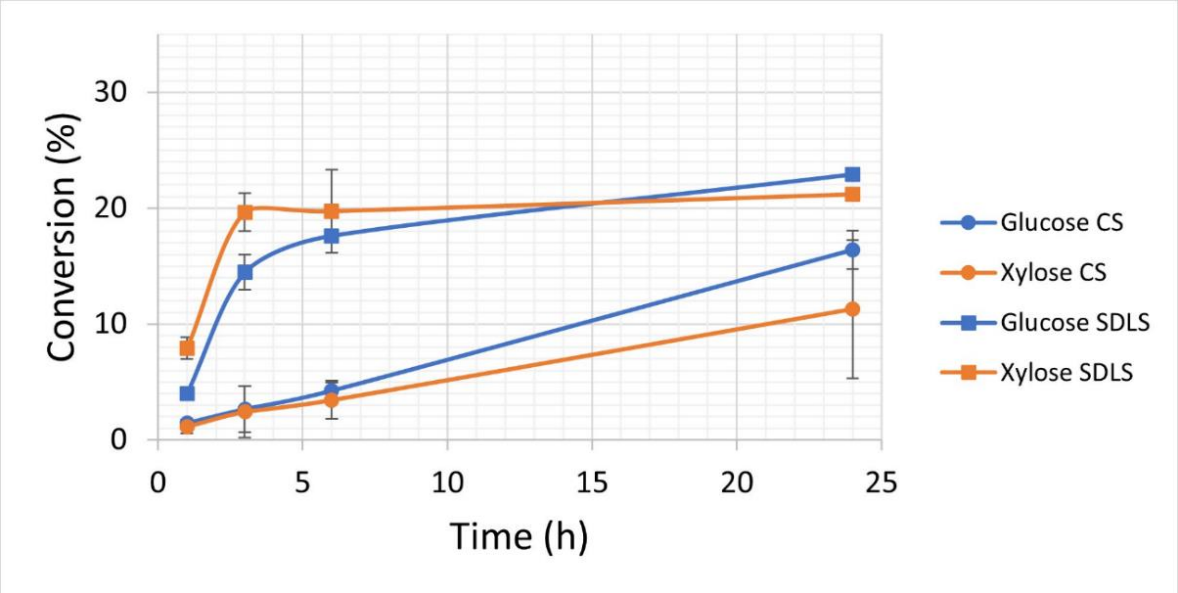
The HSEH at 15%wt solids was essential to produce significant quantities of xylose; hydrolysis yields of ~48 and 5% were obtained for xylose and glucose, respectively. Purification methods were used to remove soluble lignin and high molecular weight polyphenols, as shown by the significantly lighter color of the extract after UF (#2 in Figure 5.11). RO may have removed some organic acids and concentrated the extract – with a possible increase in sugars and polyphenols concentrations – thus increasing the color intensity of the extract (#3 in Figure 5.11). A ~54% yield of total mass of carbohydrates recovered was obtained after spray drying.

It was important to remove as much water as possible from the purified extract because residual water would adversely affect the subsequent use of this material in the synthesis of SFAEs. The esterification reaction is supposed to occur in a nonaqueous environment (GUMEL *et al*., 2011), and molecular sieves must be added to remove water present in substrates and generated as a by-product of the esterification reaction. Excess water could force the reaction equilibrium towards hydrolysis instead of esterification (GONÇALVES *et al*., 2021b). Water also influences the activity and the selectivity of the enzyme and may lead to its inhibition or inactivation (DOSSAT; COMBES; MARTY, 1999; MARTY; DOSSAT; CONDORET, 1997). For these reasons, we tried to keep the water at the lowest possible level (a minimal amount of water is necessary to ensure the hydration, stability, and catalytic activity of the enzyme (CAUGLIA; CANEPA, 2008). The spray dried powder contained about 3% moisture, and roughly 70% xylose (Table 5.7).

5.3.3.2. Enzymatic synthesis of sugar fatty acid esters

5.3.3.2.1. Comparison of spray dried lignocellulosic sugars versus commercial sugars as substrates of the esterification

The consumption of xylose and glucose during the esterification reaction is presented in Figure 5.12. Here, we compared the use of spray dried lignocellulosic sugars (SDLS) and commercial sugars (CS) for this reaction, using the uncoated N435 as biocatalyst. After 24 h, the xylose conversion obtained using SDLS was ~2-fold the conversion resulting from the use of CS. The glucose conversion was also higher with SDLS after the same period (1.4-fold). These results confirmed the better performance of the lignocellulosic sugars compared to CS as acyl acceptors in the enzymatic synthesis of SFAEs.



Reaction conditions: 60 ºC; 350 rpm; 1 mM of xylose; 24.6 mM of oleic acid; with molecular sieves. Both reactions were catalyzed by 1% w/v of the uncoated Novozyme 435 (10000 ± 589 TBU/g) in a 4 L-reactor. All experiments were performed in duplicate. Results were expressed as means + intrasample variations.

Figure 5.12. Comparison of xylose and glucose conversions with spray dried lignocellulosic sugars (SDLS) and commercial sugars (CS).

When producing SFAEs from beechwood carbohydrates (glucose and xylose), Siebenhaller *et al*. (2017) observed that roughly 0.7% of the glucose was converted to glucose–octanoate and less than 0.1% of the xylose was used to form xylose–octanoate, much less than the conversions we observed in this study. The authors used the same commercial enzyme (N435), and the reaction was conducted at 50 °C in a rotator with vortex mixer at 50 rpm for 72 h. The lower temperature and level of mixing may have contributed to the lower conversion.

In another paper, Siebenhaller *et al*. (2018) synthesized SFAEs from beechwood carbohydrates (glucose and xylose) using iCalB (lipase acrylic resin from *Candida antarctica*, Sigma-Aldrich) as the biocatalyst. The reaction was performed at 50 °C for 70 h in a rotator with vortex mixer at 50 rpm. In this study, the authors confirmed the formation of glucose and xylose esters via thin-layer chromatography, but sugars conversions to esters were not calculated.

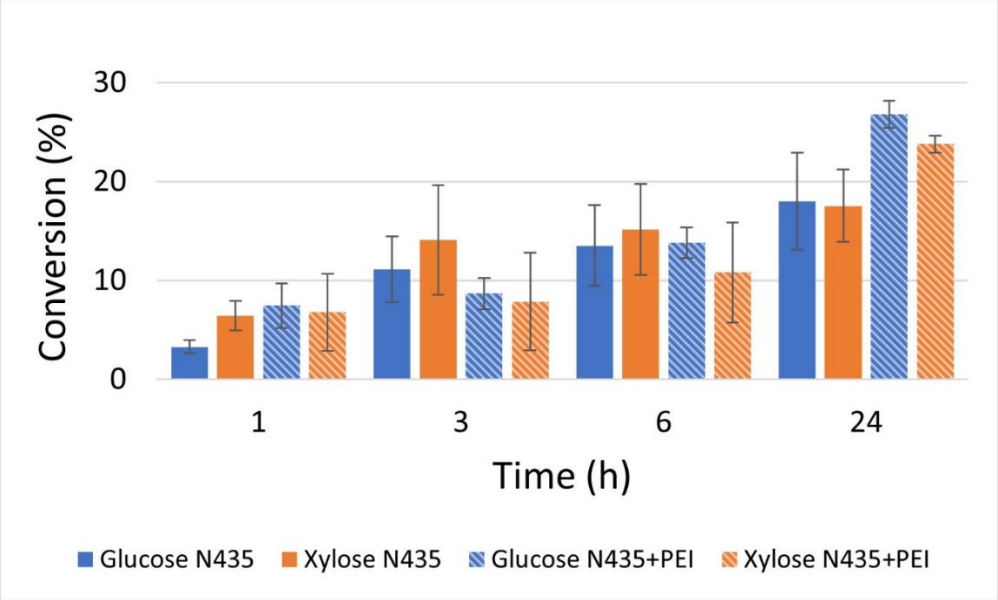
Accordingly, our results demonstrated the potential to use sustainable lignocellulosic biomass as sole sugar source to produce high value SFAEs. Moreover, SDLS appear to be better substrates than CS in the esterification reaction (in terms of sugar conversions to esters). Nonetheless, we found low conversions for both cases (xylose and glucose; CS and SDLS). The low solubility of sugars in the organic solvent (MEK), the size of the reactor (4 L), the type of agitation used (radial-flow impellers), compounds that may adsorb to the enzyme surface during the esterification reaction and block the enzyme pores (e.g., oligomers of xylan and glucan or the maltodextrin used during the SD stage), among other factors, may help explain these low sugar conversions. On the other hand, such conversions were significantly improved by adjusting the enzyme dose (see section 5.3.3.3).

5.3.3.2.2. Comparison of uncoated N435 versus PEI-coated N435 as biocatalysts of the esterification

Figure 5.13 shows the effects of using uncoated and PEI-coated N435 on xylose and glucose conversions to SFAEs. Here, we only used SDLS as the carbohydrate source of the esterification reaction. Xylose conversions were ~35% higher when using the PEI-coated biocatalyst in comparison with the uncoated N435 after 24 h-reaction. Glucose conversions were also higher with the PEI-coated N435 (by ~50%) after the same period. Until 6 h of esterification, the conversion with the PEI-coated N435 was not statistically different from the conversion obtained with the uncoated N435, according to a Tukey test performed at a 95% confidence interval, except for the glucose conversion after 1 h-reaction.

Uncoated lipases can be desorbed from the hydrophobic support under certain circumstances, resulting in product contamination by the enzyme and biocatalyst inactivation (GONÇALVES *et al.*, 2021c). Coating the N435 with PEI may prevent/reduce enzyme dissociation from the support, because the resulting intermolecularly crosslinked proteins (linked by physical intermolecular adsorption) now

must be simultaneously released from their matrix, which is much more difficult (FERNANDEZ-LAFUENTE *et al.*, 2017; FERNANDEZ-LOPEZ *et al.*, 2017). This means that coating the enzyme with this cationic polymer can reduce enzyme loss/leakage into the medium.



Reaction conditions: 60 ºC; 350 rpm; 1 mM of xylose; 24.6 mM of oleic acid; with molecular sieves. Both reactions used SDLS in a 4 L-reactor. The reaction was catalyzed by 1% w/v of the uncoated Novozyme 435 (10000 ± 589 TBU/g) and later adjusted to the same activity of PEI-coated Novozyme 435 (6042 ± 295 TBU/g). All experiments were performed in duplicate. Results were expressed as means +intrasample variations.

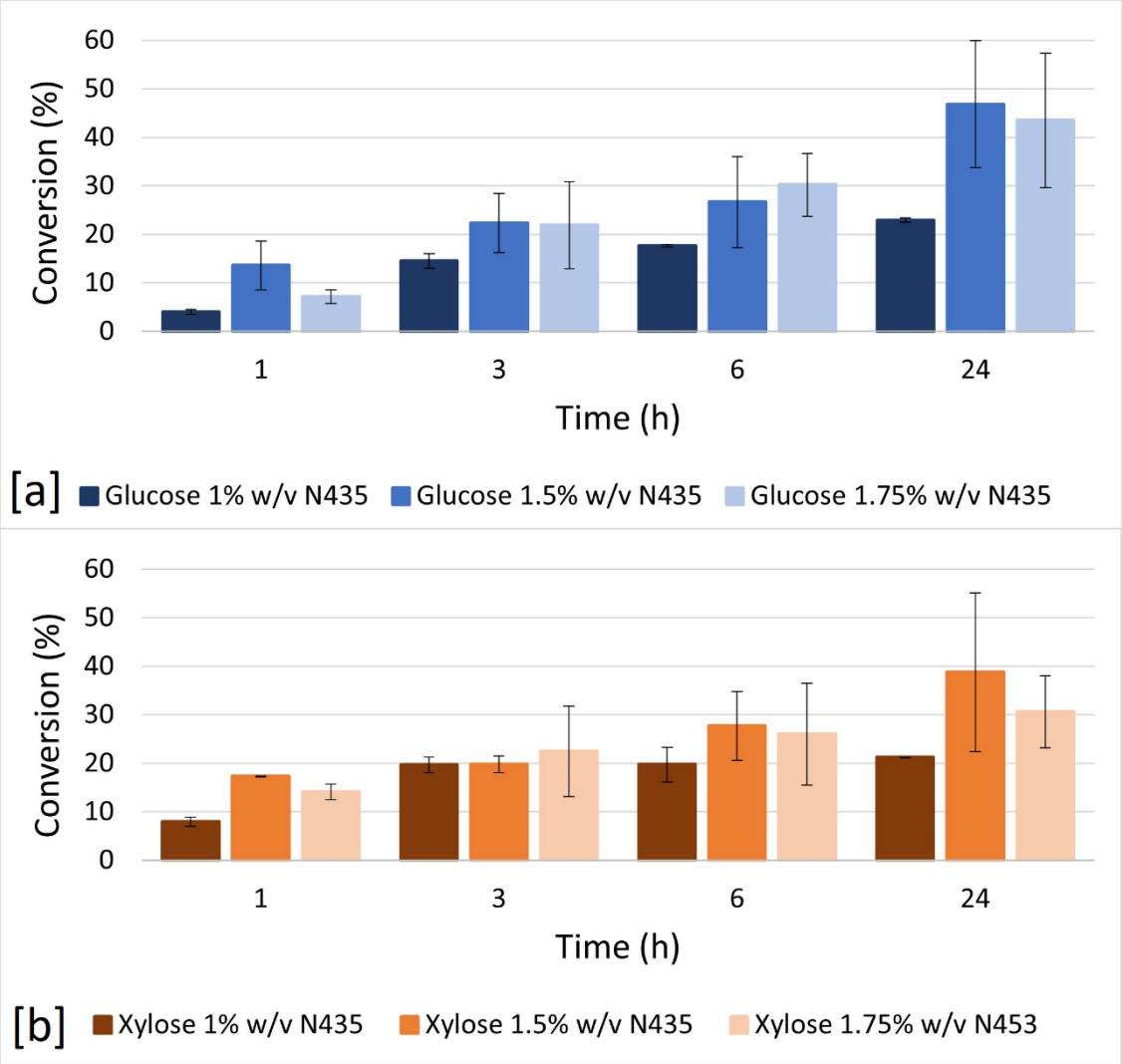
Figure 5.13. Effect of Novozyme 435 PEI-coating on glucose and xylose conversions to SFAEs.

Another consideration is the generation of a hydrophilic environment when a PEI layer is added to the enzyme/support surface, which may cause partitioning of some detrimental hydrophobic compounds (e.g., hydrophobic organic solvents) from the enzyme surroundings and thus, improve enzyme performance and support stability (VIRGEN-ORTÍZ *et al.*, 2017b). Such consequences might help to explain the higher sugar conversions observed when using the PEI-coated biocatalyst. The use of PEI for coating immobilized enzymes has been scarcely explored so far, although it is a simple way to protect enzymes from hydrophobic compounds, among other above-mentioned advantages.

5.3.3.3. Effect of N435 loading on sugar conversions to sugar fatty acid esters

The effect of N435 loading on glucose and xylose conversions to SFAEs is presented in Figure 5.14. The highest conversions (for both sugars) were obtained when using 1.5% w/v N435 (uncoated). Clearly, increasing the amount of enzyme added to the

reaction can enhance conversion rates and address the low yields noted earlier when using an enzymatic load of 1% w/v N435.



Reaction conditions: 60 ºC; 350 rpm; 1 mM of xylose; 24.6 mM of oleic acid; with molecular sieves. Both reactions used SDLS in a 4 L-reactor and were catalyzed by the uncoated Novozyme 435 (10000 ± 589 TBU/g). All experiments were performed in duplicate. Results were expressed as means + intrasample variations.

Figure 5.14. Effect of N435 loading on glucose [a] and xylose [b] conversions to sugar fatty acid esters.

At the highest enzyme loading tested (1.75% w/v), there was little additional impact on conversions; the differences between conversions at 1.5% and 1.75% are not statistically significant according to a Tukey test performed at a 95% confidence interval. Accordingly, for subsequent studies, we used 1.5% w/v N435.

Despite the benefits provided by the use of a higher enzyme dosage to the esterification reaction medium, the main challenges for enhancing SFAEs productivity still arise from the low solubility of sugars in organic solvents and the low enzymatic

activity in these media, which lead to low reaction rates and low product concentrations (WANG *et al*., 2012). Also, the high cost of enzymes can affect the cost-effectiveness of SFAEs biosynthesis. However, enzyme costs can be reduced by improving the biocatalyst's operational stability, as discussed in section 5.3.3.4.

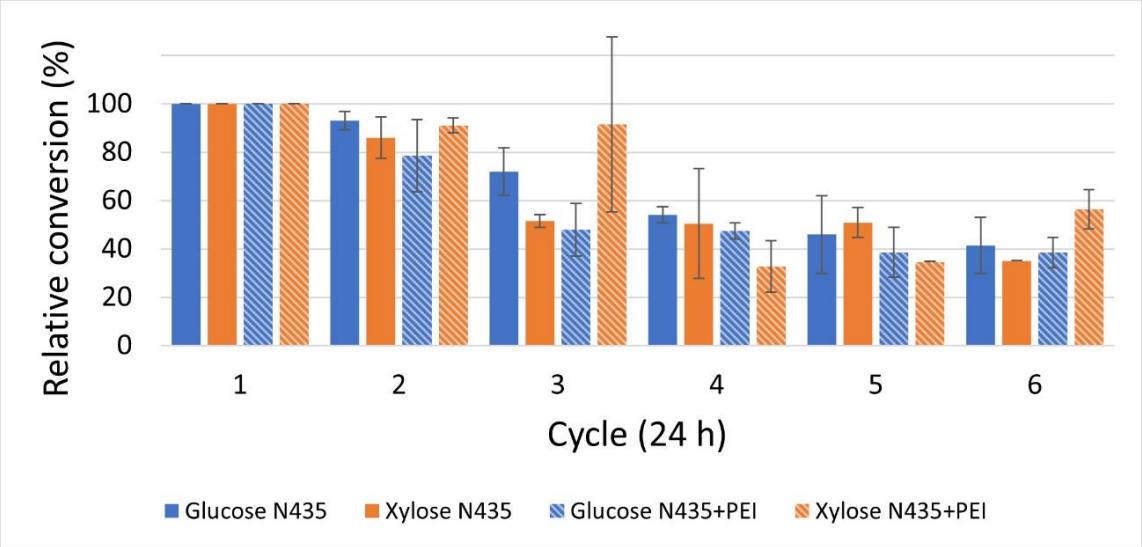
Using a fed-batch substrate feeding procedure is expected to alleviate some of the limitations with substrate solubility. Adding the substrate and/or enzymes gradually could reduce the capital cost due to a smaller reactor volume, which would be translated into lower operating and downstream processing costs with higher product concentrations (GUPTA *et al*., 2012; CAVALCANTI-MONTAÑO *et al*., 2013). Furthermore, using an organic solvent with higher polarity can increase the solubility of the sugar, but at the same time, it might decrease the enzyme activity and produce unwanted hydrolysis side reactions (CHAMOULEAU *et al*., 2001; PEDERSEN *et al*., 2003). Similarly, increasing the temperature will increase carbohydrate solubility while negatively affecting the enzyme (ARCOS; BERNABÉ; OTERO, 1998). Therefore, further research is needed to establish approaches to address these problems and facilitate the large-scale synthesis of SFAEs.

5.3.3.4. N435 operational stability in the synthesis of sugar fatty acid esters

The repeated use of N435 (uncoated and PEI-coated) for sugars (xylose and glucose) conversions to SFAEs was investigated in 6 successive batches (Figure 5.15). When using the uncoated N435, a reduction in conversion of roughly 65% and 60% for xylose and glucose was observed, respectively, after six 24 h-cycles. With the PEI-coated N435, a 44% decrease in xylose conversion and 62% reduction in glucose conversion was observed after six cycles.

Results from Figure 5.15 showed a 22% greater decrease in xylose conversion after six cycles with the uncoated N435 compared to the reduction observed with the PEI-coated enzyme. On the other hand, in cycles 4 and 5, we found lower xylose conversions when using the PEI-coated N435. Albeit, in these cycles, according to the Tukey test performed at a 95% confidence interval, we identified that the PEI-coated conversions were not statistically significantly different from those found with the uncoated N435. Some factors might help to explain the lower values (but not significant) obtained with the PEI-coated enzyme in these cycles, such as the incomplete removal of compounds adsorbed to the N435 surface during the reuse (e.g., water) or the support solubility in

MEK (MARTINS *et al*., 2013; RUEDA *et al*., 2015). To corroborate with our results, for further research it might be relevant to conduct trials with additional cycles.



Reaction conditions of each batch: 60 ºC; 350 rpm; 24 h-cycles; 1 mM of xylose; 24.6 mM of oleic acid; with molecular sieves. All reactions used SDLS in a 4 L-reactor. The reaction was catalyzed by 1.5% w/v of the uncoated Novozyme 435 (10000 ± 589 TBU/g) and later adjusted to the same activity of PEI-coated Novozyme 435 (6042 ± 295 TBU/g). All experiments were performed in duplicate. Results were expressed as means + intrasample variations.

Figure 5.15. Operational stability of uncoated and PEI-coated Novozyme 435 biocatalysts in the synthesis of SFAEs.

Overall, the higher reduction on the xylose conversion after six 24 h-batches with the uncoated N435 is likely due to the CALB desorption from the matrix of the commercial lipase, as discussed in our previous article (GONÇALVES *et al*., 2021b). In particular, residual oleic acid and/or SFAEs can lead to the CALB desorption from the support due to their surfactant properties. Indeed, at the end of the reuse cycles, we identified a 56 ± 0.4% drop in the N435 hydrolytic activity (A_H) (section 5.3.2.10) after incubation in a crude reaction product that also contained unconverted oleic acid, which confirmed N435 leakage from the matrix. Conversely, the A_H of the PEI-coated preparation did not decrease after the same process, indicating that the N435 PEI-coating reduced the desorption that has otherwise been reported when using the uncoated biocatalyst (AN *et al*., 2019; FERNANDEZ-LAFUENTE *et al*., 2014; GONÇALVES *et al*., 2021a; TENG *et al*., 2020; VESCOVI *et al*., 2016; WARD; FANG; LI, 1997; YE; HAYES; BURTON, 2014).

In a previous study, by the end of five 6 h-cycles of xylose laurate synthesis, the A_H of the uncoated N435 presented a reduction 2-fold higher in comparison to the A_H of the PEI-coated derivative after the biocatalysts' incubation in the crude reaction product (GONÇALVES *et al*., 2021c). Results from both studies have confirmed that the addition

of the PEI layer to the N435 surface reduced enzyme leakage and therefore improved N435 operational stability, facilitating its industrial implementation.

Villalba *et al*. (2016) also coated the N435 surface with PEI and reused this enzyme preparation in the alcoholysis of Camelina sativa oil to ethyl and methyl fatty acid esters after 24 h-reactions. The authors used a 3 to 1 alcohol to fatty acid molar ratio, 10% w/w biocatalyst, orbital agitation at 200 rpm, 50 ºC, and the reaction was conducted in the presence of 40% w/w t-butanol as co-solvent. They found that the conversions obtained with the PEI-coated N435 increased from cycle 1 to cycle 4 and remained constant (~100%) in the successive cycles (4 to 14). Fernandez-Lopez *et al*. (2017) reused the commercial lipase from *Rhizomucor miehei* (RML) immobilized on octyl-agarose and octyl-glyoxyl beads and coated with PEI and dextran sulfate in the hydrolysis of triacetin. After 10 reaction cycles, there was no change in the enzyme performance (initial rate and yield of diacetine). Furthermore, RML desorption from the support was greatly decreased after the enzyme coating with PEI, a result that is consistent with what was found in this research.

Finally, regarding glucose conversions, there is only a statistically significantly difference between PEI-coated and uncoated N435 results in the first cycle (according to a Tukey test performed at a 95% confidence interval), which may indicate that the coating of the N435 surface with PEI did not alter the operational stability of the enzyme for the consumption of this sugar. Nevertheless, since we are using a xylose/glucose mass ratio of 3, the amount of glucose in the reaction medium is so low that the effects of the enzyme PEI-coating on the glucose consumption over the reuse may be impossible to identify with certainty.

5.3.3.5. Characterizations – sugar fatty acid esters

5.3.3.5.1. Mass spectrometry analysis

Samples of the crude reaction product from the esterification of SDLS with oleic acid were purified (after evaporation of the MEK through distillation) according to the methodology described in Gonçalves *et al*. (2021c). Most of the unreacted sugars were removed after this process (91% of xylose and 100% of glucose). The color of the purified product was visually lighter, indicating likely removal of phenolic compounds.

The purified samples were then characterized by mass spectrometry. Mass spectra of the $[M-H]^-$ ion with m/z = 413.29 and 677.53 revealed that we produced mainly xylose mono- and dioleate, as shown in Figures S21 and S22 in the supplementary material. To

a lesser extent, we also detected the formation of glucose mono- and dioleate (m/z = 443.30 and 707.54 - Figures S23 and S24). Although N435 has been reported to selectively modify only one hydroxyl group of the sugar (PAPPALARDO *et al*., 2017) (i.e., synthesis of monoesters), the literature has shown that the solvent can change this behavior, with MEK preferentially leading to the formation of diesters instead of monoesters (LI *et al*., 2015).

Here, we found an outcome similar to those previously described by Gonçalves *et al*. (2021b; 2021c), when the formation of a mixture of SFAEs with different degrees of modification was confirmed. However, such results reported the production of mono-, di-, and tri-esters, and used CS as the carbohydrate source of the esterification reaction. In this study, using SDLS, we only synthesized mono- and di-esters, which may indicate the obtention of a slightly more purified product, now using a sustainable sugar source in an upscaled environment (from an 80 mL to a 4 L-reactor).

5.3.3.5.2. Emulsion capacity assays

The emulsion capacity (EC) of SFAEs synthesized and purified in this study (7.5%; using SDLS in a 4 L-reactor) was comparable to the EC of (1) a commercial sugar ester (10%; sucrose monolaurate) and (2) the xylose oleate synthesized in our previous study (GONÇALVES *et al*., 2021b) (6.25%; using CS in an 80 mL-reactor). These results are also comparable to the ECs of the xylose laurate (12.5%) and xylose palmitate (11.8%) described by Gonçalves *et al*. (2021c).

As previously mentioned, SFAEs have hydrophobic characteristics due to their fatty acyl chain and hydrophilic hydroxyl groups due to their sugar moiety. A higher proportion of hydrophobic moieties of the molecule leads to a lower EC (AI; XIAO; JIANG, 2021; EL-LAITHY; SHOUKRY; MAHRAN, 2011; TENG *et al*., 2020). The esters that we synthesized here are composed of a blend of mono- and di-esters (as confirmed by mass spectrometry), which means that we may have more than one fatty acid residue per sugar molecule, enhancing the product hydrophobicity and so, reducing its EC. Furthermore, when tri-esters were also produced (GONÇALVES *et al*., 2021b), the EC was a bit lower than the EC found when only mono- and di-esters were synthesized. This outcome confirms that the presence of more oleic acid residues decreases the EC and that the use of SDLS rather than CS in an upscaled environment provided a product with slightly better EC properties.

Other parameters still need to be assessed to confirm the commercial viability of the esters produced in this research. Nonetheless, EC results are an important starting point towards the future use of the purified xylose oleate (our main product) as an industrial surfactant for a wide range of practical applications, such as skin hydration products in the cosmetic and pharmaceutical sectors, oral-care products, detergents, and foods (ice cream, milk beverages, cake batter, sauces, dressings) (BIDJOU-HAIOUR; KLAI, 2013; DUMAS; BONTE, 2002; VESCOVI; DOS SANTOS; TARDIOLI, 2017).

5.3.4. Conclusions

Our primary goal in this research was to maximize xylose production yields and rates relative to those for glucose. By adjusting the enzyme dose and HSEH time, as well as purification and concentration conditions, we produced xylose and glucose in a mass ratio of ~3. These sugars were then used as substrates in the enzymatic synthesis of SFAEs and presented better esterification performance compared to commercial sugars in terms of sugars conversions to esters.

Xylose and glucose conversions obtained after 24 h-esterification using the PEI-coated biocatalyst were increased by ~35 and 50%, respectively, in comparison with the uncoated N435. The improved conversion may be related to (1) a greater resistance of the coated enzyme to activity reduction after contact with organic solvents and (2) an improved support stability due to partitioning of detrimental hydrophobic compounds from the enzyme surroundings. The addition of the PEI layer to the N435 surface also reduced enzyme desorption and therefore improved the N435 operational stability, facilitating its industrial implementation.

Further, the formation of mono- and di-esters using SDLS may indicate the production of a more purified product than the one previously obtained using CS (a blend of mono-, di-, and tri-esters). The emulsion capacity of the purified SFAEs was close to that of a commercial sugar ester. The presence of less oleic acid residues and the use of SDLS in an upscaled environment provided a product with slightly better EC properties than the one produced with CS. Overall, this research represents a pathway towards the sustainable synthesis of high value SFAEs using lignocellulosic biomass as the sole sugar source and PEI-coated N435 as an enhanced biocatalyst.

Funding: This work was supported by the “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP) [grant numbers 2016/10636-8, 2019/23908-4, and 2021/06525-

4]; in part by Natural Sciences and Engineering Research Council of Canada (NSERC) and “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) [project numbers 315092/2020-3 and 141111/2020-8].

References

AI, M.; XIAO, N.; JIANG, A. Molecular structural modification of duck egg white protein conjugates with monosaccharides for improving emulsifying capacity. **Food Hydrocolloids**, vol. 111, p. 106271, 2021. DOI https://doi.org/10.1016/j.foodhyd.2020.106271.

AN, D.; ZHANG, X.; LIANG, F.; XIAN, M.; FENG, D.; YE, Z. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A**, vol. 577, p. 257–264, 2019. DOI https://doi.org/10.1016/j.colsurfa.2019.05.079.

ANDLER, S. M.; WANG, L.; ROTELLO, V. M.; GODDARD, J. M. Influence of hierarchical interfacial assembly on lipase stability and performance in deep eutectic solvent. **Journal of Agricultural and Food Chemistry**, vol. 65, no. 9, p. 1907–1914, 2017. DOI https://doi.org/10.1021/acs.jafc.6b05372.

ARCENS, D.; GRAU, E.; GRELIER, S.; CRAMAIL, H.; PERUCH, F. 6-O-glucose palmitate synthesis with lipase: Investigation of some key parameters. **Molecular Catalysis**, vol. 460, p. 63–68, 2018. DOI https://doi.org/10.1016/j.mcat.2018.09.013.

ARCOS, J. A.; BERNABÉ, M.; OTERO, C. Quantitative enzymatic production of 6-O-acylglucose esters. **Biotechnology and Bioengineering**, vol. 57, no. 5, p. 505–509, 1998. DOI https://doi.org/10.1002/(sici)1097-0290(19980305)57:5<505::aid-bit1>3.0.co;2-k.

BASYARUDDIN, M.; RAHMAN, A.; ARUMUGAM, M. Microwave assisted enzymatic synthesis of fatty acid sugar ester in ionic liquid-tert-butanol biphasic solvent system. **Asian Journal of Chemistry**, vol. 24, no. 11, p. 5058–5062, 2012.

BEISSON, F.; TISS, A.; RIVIÈRE, C.; VERGER, R. Methods for lipase detection and assay: A critical review. **European Journal of Lipid Science and Technology**, vol. 102, no. 2, p. 133–153, 2000. DOI https://doi.org/10.1002/(sici)1438-9312(200002)102:2<133::aid-ejlt133>3.0.co;2-x.

BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. DOI https://doi.org/10.14233/ajchem.2013.13973.

CAUGLIA, F.; CANEPA, P. The enzymatic synthesis of glucosylmyristate as a reaction model for general considerations on “sugar esters” production. **Bioresource Technology**, vol. 99, no. 10, p. 4065–4072, 2008. DOI https://doi.org/10.1016/j.biortech.2007.01.036.

CAVALCANTI, R. M. F.; SILVA, D. P. D.; PAZ, M. C. F.; QUEIROZ, J. C. F. Screening, production and characterization of biosurfactants from caatinga´s filamentous Fungi. **International Journal of Pharmaceutical Science Invention**, vol. 6, no. 5, p. 23–28, 2017.

CAVALCANTI-MONTAÑO, I. D.; SUAREZ, C. A. G.; RODRÍGUEZ-ZÚÑIGA, U. F.; GIORDANO, R. L. C.; GIORDANO, R. C.; DE SOUSA JÚNIOR, R. Optimal bioreactor operational policies for the enzymatic hydrolysis of sugarcane bagasse. **BioEnergy Research**, vol. 6, p. 776–785, 2013. DOI https://doi.org/10.1007/s12155-013-9294-7.

CEPEA - Centro de Estudos Avançados em Economia Aplicada. **Ethanol**. Piracicaba, São Paulo, Brazil, 2022. Available at: https://www.cepea.esalq.usp.br/br/indicador/etanol.aspx.

CHAIWUT, P.; JIRARAT, A.; TIENSRI, N.; SANGTHONG, S. Green synthesis optimization of glucose palm oleate and its potential use as natural surfactant in cosmetic emulsion. **Cosmetics**, vol. 9, no. 4, p. 76, 2022. DOI https://doi.org/10.3390/cosmetics9040076.

CHAMOULEAU, F.; COULON, D.; GIRARDIN, M.; GHOUL, M. Influence of water activity and water content on sugar esters lipase-catalyzed synthesis in organic media. **Journal of Molecular Catalysis B: Enzymatic**, vol. 11, no. 4-6, p. 949–954, 2001. DOI https://doi.org/10.1016/S1381-1177(00)00166-1.

CHANG, P.; ZHANG, Z.; TANG, S. Lipase-catalyzed synthesis of sugar ester in mixed biphasic system of ionic liquids and supercritical carbon dioxide. **Journal of the Chinese Chemical Society**, vol. 65, no. 4, p. 452–458, 2018. DOI https://doi.org/10.1002/jccs.201700278.

CHANG, S. W.; SHAW, J. F. Biocatalysis for the production of carbohydrate esters. **New Biotechnology**, vol. 26, no. 3-4, p. 109–116, 2009. DOI https://doi.org/10.1016/j.nbt.2009.07.003.

DE LIMA, L. N.; MENDES, A. A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W.; GIORDANO, R. L. C. Performance of different immobilized lipases in the synthesis of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, 2018. DOI https://doi.org/10.3390/molecules23040766.

DOSSAT, V.; COMBES, D.; MARTY, A. Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: Influence of the glycerol production. **Enzyme and Microbial Technology**, vol. 25, no. 3-5, p. 194–200, 1999. DOI https://doi.org/10.1016/S0141-0229(99)00026-5.

DUMAS, F.; BONTE, M. **Uses of D-xylose, the esters thereof and oligosaccharides containing xylose for improving the functionality of epidermal cells**. United States: [*6.495.147 B1*], 2002.

EL-LAITHY, H. M.; SHOUKRY, O.; MAHRAN, L. G. Novel sugar esters proniosomes for transdermal delivery of vinpocetine: Preclinical and clinical studies. **European Journal of Pharmaceutics and Biopharmaceutics**, vol. 77, no. 1, p. 43–55, 2011. DOI https://doi.org/10.1016/j.ejpb.2010.10.011.

FERNANDEZ-LAFUENTE, R.; FERNANDEZ-LOPEZ, L.; VIRGEN-ORTÍZ, J. J.; PEDRERO, S. G.; LOPEZ-CARROBLES, N.; GORINES, B. C.; OTERO, C. Optimization of the coating of octyl-CALB with ionic polymers to improve stability and decrease enzyme leakage. **Biocatalysis and Biotransformation**, vol. 36, p. 47–56, 2017. DOI https://doi.org/https://doi.org/10.1080/10242422.2016.1278212.

FERNANDEZ-LAFUENTE, R.; ORTIZ, C.; FERREIRA, M. L.; BARBOSA, O.; DOS SANTOS, J. C. S.; RODRIGUES, R. C.; BERENGUER-MURCIA, Á.; BRIAND, L. E. Novozym 435: the “perfect” lipase immobilized biocatalyst? **Catalysis Science & Technology**, vol. 9, no. 10, p. 2380–2420, 2019. DOI https://doi.org/https://doi.org/10.1039/C9CY00415G.

FERNANDEZ-LAFUENTE, R.; RODRIGUES, R. C.; BARBOSA, O.; ORTIZ, C.; BERENGUER-MURCIA, Á.; TORRES, R. Amination of enzymes to improve biocatalyst performance: coupling genetic modification and physicochemical tools. **RSC Advances**, vol. 4, no. 72, p. 38350–38374, 2014. DOI https://doi.org/10.1039/c4ra04625k.

FERNANDEZ-LOPEZ, L.; PEDRERO, S. G.; LOPEZ-CARROBLES, N.; VIRGEN-ORTÍZ, J. J.; GORINES, B. C.; OTERO, C.; FERNANDEZ-LAFUENTE, R. Physical crosslinking of lipase from *Rhizomucor miehei* immobilized on octyl agarose via coating with ionic polymers: Avoiding enzyme release from the support. **Process Biochemistry**, vol. 54, p. 81–88, 2017. DOI https://doi.org/10.1016/j.procbio.2016.12.018.

FINDRIK, Z.; MEGYERI, G.; GUBICZA, L.; BÉLAFI-BAKÓ, K.; NEMESTÓTHY, N.; SUDAR, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. **Journal of Cleaner Production**, vol. 112, p. 1106–1111, 2016. DOI https://doi.org/10.1016/j.jclepro.2015.07.098.

GAONA, A.; LAWRYSHYN, Y.; SAVILLE, B. A. Enhancing biomass hydrolysis for biofuel production through hydrodynamic modeling and reactor design. **Energy Science & Engineering**, vol. 7, p. 1823–1837, 2019. DOI https://doi.org/10.1002/ese3.394.

GAONA, A.; LAWRYSHYN, Y.; SAVILLE, B. A. The effect of fed-batch operation and rotational speed on high-solids enzymatic hydrolysis of hardwood substrates. **Industrial Biotechnology**, vol. 11, no. 5, p. 1-7, 2015. DOI https://doi.org/10.1089/ind.2014.0031.

GONÇALVES, M. C. P.; ROMANELLI, J. P.; GUIMARÃES, J. R.; VIEIRA, A. C.; AZEVEDO, B. P.; TARDIOLI, P. W. Reviewing research on the synthesis of CALB-catalyzed sugar esters incorporating systematic mapping principles. **Critical Reviews in Biotechnology**, vol. 41, no. 6, p. 865–878, 2021a. DOI https://doi.org/10.1080/07388551.2021.1888071.

GONÇALVES, M. C. P.; AMARAL, J. C.; FERNANDEZ-LAFUENTE, R.; JUNIOR, R. D. S.; TARDIOLI, P. W. Lipozyme 435-mediated synthesis of xylose oleate in methyl ethyl ketone. **Molecules**, vol. 26, p. 3317, 2021b. DOI https://doi.org/https://doi.org/10.3390/molecules26113317.

GONÇALVES, M. C. P.; ANTUNES, L.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P. W. Stabilization and operational selectivity alteration of Lipozyme 435 by its coating with polyethyleneimine: Comparison of the biocatalyst performance in the synthesis of xylose fatty esters. **International Journal of Biological Macromolecules**, vol. 192, p. 665–674, 2021c. DOI https://doi.org/10.1016/j.ijbiomac.2021.10.052.

GONÇALVES, M. C. P.; ROMANELLI, J. P.; CANSIAN, A. B. M.; PUCCI, E. F. Q.; GUIMARÃES, J. R.; TARDIOLI, P. W.; SAVILLE, B. A. A review on the production and recovery of sugars from lignocellulosics for use in the synthesis of bioproducts. **Industrial Crops and Products**, vol. 186, p. 115213, 2022. DOI https://doi.org/10.1016/j.indcrop.2022.115213.

GRÜNINGER, J.; DELAVAULT, A.; OCHSENREITHER, K. Enzymatic glycolipid surfactant synthesis from renewables. **Process Biochemistry**, 2019. DOI https://doi.org/10.1016/j.procbio.2019.09.023.

GUMEL, A. M.; ANNUAR, M. S. M.; HEIDELBERG, T.; CHISTI, Y. Lipase mediated synthesis of sugar fatty acid esters. **Process Biochemistry**, vol. 46, no. 11, p. 2079–2090, 2011. DOI https://doi.org/10.1016/j.procbio.2011.07.021.

GUPTA, R.; KUMAR, S.; GOMES, J.; KUHAD, R. C. Kinetic study of batch and fed-batch enzymatic saccharification of pretreated substrate and subsequent fermentation to ethanol. **Biotechnology for Biofuels**, vol. 5, no. 16, p. 1–10, 2012. DOI https://doi.org/https://doi.org/10.1186/1754-6834-5-16.

H & Z INDUSTRY CO. **Certification of Analysis**. Jinan, Shandong, China, 2022.

HA, S. H.; HIEP, N. M.; LEE, S. H.; KOO, Y. M. Optimization of lipase-catalyzed glucose ester synthesis in ionic liquids. **Bioprocess and Biosystems Engineering**, vol. 33, p. 63–70, 2010. DOI https://doi.org/10.1007/s00449-009-0363-4.

HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, A.; SLUITER, J.; TEMPLETON, D. **Preparation of samples for compositional analysis**. Laboratory Analytical Procedure (LAP), TP-510-42620, Golden, CA, USA, 2008.

HOLLENBACH, R.; DELAVAULT, A.; GEBHARDT, L.; SOERGEL, H.; MUHLE-GOLL, C.; OCHSENREITHER, K.; SYLDATK, C. Lipase-mediated mechanoenzymatic synthesis of sugar esters in dissolved unconventional and neat reaction systems.

Sustainable Chemistry & Engineering, vol. 10, p. 10192–10202, 2022. DOI https://doi.org/10.1021/acssuschemeng.2c01727.

KUNDYS, A.; BIAŁECKA-FLORJAŃCZYK, E.; FABISZEWSKA, A.; MAŁAJOWICZ, J. *Candida antarctica* lipase B as catalyst for cyclic esters synthesis, their polymerization and degradation of aliphatic polyesters. **Journal of Polymers and the Environment**, vol. 26, no. 1, p. 396–407, 2018. DOI https://doi.org/10.1007/s10924-017-0945-1.

LI, L.; JI, F.; WANG, J.; LI, Y.; BAO, Y. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. **Enzyme and Microbial Technology**, vol. 69, p. 46–53, 2015. DOI https://doi.org/10.1016/j.enzmictec.2014.12.003.

LIANG, M. Y.; CHEN, Y.; BANWELL, M. G.; WANG, Y.; LAN, P. Enzymatic preparation of a homologous series of long-chain 6-O-acylglucose esters and their evaluation as emulsifiers. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 15, p. 3949–3956, 2018. DOI https://doi.org/10.1021/acs.jafc.8b00913.

LIN, X. S.; ZHAO, K. H.; ZHOU, Q. L.; XIE, K. Q.; HALLING, P. J.; YANG, Z. Synthesis of glucose laurate with excellent productivity. **Bioresources and Bioprocessing**, vol. 3, no. 2, p. 1–7, 2016. DOI https://doi.org/10.1186/s40643-015-0080-6.

MARTINS, A. B.; SCHEIN, M. F.; FRIEDRICH, J. L. R.; FERNANDEZ-LAFUENTE, R.; AYUB, M. A. Z.; RODRIGUES, R. C. Ultrasound-assisted butyl acetate synthesis catalyzed by Novozym 435: Enhanced activity and operational stability. **Ultrasonics Sonochemistry**, vol. 20, no. 5, p. 1155–1160, 2013. DOI https://doi.org/10.1016/j.ultsonch.2013.01.018.

MARTY, A.; DOSSAT, V.; CONDORET, J. S. Continuous operation of lipase-catalyzed reactions in nonaqueous solvents: Influence of the production of hydrophilic compounds. **Biotechnology and Bioengineering**, vol. 56, no. 2, p. 232–237, 1997. DOI https://doi.org/10.1002/(SICI)1097-0290(19971020)56:2<232::AID-BIT12>3.0.CO;2-I.

MILESSI-ESTEVES, T. S.; CORRADINI, F. A. S.; KOPP, W.; ZANGIROLAMI, T. C. An innovative biocatalyst for continuous 2G ethanol production from xylo-oligomers by *Saccharomyces cerevisiae* through simultaneous hydrolysis, isomerization, and fermentation (SHIF). **Catalysts**, vol. 9, no. 3, p. 225, 2019. DOI https://doi.org/10.3390/catal9030225.

NETA, N. A. S.; TEIXEIRA, J. A.; RODRIGUES, L. R. Sugar ester surfactants: Enzymatic synthesis and applications in food industry. **Critical Reviews in Food Science and Nutrition**, vol. 55, no. 5, p. 595–610, 2015. DOI https://doi.org/10.1080/10408398.2012.667461.

PAPPALARDO, V. M.; BOERIU, C. G.; ZACCHERIA, F.; RAVASIO, N. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, vol. 433, p. 383–390, 2017. DOI https://doi.org/10.1016/j.mcat.2017.02.029.

PEDERSEN, N. R.; WIMMER, R.; MATTHIESEN, R.; PEDERSEN, L. H.; GESSESSE, A. Synthesis of sucrose laurate using a new alkaline protease. **Tetrahedron Asymmetry**, vol. 14, no. 6, p. 667–673, 2003. DOI https://doi.org/10.1016/S0957-4166(03)00086 7.

PINO, M. S.; RODRÍGUEZ-JASSO, R. M.; MICHELIN, M.; FLORES-GALLEGOS, A. C.; MORALES-RODRIGUEZ, R.; TEIXEIRA, J. A.; RUIZ, H. A. Bioreactor design for enzymatic hydrolysis of biomass under the biorefinery concept. **Chemical Engineering Journal**, vol. 347, p. 119–136, 2018. DOI https://doi.org/10.1016/j.cej.2018.04.057.

PURI, D. J.; HEAVEN, S.; BANKS, C. J. Improving the performance of enzymes in hydrolysis of high solids paper pulp derived from MSW. **Biotechnology for Biofuels**, vol. 6, no. 1, p. 1–10, 2013. DOI https://doi.org/10.1186/1754-6834-6-107.

RECKE, V. K.; BEYRLE, C.; GERLITZKI, M.; HAUSMANN, R.; SYLDATK, C.; WRAY, V.; TOKUDA, H.; SUZUKI, N.; LANG, S. Lipase-catalyzed acylation of microbial mannosylerythritol lipids (biosurfactants) and their characterization. **Carbohydrate Research**, vol. 373, p. 82–88, 2013. DOI https://doi.org/10.1016/j.carres.2013.03.013.

RUEDA, N.; DOS SANTOS, J. C. S.; TORRES, R.; ORTIZ, C.; BARBOSA, O.; FERNANDEZ-LAFUENTE, R. Improved performance of lipases immobilized on heterofunctional octyl-glyoxyl agarose beads. **RSC Advances**, vol. 5, no. 15, p. 11212–11222, 2015. DOI https://doi.org/10.1039/c4ra13338b.

SIEBENHALLER, S.; HAJEK, T.; MUHLE-GOLL, C.; HIMMELSBACH, M.; LUY, B.; KIRSCHHÖFER, F.; BRENNER-WEIß, G.; HAHN, T.; ZIBEK, S.; SYLDATK, C. Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. **Bioresources and Bioprocessing**, vol. 4, no. 1, p. 25, 2017. DOI https://doi.org/10.1186/s40643-017-0155-7.

SIEBENHALLER, S.; KIRCHHOFF, J.; KIRSCHHÖFER, F.; BRENNER-WEIß, G.; MUHLE-GOLL, C.; LUY, B.; HAITZ, F.; HAHN, T.; ZIBEK, S.; SYLDATK, C.; OCHSENREITHER, K. Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. **Frontiers in Chemistry**, vol. 6, p. 421, 2018. DOI https://doi.org/10.3389/fchem.2018.00421.

SIÓDMIAK, T.; MANGELINGS, D.; VANDER HEYDEN, Y.; ZIEGLER-BOROWSKA, M.; MARSZAŁŁ, M. P. High enantioselective Novozym 435-catalyzed esterification of (R,S)-flurbiprofen monitored with a chiral stationary phase. **Applied Biochemistry and Biotechnology**, vol. 175, no. 5, p. 2769–2785, 2015. DOI https://doi.org/10.1007/s12010-014-1455-4.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D.; CROCKER, D. **Determination of structural carbohydrates and lignin in biomass**. Laboratory Analytical Procedure (LAP), TP-510-42618, Golden, CA, USA, 2008.

SLUITER, A.; HAMES, B.; RUIZ, R.; SCARLATA, C.; SLUITER, J.; TEMPLETON, D.; CROCKER, D. **Determination of ash in biomass**. Laboratory Analytical Procedure (LAP), TP-510-42622, Golden, CA, USA, 2005.

TAHERZADEH, M.J.; KARIMI, K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: A review. **BioResources**, vol. 2, no. 4, p. 707-738, 2007.

TENG, Y.; STEWART, S. G.; HAI, Y. W.; LI, X.; BANWELL, M. G.; LAN, P. Sucrose fatty acid esters: synthesis, emulsifying capacities, biological activities and structure-property profiles. **Critical Reviews in Food Science and Nutrition**, vol. 0, no. 0, p. 1–21, 2020. DOI https://doi.org/10.1080/10408398.2020.1798346.

VESCOVI, V.; DOS SANTOS, J. B. C.; TARDIOLI, P. W. Porcine pancreatic lipase hydrophobically adsorbed on octyl-silica: A robust biocatalyst for syntheses of xylose fatty acid esters. **Biocatalysis and Biotransformation**, vol. 35, no. 4, p. 298–305, 2017. DOI https://doi.org/10.1080/10242422.2017.1335717.

VESCOVI, V.; KOPP, W.; GUISÁN, J. M.; GIORDANO, R. L. C.; MENDES, A. A.; TARDIOLI, P.W. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octyl and multifunctional amino-glutaraldehyde spacer arms. **Process Biochemistry**, vol. 51, no. 12, p. 2055–2066, 2016. DOI https://doi.org/10.1016/j.procbio.2016.09.016.

VILLALBA, M.; VERDASCO-MARTÍN, C. M.; DOS SANTOS, J. C. S.; FERNANDEZ-LAFUENTE, R.; OTERO, C. Operational stabilities of different chemical derivatives of Novozym 435 in an alcoholysis reaction. **Enzyme and Microbial Technology**, vol. 90, p. 35–44, 2016. DOI https://doi.org/10.1016/j.enzmictec.2016.04.007.

VIRGEN-ORTÍZ, J. J.; TACIAS-PASCACIO, V. G.; HIRATA, D. B.; TORRESTIANA-SANCHEZ, B.; ROSALES-QUINTERO, A.; FERNANDEZ-LAFUENTE, R. Relevance of substrates and products on the desorption of lipases physically adsorbed on hydrophobic supports. **Enzyme and Microbial Technology**, vol. 96, p. 30–35, 2017a. DOI https://doi.org/10.1016/j.enzmictec.2016.09.010.

VIRGEN-ORTÍZ, J. J.; DOS SANTOS, J. C. S.; BERENGUER-MURCIA, Á.; BARBOSA, O.; RODRIGUES, R. C.; FERNANDEZ-LAFUENTE, R. Polyethylenimine: A very useful ionic polymer in the design of immobilized enzyme biocatalysts. **Journal of Materials Chemistry B**, vol. 5, no. 36, p. 7461–7490, 2017b. DOI https://doi.org/10.1039/c7tb01639e.

VUILLEMIN, M. E.; HUSSON, E.; LACLEF, S.; JAMALI, A.; LAMBERTYN, V.; PILARD, S.; CAILLEU, D.; SARAZIN, C. Improving the environmental compatibility of enzymatic synthesis of sugar-based surfactants using green reaction media. **Process Biochemistry**, vol. 117, p. 30–41, 2022. DOI https://doi.org/10.1016/j.procbio.2022.03.015.

WANG, X.; MIAO, S.; WANG, P.; ZHANG, S. Highly efficient synthesis of sucrose monolaurate by alkaline protease Protex 6L. **Bioresource Technology**, vol. 109, p. 7–12, 2012. DOI https://doi.org/10.1016/j.biortech.2012.01.035.

WARD, O. P.; FANG, J.; LI, Z. Lipase-catalyzed synthesis of a sugar ester containing arachidonic acid. **Enzyme and Microbial Technology**, vol. 20, no. 1, 52–56, 1997. DOI https://doi.org/10.1016/S0141-0229(96)00081-6.

WILSON, L.; FERNÁNDEZ-LORENTE, G.; FERNÁNDEZ-LAFUENTE, R.; ILLANES, A.; GUISÁN, J. M.; PALOMO, J. M. CLEAs of lipases and poly-ionic polymers: A simple way of preparing stable biocatalysts with improved properties. **Enzyme and Microbial Technology**, vol. 39, no. 4, p. 750–755, 2006. DOI https://doi.org/10.1016/j.enzmictec.2005.12.011.

YE, R.; HAYES, D. G.; BURTON, R. Effects of particle size of sucrose suspensions and pre-incubation of enzymes on lipase-catalyzed synthesis of sucrose oleic acid esters. **Journal of the American Oil Chemists' Society**, vol. 91, p. 1891–1901, 2014. DOI https://doi.org/10.1007/s11746-014-2537-8.

6. GENERAL REMARKS

The benchmark immobilized *Candida antarctica* lipase B (CALB) (Lipozyme 435/Novozyme 435, L435/N435) was proven to be efficient for xylose oleate/laurate/palmitate synthesis in methyl ethyl ketone. An excess of fatty acid markedly favored the reaction catalyzed by this enzyme preparation. However, the commercial CALB limited repeatability and stability turn the cost reduction in the large-scale production of sugar fatty acid esters (SFAEs) difficult. The addition of a polyethyleneimine (PEI) layer to the L435/N435 surface reduced enzyme desorption from the support in the presence of the crude sugar ester and therefore improved the biocatalyst's operational stability, facilitating its industrial implementation. This strategy seems to be an effective and easy-to-perform tool to tailor the biocatalyst properties.

It was also verified that in terms of carbohydrate sources used in the enzymatic synthesis of SFAEs, spray dried lignocellulosic sugars (SDLS) presented higher sugar

conversions to esters when compared to commercial sugars (CS). Further, the formation of mono- and di-esters using SDLS in an upscaled environment may indicate the production of a slightly more purified product than the one previously obtained using CS in a smaller reactor with a different configuration (a blend of mono-, di-, and tri-esters). At last, the emulsion capacities of the purified SFAEs (using both SDLS and CS) were close to that of a commercial sugar ester.

Overall, this research represents a pathway towards a more feasible and sustainable synthesis of high value SFAEs using PEI-coated commercial L435/N435 as an enhanced biocatalyst and, in the last chapter, lignocellulosic biomass as the sole sugar source of the esterification reaction.

7. SUGGESTIONS FOR FUTURE RESEARCH

Currently, many obstacles encountered in the enzymatic synthesis of SFAEs still need to be addressed, including the low rate of reaction, high cost of enzymes, low sugar solubilization rates in organic solvents, low product concentration, and the lack of understanding of the enzyme kinetics with lignocellulosic substrates. For future research addressing the topic of this study, we propose to evaluate the benefits of a fed-batch reaction to enhance the productivity of SFAEs synthesis (mostly xylose fatty acid esters). In fed-batch processes some of the abovementioned problems may be avoided. Adding the substrate and/or enzymes gradually could be translated into lower capital and operating costs due to reduced reactor volume and lower downstream processing costs due to higher product concentration. To date, we are not aware of any reports on process operation, optimization, and control of a fed-batch enzymatic esterification process to produce SFAEs, and thus, the proposed study would contribute significantly to the knowledge base for potential large-scale SFAEs production.

Regarding the enzyme kinetics study with lignocellulosic substrates, the kinetics from each batch of the fed-batch process would be compared to original data from a single batch. Additionally, mathematical modeling simulations of the reaction kinetics would be performed, allowing researchers to define a critical range where, for example, product or substrate inhibition may occur during the esterification, thus promoting a mechanistic description of the phenomena.

Also, it would be of great importance to conduct a techno-economic analysis to evaluate the technical and economic performance of this process. The sugar solubility in methyl ethyl ketone (MEK) is another parameter that still needs to be enhanced. This

could be done using, for example, another solvent in combination with MEK, or any other approaches that may arise to smooth this issue. Moreover, it would be interesting to immobilize the soluble CALB via interfacial activation and coat the derivative with PEI for further application in the synthesis of SFAEs. This way, results could be compared with the ones acquired using the PEI-coated L435/N435.

Supplementary material

Reviewing research on the synthesis of CALB-catalyzed sugar esters incorporating systematic mapping principles

Figures

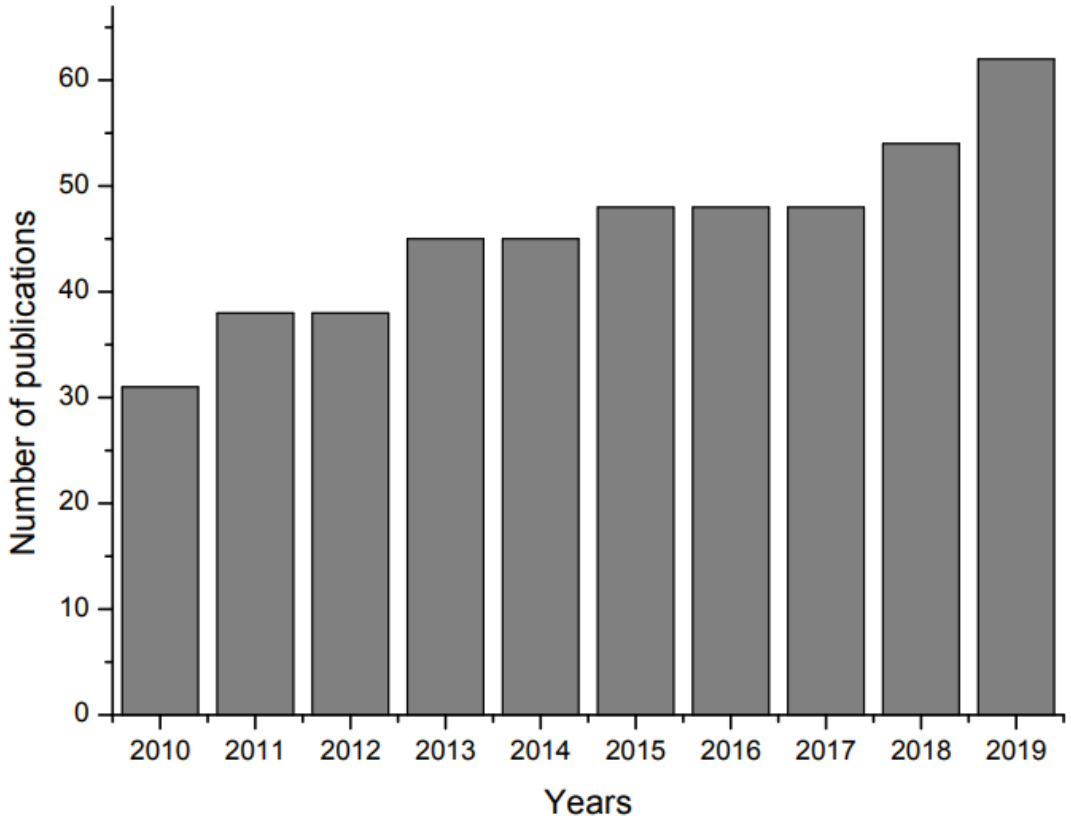


Figure S1. Annual distribution of all records retrieved through sugar fatty acid esters bibliographic searching, after duplicate removal.

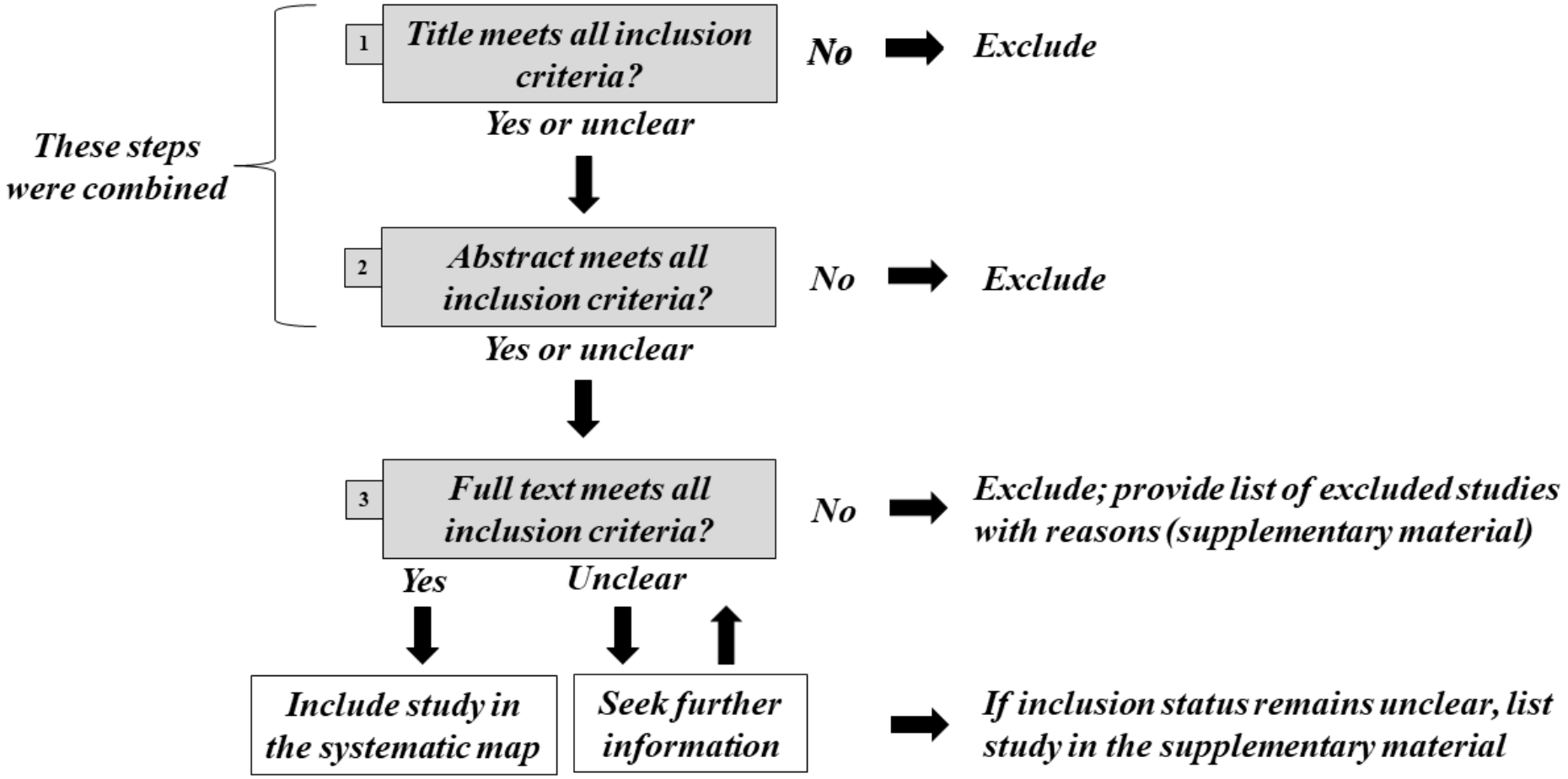
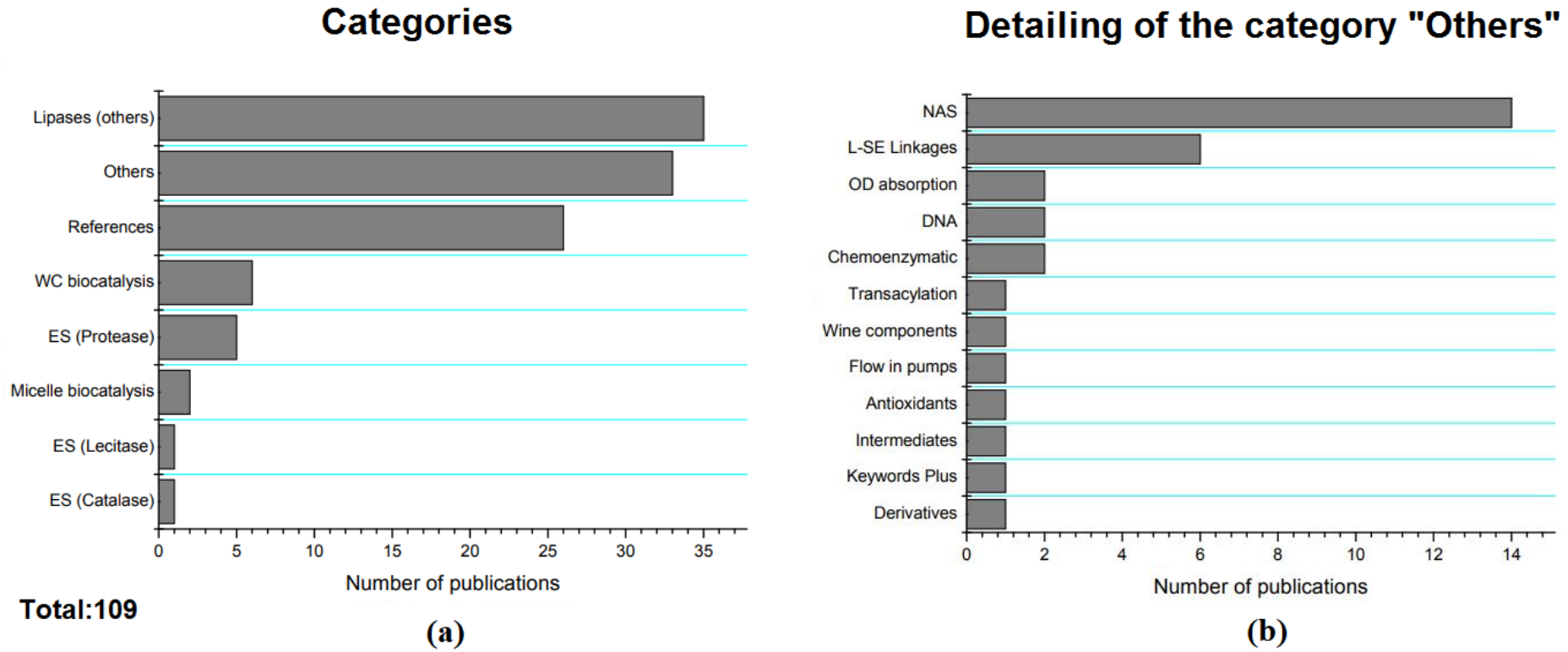


Figure S2. Stages of the literature screening process (Adapted from CEE, 2020).
Available at: http://www.environmentalevidence.org/guidelines/section-6).



Lipases (others): Enzymatic synthesis with Lipases from other sources (not Candida antarctica Lipase B); References: "Sugar esters / carbohydrate esters" appear only in article references, but are not the subject of the research; WC biocatalysis: Whole cells biocatalysis; ES (Protease): Enzymatic synthesis with Proteases; ES (Lecitase): Enzymatic synthesis with Lecitases; ES (Catalase): Enzymatic synthesis with Catalases;
In the category Others: NAS: Sugar esters (SEs) are not the article subject; L-SE Linkages: Cleavage or formation of Lignin–sugar ester linkages; OD absorption: SEs used to facilitate oral drug absorption; DNA: SEs used to promote interactions with DNA; Chemoenzymatic: SEs synthesis by chemoenzymatic routes; Transacylation: transacylation of methyl acrylate and methyl methacrylate with diols and triols; Intermediates: Synthesis of intermediates for SEs synthesis; Keywords Plus: SEs appear only in Keywords Plus, but are not the subject of the research; Derivatives: Sugar esters derivatives.

Figure S3. Records eliminated after full-text analysis (a) and detailing of the category “others” in subcategories (b).

Tables

Table S1. Search methodology for locating articles addressing the synthesis of sugar fatty acid esters. All searches were performed on May 2, 2020.

Bibliographic sources	**Search String**
Searched terms (2010-2019, Articles)	*"sugar* ester*" OR "sugar* fatty acid* ester*" OR "fatty acid* sugar* ester*" OR "carbohydrate* ester" OR "carbohydrate* esters" OR "carbohydrate* fatty acid* ester*" OR "fatty acid* carbohydrate* ester*"*
Web of Science	*Basic search:* TOPIC (Core collection: SCI-E, ESCI) ***292 records***
Scopus	*Basic search: TITLE-ABS-KEYWORDS* ***212 records***
PubMed	*Basic search: ALL FIELDS* ***153 records***
CAB Direct	*Basic search: ALL FIELDS* ***74 records***
SciELO	*Basic search: TOPIC (Citation Index)* ***No records***
	Total records: 731

Table S2. Records eliminated after title/abstract screening.

Categories	**Number of publications**
Antitumors agentes	12
Plant growth inhibitors	79
Freezing agentes	8
Reviews	74
Intestinal absorption agentes	15
Antimicrobial agentes	30
Chemical synthesis	14
Not synthesis methods	59
	Total: 291

Table S3. Interpretation of Kappa statistic.

Kappa	**Interpretation**
<0.0	No agreement
0.0 - 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

Fonte. OKWUASHI *et al*., 2012.

Table S4. Components and conditions of the reaction system for synthesis of carbohydrate esters and SEs conversions.

Acyl acceptors	Acyl donors	Solvents	Reaction Conditions	Biocatalyst	Conversions (%)	References
Mannose	Myristic acid	DMF; FOR; DMSO; PYR; T-but	60 °C, 48 h	Novozym 435	55	NOTT *et al*., 2012
Glucose	Vinyl esters	ACN; THF; DMF; DMSO; HEX	45 °C, 72 h	Immozyme CALB (ChiralVision)	88 -93	ARCENS *et al*., 2018
Glucose	Vinyl esters	2M2B	70 °C, 5 h	Novozym 435	64.5	LIN *et al*., 2016
Glucose; Xylose	Vinyl esters; Caprylic acid	DES	50 °C, 72 h	Novozym 435	4.8	SIEBENHALLER *et al*., 2017
Fructose	Oleic acid	T-but	55 °C, 54 h	Soluble CALB, Novozym 435	>70	VESCOVI *et al*., 2016
Xylose	Oleic acid, Lauric acid	T-but	55 °C, 48 h	Soluble CALB, CALB IM T2-350 (ChiralVision)	60	DE LIMA *et al*., 2016
Maltose	Vinyl esters	THF; PYR	45 °C, 36 h	Novozym 435	65-80	MA *et al*., 2018
Xylose; Arabinose	Vinyl esters	2M2B; HEX; THF	50 °C, 4 h	Novozym 435	57-75	MÉLINE *et al*., 2018
Sucrose	Oleic acid	T-but; SFS	65 °C, 240 h	Novozym 435	83	YE *et al*., 2014
Mannose	Vinyl esters	ACT	50 °C, 120 h	Novozym 435	30	JIA *et al*., 2018
Fructose	Lauric acid	IL; 2M2B	50 °C, 12 h	Novozym 435	27	LI *et al*., 2015a
Sugar derivatives	Palmitic acid	ACT; T-but	40 °C, 80 h	Chirazyme L-2; cf C2	50-70	KOBAYASHI *et al*., 2010
Glucose; Sucrose; Fructose	Vinyl esters	IL	40 °C, 12 h	Novozym 435	55	SHIN *et al*., 2019
Fructose	Palmitic acid	IL	60 °C, 14 h	Novozym 435	75	HA *et al*., 2010a
Glucose	Vinyl esters	PYR; THF; DMF; BEN; 2M2B; T-but	40 °C, 48 h	Novozym 435	47-57	LIANG *et al*., 2018
Fructose; Glucose	Vinyl esters	T-but	50 °C, 24 h	Novozym 435	34-99	PERIN and FELISBERTI, 2018
Mannose	Fluorinated acids	2M2B	80 °C, 24 h	Novozym 435	25-39	FAVRELLE *et al*., 2011
Rhamnose	Myristic acid	T-but	60 °C, 90 h	Novozym 435	25	NOTT *et al*., 2013

Maltose	Linoleic acid	IL; ACT; DMF; THF; ACN	65 °C, 72 h	Novozym 435	60	FISCHER *et al*., 2013
Sugar derivatives	Methyl hexanoate	DMSO; 2M2B	40°C, 48h	Novozym 435	NI	PÖHNLEIN *et al*., 2014
Sugar derivatives; Glucose	Vinyl esters	IL; 2M2B; DES	45°C, 24h	Novozym 435	22-30	ZHAO *et al*., 2016
Fructose; Sucrose; Lactose	Oleic acid	ETHA	40°C, 72h	Novozym 435; Lipozyme CALB L	55-84	NETA *et al*., 2012
Fructose	Lauric acid	MEK; 2M2B	40°C,12h	Novozym 435	27-54	LI *et al*., 2015b
Trehalose	Lauric acid; Ethyl laurate	SFS	110 °C, 24 h	Novozym 435	15.5	OGAWA *et al*., 2019
Glucose	Vinyl esters	IL	40 ◦C, 16 h	Novozym 435	75.8	LIN *et al*., 2015
Glucose	Vinyl esters; Lauric acid	DES	50°C, 12h	CV-CALBY (ChiralVision)	NI	ANDLER *et al*., 2017
Glucose; Xylose	FAMEs	DES	50°C, 70h	Novozym 435	NI	SIEBENHALLER *et al*., 2018a
Maltose	Myristic acid	T-but	60°C, 60h	Novozym 435	90	SUN *et al*., 2011
Glucose	Capric acid	T-but	55°C, 14.2h	Novozym 435	NI	HAN *et al*., 2011
Glucose	Vinyl esters	DES	70°C, 72h	Novozym 435	NI	PÖHNLEIN *et al*., 2015
Maltose; Glucose; Sorbitol; Xylitol	Oleic acid	ACT	NF, 42-72 h	Novozym 435	89-95	ZHANG *et al*., 2013
Glucose	Vinyl esters	IL	50°C, 48h	Novozym 435	95.5	CHANG *et al*., 2018
Honey; Agave syrup	Vinyl esters	HON; AS	50°C, 48h	Novozym 435	NI	SIEBENHALLER *et al*., 2018b
Mannose	Mercaptopropionic acid	2M2B	80°C, 24h	Novozym CRL	60	BOYÈRE *et al*., 2011
Xylose	Lauric acid; Stearic acid	MEK	60 ˚C, 72h	Novozym 435	67	BIDJOU-HAIOUR and KLAI, 2013
Glucose	Palmitic acid	IL	70°C, 48h	Novozym 435	71	FINDRIK *et al*., 2016
Sugar derivatives	Palmitic acid	HEP; TOL	50°C, 0,78 h	Fluka CALB	90.2	SUTILI *et al*., 2015
Glucose	Oleic acid	IL; T-but	60°C, 0.5h	Novozym 435	90	RAHMAN *et al*., 2012
Helicid	Vinyl esters	THF	40°C, 10h	Novozym 435	53.2	YANG *et al*., 2013
Caprolactone	MGP	T-but	60 °C, 48 h	Immozyme CALB (ChiralVision)	90	SAAT and MOHAMAD, 2019
Xylose	Capric acid	DMSO; ACT	60 °C, 24 h	Novozym 435	64	ABDULMALEK *et al*., 2016

Glucose	Lauric acid	IL	50 °C, 12 h	Novozym 435	25-59	HA *et al*., 2010b
Xylitol	Stearic acid	HEX	60 °C, 7 h	Novozym 435	96	ADNANI *et al*., 2010
Fructose; Lactose	Oleic acid; Lauric acid	T-but; 2M2B	45 or 55 °C, 72 h	Immozyme CALB (ChiralVision)	88-98	DE LIMA *et al*., 2018
Lactulose	Vinyl esters	2M2B; T-but; DMF	30 °C, 24 h	Novozym 435	87	FLORES *et al*., 2017
Sucrose	PHA	DMSO; CHL	50 °C, 24 h	Novozym 435	34	GUMEL *et al*., 2013
Sucrose; Fructose	Oleic acid	SFS	65 °C, 96 h	Novozym 435	81-83	YE *et al*., 2016
Mannose	Myristic acid	IL	NF	Novozym 435	64-70 (T), 29-44 (E)	GALONDE *et al*., 2013a
Maltose; Palatinose; Trehalose; Sucrose	Lauric acid	T-but; PYR	60 °C, 48 h	Chirazyme L-2; cf C2	NI	TAKAHASHI *et al*., 2012
Sugar derivatives	Lauric acid; Myristic acid; Palmitic acid	T-but	50 °C, 24 h	Novozym 435	NI	ARIFFIN *et al*., 2014
Arabinose	Palmitic acid	T-but	40-60 °C, ON	Novozym 435	6-20	PAPPALARDO *et al*., 2017
Lactose	Lauric acid; Palmitic acid; Caprylic acid	HEX; ACT; T-but; EA; THF; ACN	50 °C, 240 h	Novozym 435	77-93	ENAYATI *et al*., 2018
ATF; Kestose	Vinyl esters	HEX	60 °C, 96 h	Novozym 435	80	CASAS-GODOY *et al*., 2016
Sugar derivatives	Caprylic acid	ACT	50 °C, 12-72 h	IMM CALB (Roche Diagnostics)	45-55	KOBAYASHI *et al*., 2012
Glucose	N-fatty acyl glycine	THF; HEX	55 °C, 9 h	Novozym 435	60-78	AN *et al*., 2019
Sucrose	Lauric acid	2M2B; HEX; 2M2B; ISO	40 °C, 24 h	Novozym 435	NI	PUAT *et al*., 2010
Mannose	Vinyl esters	IL	80 °C, 24 h	Novozym 435	72.2	GALONDE *et al*., 2013b

ATF: Agave Tequilana Fructans; MGP: Methyl-D-glucopyranoside; PHA: bacterial polyhydroxyalkanoates; FAMEs: Fatty acid methyl esters; T-but: Tert-Butyl alcohol; IL: Ionic liquid; 2M2B: 2-Methyl-2-butanol; THF: Tetrahydrofuran; DMSO: Dimethyl sulfoxide; DES: Deep eutetic solvents; DMF: Dimethylformamide; ACN: Acetonitrile; SFS: Solvent-free system; MEK: Methyl Ethyl Ketone; PYR: Pyridine; FOR: Formamide; ACT: Acetone; HEX: Hexane; BUT: Butanol; ETHA: Ethanol; BEN: Benzene; HON: Honey; AS: Agave syrup; HEP: Heptane; TOL: Toluene; CHL: Chloroform; EA: Ethyl acetate; ISO: Isoocatane; NI: Not included; NF: Not found; ON: overnight; T: transesterification; E: esterification.

References

ABDULMALEK, E.; HAMIDON, N.F.; BASYARUDDIN, M.; RAHMAN, A. Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solventes. **Journal of Molecular Catalysis B: Enzymatic**, vol. 132, p. 1–4, 2016. DOI https://doi.org/10.1016/j.molcatb.2016.06.010.

ADNANI, A.; BASRI, M.; MALEK, E. A.; SALLEH, A. B.; RAHMAN, M. B. A.; CHAIBAKHSH, N.; RAHMAN, R. N. Z. R. A. Optimization of lipase-catalyzed synthesis of xylitol ester by Taguchi robust design method. **Industrial Crops and Products**, vol. 31, no. 2, p. 350-356, 2010. DOI: https://doi.org/10.1016/j.indcrop.2009.12.001.

AN, D.; ZHANG, X.; LIANG, F.; XIAN, M.; FENG, D.; YE, Z. Synthesis, surface properties of glucosyl esters from renewable materials for use as biosurfactants. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, vol. 577, p. 257-264, 2019. DOI https://doi.org/10.1016/j.colsurfa.2019.05.079.

ANDLER, S. M.; WANG, L. S. W.; ROTELLO, V. M.; GODDARD, J. M. Influence of hierarchical interfacial assembly on lipase stability and performance in deep eutectic solvent. **Journal of Agricultural and Food Chemistry**, vol. 65, 2017. DOI: https://doi.org/10.1021/acs.jafc.6b05372.

ARCENS, D.; GRAU, E.; GRELIER, S.; CRAMAIL, H.; PERUCH, F. 6-O-glucose palmitate synthesis with lipase: Investigation of some key parameters. **Molecular Catalysis**, vol. 460, p. 63–68, 2018. DOI https://doi.org/10.1016/j.mcat.2018.09.013.

ARIFFIN, M. F. K.; ANNUAR, M. S. M.; HEIDELBERG, T. Surfactant synthesis via lipase esterification of methyl α-d-glucopyranoside with selected aliphatic carboxylic acids. **Journal of Surfactants and Detergents**, vol. 17, no. 4, p. 683–692, 2013. DOI https://doi.org/10.1007/s11743-013-1529-2.

BIDJOU-HAIOUR, C.; KLAI, N. Lipase catalyzed synthesis of fatty acid xylose esters and their surfactant properties. **Asian Journal of Chemistry**, vol. 25, no. 8, p. 4347–4350, 2013. DOI https://doi.org/10.14233/ajchem.2013.13973.

BOYÈRE, C.; FAVRELLE, A.; BROZE, G.; LAURENT, P.; NOTT, K.; PAQUOT, M.; BLECKER, C.; JÉRÔME, C.; DEBUIGNE, A. Lipase catalysis and thiol-Michael addition: a relevant association for the synthesis of new surface-active carbohydrate esters. **Carbohydrate Research**, vol. 346, no. 14, p. 2121-2125, 2011. DOI: https://doi.org/10.1016/j.carres.2011.07.011.

CASAS-GODOY, L.; ARRIZON, A.; ARRIETA-BAEZ, D.; PLOU, F. J.; SANDOVAL, G. Synthesis and emulsifying properties of carbohydrate fatty acid esters produced from *Agave tequilana* fructans by enzymatic acylation. **Food Chemistry**, vol. 204, p. 437-443, 2016. DOI https://doi.org/10.1016/j.foodchem.2016.02.153.

CHANG, P.; ZHANG, Z.; TANG, S. Lipase-catalyzed synthesis of sugar ester in mixed biphasic system of ionic liquids and supercritical carbon dioxide. **Journal of the Chinese Chemical Society**, vol. 65, no. 4, p. 452–458, 2018. DOI https://doi.org/10.1002/jccs.201700278.

DE LIMA, L.; MENDES, A.; FERNANDEZ-LAFUENTE, R.; TARDIOLI, P.; GIORDANO, R. Performance of different immobilized lipases in the synthesis of short- and long-chain carboxylic acid esters by esterification reactions in organic media. **Molecules**, vol. 23, no. 4, p. 766, 2018. DOI https://doi.org/10.3390/molecules23040766.

DE LIMA, L. N.; VIEIRA, G. N. A.; KOPP, W.; TARDIOLI, P. W.; GIORDANO, R. L. C. Mono- and heterofunctionalized silica magnetic microparticles (SMMPs) as new carriers for immobilization of lipases, **Journal of Molecular Catalysis B: Enzymatic**, vol. 133, p. 491-499, 2016. DOI http://dx.doi.org/10.1016/j.molcatb.2017.04.002.

ENAYATI, M.; GONG, Y.; GODDARD, J. M.; ABBASPOURRAD, A. Synthesis and characterization of lactose fatty acid ester biosurfactants using free and immobilized lipases in organic solvents. **Food Chemistry**, vol. 266, p. 508–513, 2018. DOI http://dx.doi.org/10.1016/j.foodchem.2018.06.051.

FAVRELLE, A.; BOYÈRE, C.; LAURENT, P.; BROZE, G.; BLECKER, C.; PAQUOT, M.; JÉRÔME, C.; DEBUIGNE, A. Enzymatic synthesis and surface-active properties of novel hemifluorinated mannose esters. **Carbohydrate Research**, vol. 346, no. 9, p. 1161-1164, 2011. DOI: https://doi.org/10.1016/j.carres.2011.04.004.

FINDRIK, Z.; MEGYERI, G.; GUBICZA, L.; BÉLAFI-BAKÓ, K.; NEMESTÓTHY, N.; SUDAR, M. Lipase catalyzed synthesis of glucose palmitate in ionic liquid. **Journal of Cleaner Production**, vol. 112, p. 1106-1111, 2016. DOI: https://doi.org/10.1016/j.jclepro.2015.07.098.

FISCHER, F.; HAPPE, M.; EMERY, J.; FORNAGE, A.; SCHÜTZ, R. Enzymatic synthesis of 6- and 6′-O-linoleyl-α-d-maltose: From solvent-free to binary ionic liquid reaction media. **Journal of Molecular Catalysis B: Enzymatic**, vol. 90, p. 98-106, 2013. DOI: https://doi.org/10.1016/j.molcatb.2013.01.019.

FLORES, L. C.; BELTRAN, H.; ARRIETA-BAEZ, D.; REYES-DUARTE, D. Regioselective synthesis of lactulose esters by *Candida antarctica* and *Thermomyces lanuginosus* lipases. **Catalysts**, vol. 7, p. 263, 2017. DOI: https://doi.org/10.3390/catal7090263.

GALONDE, N.; NOTT, K.; RICHARD, G.; DEBUIGNE, A.; NICKS, F.; JEROME, C.; FAUCONNIER, M. L. Study of the influence of pure ionic liquids on the lipase-catalyzed (trans)esterification of mannose based on their anion and cation nature. **Current Organic Chemistry**, vol. 17, p. 763, 2013a. DOI: https://doi.org/10.2174/1385272811317070010.

GALONDE, N.; BROSTAUX, Y.; RICHARD, G.; NOTT, K.; JERÔME, C.; FAUCONNIER, M. L. Use of response surface methodology for the optimization of the lipase-catalyzed synthesis of mannosyl myristate in pure ionic liquid. **Process Biochemistry**, vol. 48, no. 12, p. 1914-1920, 2013b. DOI: https://doi.org/10.1016/j.procbio.2013.08.023.

GUMEL, A. M.; ANNUAR, S. M.; HEIDELBERG, T. Single-step lipase-catalyzed functionalization of medium-chain-length polyhydroxyalkanoates. **Journal of Chemical Technology and Biotechnology**, vol. 88, no. 7, p. 1328–1335, 2013. DOI https://doi.org/10.1002/jctb.3980.

HA, S. H.; HIEP, N. M.; KOO, Y. Enhanced production of fructose palmitate by lipase-catalyzed esterification in ionic liquids. **Biotechnology and Bioprocess Engineering**, vol. 15, p. 126–130, 2010a. DOI https://doi.org/10.1007/s12257-009-3091-3.

HA, S. H.; HIEP, N. M.; LEE, S. H.; KOO, Y. M. Optimization of lipase-catalyzed glucose ester synthesis in ionic liquids. **Bioprocess and Biosystems Engineering**, vol. 33, no. 1, p. 63-70, 2010b. DOI: https://doi.org/10.1007/s00449-009-0363-4.

HAN, J.; ANNUAR, M. S. M.; ARIFFIN, M. F. K.; GUMEL, A. M.; IBRAHIM, S.; HEIDELBERG, T.; SHARIFUDDIN, Y. Lipase-catalyzed synthesis of 6-O-D-glucosyldecanoate intert-butanol: reaction optimization and effect of mixing power input. **Biotechnology & Biotechnological Equipment**, vol. 25, no. 4, p. 2642–2651, 2011. DOI https://doi.org/10.5504/bbeq.2011.0092.

JIA, C.; WANG, H.; ZHANG, W.; ZHANG, X.; FENG, B. Efficient enzyme-selective synthesis of monolauryl mannose in a circulating fluidized bed reactor. **Process Biochemistry**, vol. 66, p. 28-32, 2018. DOI: https://doi.org/10.1016/j.procbio.2017.12.002.

KOBAYASHI, T., EHARA, T., MIZUOKA, T.; ADACHI, S. Efficient synthesis of 6-O-palmitoyl-1,2-O-isopropylidene-α-D-glucofuranose in an organic solvent system by

lipase-catalyzed esterification. **Biotechnology Letters**, vol. 32, p. 1679–1684, 2010. DOI https://doi.org/10.1007/s10529-010-0334-5.

KOBAYASHI, T.; TAKAHASHI, T.; ADACHI, S. Synthesis of 6-O-Octanoyl-1,2-O-isopropylidene-α-D-glucofuranose by lipase-catalyzed esterification in an organic solvent. **Journal of Oleo Science**, vol. 61, no. 2, p. 75–79, 2012. DOI https://doi.org/10.5650/jos.61.75.

LI, L.; JI, F.; WANG, J.; JIANG, B.; LI, Y.; BAO, Y. Efficient mono-acylation of fructose by lipase-catalyzed esterification in ionic liquid co-solvents. **Carbohydrate Research**, vol. 416, p. 51-58, 2015a. DOI https://doi.org/10.1016/j.carres.2015.08.009.

LI, L.; JI, F.; WANG, J.; LI, Y.; BAO, Y. Esterification degree of fructose laurate exerted by *Candida antarctica* lipase B in organic solvents. **Enzyme and Microbial Technology**, vol. 69, p. 46-53, 2015b. DOI https://doi.org/10.1016/j.enzmictec.2014.12.003.

LIANG, M. Y.; CHEN, Y.; BANWELL, M. G.; WANG, Y.; LAN, P. Enzymatic preparation of a homologous series of long-chain 6-o-acylglucose esters and their evaluation as emulsifiers. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 15, p. 3949–3956, 2018. DOI https://doi.org/10.1021/acs.jafc.8b00913.

LIN, X., ZHAO, K., ZHOU, Q.; XIE, K. Q.; HALLING, P. J.; YANG, Z. *Aspergillus oryzae* lipase-catalyzed synthesis of glucose laurate with excellent productivity. **Bioresources and Bioprocessing**, vol. 3, no. 1, 2016. DOI https://doi.org/10.1186/s40643-015-0080-6.

LIN, X. S.; WEN, Q.; HUANG, Z. L.; CAI, Y. Z.; HALLING, P. J.; YANG, Z. Impacts of ionic liquids on enzymatic synthesis of glucose laurate and optimization with superior productivity by response surface methodology. **Process Biochemistry**, vol. 50, no. 11, p. 1852–1858, 2015. DOI https://doi.org/10.1016/j.procbio.2015.07.019.

MA, Y. R.; BANWELL, M. G.; YAN, R.; LAN, P. Comparative study of the emulsifying properties of a homologous series of long-chain 6′-O-acylmaltose esters. **Journal of Agricultural and Food Chemistry**, vol. 66, no. 33, p. 8832–8840, 2018. DOI https://doi.org/10.1021/acs.jafc.8b02391.

MÉLINE, T.; MUZARD, M.; DELEU, M.; RAKOTOARIVONINA, H.; PLANTIER-ROYON, R.; RÉMOND, C. D-xylose and L-arabinose laurate esters: Enzymatic synthesis, characterization, and physico-chemical properties. **Enzyme and Microbial Technology**, vol. 112, p. 14–21, 2018. DOI https://doi.org/10.1016/j.enzmictec.2018.01.008.

NETA, N. A. S.; DOS SANTOS, J. C. S.; SANCHO, S. O.; RODRIGUES, S.; GONÇALVES, L. R. B.; RODRIGUES, L. R.; TEIXEIRA, J. A. Enzymatic synthesis of sugar esters and their potential as surface-active stabilizers of coconut milk emulsions. **Food Hydrocolloids**, vol. 27, no. 2, p. 324-331, 2012. DOI: https://doi.org/10.1016/j.foodhyd.2011.10.009.

NOTT, K.; BROGNAUX, A.; RICHARD, G.; LAURENT, P.; FAVRELLE, A.; JÉRÔME, C.; BLECKER, C.; WATHELET, J. P.; PAQUOT, M.; DELEU, M. (Trans)esterification of mannose catalyzed by lipase B from *Candida antarctica* in an improved reaction medium using co-solvents and molecular sieve. **Preparative Biochemistry & Biotechnology**, vol. 42, no. 4, p. 348-363, 2012. DOI https://doi.org/10.1080/10826068.2011.622330.

NOTT, K.; RICHARD, G.; LAURENT, P.; JÉRÔME, C.; BLECKER, C.; WATHELET, J. P.; PAQUOT, M.; DELEU, M. Enzymatic synthesis and surface properties of novel rhamnolipids. **Process Biochemistry**, vol. 48, no. 1, p. 133–143, 2013. DOI https://doi.org/10.1016/j.procbio.2012.11.019.

OGAWA, S.; ENDO, A.; KITAHARA, N.; YAMAGISHI, T.; AOYAGI, S.; HARA, S. Factors determining the reaction temperature of the solvent-free enzymatic synthesis of

trehalose esters. **Carbohydrate Research**, vol. 482, p. 107739, 2019. DOI: https://doi.org/10.1016/j.carres.2019.06.018.
OKWUASHI, O.; ISONG, M.; EYO, E.; EYOH, A.; NWANEKEZIE, O.; DUPE, N.; OLAYINKA, D. N.; UDOUDO, D.; OFEM, B. GIS cellular automata using artificial neural network for land use change simulation of Lagos, Nigeria. **Journal of Geography and Geology**, vol. 4, no. 2, 2012. DOI https://doi.org/10.5539/jgg.v4n2p94.
PAPPALARDO, V. M.; BOERIU, C. G.; ZACCHERIA, F.; RAVASIO, N. Synthesis and characterization of arabinose-palmitic acid esters by enzymatic esterification. **Molecular Catalysis**, vol. 433, p. 383–390, 2017. DOI https://doi.org/10.1016/j.mcat.2017.02.029.
PERIN, G. B.; FELISBERTI, M. I. Enzymatic synthesis and structural characterization of methacryloyl-D-fructose- and methacryloyl-D-glucose-based monomers and poly(methacryloyl-D-fructose)-based hydrogels. **Journal of Chemical Technology and Biotechnology**, vol. 93, no. 6, p. 1694-1704, 2018. DOI https://doi.org/10.1002/jctb.5542.
PÖHNLEIN, M.; SLOMKA, C.; KUKHARENKO, O.; GÄRTNER, T.; WIEMANN, L. O.; SIEBER, V.; SYLDATK, C.; HAUSMANN, R. Enzymatic synthesis of amino sugar fatty acid esters. **European Journal of Lipid Science and Technology**, vol. 116, no. 4, p. 423–428, 2014. DOI https://doi.org/10.1002/ejlt.201300380.
PÖHNLEIN, M., ULRICH, J., KIRSCHHÖFER, F., NUSSER, M., MUHLE-GOLL, C., KANNENGIESSER, B., WEIB, G. B.; LUY, B.; LIESE, A.; SYLDATK, C.; HAUSMANN, R. Lipase-catalyzed synthesis of glucose-6-O-hexanoate in deep eutectic solvents. **European Journal of Lipid Science and Technology**, vol. 117, no. 2, p. 161–166, 2015. DOI https://doi.org/10.1002/ejlt.201400459.
PUAT, S. B. A.; TAIB, M.; SUHAIMI, H. The catalytic properties of htab-alkanol-hydrocarbon-water microemulsion system for esterification. **Journal of Sustainability Science and Management**, vol. 5, no. 1, p. 87-93, 2010.
RAHMAN, M.; ARUMUGAM, M.; KHAIRUDDIN, N. S. K.; ABDULMALEK, E.; BASRI, M.; SALLEH, A. Microwave assisted enzymatic synthesis of fatty acid sugar ester in ionic liquid-tert-butanol biphasic solvent system. **Asian Journal of Chemistry**, vol. 24, p. 5058-5062, 2012.
SAAT, M. N.; ANNUAR, M. S. M. One-pot lipase-catalyzed esterification of ε-caprolactone with methyl-D-glucopyranoside and its elongation with free 6-hydroxyhexanoate monomer units. **Biotechnology and Applied Biochemistry**, 2019. DOI https://doi.org/10.1002/bab.1859.
SHIN, D. W.; MAI, N. L.; BAE, S. W.; KOO, Y. M. Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. **Enzyme and Microbial Technology**, vol. 126, p. 18-23, 2019. DOI: https://doi.org/10.1016/j.enzmictec.2019.03.004.
SIEBENHALLER, S; HAJEK, T; MUHLE-GOLL, C; HIMMELSBACH, M.; LUY, B.; KIRSCHHÖFER, F.; BRENNER-WEIß, G.; HAHN, T.; ZIBEK, S.; SYLDATK, C. Beechwood carbohydrates for enzymatic synthesis of sustainable glycolipids. **Bioresources and Bioprocessing**, vol. 4, no. 1, p. 25, 2017. DOI https://doi.org/10.1186/s40643-017-0155-7.
SIEBENHALLER, S.; KIRCHHOFF, J.; KIRSCHHÖFER, F.; BRENNER-WEIß, G.; MUHLE-GOLL, C.; LUY, B.; HAITZ, F.; HAHN, T.; ZIBEK, S.; SYLDATK, C.; OCHSENREITHER, K. Integrated process for the enzymatic production of fatty acid sugar esters completely based on lignocellulosic substrates. **Frontiers in Chemistry**, vol. 6, p. 421, 2018a. DOI: https://doi.org/10.3389/fchem.2018.00421.

SIEBENHALLER, S.; GENTES, J.; INFANTES, A.; MUHLE-GOLL, C.; KIRSCHHÖFER, F.; BRENNER-WEIß, G.; OCHSENREITHER, K.; SYLDATK, C. Lipase-catalyzed synthesis of sugar esters in honey and agave syrup. **Frontiers in Chemistry**, vol. 6, 2018b. DOI: https://doi.org/10.3389/fchem.2018.00024.

SUN, P.; CHEN, Y.; WANG, H.; LI, J.; GAO, J.; WANG, H.; ZHENG, X. Y,; ZHANG, S. Lipase-catalyzed synthesis and characterization of myristoyl maltose ester. **European Food Research and Technology**, vol. 233, no. 2, p. 253–258, 2011. DOI https://doi.org/10.1007/s00217-011-1490-0.

SUTILI, F. D.; NOGUEIRA, O.; LEITE, S.; MIRANDA, L.; DE SOUZA, R. Lipase immobilized in microemulsion based organogels (MBGs) as an efficient catalyst for continuous-flow esterification of protected fructose. **RSC Advances**, vol. 5, 2015. DOI https://doi.org/10.1039/c5ra04686f.

TAKAHASHI, T.; KOBAYASHI, T.; ADACHI, S. Surfactant properties of enzymatically synthesized monolauroyl disaccharides. **Food Science and Technology Research**, vol. 18, no. 2, p. 167–171, 2012. DOI https://doi.org/10.3136/fstr.18.167.

VESCOVI, V.; KOPP, W.; GUISÁN, J. M.; GIORDANO, R.; MENDES, A. A.; TARDIOLI, P. W. Improved catalytic properties of *Candida antarctica* lipase B multi-attached on tailor-made hydrophobic silica containing octil and multifunctional amino-glutaraldehyde spacer arms. **Process Biochemistry**, vol. 51, p. 2055-2066, 2016. DOI https://doi.org/10.1016/j.procbio.2016.09.016.

YANG, R.; ZHAO, X.; LIU, X. Novel and highly efficient regioselective route to helicid esters by Lipozyme TLL. **Plos One**, vol. 8, no. 11, p. 80715, 2013. DOI https://doi.org/10.1371/journal.pone.0080715.

YE, R.; HAYES, D.; BURTON, R.; LIU, A.; HARTE, F.; WANG, Y. Solvent-free lipase-catalyzed synthesis of technical-grade sugar esters and evaluation of their physicochemical and bioactive properties. **Catalysts**, vol. 6, no. 6, p. 78, 2016. https://doi.org/10.3390/catal6060078.

YE, R.; HAYES, D. G.; BURTON, R. Effects of particle size of sucrose suspensions and pre-incubation of enzymes on lipase-catalyzed synthesis of sucrose oleic acid esters. **Journal of the American Oil Chemists' Society**, vol. 91, p. 1891–1901, 2014. DOI https://doi.org/10.1007/s11746-014-2537-8.

ZHANG, H.; PENG, D.; WANG, Z.; YANG, Q.; SHEN, D.; HE, B. Lipase-catalyzed synthesis of oleic acid sugar ester and surface performance measurement. **Journal of the Chinese Cereals and Oils Association**, vol. 28, p. 109-113, 2013.

ZHAO, K. H.; CAI, Y. Z.; LIN, X. S.; XIONG, J.; HALLING, P. J.; YANG, Z. Enzymatic synthesis of glucose-based fatty acid esters in bisolvent systems containing ionic liquids or deep eutectic solvents. **Molecules**, vol. 21, no. 10, p. 1294, 2016. DOI https://doi.org/10.3390/molecules21101294.

A review on the production and recovery of sugars from lignocellulosics for use in the synthesis of bioproducts

Tables

Table S5. Search methodology for locating articles addressing the production of sugars from lignocellulosics.

Bibliographic sources	**Search String**
Searched terms (Articles)	*"lignocellulos*" AND ("sugar*" OR "carbohydrat*" OR "glycid*") AND "enzym* hydrol*" AND ("pre treat*" OR "pre-treat*")*
Web of Science	*Basic search:* TOPIC (Core collection: SCI-E, ESCI) ***177 records***
Scopus	*Basic search: TITLE-ABS-KEYWORDS* ***713 records***
CAB Direct	*Basic search: ALL FIELDS* ***83 records***
SciELO	*Basic search: TOPIC (Citation Index)* ***No records***
	Total records: 973

Table S6. Interpretation of Kappa statistic.

Kappa	**Interpretation**
<0.0	No agreement
0.0 - 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

Fonte. OKWUASHI *et al*., 2012.

Table S7. Components of the system for the obtaining of sugars from lignocellulosics and sugar-derived products.

Pretreatment methods	**Type**	**Enzymatic activities (hydrolysis)**	**Type**	**Sugar recovery methods**	**Type**	**Sugar-derived products**	**Microorganisms**	**References**
Alkaline	Chemical	Cellulase	Commercial	Washing	Physical separation	Ethanol	*Scheffersomyces shehatae* UFMG-HM 52.2	ANTUNES *et al*., 2018
H_2SO_4	Chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Centrifugation	Physical separation	GBSC	*Bacillus subtilis* CICC 10071	MA and XU, 2021
Alkaline	Chemical	Xylanase	Commercial	Washing, Drying	Physical separation	Biohydrogen	*Hypocrea lixii* SS1	SAKTHISELVAN *et al*., 2015
Milling	Physical	Cellulase, β-glucosidase	Crude	Centrifugation	Physical separation	Ethanol	*Pichia stipitis* BCC15191	BUABAN *et al*., 2010
SE	Physico-chemical	Cellulase, β-glucosidase	Commercial	NE	-	Biogas	NE	HORN *et al*., 2011
SE	Physico-chemical	Cellulase, β-glucosidase	Commercial	Filtration, Washing	Physical separation	Ethanol	*S. cerevisiae*	MANZANARES *et al*., 2012
Biological	Biological	Cellulase, β-glucosidase, Hemicellulase	Commercial	Centrifugation	Physical separation	Ethanol	*P. brevispora* NRRL-13108	SAHA *et al*., 2017
H_2SO_4	Chemical	Cellulase, β-glucosidase, Xylanase	Commercial	Filtration	Physical separation	Butanol, Ethanol	*S. cerevisiae* ATCC 96581, *Clostridium beijerinckii* B-592	NANDA *et al*., 2014
LHW	Physico-chemical	Cellulase, Hemicellulase	Commercial	Distillation, Precipitation, Evaporation, Filtration, Crystallization	Physical separation and purification	Ethanol, Xylitol	*S. cerevisiae*, *Pichia*, and *Candida*	FRANCESCHIN *et al*., 2011
H_2SO_4	Chemical	Cellulase, Hemicellulase	Commercial	Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* SyBE005	QIN *et al*., 2017
AFEX	Physico-chemical	Cellulase; Xylanase	Commercial	Centrifugation, Filtration	Physical separation	Ethanol	*S. cerevisiae* 424A	SHAO *et al*., 2010
AFEX	Physico-chemical	Cellulase, Amylase	Commercial	NE	-	Ethanol	NE	SHAO *et al*., 2013
LHW	Physico-chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Filtration, Washing	Physical separation	Ethanol	*Pachysolen tannophilus* ATCC 32691	CUEVAS *et al*., 2021

H_2SO_4	Chemical	Cellulase, β-glucosidase	Commercial	Detoxification	It can be both types	Ethanol	*S. cerevisiae*	MEHMOOD *et al*., 2009
H_2SO_4	Chemical	Cellulase, Glucanase, Hemicellulase, β-glucosidase	Commercial	Filtration, Washing	Physical separation	Lipids	*Rhodotorula mucilaginosa* 2406	AHMAD *et al*., 2016
Alkaline, H_2SO_4	Chemical	Cellulase	Commercial	Filtration, Washing	Physical separation	Ethanol	*S. cerevisiae* MTCC 11815, *P. tannophilus* NCIM 3502	JASPREET *et al*., 2019
UHP	Physico-chemical	Xylanase	Crude	Washing, Drying, Centrifugation	Physical separation	XOS	NA	SEESURIYACHAN *et al*., 2017
HC	Physico-chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	NE	-	Ethanol	*Scheffersomyces stipitis* NRRL-Y7124	HILARES *et al*., 2020
IGS	Chemical	Cellulase, Xylanase	Commercial	Filtration, Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* LPB 2705	VALLADARES-DIESTRA *et al*., 2021
Alkaline, H_2SO_4	Chemical	Cellulase, β-glucosidase	Commercial	Drying	Physical separation	Ethanol	*S. cerevisiae* SR8	SARATALE and OH, 2015
LHW, H_2SO_4, SE	Physico-chemical, Chemical	NE	NE	NE	-	Ethanol	*S. cerevisiae*, *Pichia stipitis*	OLIVEIRA *et al*., 2016
Alkaline, FO, FBC	Chemical	Glucanase, Pectinase, Hemicellulase, Xylanase, β-glucosidase	Commercial	Centrifugation, Filtration, Washing, Chromatography	Physical separation and purification	Biohydrogen	NE	KUCHARSKA *et al*., 2020
LHW	Physico-chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Drying	Physical separation	Ethanol	*S. cerevisiae* SA-1, *Pichia stipitis* NRRLY-7124	OLIVEIRA *et al*., 2021
EDA	Chemical	Cellulase	Commercial	Centrifugation, Washing, Drying	Physical separation	Lactic acid	*Bacillus coagulans* LA-15-2	CHEN *et al*., 2019
Alkaline, H_2SO_4	Chemical	Xylanase	Crude, Commercial	Filtration	Physical separation	Lactic acid	*Lactobacillus* spp.	WISCHRAL *et al*., 2019
Alkaline	Chemical	Cellulase, Cellobiase	Commercial	Filtration, Washing, Centrifugation	Physical separation	Ethanol	*S. cerevisiae* ATCC 24859	WANG and CHENG, 2011
SE, H_2SO_4, CaO	Physico-chemical, Chemical	Cellulase, Xylanase	Commercial	Drying, Centrifugation, Washing	Physical separation	Ethanol	*S. cerevisiae*	DENG *et al*., 2020

Alkaline, GI	Chemical, Physico-chemical	Cellulase, β-glucosidase, Hemicellulase, Xylanase	Commercial	Filtration, Washing, Drying	Physical separation	Lipids	*C. protothecoides* 25	JOE *et al*., 2015
H_2SO_4	Chemical	Glucanase, β-glucosidase, Xylanase	Crude	Centrifugation, Detoxification	Physical separation or both types	Ethanol	*S. cerevisiae* (MTCC 3089, DSM 1334), *Zymomonas mobilis* NCIM 5134, *Pichia stipitis* NCIM 3498, *C. shehatae* NCIM 3500	KAUSHAL *et al*., 2016
Alkaline, H_2SO_4	Chemical	Cellulase, β-glucosidase	Commercial	Detoxification, Filtration, Washing, Centrifugation	Physical separation or both types	Ethanol	*S. shehatae* UFMG HM 52.2	CHANDEL *et al*., 2014
Alkaline	Chemical	Cellulase	Commercial	NE	-	FAMEs	*Schizochytrium* sp. DT3	GUPTA *et al*., 2015
Alkaline	Chemical	Cellulase, Hemicellulase	Commercial, Crude	Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* ATCC 24858	KAMOLDEEN *et al*., 2017
SE, H_2SO_4	Physico-chemical, Chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Filtration	Physical separation	Ethanol	*S. cerevisiae* KE6-12.A	WANG *et al*., 2016
Alkaline	Chemical	Cellulase, Glucanase, Cellobiase	Commercial	Centrifugation, Filtration, Washing, Drying	Physical separation	1.3-propanediol, 2.3-butanediol	*K. pneumoniae* AJ4	HONG *et al*., 2015
SE, Alkaline	Physico-chemical, Chemical	Cellulase, Xylanase	Commercial	Washing	Physical separation	Ethanol	*Escherichia coli* KO11	YANG *et al*., 2014
Alkaline, IL	Chemical	Cellulase, Xylanase	Commercial	Washing, Drying, Centrifugation	Physical separation	Ethanol	*S. cerevisiae*, *Pichia stipitis*	ZHU *et al*., 2012
H_2SO_4, SE, AFEX, SAA, LHW, Alkaline	Chemical, Physico-chemical	Cellulase	Commercial	Washing	Physical separation	Ethanol	*Zymomonas mobilis*	TAO *et al*., 2011
SE	Physico-chemical	Cellulase, β-glucosidase	Commercial	Washing, Drying	Physical separation	Ethanol	*S. cerevisiae*	HIGERDAL *et al*., 1988
AFEX	Physico-chemical	Cellulase, Xylanase	Commercial	Drying, Centrifugation, Filtration	Physical separation	Ethanol	*Zymomonas mobilis* 2032	ZHANG *et al*., 2020
CH_3COOH	Chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Washing, Centrifugation, Drying	Physical separation	XOS	NA	WANG *et al*., 2021
Ultrasonic	Physico-chemical	Xylanase	Crude	NE	-	XOS	NA	YANG *et al*., 2011

SE, Alkaline, H_2SO_4	Physico-chemical, Chemical	Cellulase, Xylanase, Pectinase, Mannanase, Laccase	Crude	Washing, Drying, Filtration, Centrifugation	Physical separation	Ethanol	*S. cerevisiae*, *C. tropicalis*	SHANKAR *et al*., 2019
Alkaline, SAA	Chemical	Cellulase, β-glucosidase	Commercial	Filtration, Washing, Drying	Physical separation	Ethanol, Methane	*Pichia stipitis* CBS 6054	ZUO *et al*., 2012
H_2SO_4	Chemical	Cellulase	Commercial	Filtration, Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* CICC 31014	CAI *et al*., 2012
Alkaline	Chemical	Cellulase, Xylanase	Commercial	Filtration, Washing, Drying, Centrifugation	Physical separation	XOS	NA	LI *et al*., 2019
LHW	Physico-chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Drying, Evaporation, Centrifugation	Physical separation and purification	Ethanol	*S. cerevisiae* SA-1, *Scheffersomyces stipitis* NRRLY-7124	OLIVEIRA *et al*., 2020
H_2SO_4	Chemical	NE	NE	Filtration	Physical separation	Succinic acid	*Actinobacillus succinogenes* ATCC 55618	SALVACHÚA *et al*., 2016
H_2SO_4, Alkaline	Chemical	Cellulase, Amylase, Glucoamylase	Commercial	Filtration, Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* JP1, *Scheffersomyces stipitis* CBS5774	BARCELOS *et al*., 2016
H_2SO_4	Chemical	Cellulase, β-glucosidase, Hemicellulase, Xylanase	Commercial	Washing, Drying, Centrifugation	Physical separation	Butanol	*Clostridium acetobutylicum* NRRL B-591	FARMANBORDAR *et al*., 2020
SE, Alkaline	Physico-chemical, Chemical	Cellulase, β-glucosidase, Hemicellulase Xylanase,	Commercial	Washing	Physical separation	Ethanol	*S. cerevisiae*, *Pichia stipitis*	CHU *et al*., 2018
Milling	Physical	Cellulase, Glucanase, Pectinase, Mannanase, Xyloglucanase, Laminarase, β-glucosidase, β-xylosidase, *αL*-	Commercial	NE	-	Lactic acid	Several *Lactobacillus* strains	CIZEIKIENE *et al*., 2018

IL	Chemical	*arbf*, Amylase, Protease	Commercial	Centrifugation, Washing, Drying	Physical separation	Lipids	*Trichosporon fermentans* CICC 1368	REN *et al*., 2016
LHW, Alkaline, H_2SO_4	Physico-chemical, Chemical	Cellulase, β-glucosidase, Xylanase	Commercial	Washing, Drying, Centrifugation, Filtration	Physical separation	Ethanol	*S. cerevisiae* UFLA CA11, *S. cerevisiae* CAT-1, *Kluyveromyces marxianus* ATCC-36907	DA SILVA *et al*., 2018
H_2SO_4, HCl, HNO_3, CH_3COOH, EDTA	Chemical	Cellulase, β-glucosidase, Hemicellulase, Glucanase	Commercial	Filtration, Washing, Drying, Centrifugation	Physical separation	Ethanol	*P. stipitis* DSM 3651, *S. cerevisiae*	MENDES *et al*., 2014
H_2SO_4, Alkaline	Chemical	Cellulase	Crude	Washing, Detoxification	Physical separation or both types	Ethanol	*Pichia stipitis* NCIM 3499, *S. cerevisae* HAU	KAUR and KUHAD, 2019
H_2SO_4	Chemical	NE	NE	Detoxification	It can be both types	Xylitol	*Candida guilliermondii* FTI 20037	MORAES *et al*., 2020
Alkaline	Chemical	Glucanase, Hemicellulase, β-glucosidase, Xylanase, Pectinase	Commercial	Drying, Centrifugation, Washing, Drying	Physical separation	Ethanol	*S. cerevisiae* Y35	BANERJEE *et al*., 2012
SE	Physico-chemical	Cellulase, β-glucosidase, Hemicellulase	Commercial	Centrifugation	Physical separation	Ethanol, Xylitol	*S. cerevisiae* KE6-12	KOPPRAM *et al*., 2013

H_2SO_4: Sulfuric acid acidolysis; SE: Steam explosion; LHW: Liquid hot water; AFEX: Ammonia fibre expansion; IL: Ionic liquid; AHP: Alkaline hydrogen peroxide; SWH: Subcritical water hydrolysis; SAA: Soaking in aqueous ammonia; CH_3COOH: Acetic acid acidolysis; SO_2: Sulfur dioxide-impregnated steam explosion; UHP: Ultra-high pressure; HC: Hydrodynamic cavitation; IGS: Imadazole green solvent; FO: Fenton oxidation; FBC: Fenton-bipyridine catalyzed; EDA: Ethylenediamine; CaO: Calcium oxide; GI: Gamma irradiation; SPORL: Sulfite pretreatment to overcome recalcitrance of lignocelluloses; HCl: hydrochloric acid acidolysis; HNO_3: Nitric acid acidolysis; EDTA: Ethylenediaminetetra acetic acid; αL-arbf: αL-arabinofuranosidase; XOS: Xylooligosaccharides; GBSC: Growth of Bacillus subtilis cells; FAMEs: fatty acid methyl esters; NE: Not specified; NA: not applied.

References

AHMAD, F. B.; ZHANG, Z.; DOHERTY, W. O. S.; O'HARA, I. M. Evaluation of oil production from oil palm empty fruit bunch by oleaginous micro-organisms. **Biofuels, Bioproducts & Biorefining**, vol. 10, no. 4, p. 378–392, 2016. DOI https://doi.org/10.1002/bbb.1645.

ANTUNES, F. A. F.; CHANDEL, A. K.; BRUMANO, L. P.; HILARES, R. T.; PERES, G. F. D.; AYABE, L. E. S.; SORATO, V. S.; SANTOS, J. R.; SANTOS, J. C.; DA SILVA, S. S. A novel process intensification strategy for second-generation ethanol production from sugarcane bagasse in fluidized bed reactor. **Renewable Energy**, vol. 124, p. 189–196, 2018. DOI https://doi.org/10.1016/j.renene.2017.06.004.

BANERJEE, G.; CAR, S.; LIU, T.; WILLIAMS, D. L.; MEZA, S. L.; WALTON, J. D.; HODGE, D. B. Scale-up and integration of alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis, and ethanolic fermentation. **Biotechnology and Bioengineering**, vol. 109, no. 4, p. 922–931, 2012. DOI https://doi.org/10.1002/bit.24385.

BARCELOS, C. A.; MAEDA, R. N.; ANNA, L. M. M. S.; PEREIRA, N. Sweet sorghum as a whole-crop feedstock for ethanol production. **Biomass and Bioenergy**, vol. 94, p. 46–56, 2016. DOI https://doi.org/10.1016/j.biombioe.2016.08.012.

BUABAN, B.; INOUE, H.; YANO, S.; TANAPONGPIPAT, S.; RUANGLEK, V.; CHAMPREDA, V.; PICHYANGKURA, R.; RENGPIPAT, S.; EURWILAICHITR, L. Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. **Journal of Bioscience and Bioengineering**, vol. 110, no. 1, p. 18–25, 2010. DOI https://doi.org/10.1016/j.jbiosc.2009.12.003.

CAI, B. Y.; GE, J. P.; LING, H. Z.; CHENG, K. K.; PING, W. X. Statistical optimization of dilute sulfuric acid pretreatment of corncob for xylose recovery and ethanol production. **Biomass and Bioenergy**, vol. 36, p. 250–257, 2012. DOI https://doi.org/10.1016/j.biombioe.2011.10.023.

CHANDEL, A. K.; ANTUNES, F. A.; ANJOS, V.; BELL, M. J. V; RODRIGUES, L. N.; POLIKARPOV, I.; DE AZEVEDO, E. R.; BERNARDINELLI, O. D.; ROSA, C. A.; PAGNOCCA, F. C.; DA SILVA, S. S. Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid-base pretreatment and ethanol production by *Scheffersomyces shehatae* and *Saccharomyces cerevisiae*. **Biotechnology for Biofuels**, vol. 7, p. 63, 2014. DOI https://doi.org/https://doi.org/10.1186/1754-6834-7-63.

CHEN, H.; HUO, W.; WANG, B.; WANG, Y.; WEN, H.; CAI, D.; ZHANG, C.; WU, Y.; QIN, P. L-lactic acid production by simultaneous saccharification and fermentation of dilute ethylediamine pre-treated rice straw. **Industrial Crops and Products**, vol. 141, 2019. DOI https://doi.org/10.1016/j.indcrop.2019.111749.

CHU, Q.; SONG, K.; BU, Q.; HU, J.; LI, F.; WANG, J.; CHEN, X.; SHI, A. Two-stage pretreatment with alkaline sulphonation and steam treatment of *Eucalyptus woody* biomass to enhance its enzymatic digestibility for bioethanol production. **Energy Conversion and Management**, vol. 175, p. 236–245, 2018. DOI https://doi.org/10.1016/j.enconman.2018.08.100.

CIZEIKIENE, D.; JUODEIKIENE, G.; DAMASIUS, J. Use of wheat straw biomass in production of L-lactic acid applying biocatalysis and combined lactic acid bacteria strains belonging to the genus *Lactobacillus*. **Biocatalysis and Agricultural Biotechnology**, vol. 15, p. 185–191, 2018. DOI https://doi.org/10.1016/j.bcab.2018.06.015.

CUEVAS, M.; GARCÍA MARTÍN, J. F.; BRAVO, V.; SÁNCHEZ, S. Ethanol production from olive stones through liquid hot water pre-treatment, enzymatic hydrolysis and fermentation. Influence of enzyme loading, and pretreatment temperature

and time. **Fermentation**, vol. 7, no. 1, 2021. DOI https://doi.org/10.3390/fermentation7010025.
DA SILVA, F. L.; CAMPOS, A. O.; DOS SANTOS, D. A.; MAGALHÃES, E. R. B.; DE MACEDO, G. R.; DOS SANTOS, E. S. Valorization of an agroextractive residue—Carnauba straw—for the production of bioethanol by simultaneous saccharification and fermentation (SSF). **Renewable Energy**, vol. 127, p. 661–669, 2018. DOI https://doi.org/10.1016/j.renene.2018.05.025.
DENG, J.; ZHU, X.; CHEN, P.; HE, B.; TANG, S. W.; ZHAO, W.; LI, X.; ZHANG, R.; LV, Z.; KANG, H.; YU, L.; PENG, L. Mechanism of lignocellulose modification and enzyme disadsorption for complete biomass saccharification to maximize bioethanol yield in rapeseed stalks. **Sustainable Energy and Fuels**, vol. 4, no. 2, p. 607–618, 2020. DOI https://doi.org/10.1039/c9se00906j.
FARMANBORDAR, S.; AMIRI, H.; KARIMI, K. Synergy of municipal solid waste co-processing with lignocellulosic waste for improved biobutanol production. **Waste Management**, vol. 118, p. 45–54, 2020. DOI https://doi.org/10.1016/j.wasman.2020.07.053.
FRANCESCHIN, G.; SUDIRO, M.; INGRAM, T.; SMIRNOVA, I.; BRUNNER, G.; BERTUCCO, A. Conversion of rye straw into fuel and xylitol: A technical and economical assessment based on experimental data. **Chemical Engineering Research and Design**, vol. 89, no. 6, p. 631–640, 2011. DOI https://doi.org/10.1016/j.cherd.2010.11.001.
GUPTA, A.; ABRAHAM, R. E.; BARROW, C. J.; PURI, M. Omega-3 fatty acid production from enzyme saccharified hemp hydrolysate using a novel marine thraustochytrid strain. **Bioresource Technology**, vol. 184, p. 373–378, 2015. DOI https://doi.org/10.1016/j.biortech.2014.11.031.
HIGERDAL, B.; TJERNELD, F.; ZACCHI, G. Production of ethanol from lignocellulosic materials. **Animal Feed Science and Technology**, vol. 21, p. 175–182, 1988. DOI https://doi.org/10.1016/0377-8401(88)90098-3.
HIGGINS, J. P. T. **Handbook for Systematic Reviews of Interventions**. Version 5.1.0. The Cochrane Collaboration, 2011.
HILARES, R. T.; DIONÍZIO, R. M.; MUÑOZ, S. S.; PRADO, C. A.; DE SOUSA JÚNIOR, R.; DA SILVA, S. S.; SANTOS, J. C. Hydrodynamic cavitation-assisted continuous pre-treatment of sugarcane bagasse for ethanol production: Effects of geometric parameters of the cavitation device. **Ultrasonics Sonochemistry**, vol. 63, p. 104931, 2020. DOI https://doi.org/10.1016/j.ultsonch.2019.104931.
HONG, E.; KIM, D.; KIM, J.; KIM, J.; YOON, S.; RHIE, S.; HA, S.; RYU, Y. Optimization of alkaline pretreatment on corn stover for enhanced production of 1.3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae* AJ4. **Biomass and Bioenergy**, vol. 77, p. 177–185, 2015. DOI https://doi.org/10.1016/j.biombioe.2015.03.016.
HORN, S. J.; ESTEVEZ, M. M.; NIELSEN, H. K.; LINJORDET, R.; EIJSINK, V. G. H. Biogas production and saccharification of Salix pretreated at different steam explosion conditions. **Bioresource Technology**, vol. 102, no. 17, p. 7932–7936, 2011. DOI https://doi.org/10.1016/j.biortech.2011.06.042.
JASPREET, K.; TAGGAR, M. S.; KOCHER, G. S. Fermentation of enzymatically saccharified rice straw hydrolysate for bioethanol production. **Research Journal of Biotechnology**, vol. 14, no. 12, p. 98–103, 2019.
JOE, M. H.; KIM, J. Y.; LIM, S.; KIM, D. H.; BAI, S.; PARK, H.; LEE, S. G.; HAN, S. J.; CHOI, J. I. Microalgal lipid production using the hydrolysates of rice straw pretreated with gamma irradiation and alkali solution. **Biotechnology for Biofuels**, vol. 8, no. 1, 2015. DOI https://doi.org/10.1186/s13068-015-0308-x.

KAMOLDEEN, A. A.; LEE, C. K.; WAN ABDULLAH, W. N.; LEH, C. P. Enhanced ethanol production from mild alkali-treated oil-palm empty fruit bunches via co-fermentation of glucose and xylose. **Renewable Energy**, vol. 107, p. 113–123, 2017. DOI https://doi.org/10.1016/j.renene.2017.01.039.

KAUR, A.; KUHAD, R. C. Valorization of rice straw for ethanol production and lignin recovery using combined acid-alkali pre-treatment. **Bioenergy Research**, vol. 12, no. 3, p. 570–582, 2019. DOI https://doi.org/10.1007/s12155-019-09988-3.

KAUSHAL, R.; SHARMA, N.; DOGRA, V. Molecular characterization of glycosyl hydrolases of *Trichoderma harzianum* WF5 - A potential strain isolated from decaying wood and their application in bioconversion of poplar wood to ethanol under separate hydrolysis and fermentation. **Biomass and Bioenergy**, vol. 85, p. 243–251, 2016. DOI https://doi.org/10.1016/j.biombioe.2015.12.010.

KOPPRAM, R.; NIELSEN, F.; ALBERS, E.; LAMBERT, A.; WÄNNSTRÖM, S.; WELIN, L.; ZACCHI, G.; OLSSON, L. Simultaneous saccharification and co-fermentation for bioethanol production using corncobs at lab, PDU and demo scales. **Biotechnology for Biofuels**, vol. 6, no. 1, 2013. DOI https://doi.org/10.1186/1754-6834-6-2.

KUCHARSKA, K.; RYBARCZYK, P.; HOŁOWACZ, I.; KONOPACKA-ŁYSKAWA, D.; SŁUPEK, E.; MAKOŚ, P.; CIEŚLIŃSKI, H.; KAMIŃSKI, M. Influence of alkaline and oxidative pre-treatment of waste corn cobs on biohydrogen generation efficiency via dark fermentation. **Biomass and Bioenergy**, vol. 141, 2020. DOI https://doi.org/10.1016/j.biombioe.2020.105691.

LI, H.; CHEN, X.; XIONG, L.; LUO, M.; CHEN, X.; WANG, C.; HUANG, C.; CHEN, X. Stepwise enzymatic hydrolysis of alkaline oxidation treated sugarcane bagasse for the co-production of functional xylo-oligosaccharides and fermentable sugars. **Bioresource Technology**, vol. 275, p. 345–351, 2019. DOI https://doi.org/10.1016/j.biortech.2018.12.063.

MA, X.; XU, Y. A remixed-fermentation technique for the simultaneous bioconversion of corncob C6 and C5 sugars to probiotic *Bacillus subtilis*. **Applied Biochemistry and Biotechnology**, vol. 193, no. 8, p. 2580–2590, 2021. DOI https://doi.org/10.1007/s12010-021-03560-6.

MANZANARES, P.; BALLESTEROS, I.; NEGRO, M. J.; OLIVA, J. M.; GONZALEZ, A.; BALLESTEROS, M. Biological conversion of forage sorghum biomass to ethanol by steam explosion pretreatment and simultaneous hydrolysis and fermentation at high solid content. **Biomass Conversion and Biorefinery**, vol. 2, no. 2, p. 123–132, 2012. DOI https://doi.org/10.1007/s13399-012-0040-8.

MEHMOOD, S.; GULFRAZ, M.; FATIMA RANA, N.; AHMAD, A.; KIAER AHRING, B.; MINHAS, N.; MALIK, M. F. Ethanol production from sorghum bicolor using both separate and simultaneous saccharification and fermentation in batch and fed batch systems. **African Journal of Biotechnology**, vol. 8, no. 12, p. 2857–2865, 2009. DOI http://www.academicjournals.org/AJB.

MENDES, C. V. T.; ROCHA, J. M. S.; CARVALHO, M. G. V. S. Valorization of residual streams from pulp and paper mills: Pretreatment and bioconversion of primary sludge to bioethanol. **Industrial and Engineering Chemistry Research**, vol. 53, no. 50, p. 19398–19404, 2014. DOI https://doi.org/10.1021/ie503021y.

MORAES, E. J. C.; SILVA, D. D. V.; DUSSÁN, K. J.; TESCHE, L. Z.; SILVA, J. B. A.; RAI, M.; FELIPE, M. G. A. Xylitol-sweetener production from barley straw: optimization of acid hydrolysis condition with the energy consumption simulation. **Waste and Biomass Valorization**, vol. 11, no. 5, p. 1837–1849, 2020. DOI https://doi.org/10.1007/s12649-018-0501-9.

NANDA, S.; DALAI, A. K.; KOZINSKI, J. A. Butanol and ethanol production from lignocellulosic feedstock: Biomass pretreatment and bioconversion. **Energy Science and Engineering**, vol. 2, no. 3, p. 138–148, 2014. DOI https://doi.org/10.1002/ese3.41.

OKWUASHI, O.; ISONG, M.; EYO, E.; EYOH, A.; NWANEKEZIE, O.; OLAYINKA, D. N.; UDOUDO, D. O.; OFEM, B. GIS cellular automata using artificial neural network for land use change simulation of Lagos, Nigeria. **Journal of Geography and Geology**, vol. 4, no. 2, p. 94–101, 2012. DOI https://doi.org/10.5539/jgg.v4n2p94.

OLIVEIRA, C. M.; CRUZ, A. J. G.; COSTA, C. B. B. Improving second generation bioethanol production in sugarcane biorefineries through energy integration. **Applied Thermal Engineering**, vol. 109, p. 819–827, 2016. DOI https://doi.org/10.1016/j.applthermaleng.2014.11.016.

OLIVEIRA, T. C. G.; HANLON, K. E.; INTERLANDI, M. A.; TORRES-MAYANGA, P. C.; SILVELLO, M. A. C.; LACHOS-PEREZ, D.; TIMKO, M. T.; ROSTAGNO, M. A.; GOLDBECK, R.; FORSTER-CARNEIRO, T. Subcritical water hydrolysis pretreatment of sugarcane bagasse to produce second generation ethanol. **Journal of Supercritical Fluids**, vol. 164, 2020. DOI https://doi.org/10.1016/j.supflu.2020.104916.

OLIVEIRA, T. C. G.; INTERLANDI, M. A.; HANLON, K. E.; TORRES-MAYANGA, P. C.; SILVELLO, M. A. C.; TIMKO, M. T.; ROSTAGNO, M. A.; GOLDBECK, R.; FORSTER-CARNEIRO, T. Integration of subcritical water and enzymatic hydrolysis to obtain fermentable sugars and second-generation ethanol from sugarcane straw. **Bioenergy Research**, 2021. DOI https://doi.org/10.1007/s12155-021-10274-4.

QIN, L.; LI, X.; LIU, L.; ZHU, J. Q.; GUAN, Q. M.; ZHANG, M. T.; LI, W. C.; LI, B. Z.; YUAN, Y. J. Dual effect of soluble materials in pretreated lignocellulose on simultaneous saccharification and co-fermentation process for the bioethanol production. **Bioresource Technology**, vol. 224, p. 342–348, 2017. DOI https://doi.org/10.1016/j.biortech.2016.11.106.

REN, H.; ZONG, M. H.; WU, H.; LI, N. Utilization of seawater for the biorefinery of lignocellulosic biomass: ionic liquid pretreatment, enzymatic hydrolysis, and microbial lipid production. **ACS Sustainable Chemistry and Engineering**, vol. 4, no. 10, p. 5659–5666, 2016. DOI https://doi.org/10.1021/acssuschemeng.6b01562.

SAHA, B. C.; KENNEDY, G. J.; QURESHI, N.; COTTA, M. A. Biological pretreatment of corn stover with *Phlebia brevispora* NRRL-13108 for enhanced enzymatic hydrolysis and efficient ethanol production. **Biotechnology Progress**, vol. 33, no. 2, p. 365–374, 2017. DOI https://doi.org/10.1002/btpr.2420.

SAKTHISELVAN, P.; NAVEENA, B.; GOPINATH, K. P.; PARTHA, N. Bio-hydrogen production by enzymic hydrolysis of sunflower oil sludge. **Clean - Soil, Air, Water**, vol. 43, no. 11, p. 1547–1555, 2015. DOI https://doi.org/10.1002/clen.201300765.

SALVACHÚA, D.; MOHAGHEGHI, A.; SMITH, H.; BRADFIELD, M. F. A.; NICOL, W.; BLACK, B. A.; BIDDY, M. J.; DOWE, N.; BECKHAM, G. T. Succinic acid production on xylose-enriched biorefinery streams by *Actinobacillus succinogenes* in batch fermentation. **Biotechnology for Biofuels**, vol. 9, no. 1, 2016. DOI https://doi.org/10.1186/s13068-016-0425-1.

SARATALE, G. D.; OH, M. K. Improving alkaline pretreatment method for preparation of whole rice waste biomass feedstock and bioethanol production. **RSC Advances**, vol. 5, no. 118, p. 97171–97179, 2015. DOI https://doi.org/10.1039/c5ra17797a.

SEESURIYACHAN, P.; KAWEE-AI, A.; CHAIYASO, T. Green and chemical-free process of enzymatic xylooligosaccharide production from corncob: Enhancement of the yields using a strategy of lignocellulosic destructuration by ultra-high pressure pretreatment. **Bioresource Technology**, vol. 241, p. 537–544, 2017. DOI https://doi.org/10.1016/j.biortech.2017.05.193.

SHANKAR, K.; KULKARNI, N. S.; JAYALAKSHMI, S. K.; SREERAMULU, K. Saccharification of the pretreated husks of corn, peanut and coffee cherry by the lignocellulolytic enzymes secreted by *Sphingobacterium sp*. ksn for the production of bioethanol. **Biomass and Bioenergy**, vol. 127, 2019. DOI https://doi.org/10.1016/j.biombioe.2019.105298.

SHAO, Q; ZHU, L.; LIU, J. Enzymatic hydrolysis and fermentability of AFEX pretreated whole crop plants for ethanol. **Acta Energia e Solaris Sinica**, vol. 34, no. 6, p. 1097–1102, 2013.

SHAO, Q.; CHUNDAWAT, S. P.; KRISHNAN, C.; BALS, B.; SOUSA, L. D. C.; THELEN, K. D.; DALE, B. E.; BALAN, V. Enzymatic digestibility and ethanol fermentability of AFEX-treated starch-rich lignocellulosics such as corn silage and whole corn plant. **Biotechnology for Biofuels**, vol. 3, p. 12, 2010. DOI https://doi.org/10.1186/1754-6834-3-12.

TAO, L.; ADEN, A.; ELANDER, R. T.; PALLAPOLU, V. R.; LEE, Y. Y.; GARLOCK, R. J.; BALAN, V.; DALE, B. E.; KIM, Y.; MOSIER, N. S.; LADISCH, M. R.; FALLS, M.; HOLTZAPPLE, M. T.; SIERRA, R.; SHI, J.; EBRIK, M. A.; REDMOND, T.; YANG, B.; WYMAN, C. E.; HAMES, B.; THOMAS, S.; WARNER, R. E. Process and technoeconomic analysis of leading pretreatment technologies for lignocellulosic ethanol production using switchgrass. **Bioresource Technology**, vol. 102, no. 24, p. 11105–11114, 2011. DOI https://doi.org/10.1016/j.biortech.2011.07.051.

VALLADARES-DIESTRA, K. K.; VANDENBERGHE, L. P. S.; TORRES, L. A. Z.; NISHIDA, V. S.; ZANDONÁ FILHO, A.; WOICIECHOWSKI, A. L.; SOCCOL, C. R. Imidazole green solvent pre-treatment as a strategy for second-generation bioethanol production from sugarcane bagasse. **Chemical Engineering Journal**, vol. 420, no. 2, p. 127708, 2021. DOI https://doi.org/10.1016/j.cej.2020.127708.

WANG, J.; XU, Y.; MENG, X.; PU, Y.; RAGAUSKAS, A.; ZHANG, J. Production of xylo-oligosaccharides from poplar by acetic acid pretreatment and its impact on inhibitory effect of poplar lignin. **Bioresource Technology**, vol. 323, 2021. DOI https://doi.org/10.1016/j.biortech.2020.124593.

WANG, R.; UNREAN, P.; FRANZÉN, C. J. Model-based optimization and scale-up of multi-feed simultaneous saccharification and co-fermentation of steam pre-treated lignocellulose enables high gravity ethanol production. **Biotechnology for Biofuels**, vol. 9, no. 1, 2016. DOI https://doi.org/10.1186/s13068-016-0500-7.

WANG, Z.; CHENG, J. J. Lime pretreatment of coastal bermudagrass for bioethanol production. **Energy and Fuels**, vol. 25, no. 4, p. 1830–1836, 2011. DOI https://doi.org/10.1021/ef2000932.

WISCHRAL, D.; ARIAS, J. M.; MODESTO, L. F.; PASSOS, D. F.; PEREIRA, N. Lactic acid production from sugarcane bagasse hydrolysates by *Lactobacillus pentosus*: Integrating xylose and glucose fermentation. **Biotechnology Progress**, vol. 35, no. 1, 2019. DOI https://doi.org/10.1002/btpr.2718.

YANG, B. Y.; CHENG, M. H.; KO, C. H.; WANG, Y. N.; CHEN, W. H.; HWANG, W. S.; YANG, Y. P.; CHEN, H. T.; CHANG, F. C. Potential bioethanol production from Taiwanese chenopods (*Chenopodium formosanum*). **Energy**, vol. 76, p. 59–65, 2014. DOI https://doi.org/10.1016/j.energy.2014.03.046.

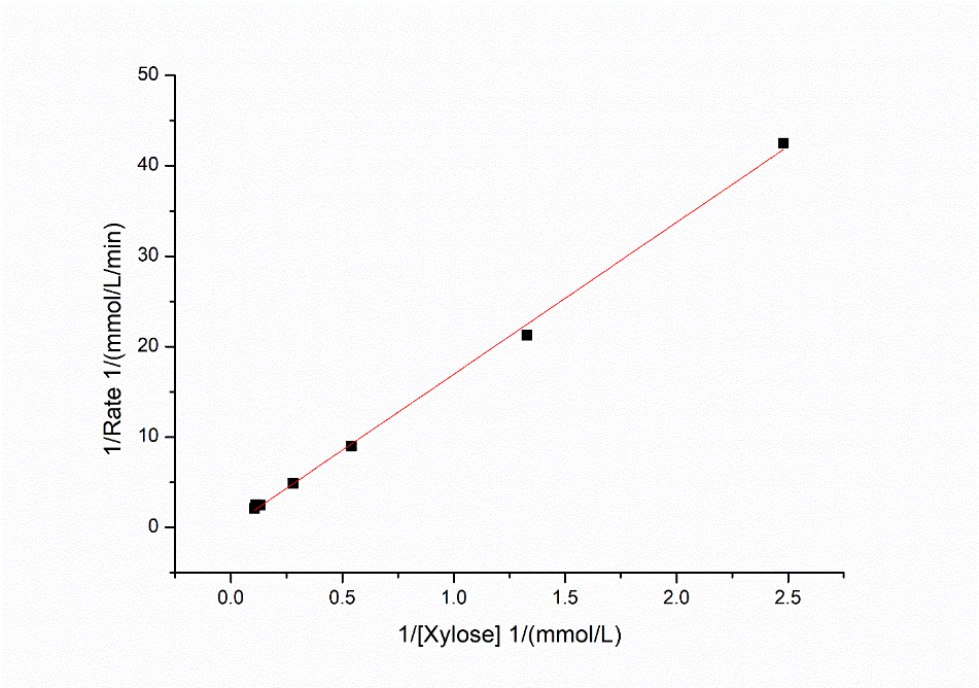
YANG, H.; WANG, K.; SONG, X.; XU, F. Production of xylooligosaccharides by xylanase from *Pichia stipitis* based on xylan preparation from triploid *Populas tomentosa*. **Bioresource Technology**, vol. 102, no. 14, p. 7171–7176, 2011. DOI https://doi.org/10.1016/j.biortech.2011.03.110.

ZHANG, Y.; SERATE, J.; XIE, D.; GAJBHIYE, S.; KULZER, P.; SANFORD, G.; RUSSELL, J. D.; MCGEE, M.; FOSTER, C.; COON, J. J.; LANDICK, R.; SATO, T. K.

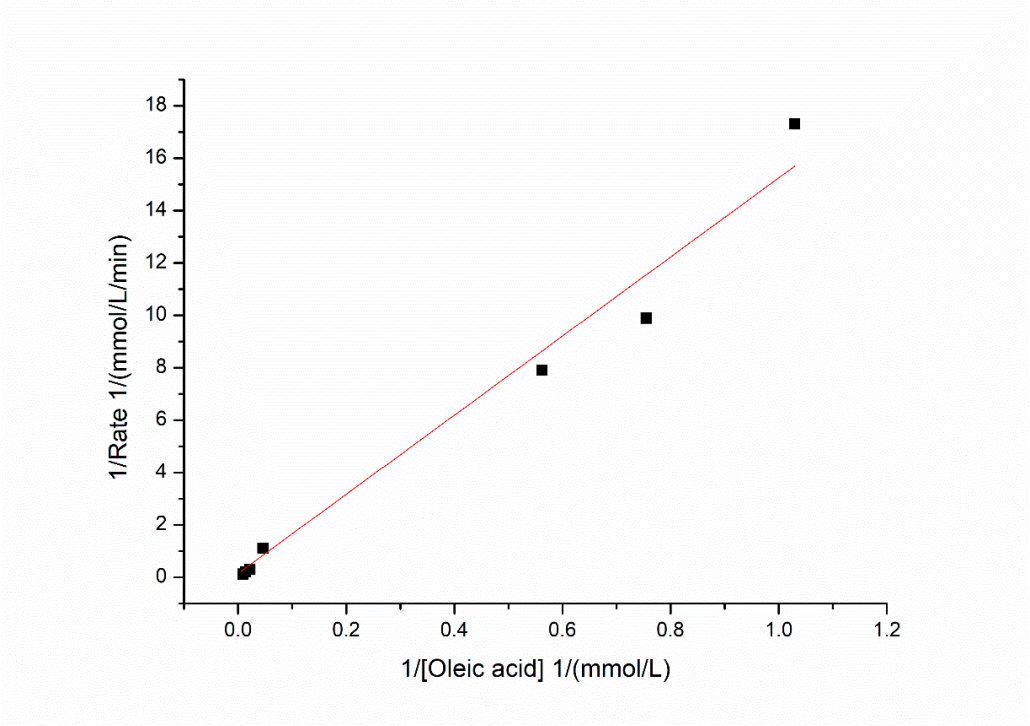
Production of hydrolysates from unmilled AFEX-pretreated switchgrass and comparative fermentation with *Zymomonas mobilis*. **Bioresource Technology Reports**, vol. 11, 2020. DOI https://doi.org/10.1016/j.biteb.2020.100517.
ZHU, Z.; ZHU, M.; WU, Z. Pretreatment of sugarcane bagasse with NH4OH-H2O2 and ionic liquid for efficient hydrolysis and bioethanol production. **Bioresource Technology**, vol. 119, p. 199–207, 2012. DOI https://doi.org/10.1016/j.biortech.2012.05.111.
ZUO, Z.; TIAN, S.; CHEN, Z.; LI, J.; YANG, X. Soaking pretreatment of corn stover for bioethanol production followed by anaerobic digestion process. **Applied Biochemistry and Biotechnology**, vol. 167, no. 7, p. 2088–2102, 2012. DOI https://doi.org/10.1007/s12010-012-9751-3.

Lipozyme 435-mediated synthesis of xylose oleate in methyl ethyl ketone

Figures and Table



Conditions: 60 °C, 200 rpm, 15 min, enzyme load of 0.23% w/v, with molecular sieves.
Figure S4. Lineweaver-Burk plot for the initial reaction rates of xylose consumption for L435 at 1.4 mM of oleic acid.

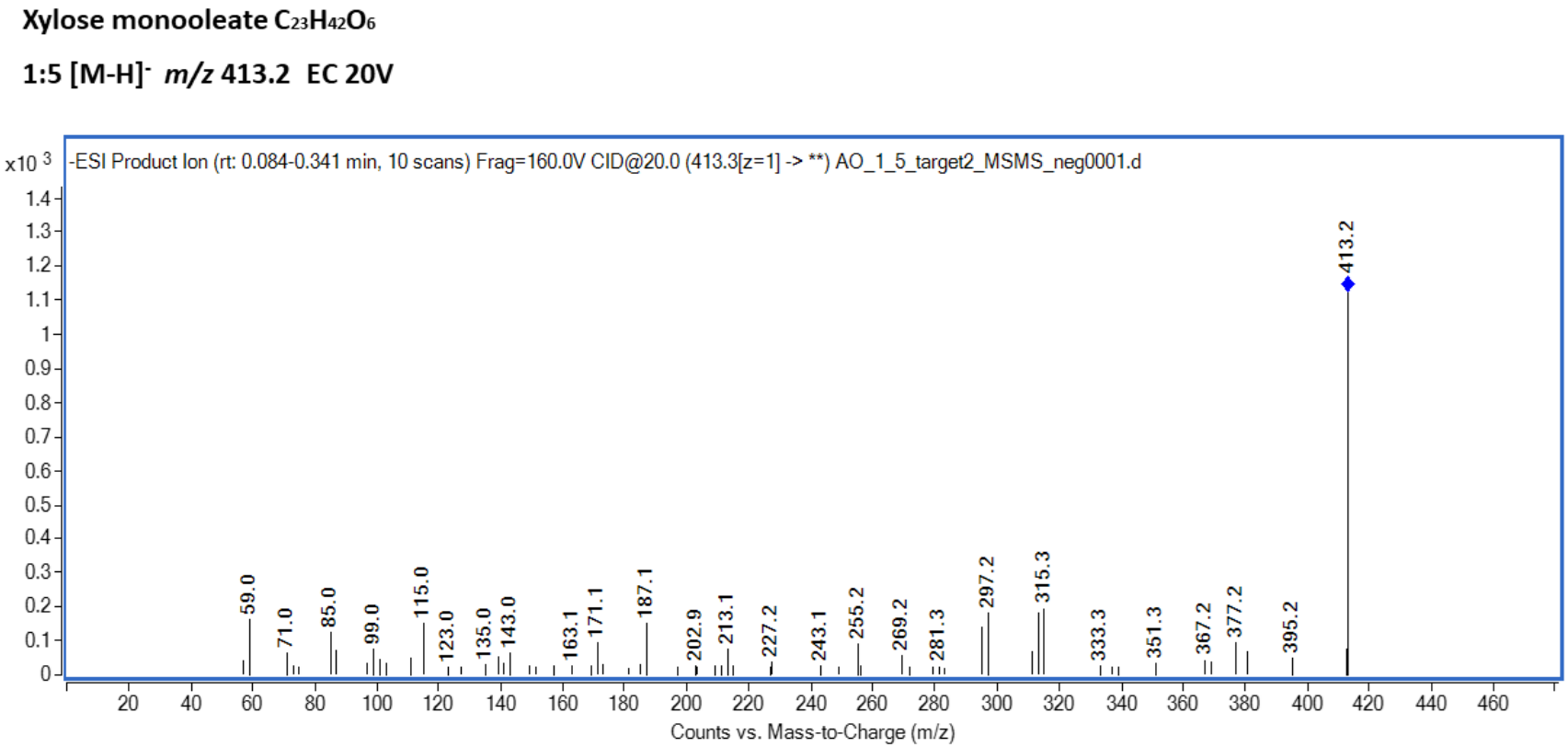


Conditions: 60 °C, 200 rpm, 15 min, enzyme load of 0.23% w/v, with molecular sieves.

Figure S5. Lineweaver-Burk plot for the initial reaction rates of oleic acid consumption for L435 at 7 mM of xylose.

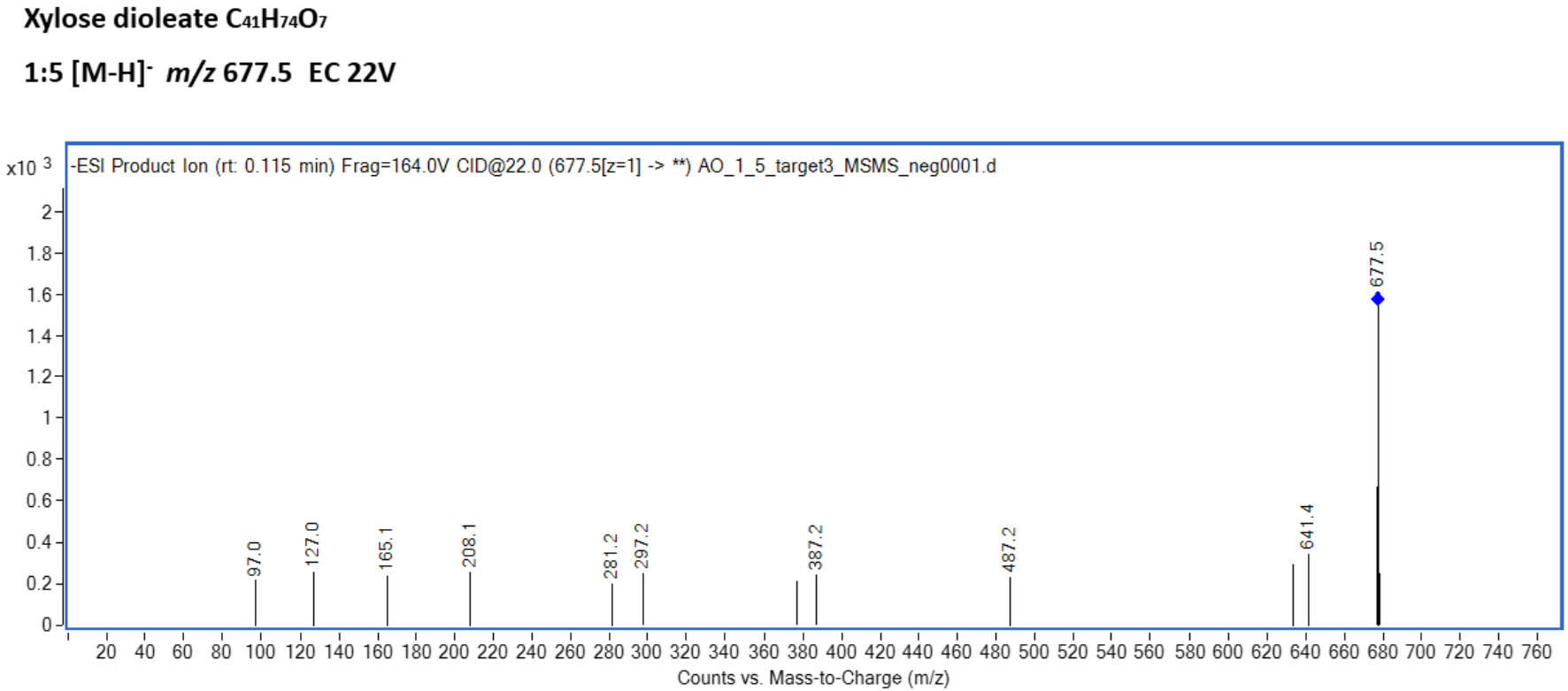
Table S8. Kinetic constants of the esterification reaction catalyzed by L435.
Reaction conditions: 15 min-reaction, 60 °C, 200 rpm, enzyme load of 0.23% w/v, xylose: oleic acid molar ratio of 1:5, with molecular sieves.

Parameters	
V_{max} (mmol/L/min)	6.59 ± 0.21
K_m ($_{Xyl}$) (mmol/L)	97.22
K_m ($_{OA}$) (mmol/L)	113.24



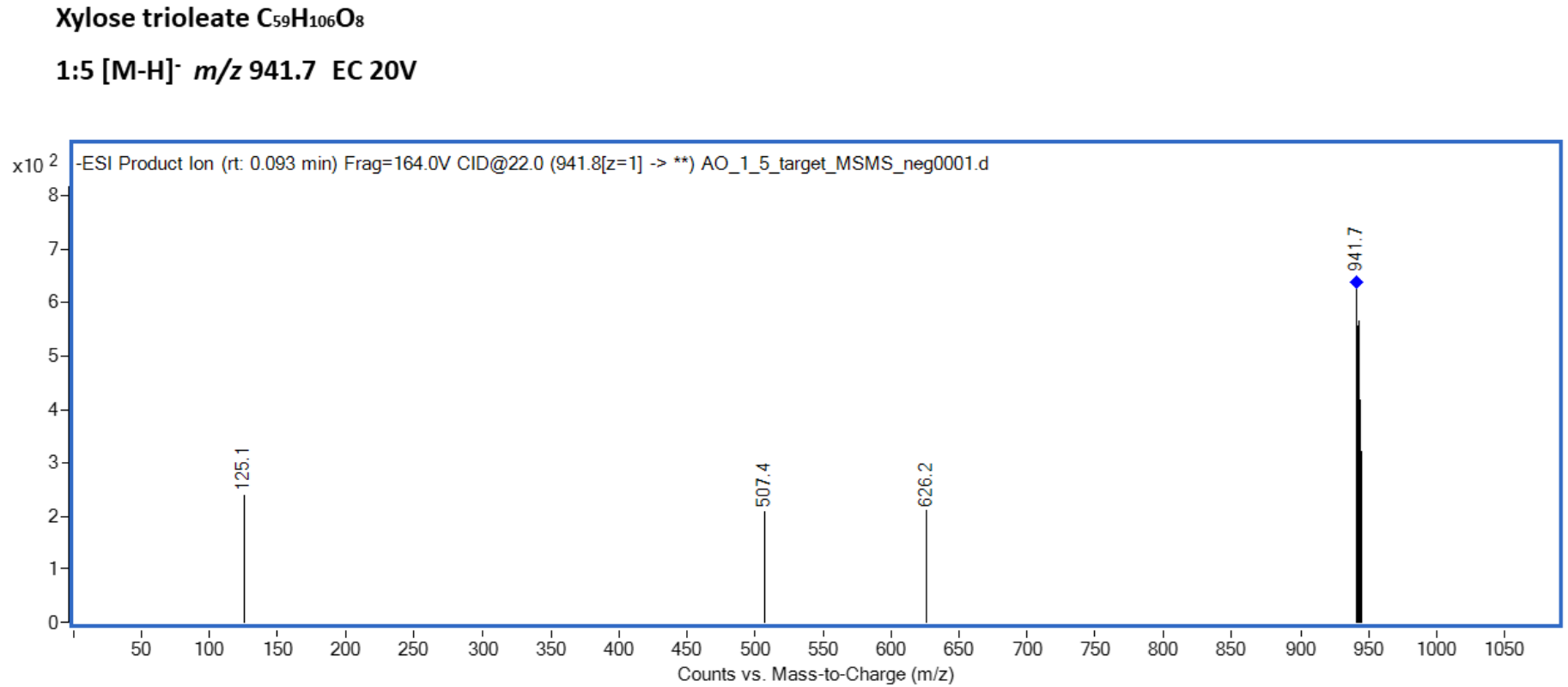
Xylose: oleic acid molar ratio of 1:5.

Figure S6. Mass spectrum of xylose monooleate with m/z = 413.2.



Xylose: oleic acid molar ratio of 1:5.

Figure S7. Mass spectrum of xylose dioleate with m/z = 677.5.

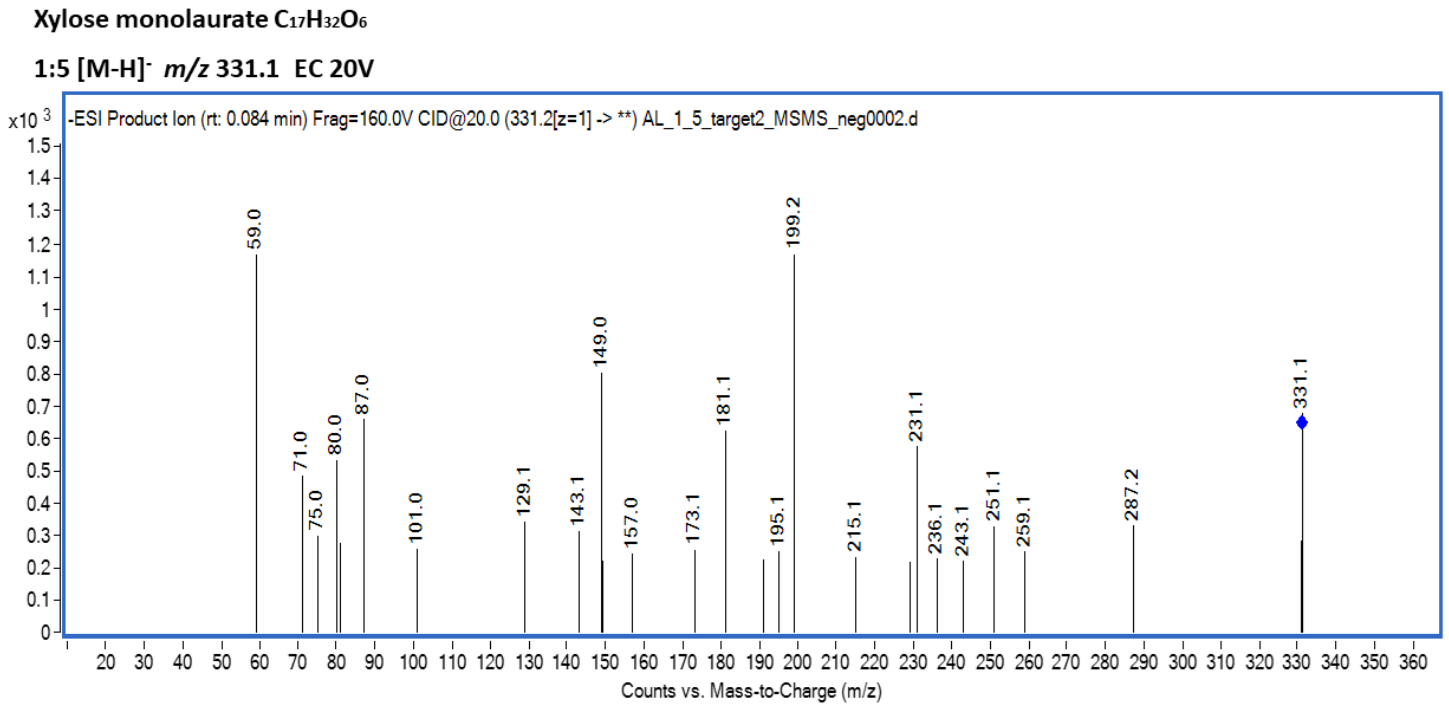


Xylose: oleic acid molar ratio of 1:5.

Figure S8. Mass spectrum of xylose trioleate with m/z = 941.7

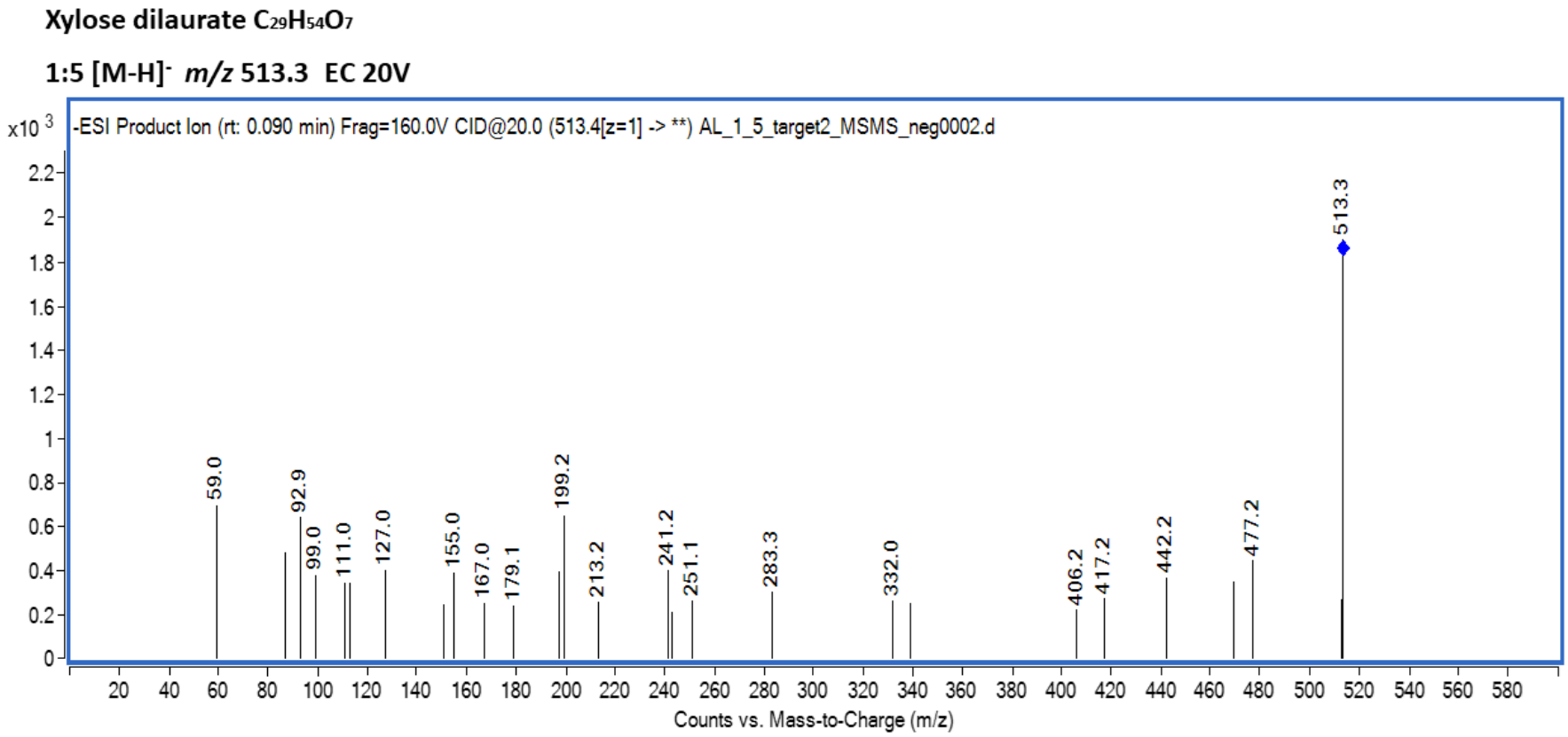
Stabilization of Lipozyme 435 by its coating with polyethyleneimine: comparison of the biocatalyst performance in the synthesis of xylose fatty esters

Figures



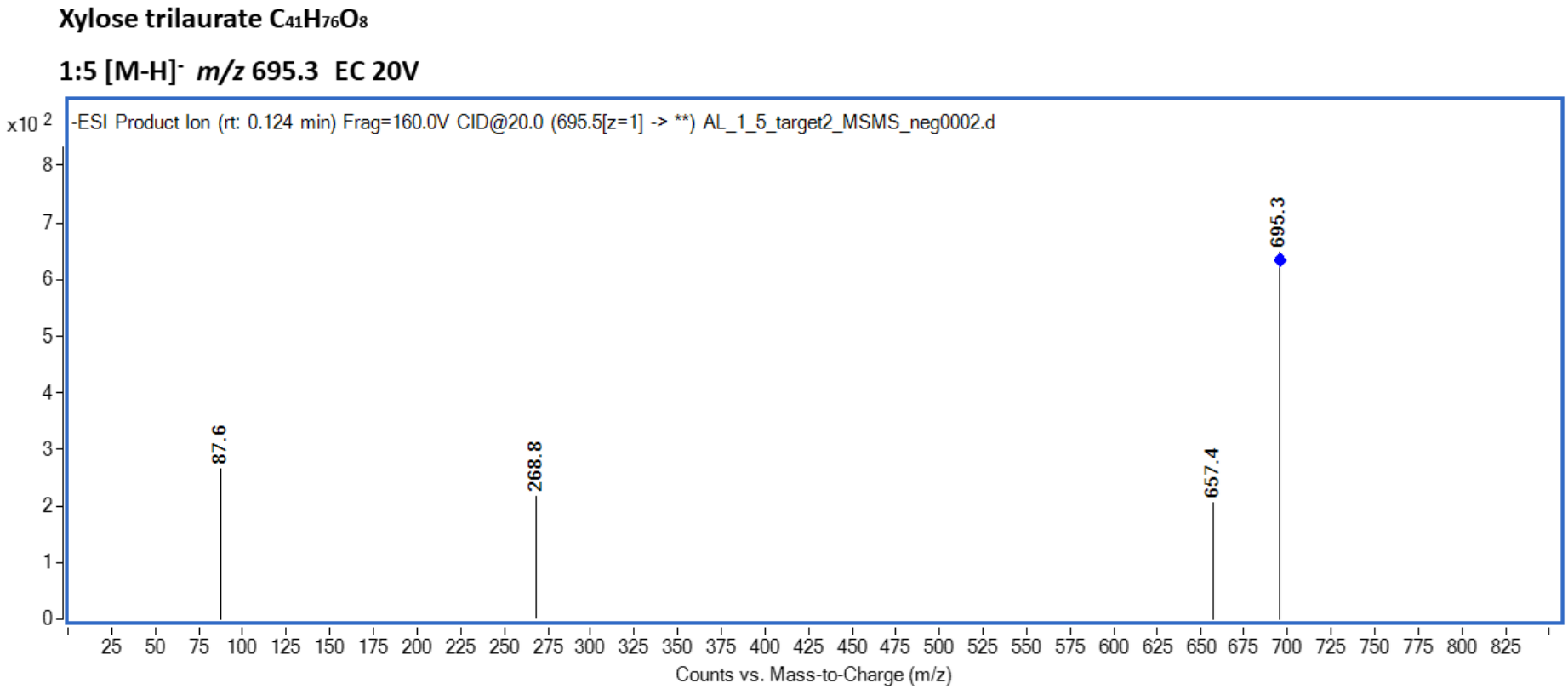
Xylose: lauric acid molar ratio of 1:5.

Figure S9. Mass spectrum of xylose monolaurate with m/z = 331.1.



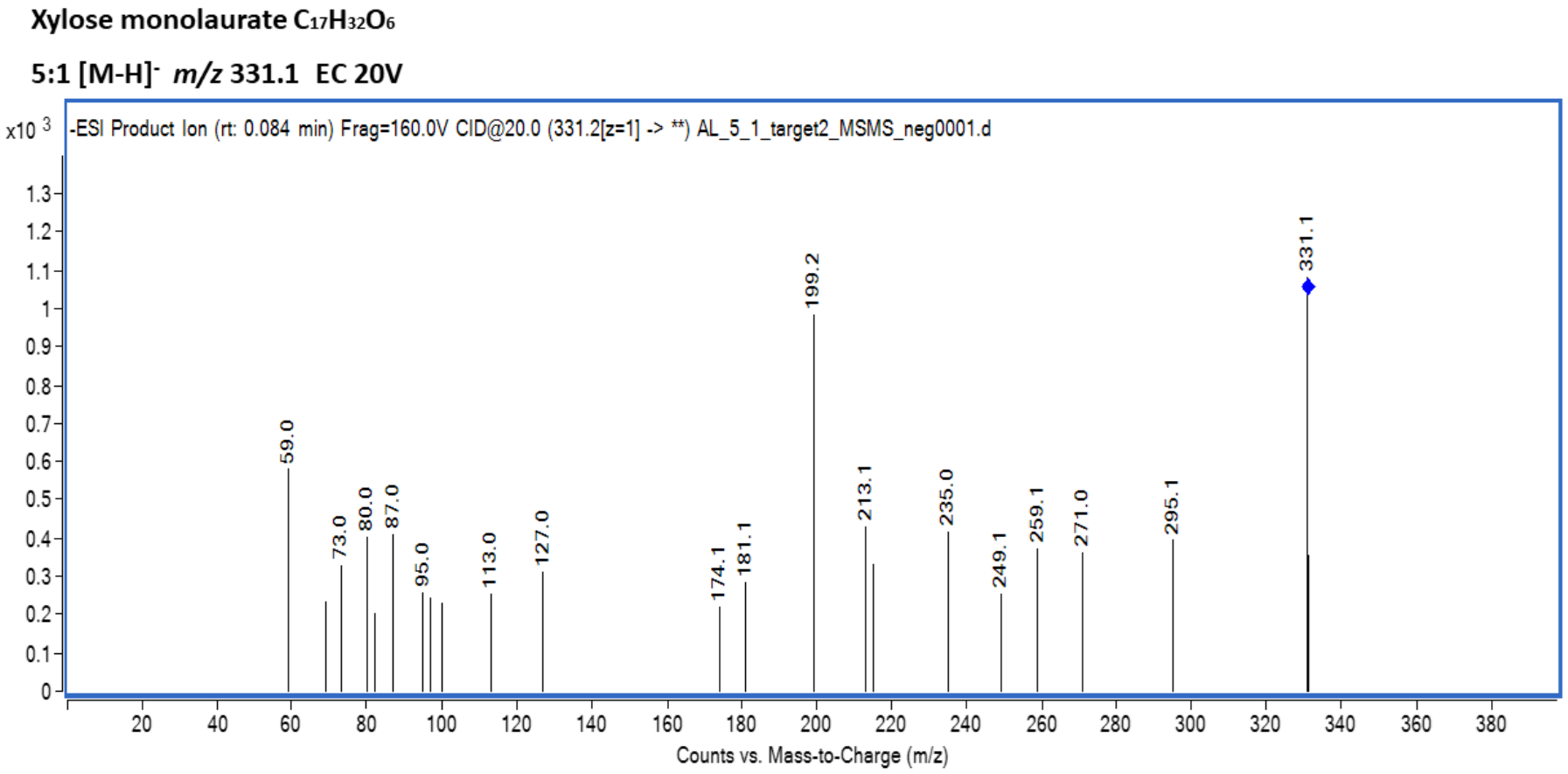
Xylose: lauric acid molar ratio of 1:5.

Figure S10. Mass spectrum of xylose dilaurate with m/z = 513.3.



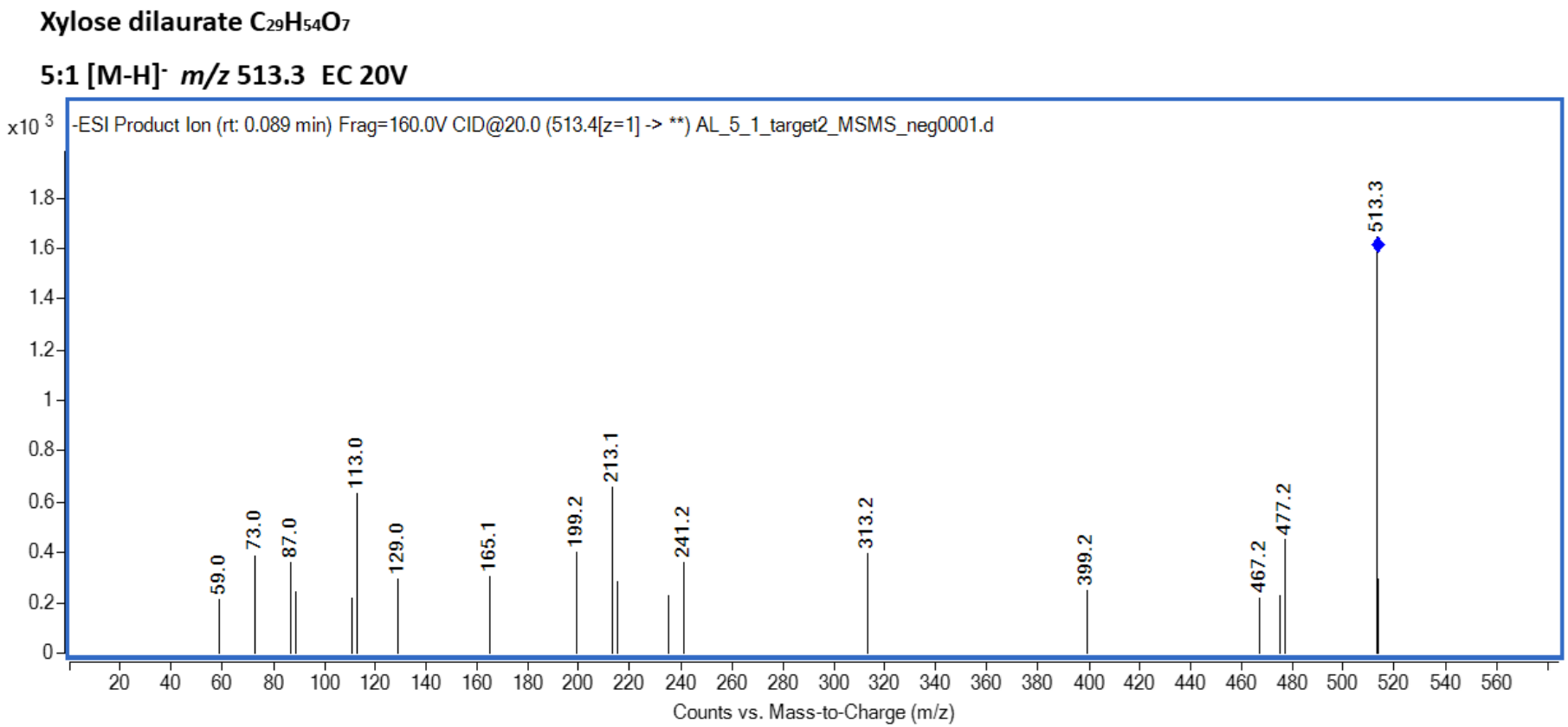
Xylose: lauric acid molar ratio of 1:5.

Figure S11. Mass spectrum of xylose trilaurate with m/z = 695.3.



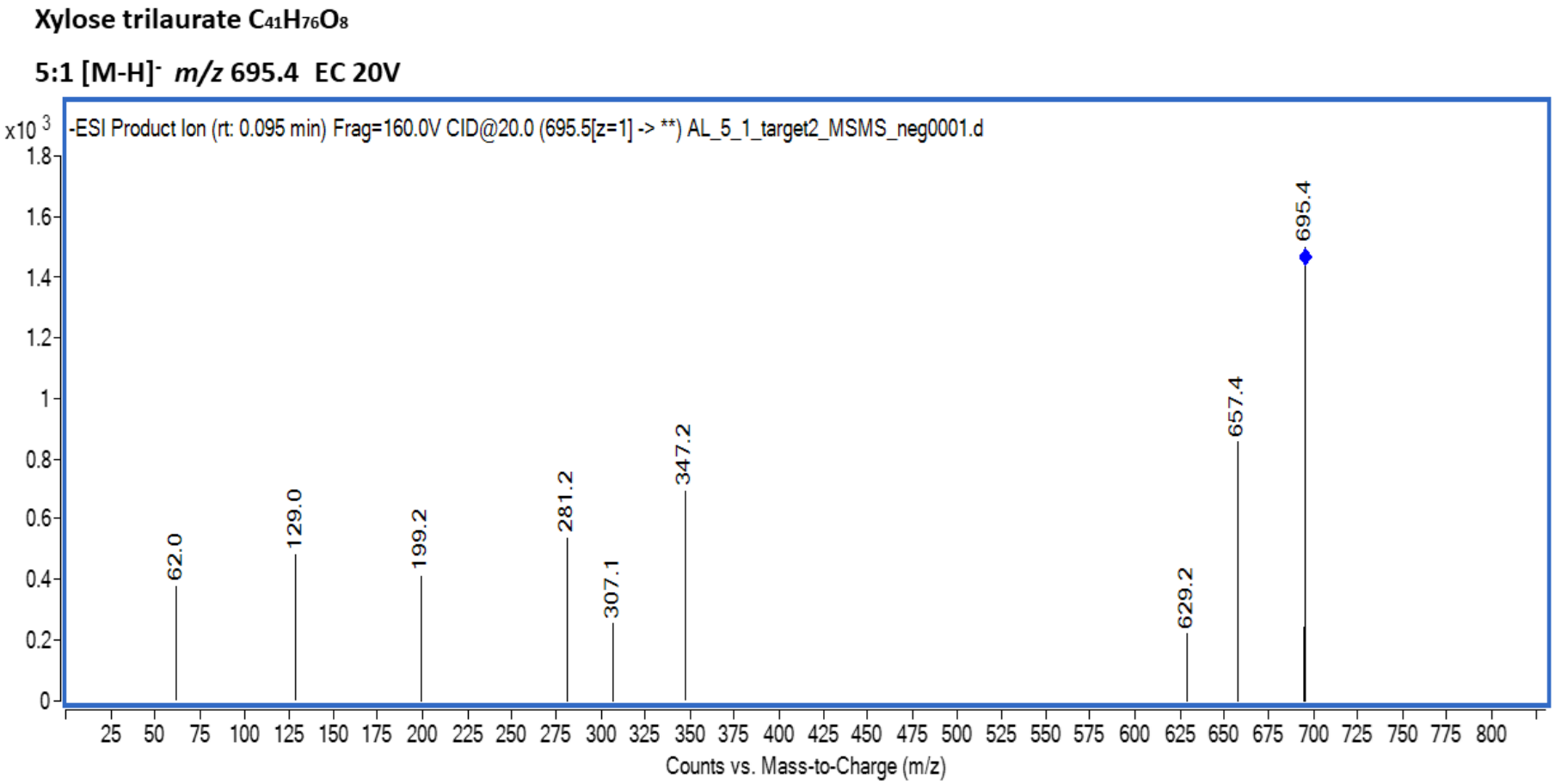
Xylose: lauric acid molar ratio of 5:1.

Figure S12. Mass spectrum of xylose monolaurate with m/z = 331.1.



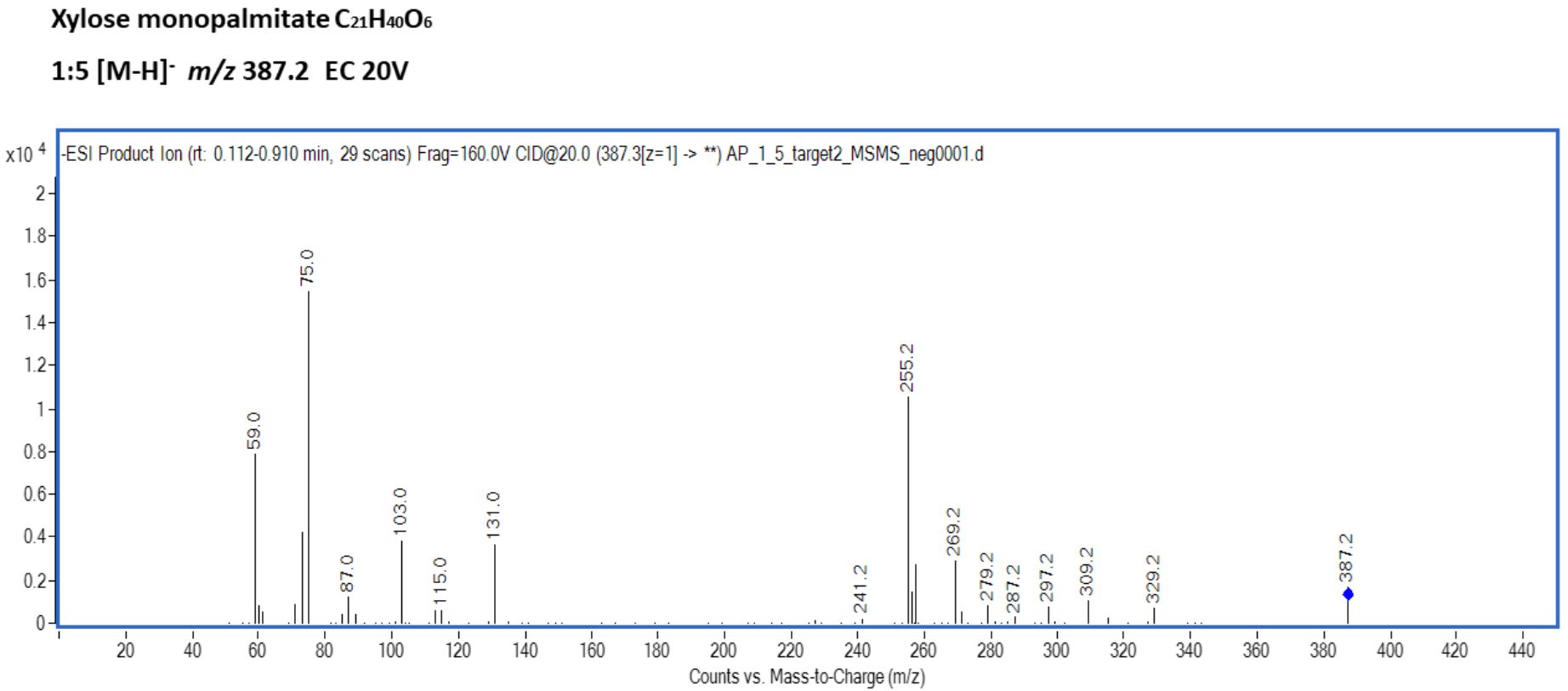
Xylose: lauric acid molar ratio of 5:1.

Figure S13. Mass spectrum of xylose dilaurate with m/z = 513.3.



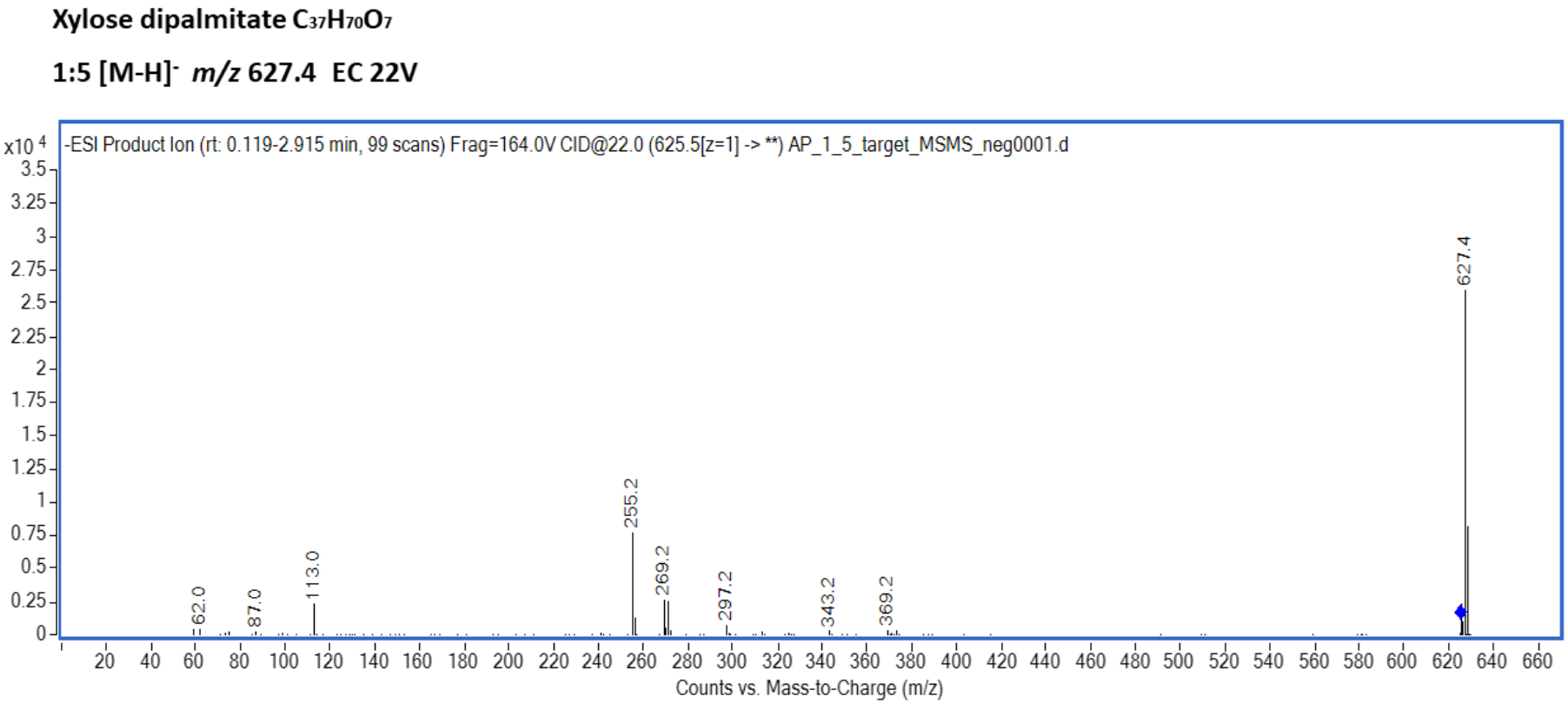
Xylose: lauric acid molar ratio of 5:1.

Figure S14. Mass spectrum of xylose trilaurate with m/z = 695.4.



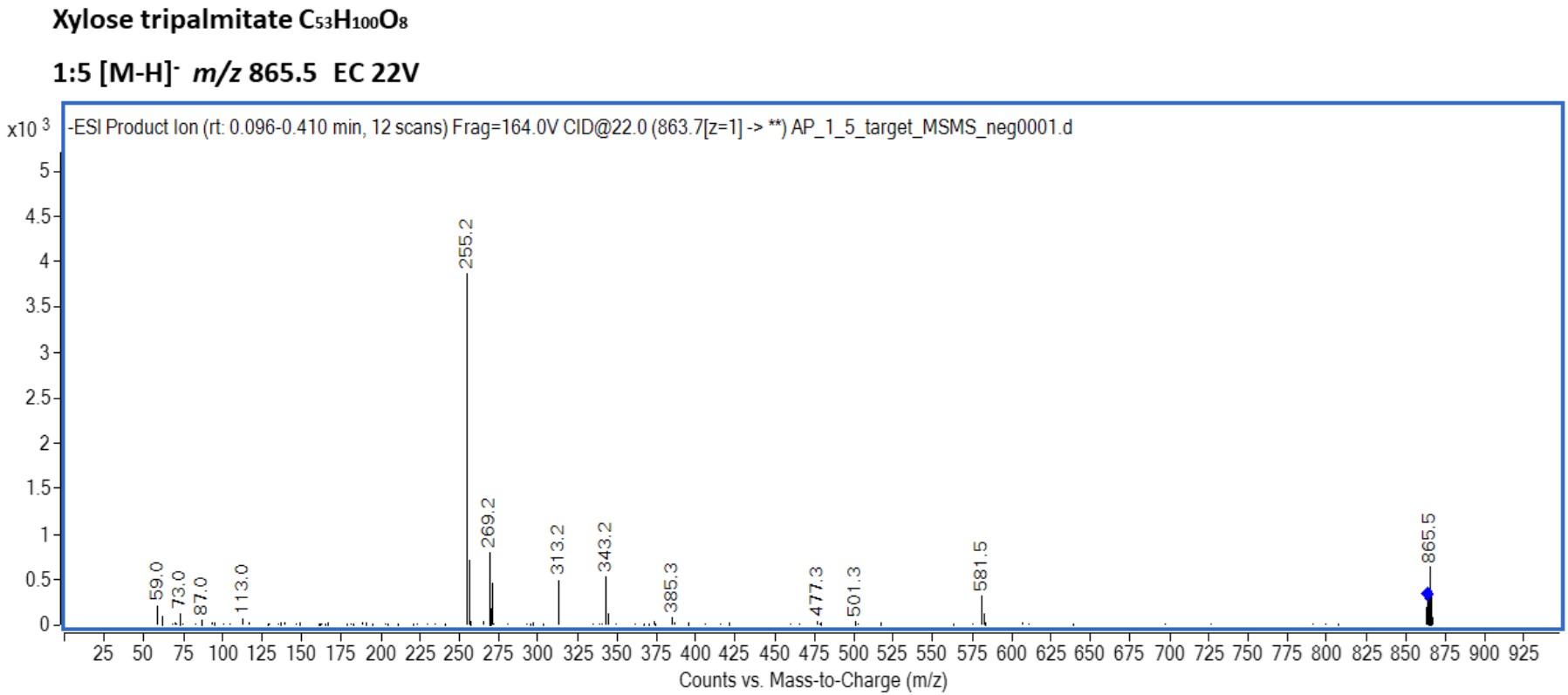
Xylose: palmitic acid molar ratio of 1:5.

Figure S15. Mass spectrum of xylose monopalmitate with m/z = 387.2.



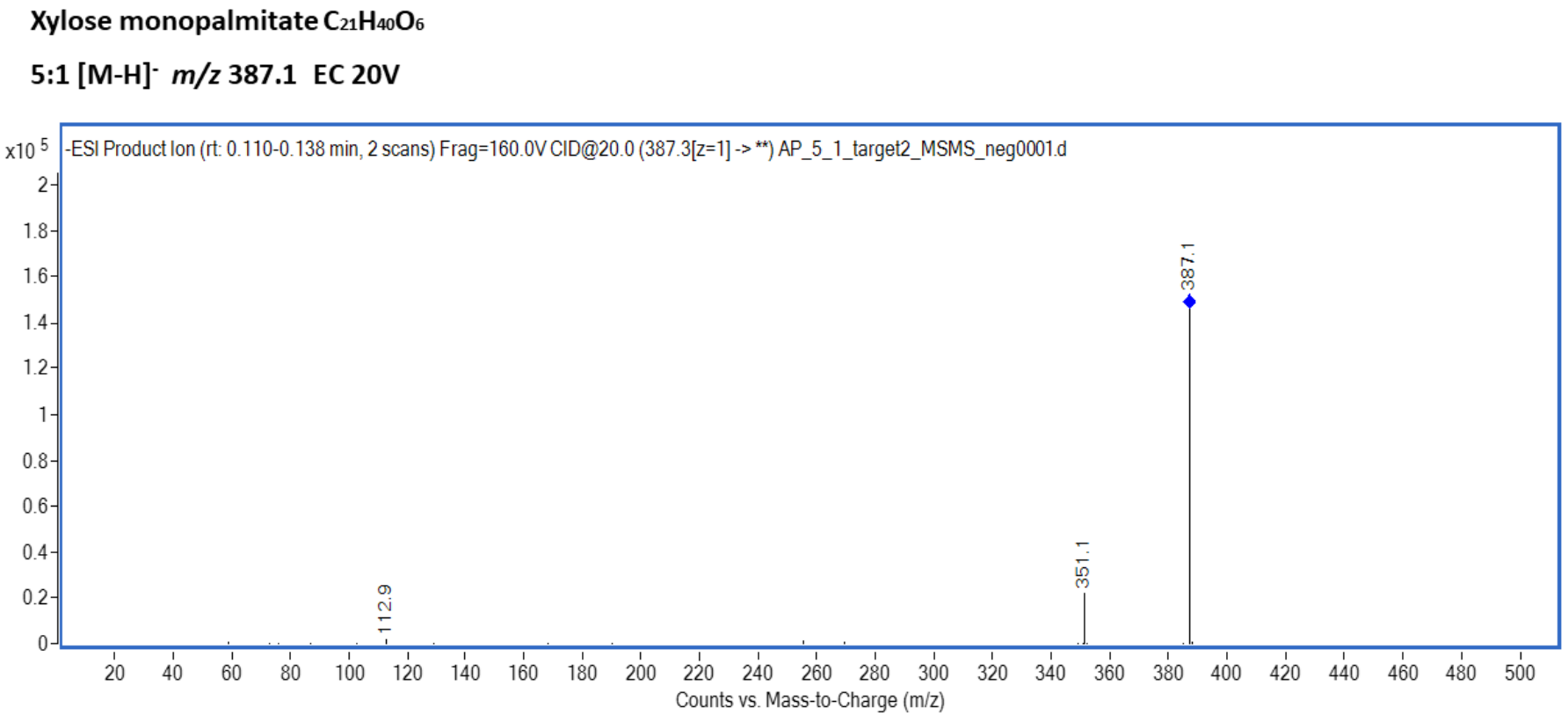
Xylose: palmitic acid molar ratio of 1:5.

Figure S16. Mass spectrum of xylose dipalmitate with m/z = 627.4.



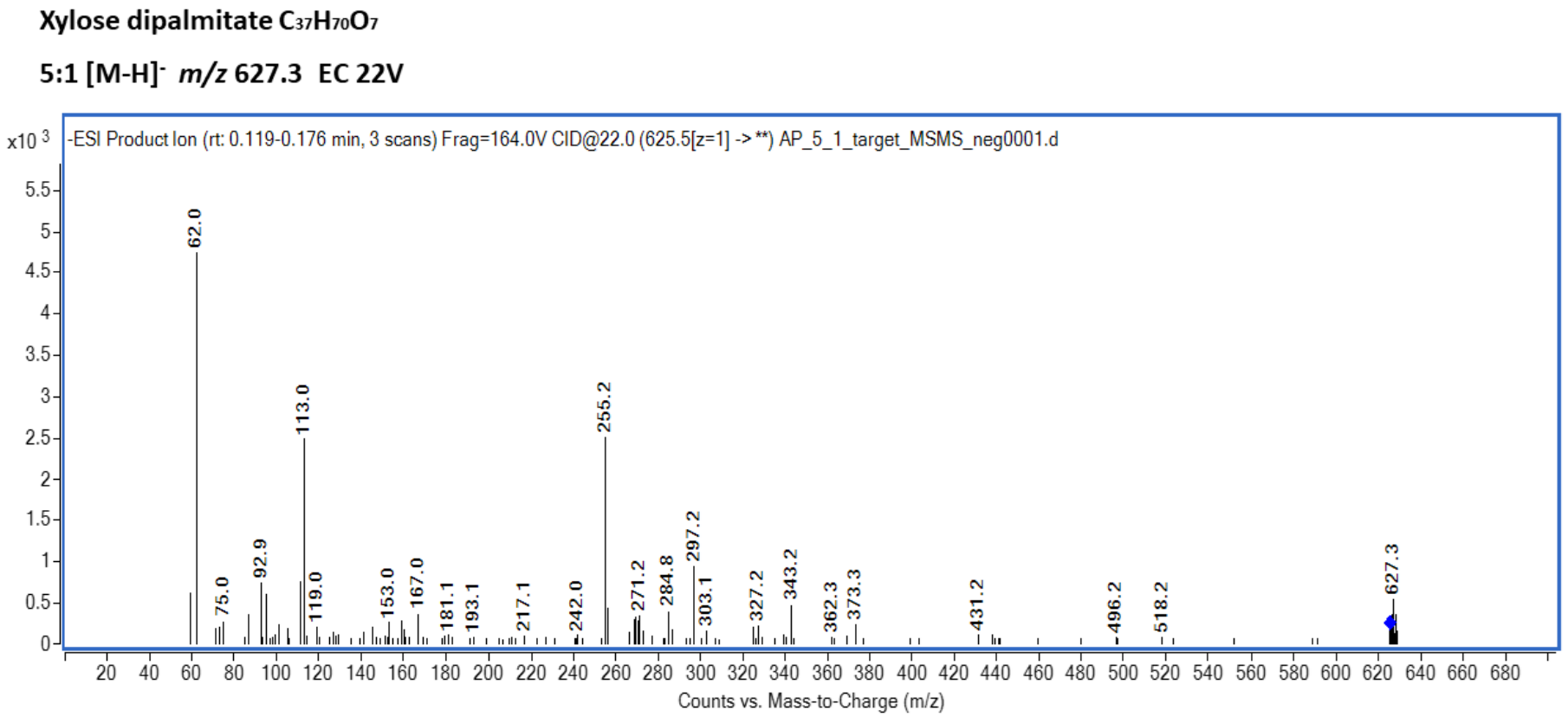
Xylose: palmitic acid molar ratio of 1:5.

Figure S17. Mass spectrum of xylose tripalmitate with m/z = 865.5.



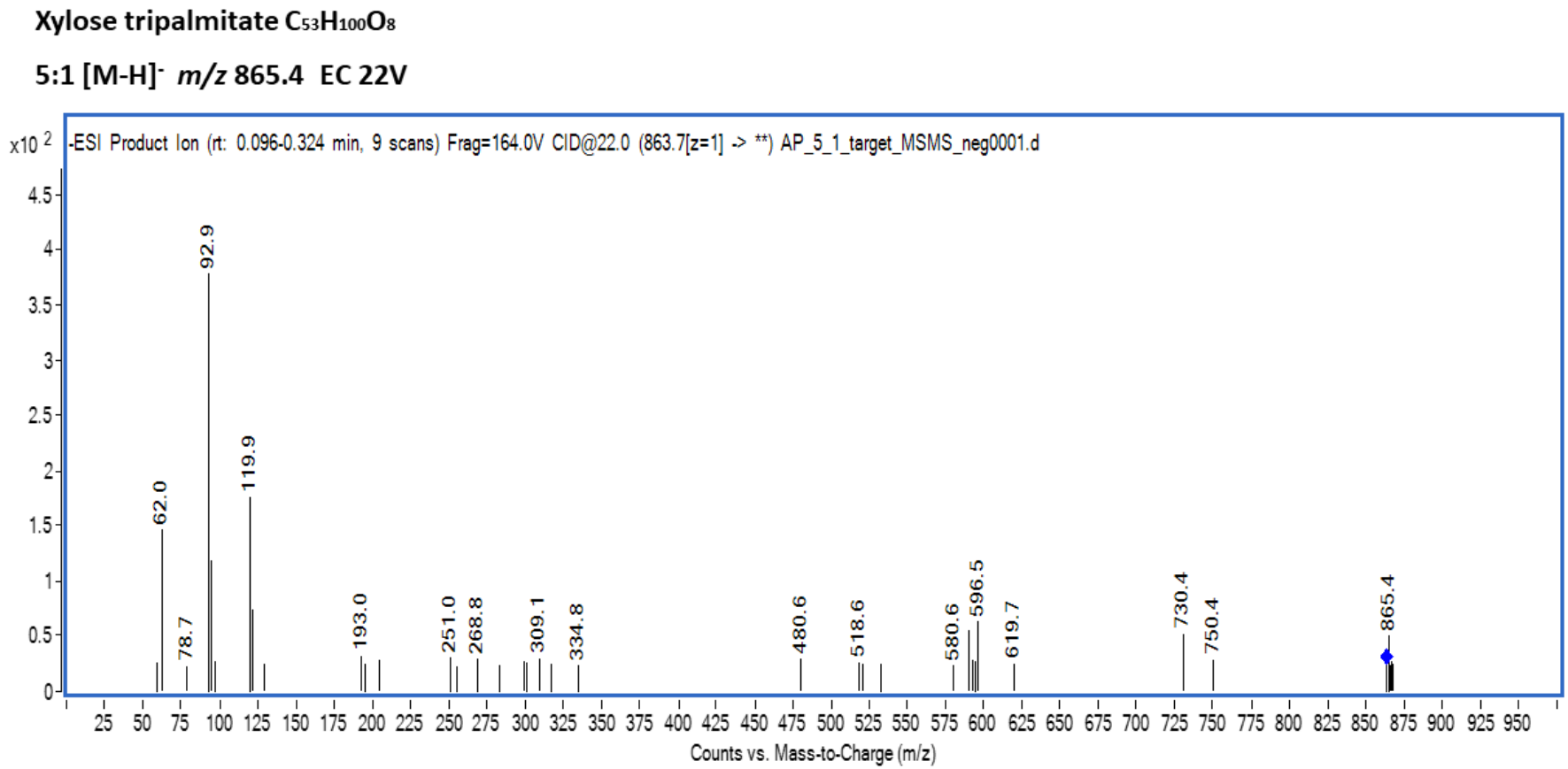
Xylose: palmitic acid molar ratio of 5:1.

Figure S18. Mass spectrum of xylose monopalmitate with m/z = 387.1.



Xylose: palmitic acid molar ratio of 5:1.

Figure S19. Mass spectrum of xylose dipalmitate with m/z = 627.3.



Xylose: palmitic acid molar ratio of 5:1.

Figure S20. Mass spectrum of xylose tripalmitate with m/z = 865.4.

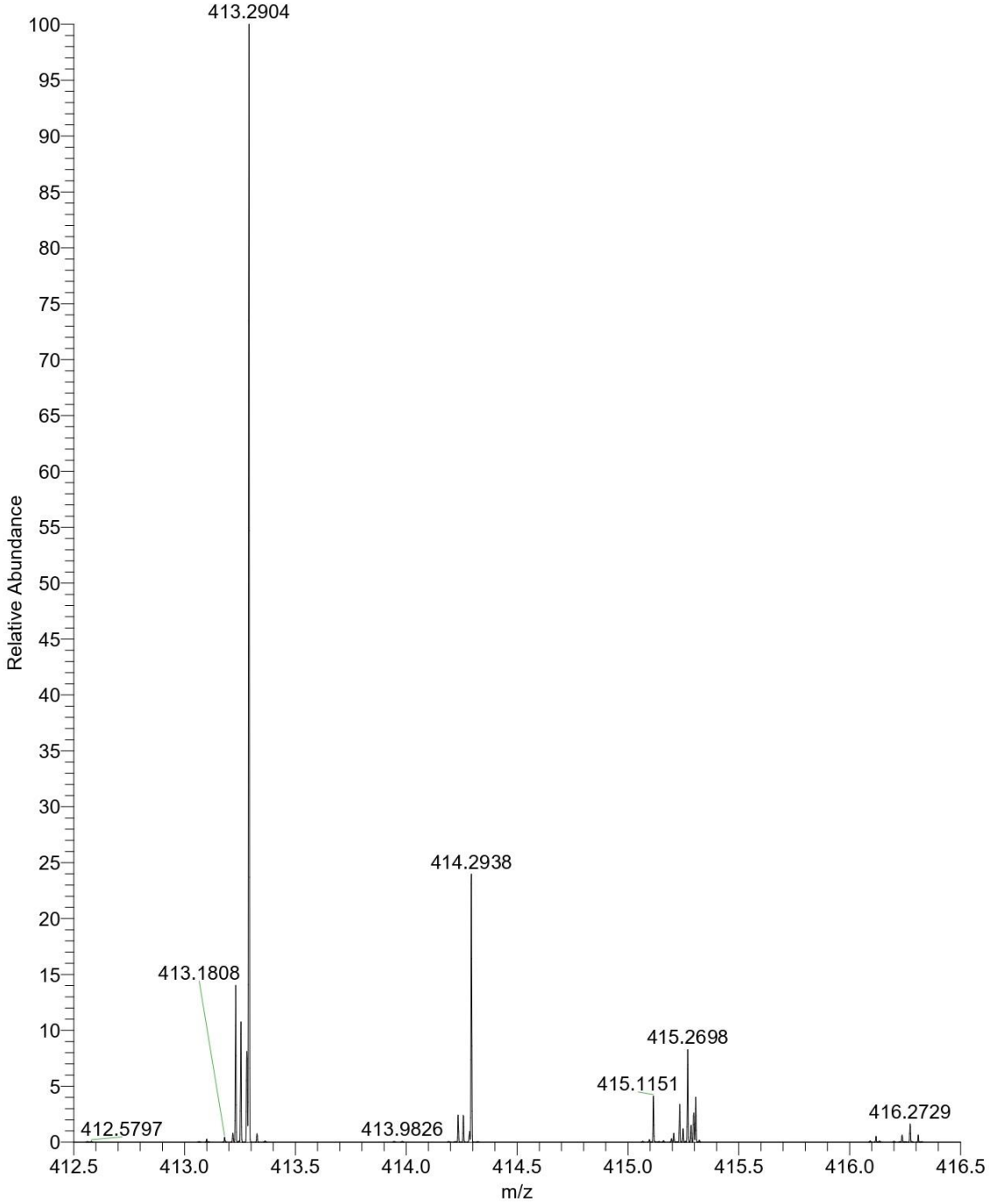


Figure S21. Mass spectrum of xylose monooleate with m/z = 413.29.

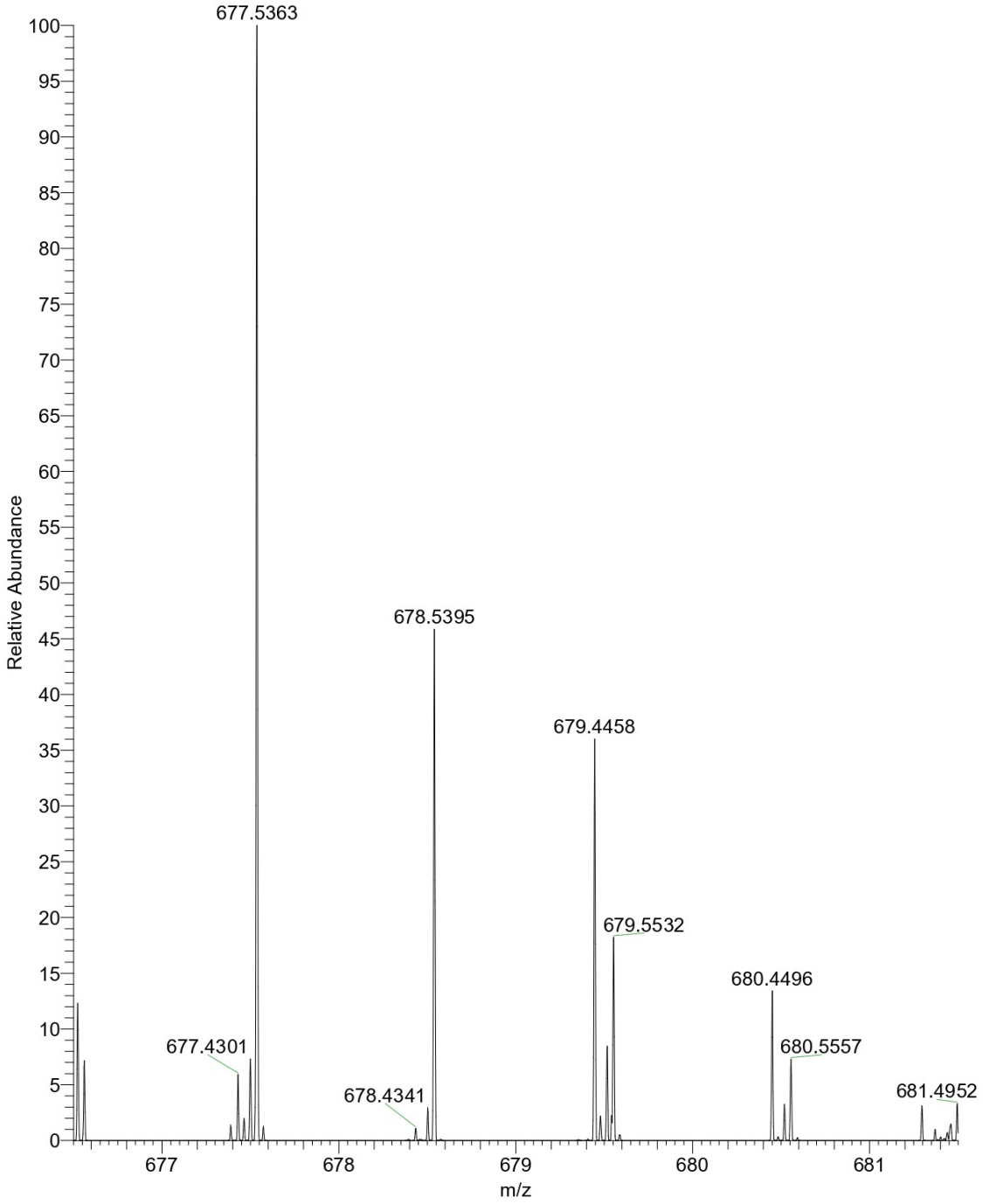


Figure S22. Mass spectrum of xylose dioleate with m/z = 677.53.

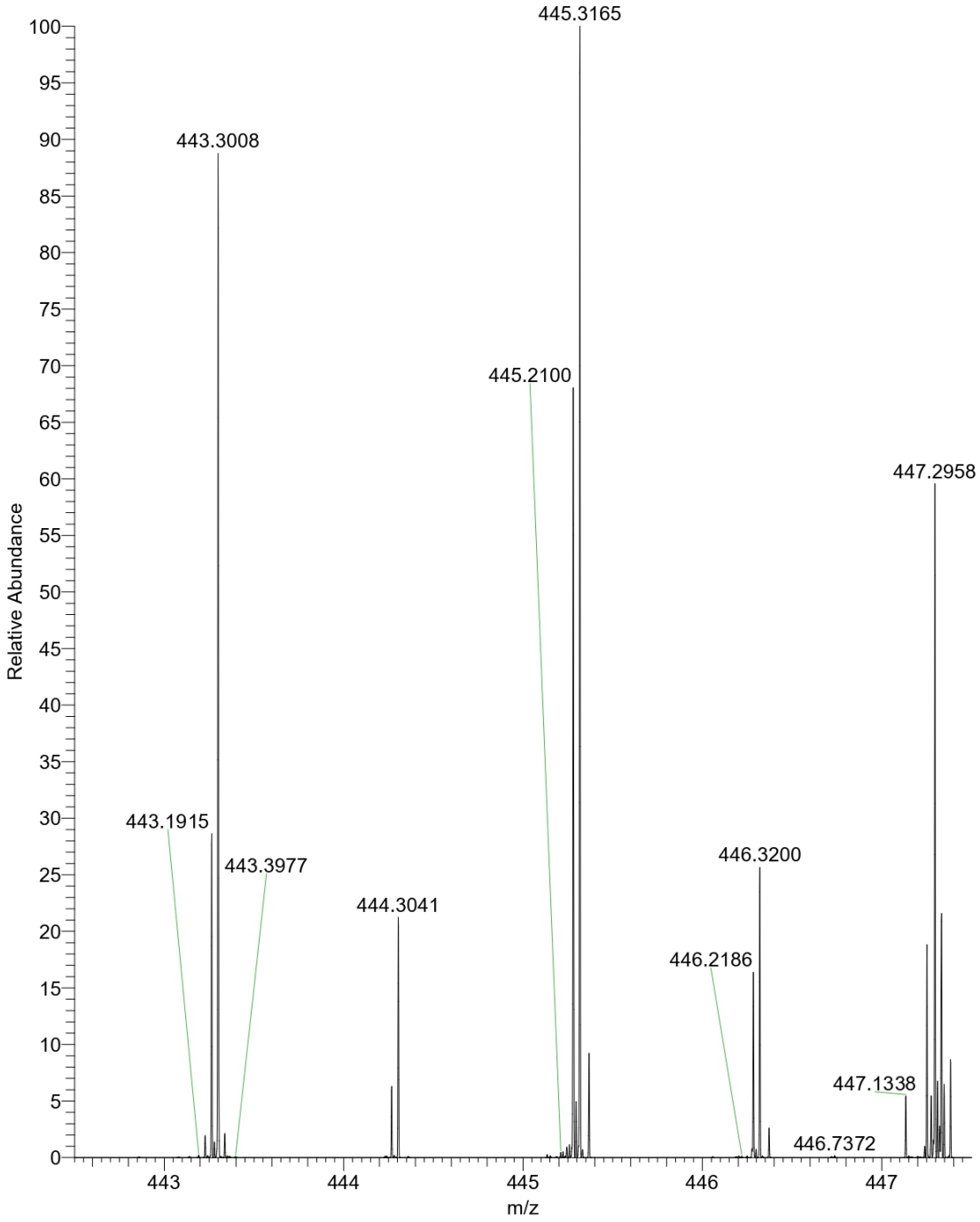


Figure S23. Mass spectrum of glucose monooleate with m/z = 443.30.

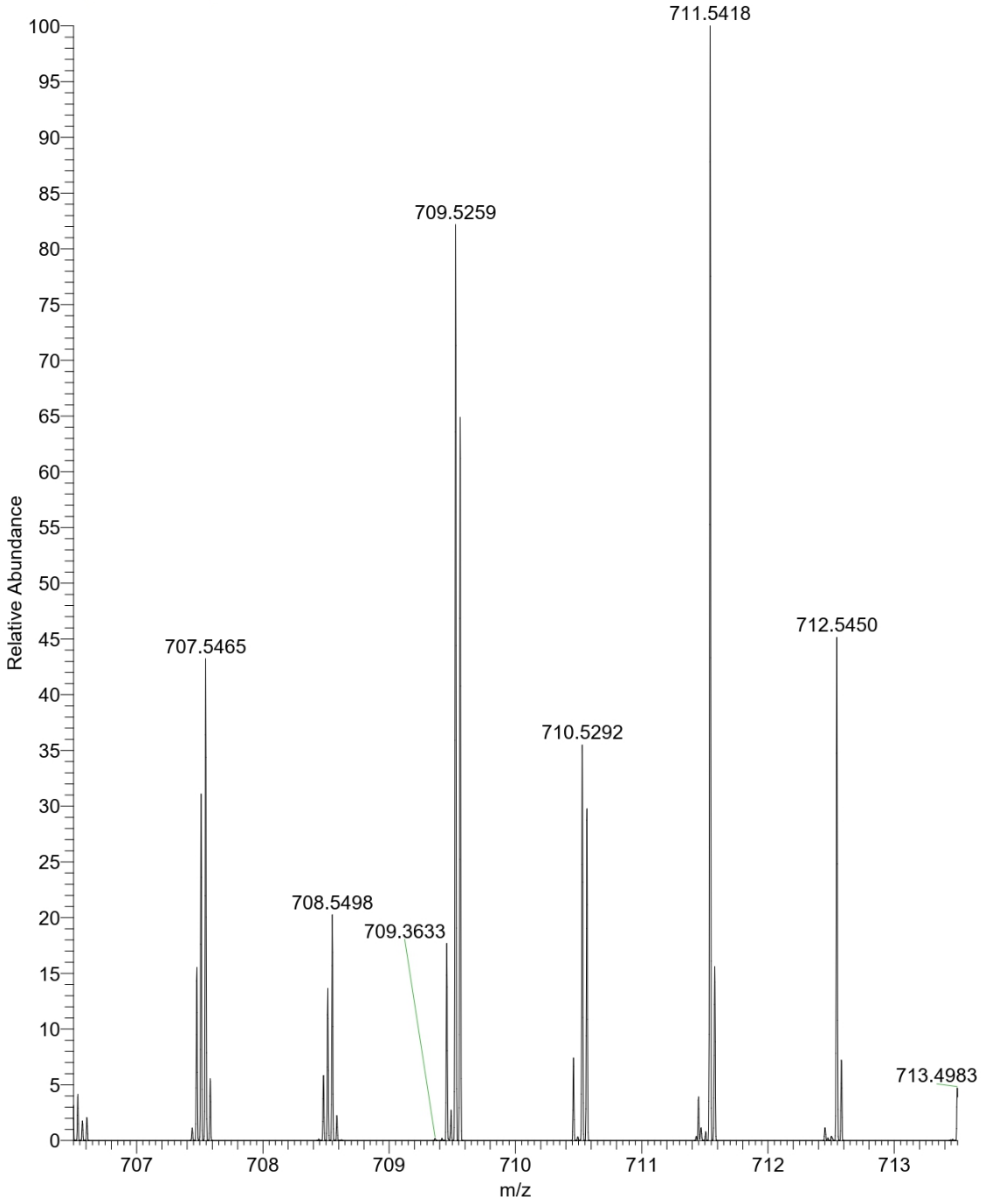


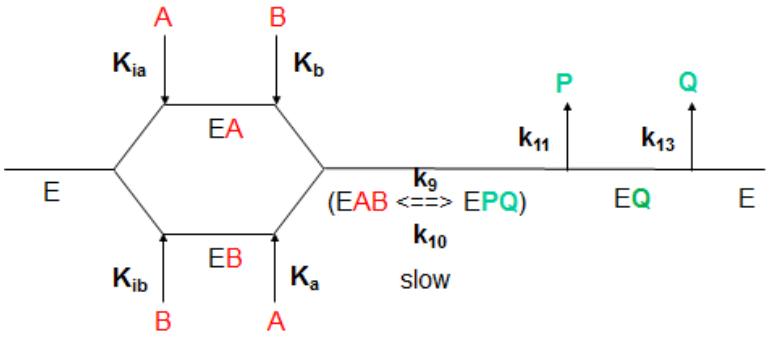
Figure S24. Mass spectrum of glucose dioleate with m/z = 707.54.

APPENDIX

Ping Pong Bi Bi mechanism

For this mechanism, the enzyme must bind substrate A first, followed by the release of product P and the formation of the enzyme species E'. This is followed by binding of substrate B to E' and the breakdown of the E'B complex to free enzyme E and the second product Q. Thus, for ping pong mechanisms, no ternary complex is formed.

Considering a bi-bi reaction for a "simple" case in which the rapid equilibrium assumption defines the binding of substrates A and B:



There would appear to be two types of "effective" dissociation constants for reactant A. One describes the binding of A to E (K_{i_A}) and the other the binding of A to EB (K_A). Using mass balance for E and the relationship:

$$v = K_{cat}.[EAB] \quad (1)$$

The following initial rate equation can be derived:

$$K_{i_A} = \frac{[E].[A]}{[EA]} \quad (2)$$

$$K_{i_B} = \frac{[E].[B]}{[EB]} \quad (3)$$

$$K_A = \frac{[EB].[A]}{[EAB]} \quad (4)$$

$$K_B = \frac{[EA].[B]}{[EAB]} \quad (5)$$

$$[E]_0 = [E] + [EA] + [EB] + [EAB] \quad (6)$$

$$[EA] = \frac{K_B.[EAB]}{[B]} \quad (7)$$

$$[EB] = \frac{K_A.[EAB]}{[A]} \quad (8)$$

$$[E] = \frac{K_{i_A}.[EA]}{[A]} = \frac{K_{i_A}.K_B.[EAB]}{[AB]} \quad (9)$$

$$[E]_0 = \frac{K_{i_A}.K_B.[EAB]}{[AB]} + \frac{K_B.[EAB]}{[B]} + \frac{K_A.[EAB]}{[A]} + [EAB] \quad (10)$$

$$[E]_0 = \left(\frac{K_{i_A}.K_B}{[AB]} + \frac{K_B}{[B]} + \frac{K_A}{[A]} + 1\right).[EAB] \quad (11)$$

$$v = K_{cat}.[EAB] = \frac{K_{cat}.[E]_0}{\frac{K_{i_A}.K_B}{[AB]} + \frac{K_B}{[B]} + \frac{K_A}{[A]} + 1} = \frac{K_{cat}.[E]_0.[AB]}{K_{i_A}.K_B + K_B.[A] + K_A.[B] + [AB]} \quad (12)$$

$$v = \frac{v_{máx}.[AB]}{K_{i_A}.K_B + K_B.[A] + K_A.[B] + [AB]} \quad (13)$$

Where $v_{máx} = K_{cat}.[E]_0$.

Note that K_{i_B} does not appear in the final equation, since the final concentration of [EAB] can be derived from the path [E] to [EA] to [EAB] or from the path [E] to [EB] to [EAB]. Assuming rapid equilibrium:

$$K_{i_A}.K_B = K_{i_B}.K_A = 0 \quad (14)$$

The following equation can be derived:

$$v = \frac{v_{máx}.[AB]}{K_B.[A] + K_A.[B] + [AB]} \quad (15)$$

REFERENCES

MARANGONI, A. Enzyme kinetics: A modern approach. 1.ed. John Wiley & Sons, Inc., Hoboken, New Jersey, 2003.

JAKUBOWSKI, H. Multi-Substrate Ping-Pong Mechanisms. College of St. Benedict/St. John's University. Available at: <https://bio.libretexts.org/Courses/Ouachita_Baptist_University/Reyna_Cell_Biology/03%3A_(T1)Enzymes_-/3.07%3A_More_Complicated_Enzymes/3.7.02%3A_Multi-Substrate_Ping-Pong_Mechanisms>. Access at: March 24, 2023.