Universidade Federal de Minas Gerais
Escola de Engenharia
Departamento de Engenharia Química
Programa de Pós-Graduação em Engenharia Química

Preparação e caracterização de biocatalisadores heterogêneos para a produção de biolubrificante

Iara Camila de Almeida Bolina

Orientador: Prof. Dr. Adriano Aguiar Mendes
Coorientadora: Profa. Dra Érika Cristina Cren

Belo Horizonte
2020

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Tese apresentada ao Programa de Pós-Graduação em Engenharia Química da Escola de Engenharia da Universidade Federal de Minas Gerais como um dos requisitos para a obtenção do título de doutor em Engenharia Química.

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FOLHA DE APROVAÇÃO

"PREPARAÇÃO E CARACTERIZAÇÃO DE BIOCATALISADORES HETEROGÊNEOS PARA A PRODUÇÃO DE BIOLUBRIFICANTE"

Iara Camila de Almeida Bolina

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Engenharia Química da Escola de Engenharia da Universidade Federal de Minas Gerais, como parte dos requisitos à obtenção do título de **DOUTORA EM ENGENHARIA QUÍMICA**.

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RESUMO

Nos últimos anos, os biolubrificantes vêm ganhando destaque em comparação com os lubrificantes convencionais à base de petróleo. Essa tendência é observada em quase todos os setores que contam com esses produtos, independentemente de suas aplicações. Os fatores que contribuem para essa tendência de crescimento são os problemas ambientais causados pelos lubrificantes convencionais e o esgotamento das reservas de petróleo que aumentam a necessidade de lubrificantes renováveis e biodegradáveis. Além disso, os biolubrificantes podem ser produzidos utilizando diversos tipos de matéria-prima oleaginosa e por meio de reações químicas distintas que podem ser eficientemente catalisadas por lipases imobilizadas, o que os torna bastante atrativos no contexto da Química Verde. Assim, suportes iônicos foram preparados via sequencial funcionalização da sílica de casca de arroz com (3- Glicidilóxipropil)trimetóxissilano (GPTMS) (Epx-SiO_2) e ativação com glicina (Gli-Epx-SiO_2) a fim de imobilizar a lipase de *Thermomyces lanuginosus* (TLL) por adsorção e posteriormente empregar o biocatalisador obtido na síntese de biolubrificante. A sílica da casca de arroz (RHS) foi selecionada como suporte com o objetivo de comparar o seu desempenho frente ao uso da sílica comercial (Immobead S60S). A sequencial funcionalização/ativação dos suportes à base de SiO_2 foram confirmados por análises de MFA, MEV e Fisissorção de N_2. A capacidade máxima de adsorção de TLL de 14,8 ± 0,1 mg/g e 16,1 ± 0,6 mg/g usando, respectivamente, RHS e Immobead S60S como suporte foi observada. O modelo de isotermas de Sips foi o que se ajustou melhor aos dados experimentais de adsorção de TLL. As atividades catalíticas da TLL imobilizada nos suportes foram analisadas por hidrólise da emulsão de azeite de oliva e síntese de estearato de butila via reação de esterificação. A atividade hidrolítica do biocatalisador preparado a partir do suporte de sílica comercial (357,6 ± 11,2 IU/g) foi ligeiramente superior ao Gli-Epx-SiO_2 preparado com RHS (307,4 ± 7,2 IU/g). Por outro lado, foi similar para ambos os biocatalisadores a atividade catalítica na reação de esterificação (conversão em torno de 90% em 9-10h de reação) e a estabilidade operacional após 6 ciclos consecutivos de síntese de estearato de butila (biolubrificante) em sistemas descontínuos.

Palavras-chave: Sílica. Funcionalização. Suportes iônicos. Imobilização de lipase. Propriedades catalíticas. Biolubrificantes.

ABSTRACT

In recent years, biolubricants have been gaining prominence in comparison with conventional petroleum-based lubricants. This trend is observed in almost all sectors relying on these products, regardless of their applications. The factors that contribute to such growing trend are the environmental problems caused by coventional lubricants and the depletion of oil reserves that have increased the need for renewable and biodegradable lubricants. In addition, biolubricants can be produced using several types of oleaginous feedstock and distinct chemical reactions that can be efficiently catalyzed by lipases, which make them quite attractive in the context of Green Chemistry. Thereby, ion-exchange supports have been prepared via sequential functionalization of silica-based materials with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) (Epx-SiO_2) and activation with glycine (Gly-Epx-SiO_2) in order to immobilize lipase from *Themomyces lanuginosus* (TLL) via adsorption and then use the biocatalyst obtained in the synthesis of biolubricant. Rice husk silica (RHS) was selected as support with the aim of comparing its performance with commercial silica (Immobead S60S). Sequential functionalization/activation of SiO_2-based supports has been confirmed by AFM, SEM and N_2 adsorption-desorption analyses. Maximum TLL adsorption capacities of 14.8 ± 0.1 mg/g and 16.1 ± 0.6 mg/g using RHS and Immobead S60S as supports, respectively, have been reached. The Sips isotherm model has been used, which was well fitted to experimental data on TLL adsorption. Catalytic activities of immobilized TLL were assayed by olive oil emulsion hydrolysis and butyl stearate synthesis via an esterification reaction. Hydrolytic activity of the biocatalyst prepared with a commercial support (357.6 ± 11.2 IU/g) was slightly higher than that of Gly-Epx-SiO_2 prepared with RHS (307.4 ± 7.2 IU/g). On the other hand, both biocatalysts presented similar activity (around 90% conversion within 9-10 h of reaction) and operational stability after 6 consecutive cycles of butyl stearate synthesis in batch systems.

Keywords: Silica. Functionalization. Ion-exchanger supports. Lipase immobilization. Catalytic properties. Biolubricants.

APRESENTAÇÃO

Esta tese compreende aa preparação, caracterização e imobilização de lipase em partículas de sílica funcionalizadas e aplicação dos biocatalisadores preparados na produção de ésteres graxos com propriedades lubrificantes.

O texto está dividido em três capítulos, além da introdução e dos anexos.

Na Introdução discorre-se sobre a motivação e proposta do projeto, tratando do preparo e aplicação de biocatalisadores heterogêneos na produção de biolubrificantes, além dos objetivos gerais e específicos.

O capítulo 1 traz uma Revisão de Literatura que aborda sobre biolubrificantes, rota de produção dos mesmos, lipases, imobilização de enzimas em suportes porosos enfatizando aqueles à base de sílica e por fim aborda a importância e a contribuição do processo de funcionalização/ativação de suportes para a melhora do desempenho de biocatalisadores heterogêneos. Dessa forma, esse capítulo embasa a pesquisa, as discussões e as considerações abordadas.

O capítulo 2 contempla um artigo de revisão intitulado "Biolubricant production using lipases as catalysts: Current scenario and future perspectives" submetido no periódico indexado BioEnergy Research. Esse artigo fornece uma visão geral sobre os biolubrificantes, detalhando suas principais aplicações e usos, potenciais matérias-primas, rotas de produção e a ascensão da catálise enzimática heterogênea como uma rota promissora para a produção de biolubrificantes com alta biodegradabilidade e viabilidade econômica.

No capítulo 3 apresenta-se um artigo intitulado "Preparation of ion-exchange supports via activation of epoxy-SiO_2 with glycine to immobilize a microbial lipase – Use of biocatalysts in hydrolysis and esterification reactions" publicado no periódico indexado International Journal of Biological Macromolecules. O artigo aborda a preparação e caracterização de partículas de sílica funcionalizadas e emprego desse suporte na imobilização via adsorção física de lipase de *Thermomyces lanuginosus* com posterior aplicação na síntese de biolubrificantes.

O Anexo I traz onze trabalhos com os resultados adicionais e parciais obtidos no projeto que foram divulgados nos seguintes eventos científicos: 1 trabalho no XXI Simpósio Nacional de Bioprocessos (XXI SINAFERM) realizado entre os dias 03 a 06/09/2017 na cidade de Aracajú – SE; 2 trabalhos no XIII Seminário Brasileiro de Tecnologia Enzimática (XIII ENZITEC), que realizado entre os dias 16 a 19/09/2018 na

cidade de Florianópolis – SC; 2 trabalhos no XXII Congresso Brasileiro de Engenharia Química (XXII COBEQ) que realizado entre os dias 23 a 26/09/2018 na cidade de São Paulo – SP; 6 trabalhos no XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) realizado entre os dias 28 a 31/07/2019 na cidade de Uberlândia – MG .

Por fim, o Anexo II contempla três artigos científicos realizados em coautoria e publicados nos seguintes periódicos indexados: Chemical Engineering Research and Design, Enzyme and Microbial Technology e Materials Chemistry and Physics. Esses trabalhos abrangem áreas afins ao projeto de doutorado e foram realizados em conjunto com o Laboratório de Bioprocessos da Universidade Federal de Alfenas.

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INTRODUÇÃO

Biolubrificantes são ésteres graxos que tem se mostrado uma alternativa promissora na substituição dos óleos lubrificantes de origem fóssil (Monteiro et al., 2019; Mobarak et al., 2014). Os ésteres lubrificantes podem ser produzidos pela reação de esterificação de um álcool com um ácido carboxílico ou pela reação transesterificação de óleos e gorduras com alcoois na presença de catalisadores químicos e enzimáticos (Alves et al., 2017b). Porém, diversas limitações como alto valor energético requerido, longos tempos de reação e geração de resíduos químicos são reportadas para a rota química (Cirujano et al., 2015; Bouzidi et al., 2012; Al-Arafi e Salimon, 2011). Com o propósito de solucionar esses problemas, a aplicação de enzimas (lipases) é uma alternativa promissora para a produção destes ésteres devido à demanda crescente por produtos ambientalmente sustentáveis (Alves et al., 2017b; Khan et al., 2015; Ungcharoenwiwat e Kittikun, 2013).

Lipases (triacilglicerol éster acilhidrolases – EC 3.1.1.3) são enzimas hidrolíticas amplamente empregadas em processos industriais porque podem catalisar diferentes reações químicas em meios aquosos (hidrólise de ésteres) e não aquosos (síntese de ésteres) (Gholamzadeh et al., 2017; Vescovi et al., 2017; Kapoor e Gupta, 2012). A partir dessas reações é possível obter produtos de elevado valor agregado para as indústrias química, farmacêutica e de alimentos, dentre os mais importantes estão os biolubrificantes, biodiesel, emulsificantes, fragrâncias e aromas, polímeros, resolução de misturas racêmicas, etc. (Alves et al., 2017a; Salihu e Alam, 2015; Homaei et al., 2013).

A aplicação de lipases nas formas solúvel (formulações líquidas) ou agregadas (formulações em pó) em processos industriais é limitada devido à sua difícil recuperação do meio reacional e posterior reutilização ao final do processo, sensibilidade à alta temperatura, solventes orgânicos e pH extremos (Homaei et al., 2013; Adlercreutz, 2013). Estas limitações podem ser amenizadas ou até superadas pelo uso de diferentes protocolos de imobilização, como adsorção física (interações iônicas, hidrofóbicas e ponte de hidrogênio), encapsulação, ligação covalente e enzimas reticuladas – *crosslinked enzymes aggregates* (CLEAs) (Garmroodi et al., 2016; Ganguly e Nandi, 2015; Homaei et al., 2013). Dentre estes diferentes métodos de imobilização, a adsorção física é o método mais empregado na imobilização de lipases porque em alguns casos melhora sensivelmente a sua estabilização e atividade catalítica em meios não-aquosos e permite regenerar o

suporte após a inativação de lipases por dessorção empregando agentes químicos como tampão, tensoativos, ureia, guanidina, etc (Virgen-Ortiz et al., 2017; Rueda et al., 2016).

A triagem e a seleção do suporte que será utilizado na imobilização de lipases é um ponto crucial na preparação de biocatalisadores heterogêneos, uma vez que, o mesmo deve apresentar alta atividade catalítica e mínima limitação difusional das moléculas dos reagentes e/ou produtos em seu microambiente (Alves et al., 2017a). Atualmente, existe uma gama de materiais, orgânicos e inorgânicos, que são utilizados como suportes porosos para a imobilização de lipases. Os materiais inorgânicos como sílica (Gholamzadeh et al., 2017; Vescovi et al., 2017), alumina (Kumar et al., 2013), titânio (Zucca e Sanjust, 2014), materiais cerâmicos (Jean et al., 2014) e partículas magnéticas (Monteiro et al., 2019) têm sido amplamente empregados devido às suas importantes características como alta estabilidade em solventes orgânicos e ao ataque microbiano, fácil funcionalização, elevada área superficial e/ou tamanho de poros e excelentes propriedades mecânicas (Gholamzadeh et al., 2017; Zucca e Sanjust, 2014; Kumar et al., 2013).

Nos últimos anos, os suportes à base de sílica têm ganhado grande destaque devido à sua flexibilidade para o uso de diferentes mecanismos e protocolos de imobilização, o que resulta em biocatalisadores mais estáveis, resistentes e versáteis (Homaei et al., 2013). Estes materiais são comercializados por diferentes companhias internacionais e podem ser obtidos através da técnica sol-gel usando precursores de alcóxissilanos, ou preparados por processos hidrotérmicos a partir de resíduos do setor agroindustrial como bagaço de cana, casca de arroz, folhas de bambu, cascas de amendoim, folhas de romã, dentre outros (Vescovi et al., 2017; Vescovi et al., 2016; Vaibhav et al., 2015; Carvalho et al., 2015). Em razão do seu baixo custo, propriedades físico-químicas atraentes e fácil aquisição, sílica obtida a partir de resíduos agroindustriais pode ser usada em diferentes setores como na construção civil, como adsorvente de poluentes de resíduos industriais, na produção de baterias (Cui et al., 2017; Shen, 2017; Zhang et al., 2017; Mor et at., 2016; Noushad et al., 2016; Liu et al., 2015; Salazar-Carreño e García-Cáceres, 2015). Entretanto, sua aplicação como suporte para preparar biocatalisadores ativos e estáveis ainda é pouco reportada pela literatura especializada.

No presente projeto de doutorado, partículas de sílica foram obtidas a partir do tratamento hidrotérmico de casca de arroz, um resíduo agroindustrial comumente encontrado em nosso país, e funcionalizadas com o intuito de obter um suporte iônico capaz de interagir via adsorção física com grupos carboxilato e amino da estrutura de

lipases. A estratégia adotada neste projeto para a preparação do suporte iônico consistiu na sequencial funcionalização da sílica de casca de arroz com (3-Glicidilóxipropil)trimetóxissilano (GPTMS) para a inserção de grupos epóxi na superfície do suporte (Epx-SiO_2), seguido por ativação com glicina para a introdução dos grupos iônicos (Gli-Epx-SiO_2). Sílica comercial (Immobead S60S) foi também usada como fonte de sílica e os resultados obtidos foram comparados com o material obtido a partir da casca de arroz preparado em laboratório. Os suportes quimicamente modificados foram caracterizados por diferentes técnicas como microscopia de força atômica (MFA), microscopia eletrônica de varredura (MEV) e fisissorção de nitrogênio (determinação de área superficial, diâmetro e volume de poros). A preparação de suporte iônico via ativação de Epx-SiO_2 com glicina foi inicialmente reportada por Bolivar e Nidetzky (2012) para a imobilização da enzima $_D$-Amino acid oxidase de *Trigonopsis variabilis* (*Tv*DAO), uma enzima aplicada na produção de intermediários da indústria farmacêutica e de química fina. A inovação do presente projeto foi a utilização de suporte funcionalizado de forma semelhante na imobilização da lipase de *Thermomyces lanuginosus* (TLL) para posterior uso como catalisador heterogêneo na síntese de estearato de butila (biolubrificante) via esterificação. TLL foi usada como lipase modelo devido à sua alta atividade catalítica em reações em meio orgânico na síntese de diferentes ésteres (Fernandez-Lafuente, 2010; Alves et al., 2017a, b).

Neste contexto, o objetivo geral deste trabalho foi a preparação e caracterização de suportes iônicos obtidos a partir da funcionalização de sílica de casca de arroz e sílica comercial (Immobead S60S) e suas aplicações na síntese de éster lubrificante (estearato de butila) por reação de esterificação em meio orgânico. O objetivo geral do projeto foi alcançado mediante a execução dos seguintes objetivos específicos:

- Obtenção de partículas de sílica e sequencial funcionalização/ativação para a obtenção de suportes iônicos;

- Caracterização dos suportes por diferentes técnicas;

- Aplicação dos suportes obtidos na imobilização de TLL. Avaliação da influência dos parâmetros como pH e concentração inicial de proteína (enzima) no processo de imobilização;

- Determinação da atividade catalítica do biocatalisador preparado em reações de hidrólise de óleo;

- Estudos de isoterma de adsorção com o intuito de avaliar o processo de adsorção física da enzima;

- Aplicação dos biocatalisadores heterogêneos preparados na síntese de estearato de butila por esterificação em meio orgânico;

- Testes de estabilidade operacional do biocatalisador (reuso) após sucessivos ciclos de reação de esterificação.

- Elaboração de trabalho de revisão de literatura com o intuito de elucidar o conhecimento sobre a produção de biolubrificante catalisada por biocatalisadores heterogêneos.

CAPÍTULO 1

REVISÃO DE LITERATURA

1.1 Biolubrificantes

Os lubrificantes são compostos orgânicos capazes de controlar ou reduzir o atrito entre duas superfícies móveis, além de diminuir o aquecimento e o desgaste das peças mecânicas através da formação de uma película de proteção (Fernandes et al., 2018; Bassi et al., 2016). Eles podem ser encontrados no mercado na forma sólida, líquida ou gasosa (Mobarak et al., 2014). Segundo a Grand View Research, Inc. (2020), o mercado global de lubrificantes em 2018 foi de US$ 128,51 bilhões. Somente nos Estados Unidos foram comercializados 2.260 quilotoneladas de lubrificantes, sendo que a distribuição do consumo se concentra nos setores de lubrificantes automotivos (56%), lubrificantes industriais (32%) e no processamento de óleos (12%). Ademais, a projeção de crescimento mundial até 2022 é de 43,87 milhões de toneladas a uma taxa de 2,4% ao ano.

Via de regra, são utilizados para formulação de lubrificantes três tipos de fluidos base: óleos minerais, óleos vegetais e lubrificantes sintéticos (Nagendramma e Kaul, 2012). O óleo mineral é derivado do petróleo e largamente utilizado como lubrificante em motores de combustão interna, contudo esse cenário vem sofrendo mudanças severas dada a necessidade do uso de técnicas agressivas para a extração do petróleo devido à escassez das suas reservas naturais, alto custo do transporte e dos processos para o seu refino (Monteiro et al., 2019; Mobarak et al., 2014). Dessa maneira, os óleos lubrificantes sintéticos ganharam cada vez mais espaço no mercado, principalmente a partir da Segunda Guerra Mundial, pois podem ser produzidos a partir da combinação química de componentes de baixa massa molecular para formar compostos de alta massa molecular com estrutura e propriedades desejadas (Fernandes et al., 2018; Bassi et al., 2016; Mobarak et al., 2014).

A atenção e a responsabilidade sobre questões ambientais é um ponto crítico para a indústria e órgãos públicos. Na esfera dos lubrificantes, a preocupação consiste em questões relacionadas à grande quantidade que é descartada no meio ambiente de maneira inadequada, uma vez que cerca de metade de todo o lubrificante usado no mundo contamina diretamente os corpos hídricos, o solo e a atmosfera (Zainal et al. 2018; Fernandes et al., 2018; Nagendramma e Kaul, 2012). Na água, eles formam uma película

de óleo sobre a superfície, o que impede a entrada da luz solar e do oxigênio que são necessários para a manutenção da vida do ecossistema aquático; quando depositados no solo diminuem sua capacidade de filtração e troca iônica, bem como sua fertilidade; e quando queimados liberam gases tóxicos ao meio ambiente (Castro et al., 2015; Mobarak et al., 2014). Segundo o Sindicato Nacional da Indústria do Rerrefino de Óleos Minerais, no ano de 2017 o Brasil foi o sexto maior consumidor de óleos lubrificantes do mundo e, consequentemente, o sexto maior produtor de óleo lubrificante usado ou contaminado, responsabilizando-se pelo consumo de aproximadamente 1,3 bilhões de litros de óleo lubrificante por ano (SINDIRREFINO, 2018). Dessa maneira, a necessidade de desenvolvimento de novas tecnologias para a produção de lubrificantes sintéticos biodegradáveis (biolubrificantes) é de extrema importância.

Biodegradabilidade é a capacidade de um material ser degradado por microrganismos. Um lubrificante é classificado como biodegradável se seu percentual de degradação em testes padrões excede determinado nível. O percentual de biodegradabilidade é influenciado pelo óleo base dos lubrificantes e depende da composição química do composto. A partir disso, é observado que os lubrificantes provenientes de óleos vegetais são os que melhor atendem aos requisitos de biodegradabilidade (90-98%) (Monteiro et al., 2019; Mobarak et al., 2014).

Quando comparado com lubrificantes minerais e semi-sintéticos, os biolubrificantes apresentam as seguintes vantagens: menor toxicidade, biodegradabilidade, alta lubrificação, produção a partir de fontes renováveis, alto ponto de fulgor, menor volatilidade, maior índice de viscosidade e baixo ponto de fluidez que garante excelente lubrificação em arranques à frio (Monteiro et al., 2019; Bassi et al., 2016; Silva et al., 2015). Outra vantagem dos biolubrificantes é a variedade de óleos vegetais que podem ser utilizados para a sua produção incluindo linhaça, palma, girassol, coco, soja, oliva, mamona e colza (Saboya et al., 2017; Silva et al., 2015; Mobarak et al., 2014). Todavia, os óleos vegetais apresentam algumas desvantagens como instabilidade oxidativa e hidrolítica, limitações lubrificantes em baixas temperaturas e baixa compatibilidade com tintas e selantes (Fernandes et al., 2018; Silva et al., 2015; Mobarak et al., 2014). Entretanto, dependendo da rota de produção escolhida para a obtenção de um biolubrificante e da possibilidade de realizar modificações químicas nos óleos vegetais podem-se eliminar os aspectos desvantajosos (Zainal et al., 2018; Silva et al., 2015; Tao et al., 2014).

1.2 Matérias-primas para a produção de biolubrificantes

Atualmente, os lubrificantes têm aplicações e funções variadas, a saber: graxa, fluido hidráulico, óleo de engrenagem e motosserra, óleo para barras e correntes, óleo para motor de dois tempos, óleo de bomba de vácuo e aplicações na indústria de cosméticos (Singh et al., 2019; Zainal et al., 2018; Rudnick, 2013). Dessa forma, a escolha da matéria-prima que será empregada como base para a produção desse produto é de suma importância, para que cada finalidade de uso seja alcançada.

Os biolubrificantes que atualmente estão disponíveis no mercado são compostos total ou parcialmente de óleos e gorduras de origem biológica, estes produtos atendem aos requisitos das normas internacionais em termos de renovabilidade, biodegradabilidade, toxicidade e desempenho técnico (Chan et al., 2018; Panchal et al., 2017; Sethuramiah e Kumar, 2016).

As propriedades físicas e tribológicas (caracteriza a lubrificação) dos biolubrificantes dependem essencialmente da estrutura molecular dos seus compostos constituintes (Chan et al., 2018; Bart et al., 2013). A maioria dos biolubrificantes usados nos dias de hoje apresentam regiões polares e não polares nas moléculas do produto. A região polar é responsável pela adsorção ou adesão à superfície deslizante, enquanto a região não polar é responsável pela resistência e oleosidade do fluido lubrificante. Para a produção do biolubrificante a matéria-prima base mais simples é o óleo vegetal, o qual é denominado de triacilglicerídeo, pois, bioquimicamente consiste em três ácidos graxos de cadeia linear ligados a um glicerol por ligação éster (Ruggiero et al., 2017; Sethuramiah e Kumar, 2016; Quinchia et al, 2014; Bart et al., 2013).

A capacidade geral dos óleos naturais de aderir às superfícies metálicas, motivo pelo qual podem ser empregados como lubrificantes naturais, é ditada pelos ácidos graxos presentes em sua estrutura. Esses ácidos graxos estabelecem um filme de monocamada na superfície metálica usando seu grupo carbonila polar compactado, o que permite reduzir o atrito e o desgaste das peças e estruturas, minimizando o contato de metal com metal (Reeves et al., 2017). Além disso, os óleos naturais derivados de óleos vegetais apresentam características semelhantes aos lubrificantes à base de petróleo usados em aplicações industriais para estampagem e modelagem de metais, pois possuem viscosidade e tensão superficial comparáveis aos de origem fóssil (Reeves et al., 2017; Lovell et al.,2010).

A gama de óleos vegetais empregados na produção de biolubrificantes é diversificada e difere de uma região para outra devido a fatores climáticos e geográficos (Zainal et al., 2018; Atabani et al., 2013). Por exemplo, os óleos de colza e de girassol são frequentemente usados na Europa, enquanto os óleos de palma (dendê) e coco são usados principalmente na Ásia. Por outro lado, a principal matéria-prima dos Estados Unidos para produzir os biolubrificantes é a soja (Zainal et al., 2018; Saboya et al., 2017; Silva et al., 2015; Mobarak et al., 2014). Segundo a Companhia Nacional de Abastecimento (Conab), a safra brasileira de soja 2019/2020 tem produção estimada em 122,2 milhões de toneladas, um aumento de 6,3% em relação à safra 2018/2019, o que mantem o Brasil como o segundo maior produtor mundial dessa oleaginosa (CONAB, 2020). Em consequência dessa elevada produção agrícola, é possível obter no país grandes quantidades de óleo, dado que para cada tonelada de soja esmagada são extraídos aproximadamente 190 kg de óleo bruto (ABIOVE, 2020). A Tabela 1.1 lista algumas matérias-primas oleaginosas utilizadas na produção de biolubrificantes, condições edafoclimáticas e maiores produtores.

Os óleos vegetais podem ser classificados como óleos comestíveis e não comestíveis (Singh et al, 2019). Dessa maneira, é desejável priorizar o emprego dos óleos não comestíveis e/ou usados como matérias-primas para a produção dos lubrificantes renováveis, uma vez que esses óleos não competem com a indústria de alimentos e/ou podem ser obtidos a partir do uso dos óleos comestíveis ou resíduos. Outro fator relevante, é que essa prática pode diminuir o custo total de produção do lubrificante renovável em até 80% comparado ao produzido a partir de óleos virgens coméstíveis (Sarno et al., 2019; Chan et al., 2018; Zainal et al., 2018).

Outras matérias-primas menos usuais que podem ser empregadas na produção dos biolubrificantes são os óleos de origem microbiana e os óleos obtidos a partir de plantas geneticamente modificadas (OMGs) (Reeves et al., 2017). Nesses últimos, as plantas são manipuladas para a alteração da sequência de genes dentro dos organismos, fazendo com que um gene fique inoperante ou anexando um gene a uma região identificada do DNA que inicia a transcrição de um gene em particular, agindo como um "originador de expressão gênica". Os óleos vegetais geneticamente modificados geralmente se concentram em melhorar as estabilidades térmica e oxidativa dos óleos naturais. O objetivo dessas manipulações é criar óleos com graus mais altos de saturação, pois assim o óleo será menos suscetível à deterioração melhorando as propriedades de fluxo a frio dos lubrificantes produzidos (Reeves et al., 2017; Mendonza et al., 2011).

Tabela 1.1 Levantamento bibliográfico das matérias-primas oleaginosas utilizadas na produção de biolubrificantes, condições edafoclimáticas, maiores produtores atuais e aplicação específicas dos biolubrificantes (Zainal et al., 2018; Panchal et al., 2017).

Oleaginosas	Condições Edafoclimáticas	Maiores Produtores	Aplicações Específicas
Canola	É bem adaptada aos frios extremos das zonas temperadas e cresce bem em solos férteis e bem drenados. A canola requer cerca de 406-460 mm de água durante a estação de crescimento.	Europa, Índia, Canadá, China e Austrália.	Óleos hidráulicos, fluidos de transmissão de trator, lubrificantes para a indústria de alimentos, lubrificantes de barras de corrente.
Coco	Esta planta prospera bem em solos arenosos e é altamente tolerante à salinidade. Os coqueiros colonizam os litorais das regiões tropicais, pois preferem áreas com luz solar abundante e chuvas regulares (1500-2500 mm por ano).	Indonésia, Índia, Filipinas e Sri Lanka.	Óleos para motores à gás.
Colza	A colza está adaptada à climas marítimos amenos, onde as temperaturas médias variam de 10 à 30 °C. No entanto, a temperatura ideal dessa é em torno de 20 °C.	Europa, Canadá, Estados Unidos, Austrália, China e Índia.	Lubrificantes para motosserras, equipamentos agrícolas e compressores de ar.
Girassol	Os girassóis precisam de luz solar constante e são altamente tolerantes à seca. Ademais, crescem em solos férteis, úmidos e bem drenados.	Rússia, Ucrânia, Argentina e Europa.	Graxa e substitutos de diesel combustível.
Mamona	A precipitação constante e o solo drenado, profundo e fértil são necessários para o bom crescimento.	Cuba, Brasil, China, Índia, Itália e França.	Lubrificantes para engrenagens e graxas.
Oliva	A oliveira prefere clima quente e áreas ensolaradas sem sombra, pois tolera bem as condições de seca devido ao seu sistema radicular firme e extenso.	Espanha, Itália e Grécia.	Lubrificantes para automóveis.

Palma	A palma é caracterizada por ser cultivada em solos profundos, de textura argilosa, boa drenagem e com pH entre 4,5 e 6. Ademais, as temperaturas médias superiores a 24 °C garantem uma alta produtividade da planta.	Malásia e Indonésia.	Lubrificante para laminação e graxa
Soja	As condições ideias para o plantio é em regiões com verões quentes. A temperatura média recomendada está entre 20 e 30 °C.	Estados Unidos, Brasil, Argentina, China e Índia.	Lubrificantes para tintas de impressão, revestimentos, óleo hidráulico, sabonetes, detergentes, xampus, desinfetantes e plastificantes.

1.3 Rotas para produção de biolubrificantes

A seleção da matéria-prima lipídica empregada para a produção dos ésteres lubrificantes é um parâmetro de processo extremamente relevante a ser estudado, além dele, a escolha adequada da rota de produção que será empregada na síntese desses compostos também é de suma importância (Silva et al., 2015; Tao et al., 2014).

Os ésteres são compostos químicos frequentemente usados na manufatura de plastificantes, tintas, aromas, produtos farmacêuticos, cosméticos, combustíveis líquidos e lubrificantes (Wang et al., 2016; Chowdhury et al., 2014). Esses últimos são ésteres alquílicos que se originam a partir da reação de ácidos carboxílicos e alcoois de cadeia longa, ou seja, compostos com 8 ou mais carbonos (Lima et al., 2018b; Chowdhury et al., 2014; Rudnick, 2013). Os radicais do éster influenciam diretamente nas propriedades físico-químicas dos biolubrificantes, sendo que um dos requisitos mínimos para ser um bom lubrificante é permanecer líquido em uma ampla faixa de temperatura de forma que os limites são determinados pelo ponto de fluidez e pelo ponto de fulgor (Saboya et al., 2017).

Os biolubrificantes podem ser produzidos a partir de várias reações químicas como a transesterificação de óleos/gorduras e ésteres monoalquílicos (biodiesel), esterificação direta de ácidos graxos livres (AGL), epoxidação sequencial de lipídeos e hidroesterificação, um processo seriado de hidrólise de óleos/gorduras em AGL seguido

pela reação de esterificação direta dos AGL em meio orgânico (Valle et al., 2018; Bassi et al., 2016; Bressani et al., 2015).

A reação de esterificação é uma das rotas mais utilizadas para a produção de biolubrificantes, entretanto em baixas temperaturas o tempo reacional é muito longo. Dessa forma, para solucionar esse problema a temperatura da reação é aumentada e catalisadores químicos são empregados, que podem ser ácidos ou básicos, homogêneos ou heterogêneos. Os principais catalisadores químicos utilizados na catálise homogênea são os ácidos fosfórico, clorídrico, *p*-toluenosulfônico e sulfúrico; entre as bases mais comuns estão o hidróxido de sódio, hidróxido de potássio e etóxido e metóxido de sódio (Saboya et al.,2017; Silva et al., 2013). Entretanto, a catálise homogênea apresenta alguns problemas operacionais como a possível corrosão dos reatores, dificuldade na separação entre produtos e catalisadores demandando alto volume de água para purificá-los (Saboya et al., 2017; Li et al., 2016).

A catálise heterogênea surgiu como uma alternativa promissora, frente à catálise homogênea, devido à facilidade de separação dos produtos, além de reduzirem a produção de águas residuais e os catalisadores poderem ser reciclados para posterior utilização em outros ciclos reacionais (Saboya et al., 2017). Dentre os exemplos de catalisadores químicos heterogêneos mais utilizados estão as resinas de troca catiônica e ácidos sólidos tais como ZrO_2/TiO_2 (Boffito et al., 2013), ZrO_2/WO_3 (Saboya et al., 2016), ZrO_2/SiO_2 (Kuzminska et al., 2014), heteropoliácidos (Kuzminska et al., 2014) e zeólitas (Doyle et al., 2016). Todavia, devido às limitações de transferência de massa, a catálise química heterogênea também traz sérios problemas porque exige condições reacionais mais severas para alcançar altos índices de conversão de produto (Saboya et al., 2017).

Com o objetivo de melhorar as condições reacionais e o percentual de conversão da reação de esterificação para a produção de biolubrificantes, as lipases têm sido muito pesquisadas em consequência das brandas condições reacionais demandadas, alta especificidade frente aos substratos, alto grau de pureza dos produtos obtidos o que faz com que a geração de subprodutos e efluentes seja reduzida proporcionando a realização de processos ambientalmente favoráveis. Em função dessas vantagens a produção e comercialização de lipases estão em ascensão, resultando no desenvolvimento de tecnologias alternativas consistentes para utilização no setor industrial (Thangaraj e Solomon, 2019; Bassi et al., 2016; Lage et al., 2016; Silva et al., 2015).

No entanto, o emprego de lipases na sua forma livre, ou seja, via catálise homogênea não é interessante, devido ao alto custo da enzima, alta sensibilidade em

solventes e altas temperaturas, impossibilidade de reuso do catalisador e possível contaminação do produto (Thangaraj e Solomon, 2019; Alves et al., 2017a; Alves et al., 2017b). A fim de ultrapassar essas barreiras, os protocolos de imobilização de lipases para obtenção de catalisadores heterogêneos são indicados e empregados em pesquisas científicas (Lima et al., 2018a; Vescovi et al., 2017).

1.4 Lipases

Enzimas são biocatalisadores de natureza proteica, que reduzem a energia de ativação de uma reação química, sem alterar o seu equilíbrio e consequentemente provocam o aumento da velocidade reacional (Homaei et al., 2013). A partir da participação das enzimas, a catálise ocorre em condições brandas e na maioria das vezes em soluções aquosas a pressão atmosférica e temperatura ambiente. Dessa forma, o consumo de energia e a formação de subprodutos indesejáveis são reduzidos devido à sua elevada especificidade, o que reduz a quantidade de resíduos gerados e aumenta o rendimento do processo (Thangaraj e Solomon, 2019; Homaei et al., 2013; Fernandes-Lafuente, 2010).

As lipases (triacilglicerol acil hidrolases - EC 3.1.1.3) são para o setor industrial um grupo importante de enzimas, devido à sua capacidade de clivar ligações ésteres de óleos e gorduras na interface água/óleo, com subsequente liberação de ácidos graxos livres e glicerol (Lage et al., 2015; He et al. 2010). Além disso, as lipases também podem catalisar as reações de esterificação, transesterificação e aminólise (síntese de amidas) em meios não-aquosos (Javed et al., 2018; Carvalho et al., 2015; Adlercreutz, 2013). As lipases são produzidas a partir de fontes animais, vegetais e microbianas, sendo a última fonte a mais empregada industrialmente devido à sua simplicidade na manipulação genética e condições de cultivo (Filho et al., 2019; Lage et al., 2015; Adlercreutz, 2013). As lipases microbianas mais comuns são as obtidas por: *Bacillus* sp., *Candida* sp., *Candida rugosa*, *Candida antartica*, *Burkholderia cepacia*, *Pseudomonas alcaligenes*, *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., *Mucor sp.*, *Geotrichum* sp., *Tulopis* sp. e *Thermomyces lanuginosus* (Carvalho et al., 2015).

A lipase de *Thermomyces lanuginosus* (TLL) é a enzima responsável pela atividade lipolítica da Lipolase®, uma preparação de lipase solúvel comercializada pela Novozymes, que tambem produz a sua forma imobilizada denominada Lipozyme TL IM®. A TLL é uma proteína formada por uma cadeia de 269 aminoácidos, massa

molecular de 31,7 kDa, atividade ótima em torno do pH 9 com o ponto isoelétrico de 4.4 e temperatura ótima variando de 55 a 60 °C (Lage et al., 2015; Miranda et al., 2014). Além das características acima citadas, a TLL possui uma tampa (*lid*) cobrindo o seu sítio ativo, que quando a enzima se encontra na interface óleo-água esta *lid* sofre uma mudança conformacional, controlando o acesso das moléculas de substrato ao sítio ativo da enzima, conforme ilustrada pela Figura 1 (Willems et al., 2018; Skjold-Jorgensen et al., 2017; Fernandes-Lafuente, 2010). Ademais, a TLL é biotecnologicamente relevante podendo ser utilizada em diferentes tipos de indústria como alimentos, química fina, tratamento de águas residuais, cosméticos, papel e celulose, farmacêutica, biocombustíveis (biodiesel) e biolubrificantes (Willems et al., 2018; Fernandes-Lafuente, 2010).

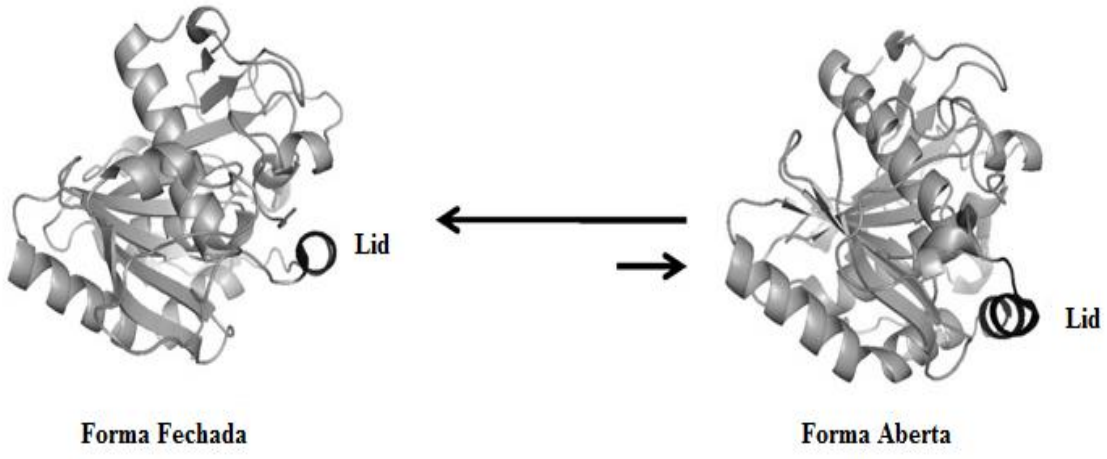

Figura 1.1 Estrutura das formas aberta e fechada da lipase de *Thermomyces lanuginosus* (Fernandes-Lafuente, 2010).

1.5 Imobilização de lipases

É inegável que o emprego das lipases como catalisadores em processos indústrias é uma alternativa promissora, contudo, as mesmas possuem desvantagens quando empregadas na forma solúvel ou na forma de preparados em pó (forma livre) devido ao alto custo financeiro para a sua aquisição, baixas estabilidades operacional e de armazenamento (Barbosa et al., 2014a; Poppe et al., 2013). Em consequência disso, as técnicas de imobilização de lipases por diferentes protocolos são amplamente empregadas (Alves et al., 2017a; Adlercreutz, 2013).

Muitos protocolos de imobilização baseiam-se no confinamento da enzima em um suporte sólido, que pode ser poroso ou não, criando um sistema de catálise heterogênea que possibilite a posterior reutilização do biocatalisador (Alves et al., 2017a). Além disso,

existem protocolos que não utilizam suportes para a imobilização da enzima e empregam agentes reticuladores para a modificação da mesma, a fim de obter o biocatalisador heterogêneo de interesse. De um modo geral, a forma imobilizada da enzima simula a sua condição natural nas células vivas, onde a maioria delas está ligada a estruturas celulares do citoesqueleto, membrana e organelas (Homaei et al., 2013). Para ser considerado um bom biocatalisador, a estabilidade operacional deve ser satisfatória e a atividade catalítica e conformação nativa da enzima pós imobilização não podem sofrer muitas alterações (Barbosa et al., 2014a; Mendes et al., 2013). Dessa maneira, é de extrema importância a realização de pesquisas científicas e trabalhos tecnológicos dedicados à elaboração de protocolos de imobilização. A revisão de literatura feita por Filho et al. (2019), ressalta que as pesquisas feitas sobre os diferentes métodos de imobilização de enzimas no período de 2013 a 2018 mais que dobrou, sendo que, a imobilização por adsorção teve destaque com 61 trabalhos publicados, seguida pelo protocolo de ligação covalente com 25 artigos científicos.

As principais vantagens obtidas pelo processo de imobilização são:

- aumento da estabilidade térmica do biocatalisador;
- aumento da resistência à mudanças do meio reacional;
- enzimas imobilizadas são mais robustas do que a sua forma livre;
- permite a aplicação em reatores com maior controle do processo;
- possibilita o uso em processos contínuos;
- possibilita o uso de elevadas concentrações de enzimas;
- permite o reuso do biocatalisador por vários ciclos sem perda significativa da sua atividade catalítica.

As possíveis desvantagens dos protocolos de imobilização são:

- alteração da conformação nativa da enzima;
- custo do suporte;
- pode apresentar limitações de transferência de massa;
- pode apresentar efeitos de partição e eletrostáticos;
- perda de atividade durante o processo de imobilização.

A seleção do protocolo de imobilização para cada enzima deve ser fundamentada em parâmetros como: atividade catalítica, efeito limitante difusional, características de regeneração e inativação da enzima, custos do método de imobilização, características do

suporte, toxicidade dos reagentes utilizados e análise das propriedades catalíticas finais desejadas (Carvalho et al., 2015; Mendes et al., 2013).

As lipases podem ser imobilizadas de muitas maneiras, conforme mostrado na Figura 1.2, tais como adsorção física em suportes hidrofóbicos ou iônicos, encapsulação, ligação covalente e enzimas reticuladas – *crosslinked enzymes aggregates* (CLEAs) (Gholamzadeh et al., 2017; Vescovi et al., 2017). No presente trabalho, o método de adsorção física foi empregado na imobilização de lipase de *Thermomyces lanuginosus* e as principais características deste e outros métodos são relatadas a seguir.

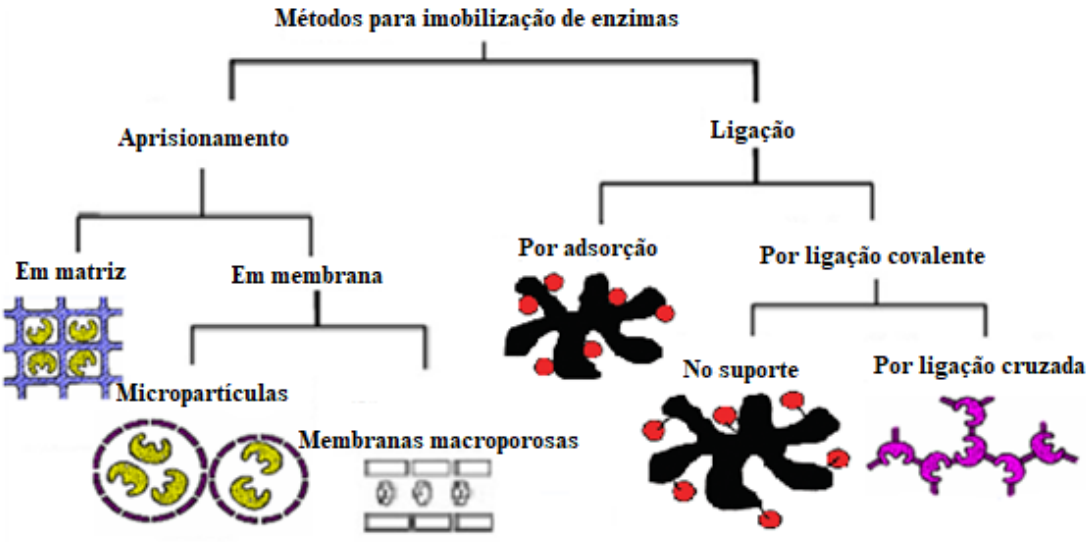

Figura 1.2 Protocolos para imobilização de enzimas (Dalla-Vecchia et al., 2004).

1.5.1 Imobilização de lipases por adsorção física

A adsorção física é o método mais simples utilizado para a imobilização de lipases (Jesionowski et al., 2014; Homaei et al., 2013). Essa técnica tem como base as interações que incluem as forças de van der Waals, ligação de hidrogênio, interações iônicas e hidrofóbicas para confinar a enzima na superfície do suporte (Filho et al., 2019; Jesionowski et al., 2014). Em geral, para a adsorção ocorrer a solução enzimática é colocada em contato com o material sólido, em um meio reacional favorável, para que as interações enzima-suporte sejam estabelecidas (Liu et al., 2018; Adlercreutz, 2013).

Esse protocolo de imobilização é de baixo custo, preserva a conformação tridimensional da enzima e permite fácil reuso do suporte após vários ciclos de catálise,

necessitando apenas de agentes químicos que promovam a dessorção da enzima tais como: ureia, guanidina e tensoativos (Virgen-Ortíz et al., 2016; Jesionowski et al., 2014). No entanto, a imobilização via adsorção abrange interações relativamente fracas e reversíveis, dessa maneira, as enzimas podem se desprender do suporte (processo de lixiviação) precocemente contaminando o meio reacional e o biocatalisador heterogêneo perde a sua atividade catalítica (Ding et al., 2019; Homaei et al., 2013).

Uma gama de materiais pode ser utilizada como suportes na adsorção física, mas algumas condições devem ser satisfeitas para o sucesso desse protocolo, dentre elas a afinidade da enzima pelo suporte é a mais importante. Essa atração é assegurada pela presença de grupos ativos específicos que se localizam na estrutura do suporte permitindo que ocorram as interações que retêm a enzima em sua superfície (Sirisha et al., 2016; Jesionowski et al., 2014; Gustafsson et al., 2012; Wu et al., 2012). No entanto, se essa interação entre adsorvato e adsorvente for insuficiente, pode-se ocasionar a redução drástica da atividade e/ou estabilidade catalítica do biocatalisador (Tao et al., 2014; Adlercreutz, 2013). Dessa maneira, a preparação de suportes modificados e funcionalizados com agentes intermediários, tais como polímeros funcionalizados, aminoácidos, líquidos iônicos e agentes alcóxissilanos tem sido relatado na literatura especializada como uma alternativa para melhorar as interações da enzima com o suporte favorecendo o alto desempenho do biocatalisador heterogêneo (Gholamzadeh et al., 2017; Virgen-Ortíz et al., 2016; Jesionowski et al., 2014).

Ferreira e colaborares (2018) imobilizaram lipase de *Geotrichum candidum* por diferentes protocolos, tais como adsorção por ativação interfacial, adsorção por interações iônicas e ligação covalente multipontual. Dentre os biocatalisadores preparados, o imobilizado via adsorção por interações iônicas obteve o maior rendimento de imobilização (98,82 ± 1,71%) e atividade hidrolítica (45,40 ± 0,19 UI/g de suporte). O processo de imobilização foi capaz de reduzir a energia de ativação aparente necessária para a formação do complexo enzima-substrato em cerca de 50%, melhorando assim a propriedade catalítica da enzima em meio aquoso. Outros parâmetros foram avaliados e mostraram que a imobilização melhorou a estabilidade térmica e consequentemente a meia-vida da enzima em toda a faixa de temperatura avaliada (35-50°C) demonstrando que este biocatalisador tem potencial para diferentes aplicações de interesse industrial.

A fim de melhorar o desempenho da sílica comercial como suporte para imobilização por adsorção da lipase de *Pseudomonas fluorescens*, Lima et al. (2018a) modificaram quimicamente a superfície da sílica com grupamentos octil. O biocatalisador

produzido foi empregado na síntese de ésteres com propriedades aromáticas e apresentou desempenho e estabilidade operacional semelhantes aos catalisadores comerciais preparados via imobilização covalente com a mesma lipase e com outras lipases de *Candida antarctica* tipo B e *Thermomyces lanuginosus*.

Lipase de *Burkholderia ambifaria* foi imobilizada via adsorção por Gao et al. (2018) em suporte de dióxido de titânio modificado quimicamente para a síntese do éster de aroma acetato de cinamilo. O suporte mesoporoso foi funcionalizado com o agente silano 3(fenil amino) propil trimetoxi silano e a sua a atividade catalítica alcançou 6119,2 UI/g de suporte e atividade recuperada reteve 221,6%. Além disso, a atividade catalítica e estabilidade em pH e térmica da lipase imobilizada foram melhoradas em relação a sua forma livre. A utilização do biocatalisador reteve mais de 80% da atividade após dez ciclos reacionais indicando excelente potencial para a aplicação em grande escala.

Miguez et al. (2018) funcionalizaram sílica da casca de arroz com o agente silano (3 amino propril) trietoxi silano (3-APTES) a fim de imobilizar lipase de *Thermomyces lanuginosus* via adsorção por interações iônicas e posteriormente empregar o biocatalisador preparado na síntese do éster cosmético oleato de decila. Sob condições experimentais ideais, foi observada uma porcentagem máxima de conversão de éster de 87% após 105 e 180 min de tempo de reação nos sistemas com solvente orgânico e sem solvente, respectivamente, além disso, testes de reuso mostraram que o biocatalisador reteve toda a sua atividade original após oito ciclos sucessivos de reação em um sistema sem solvente.

1.6 Suportes para imobilização de lipases

A escolha do suporte a ser utilizado no protocolo de imobilização é um ponto de extrema importância no processo, visto que a interação entre o suporte e a enzima irá proporcionar ao biocatalisador heterogêneo todas as suas propriedades bioquímicas, químicas, mecânicas e cinéticas (Alves et al., 2017a; Carvalho et al., 2015). Desse modo, se a matriz for escolhida de forma correta pode-se aumentar o rendimento de imobilização, tempo de meia-vida, estabilidade operacional e térmica da enzima. Entretanto, se a escolha não for adequada, poderá prejudicar a estabilidade e o desempenho global do derivado imobilizado (Barbosa et al., 2014b; Carvalho et al., 2015). A Figura 1.3 ilustra a interação entre o suporte e a enzima para compor as propriedades globais do biocatalisador.

As características básicas para a escolha de um material para ser o suporte no protocolo de imobilização são: apresentar grande área superficial, ser inerte, não ser solúvel nas condições reacionais, alta porosidade, resistência a ataque microbiano, permitir fixação da proteína sem alterar a atividade catalítica e o equilíbrio da reação na qual esteja sendo aplicada (Alves et al., 2017a; Mohamad et al., 2015). Em seu estudo Carvalho et al. (2015) sumarizaram as características que devem ser analisadas para a escolha de um suporte, são elas:

- Características químicas: analisa a composição química e da superfície do material, grupos funcionais, estabilidade química, natureza hidrofóbica ou hidrofílica.
- Características mecânicas: observa a capacidade de compressão e compactação da matriz, tamanho da partícula, diâmetro do poro, área superficial, volume, abrasão para reatores agitados e velocidade de sedimentação para reatores do tipo leito fluidizado.
- Características morfológicas: estuda a estrutura porosa ou gel, rugosidade e área superficial da matriz.

Outro fator de extrema importância para a escolha do material que será o suporte é o custo financeiro, pois o seu preço é uma variável que influencia diretamente na viabilidade econômica do protocolo de imobilização e na sua futura aplicação em escala industrial (Siricha et al., 2016; Scherer, et al., 2012).

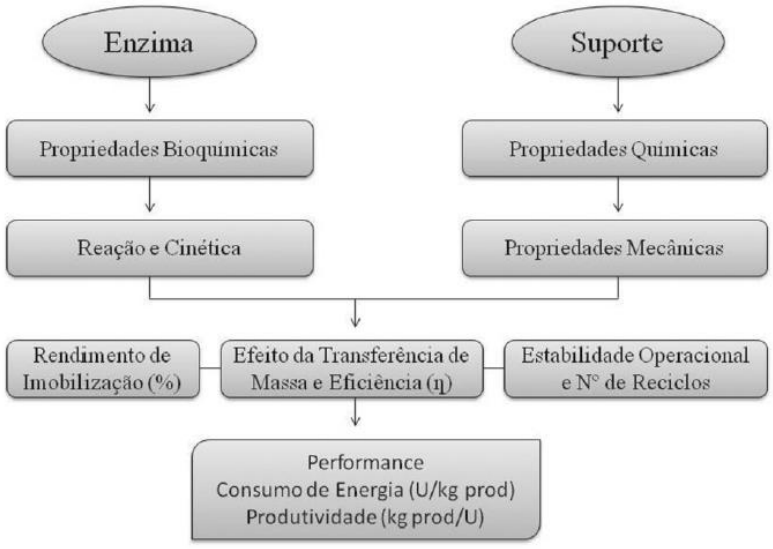

Figura 1.3 Características intrínsecas do suporte e da enzima que influenciam nas propriedades globais do biocatalisador (Carvalho et al., 2015).

A classificação dos suportes é muita ampla, pode-se usar como critério a composição química (orgânico ou inorgânico), a natureza (sintética ou não sintética), a morfologia, porosidade e tamanho dos poros (Alves et al., 2017a; Mohamad et al., 2015). Os materiais inorgânicos, são melhores para uso industrial por mostrarem alta resistência mecânica, estabilidade em ampla faixa de pressões, temperaturas e valores de pH, elevada rigidez, resistência à solventes orgânicos e ao ataque microbiano. Os suportes sintéticos se apresentam de várias formas físicas e estruturas químicas, podendo ser combinados para a produção de uma matriz específica. Por outro lado, os naturais apresentam algumas vantagens quando comparados aos sintéticos, como baixo custo e facilidade de degradação sem causar danos ao meio ambiente. A distribuição, tamanho e morfologia dos poros do suporte influenciam diretamente no sucesso do biocatalisador, visto que modulam a transferência de massa interna e externa do processo reacional. Os materiais porosos são distribuídos em quatro categorias de acordo com o diâmetro dos poros, são elas: microporosos (poros menores que 2 nm), mesoporosos (poros entre 2 e 50 nm), megaporosos (poros entre 50 a 200 nm) e gigaporosos (poros maiores que 200 nm). O uso de suportes megaporosos e mesoporosos são adequados para imobilização de enzimas pois permitem o fácil acesso do substrato ao sítio ativo da enzima (Alves et al., 2017a; Siricha et al., 2016; Carvalho et al., 2015; Mohamad et al., 2015; Barbosa et al., 2014b; Scherer et al., 2012).

São reportados na literatura especializada vários compostos que podem ser empregados como suporte para a imobilização de enzimas. A Tabela 1.2 lista alguns materiais utilizados e as suas características para a imobilização de lipases por diferentes protocolos.

No intuito de melhorar os rendimentos dos derivados e a viabilidade do uso dos protocolos de imobilização, faz-se necessário o desenvolvimento adequado de técnicas de aprimoramento de materiais, por meio de modificações químicas dos suportes com aditivos e/ou estabilizantes, que combinem desempenho tecnológico com renovação e sustentabilidade econômica (Siricha et al., 2016; Barbosa et al., 2014b; Scherer et al., 2012).

Tabela 1.2 Levantamento bibliográfico de suportes utilizados para a imobilização de lipases via diferentes protocolos

Natureza	Classe do suporte	Suporte	Lipase	Protocolo de imobilização	Referência
Natural	Orgânico	Fibra de seda modificada	*Candida sp* 99-125	Adsorção Física	Tao et al., (2014)
		Casca de arroz	*Thermomyces lanuginosus*	Ligação Covalente	Lima et al., (2018b)
			Pseudomonas cepacia	Adsorção Física/ Ligação Covalente	Abdulla et al., (2017)
		Bagaço da cana-de-açúcar	*Thermomyces lanuginosus*	Ligação Covalente	Mendes et al., (2013)
		Talos de milho	*Pseudomonas aeruginosa*	Adsorção Física	Lv et al., (2013)
		Membrana da casca do ovo	*Pseudomonas cepacia*	Adsorção Física/ Ligação Covalente	Abdulla et al., (2017)
		Combinado de quitina e amido	Lipase de sementes de girassol	Ligação Covalente	Ozacar et al. (2018)
	Inorgânico	Argila	Pâncreas de porco	Adsorção Física	Scherer et al., (2012)
		Sílica	*Candida antarctica* tipo B	Adsorção Física/ Ligação Covalente	Vescovi et al., (2016)
			Thermomyces lanuginosus	Adsorção Física	Miguez et al. (2018) e Gama et al. (2019)

Natureza	**Classe do suporte**	**Suporte**	**Lipase**	**Protocolo de imobilização**	**Referência**
Sintético	Orgânico	Poli (GMA-*co*-etileno dimetacrilato [EDMA])	*Candida antarctica* tipo B	Ligação Covalente	Mugo e Ayton, (2013)
		Poli-hidróxibutirato	*Thermomyces lanuginosus*	Adsorção Física	Miranda et al., (2014)
		Poli- (estireno-divinilbenzeno)	*Thermomyces lanuginosus*	Adsorção Física	Alves et al., (2017a)
		Poli-metacrilato	*Thermomyces lanuginosus*	Adsorção Física	Bassi et al., (2016)
		Nanocristais de celulose	*Pseudomonas cepacia*	Ligação Covalente	Cao et al., (2016)
	Inorgânico	Sílica	*Pseudomonas fluorescens*	Adsorção Física	Lima et al., (2018a)
			Rhizopus oryzae	Ligação Covalente	Ashjari et al., (2015)
			Thermomyces lanuginosus	Ligação Covalente	Kumar et al., (2018)
		Óxido de Titânio (TiO_2)	*Burkholderia ambifaria*	Adsorção Física	Gao et al., (2018)
		Óxido de Alumínio (Al_2O_3)	*Bacillus sp*	Ligação Covalente	Kumar et al., (2013)
		Nanopartículas magnéticas	*Candida rugosa*	Adsorção Física	Yi et al., (2017)
		Nanofolhas de carboneto de titânio	*Candida rugosa*	Ligação Covalente	Ding et al., (2019)

1.6.1 Aproveitamento de resíduos agroindústriais para a produção de suportes para imobilização enzimática

Nos últimos anos, especial atenção vem sendo dada para minimização ou reaproveitamento de resíduos sólidos gerados nos diferentes processos industriais. A indústria de alimentos produz, ao longo de sua cadeia produtiva, uma grande quantidade de resíduos agroindustriais, dentre eles estão, cascas, caroços, sementes, bagaços e tortas gerando prejuízo financeiro e inúmeros problemas ambientais, devido à poluição do solo e rios. Estima-se que ao longo da cadeia produtiva, o aproveitamento das matérias-primas vegetais não ultrapasse os 85%, e que os resíduos gerados cheguem a 30% (Nascimento Filho e Franco, 2015; Vaibhav et al., 2015).

Em sua grande maioria, os resíduos agroindustriais são reaproveitados como adubo ou ração animal, entretanto, podem ser utilizados para a extração de biocompostos, minerais e óxidos passíveis de recuperação e aproveitamento, viabilizando o uso sustentável das matérias-primas agroindustriais (Vaibhav et al., 2015; Sousa et al., 2011). Dessa maneira, o emprego de resíduos agroindustriais como matriz para suportes no processo de imobilização é muito interessante. Na literatura especializada é relatado a aplicação de vários resíduos para esse fim, tais como fibra de coco verde, bagaço de cana-de-açúcar, casca de arroz, casca de amendoim, membrana da casca do ovo e talos de milho (Lima et al., 2018b; Abdulla et al., 2017; Vaibhav et al., 2015; Mendes et al., 2013; Lv et al., 2013).

Esses resíduos podem ser utilizados *in natura*, passando por processos físicos de moagem, peneiramento e lavagem, ou quimicamente modificados por processos hidrotérmicos e/ou usando diferentes agentes funcionalizantes, como precursores alcóxissilanos (Vaibhav et al., 2015).

O dióxido de silício (SiO_2), mais conhecido como sílica, é um óxido que apresenta excelentes características para o uso como suporte em protocolos de imobilização enzimática (Siricha et al., 2016). No mercado internacional, o custo de partículas de sílica pode variar de R$ 1166,00/kg a R$ 978,00 o grama (Sigma-Aldrich, 2020). Vaibhav e colaboradores (2015) realizaram um trabalho com o objetivo de extrair sílica de quatro fontes naturais: casca de arroz, folhas de bambu, bagaço de cana e casca de amendoim. Todos esses resíduos foram submetidos a um tratamento hidrotérmico ácido para a obtenção da sílica pura que resultou em um rendimento de 52% a 78% e pureza de 98%. Os preparados foram caracterizados por análises de difração de raio-X, microscopia

eletrônica de varredura e espectroscopia de infravermelho com transformada de Fourier e os autores concluíram que as cinzas obtidas dos diferentes materiais residuais seriam uma matéria-prima promissora de baixo custo para a preparação de sílica superfina.

Abdulla e colaboradores (2017) estudaram a casca de arroz e a membrana da casca do ovo como alternativas de suportes para a imobilização da lipase de *Burkholderia cepacia* por protocolo de adsorção. As eficiências dos processos de imobilizações na casca do arroz e na membrana da casca do ovo foram, respectivamente, 81% e 87%. As atividades hidrolíticas foram satisfatórias para os dois tipos de biocatalisadores obtidos, 40 UI/ g de suporte para a proteína imobilizada na casca de arroz e 80 UI/ g para a mesma imobilizada na membrana da casca do ovo, além disso, ambos os biocatalisadores mantiveram as atividades catalíticas iniciais após dez ciclos de reutilização.

O bagaço de cana foi o resíduo utilizado por Mendes et al. (2013) para imobilizar via ligação covalente a lipase de *Thermomyces lanuginosus*. O material foi modificado quimicamente com epicloridrina, glicidol e glutaraldeído obtendo atividades hidrolíticas dos derivados variando de 79,5 a 191,8 UI/g de suporte. A enzima solúvel possuía tempo de meia-vida inferior a 4 min a 70°C, os biocatalisadores obtidos apresentaram um tempo de meia vida acima de 50 minutos na mesma temperatura, comprovando uma melhora expressiva na estabilidade térmica da enzima.

Talos de milho foram modificados hidrotermicamente por Lv et al. (2013) para a obtenção de um suporte usado para imobilizar a lipase de *Pseudomonas aeruginosa* por adsorção física. A estrutura do suporte foi estudada pelas técnicas de difração de raio-X, microscopia eletrônica de varredura e espectroscopia de infravermelho com transformada de Fourier. A capacidade de adsorção do suporte foi de 5,15 mg de proteína/ g de suporte e a atividade relativa total de 78,23% após ter sido reutilizado em três ciclos.

1.6.2 A casca de arroz como matéria-prima para a produção de sílica

O arroz (*Oryza sativa* L.) é um grão que é considerado o alimento base para quase metade da população mundial, além desse fato mais de um bilhão de famílias da Ásia, África e América do Sul tem esse grão como principal fonte de renda e emprego. Os campos de arroz cobrem mais de 9% do total das terras aráveis do planeta, isso representa cerca de 1% da superfície terrestre (Kumar et al., 2016a; Oli et al., 2014). A demanda pelo grão está em franco crescimento, segundo a FAO (Organização das Nações Unidas para Alimentação e Agricultura) na safra 2019/2020, a produção mundial de arroz

beneficiado foi de aproximadamente 494,2 milhões de toneladas. Além disso, só no Brasil a produção de arroz gira em torno de 10,5 milhões de toneladas (safra 2019/2020) (Conab, 2020).

O arroz pode ser comercializado de diversas formas, tais como grãos, farinhas e produtos fermentados e para produzi-los a grande maioria das indústrias utilizam moinhos modernos (Kumar et al., 2016b; Oli et al., 2014). No entanto, durante o processamento de moagem do grão uma grande quantidade de biomassa sólida é obtida, em média para cada 100 kg de arroz produzido são gerados 22 kg de casca (Kumar et al., 2016a; Kumar et el., 2015; Oli et al., 2014). A casca de arroz é composta por 3% de umidade, 35% de celulose, 25% de hemicelulose, 20% de lignina e 17% de cinzas (Liu et al., 2012). Os destinos para uma parte do volume total da casca de arroz produzida são o uso como ração animal ou emprego como combustível para gerar energia para os moinhos e fornos, ação esta que gera grande quantidade de cinzas; a outra parte do montante da casca de arroz produzida é descartada como resíduo em aterros sanitários provocando a poluição da atmosfera e de corpos hídricos (Kumar et al., 2016b; Zhang et al., 2015; Ayswarya et al., 2012).

As cinzas da casca de arroz são ricas em sílica, podendo conter de 55 a 97% desse óxido em sua composição, dependendo das condições em que o arroz é cultivado (Ayswarya et al., 2012). Apesar da sílica ser o componente predominante nas cinzas, traços de outros elementos como potássio, magnésio, cálcio e sódio também estão presentes (Kumar et al., 2016a). Dessa maneira, atualmente, a casca de arroz e as cinzas da casca de arroz vêm sendo estudadas para serem aplicadas em outros fins mais úteis e com maior valor agregado, tais como suportes para catalisadores, componente em cimentos e concreto, adsorventes para corantes orgânicos, fertilizantes, produção de baterias, geopolímeros e produção de sílica pura e sílica gel (Zhang et al., 2015; Soltani et al., 2015).

A sílica tem elevado potencial para ser aplicado como suporte nos protocolos de imobilização enzimática pois permite modificação da sua superfície, apresenta excelentes estabilidades térmica e mecânica, além de ser segura do ponto de vista microbiológico e toxicológico (Carvalho et al., 2015; Carvalho et al., 2014; Zucca e Sanjust, 2014). Para o uso em métodos de imobilização, a sílica adquirida comercialmente é, em sua maioria, produzida pela onerosa técnica sol-gel e na superfície da sílica gel existem grupos hidroxilas ligados quimicamente que reagem com compostos que atuam como funcionalizantes do suporte ou reagem diretamente com os grupos funcionais da enzima

(Carvalho et al., 2015; Bernal et al., 2014). A sílica obtida das cinzas da casca de arroz apresenta-se de duas formas, com estrutura cristalina quando o processo de queima é feito em temperaturas variando de 800 a 1200 °C e na forma amorfa quando a queima acontece em baixas temperaturas (500-600ºC), essa última estrutura é altamente reativa devido ao seu tamanho fino e grande área de superfície (Le et al., 2015). Os processos de obtenção da sílica da casca de arroz podem seguir diferentes protocolos, como os que estão sumarizados na Tabela 1.3 (Kumar et al., 2016a).

Tabela 1.3 Diferentes metodologias para obtenção de sílica da casca de arroz (Kumar et al., 2016a)

Metodologia	Características da Sílica e/ou Protocolos
Neutralização com dióxido de carbono (CO_2) e processos de separação por precipitação	Rendimento de sílica 72-98%
Método de carbonatação com bicarbonato de sódio (Na_2CO_3) e processos de separação por precipitação	A sílica apresentou rendimento de 84,5%, estrutura amorfa e alta pureza.
Extração alcalina seguida de precipitação ácida	A sílica obtida foi pura, mínima contaminação mineral e rendimento de 91%
Método de degradação hidrotérmica	Sílica superfina e com diâmetro de 50 nm

A casca de arroz foi utilizada por He e colaboradores (2016) para imobilizar enzimas produzidas por *Aspergillus niger* via ligação covalente para catalisar a degradação da micotoxina zearalenona. O biocatalisador exibiu alta estabilidade, atividade catalítica e resistência a desnaturação térmica em comparação com a enzima livre. Além disso, a 90 °C o biocatalisador reteve 70% da taxa de remoção da micotoxina e foi estável durante um mês de armazenamento.

Corici et al. (2016) utilizaram a casca de arroz moída para imobilizar a protease de *Bacillus licheniformis* e as lipases de *Candida antarctica* e *Thermomyces lanuginosus* e posteriormente aplicar essas lipases na síntese do éster laurato de propila e a protease na reação de hidrólise da caseína. Diferentes funcionalizações e protocolos de

imobilizações foram reportados na literatura especializada e os dados obtidos indicaram um grande potencial para a imobilização de enzimas na casca de arroz e futura substituição dos suportes convencionais por materiais que atendam melhor aos custos e as exigências ambientais da indústria.

Machado e colaboradores (2019) extraíram sílica da casca de arroz por processo hidrotérmico e funcionalizaram com trietóxi (octil) silano - OCTES e (3 amino propril) trietoxi silano- 3-APTES, com o objetivo de usá-las como suporte para imobilizar lipase do *Thermomyces lanuginosus* via adsorção. Os suportes foram caracterizados por distribuição granulométrica e análises elementares, difração de raio-X, fisissorção de nitrogênio, microscopia eletrônica de varredura e força atômica, a fim de confirmar sua funcionalização. A carga máxima de proteínas imobilizadas foi de 12,3 ± 0,1 mg/g de suporte para a Amino-SiO_2 e 21,9 ± 0,1 mg/g para a Octil-SiO_2. No entanto, esses biocatalisadores apresentaram atividade catalítica semelhante na hidrólise da emulsão de azeite de oliva (entre 630 e 645 UI/g).

1.6.3 Funcionalização e ativação de sílica para uso como suporte na imobilização de lipases

O uso de sílica como suporte para imobilização de lipases é extremamente atrativo. Entretanto, este tipo de suporte quando usado na forma pura apresenta algumas limitações, dentre elas a capacidade de acúmulo excessivo de substâncias e moléculas hidrofílicas em torno da sua estrutura. Esse fato ocorre devido à presença de grupos silanois (Si-OH) na composição da sílica que interagem via ponte de hidrogênio com moléculas de água ou outros compostos hidrofílicos presentes no meio reacional onde é utilizado (Saliba et al., 2016). O acúmulo destes compostos na superfície do suporte favorece a dessorção da lipase, além de contribuir para a formação de interações indesejadas entre enzima e suporte, comprometendo o processo de transferência de massa e a atividade catalítica do biocatalisador. Com o intuito de solucionar esses problemas, protocolos de modificações químicas com agentes silanos para introduzir grupamentos específicos e a ativação da superfície do suporte com a inserção de grupos iônicos a fim imobilizar a enzima e obter biocatalisadores heterogêneos mais estáveis e ativos são reportados na literatura especializada (Xiang et al., 2018; Yi et al., 2018; Siricha et al., 2016; Motevalizadeh et al., 2013; Zhou et al., 2012; Bolivar e Nidetzky, 2012).

A melhora do desempenho de biocatalisadores heterogêneos que se apresentam como suportes iônicos funcionalizados é devido à intensa interação entre os grupos carboxilato (resíduos de C-terminal e aspartato/glutamato) ou amino (resíduos N-terminal e arginina/histidina/lisina) das estruturas enzimáticas com os grupos iônicos adicionados na superfície dos suportes (Bernal et al., 2018; Vescovi et al., 2016). Esses grupamentos que promovem a funcionalização e ativação do suporte podem ser introduzidos na sua superfície da matriz via modificação química reversível (adsorção física) ou irreversível (ligação covalente) (Gholamzadeh et al., 2017; Bolivar e Nidetzky, 2012). A funcionalização pode ser feita por polímeros, aminoácidos, líquidos iônicos, agentes alcóxissilanos, etc (Xiang et al., 2018; Yi et al., 2018; Motevalizadeh et al., 2015; Mendes et al., 2011). Nos grupos iônicos, a ligação covalente acontece entre os grupamentos nucleofílicos dos agentes funcionalizantes e os eletrofílicos da superfície do suporte, por exemplo grupos epóxi podem ser previamente introduzidos via funcionalização com (3-Glicidilóxipropil)trimetóxissilano (GPTMS) em suporte inorgânicos ou epicloridrina em suportes orgânicos (Bernal et al., 2018; Motevalizadeh et al., 2015; Bolivar e Nidetzky, 2012).

A ativação da sílica pode ocorrer em uma única reação ou por duas ou mais reações, como por exemplo, no trabalho realizado por Bolivar e Nidetzky (2012) em que, inicialmente, os autores funcionalizaram a sílica com GPTMS para a inserção de grupos epóxi e em seguida ativaram o material com glicina para a introdução dos grupos iônicos a fim de imobilizar a enzima $_{D}$-Amino acid oxidase de *Trigonopsis variabilis* (*Tv*DAO). Entretanto, ainda não são reportados na literatura especializada estudos que utilizam a casca de arroz para a obtenção de partículas de sílica funcionalizadas com GPTMS e ativadas com glicina como suporte em protocolos de imobilização de lipase.

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CAPÍTULO 2

"Biolubricant production using lipases as catalysts: Current scenario and future perspectives" submetido no periódico indexado BioEnergy Research.

Abstract

In recent years, biolubricants have been gaining prominence in comparison with conventional petroleum-based lubricants. This trend is observed in almost all sectors relying on these products, regardless of their applications. The factors that contribute to such growing trend are the environmental problems caused by conventional lubricants and the depletion of oil reserves that have increased the need for renewable and biodegradable lubricants. Biolubricants have several advantages over mineral oil lubricants such as high biodegradability, low toxicity, excellent lubricating performance and minimal impact on the environment and human health. In addition, biolubricants can be produced using several types of oleaginous feedstock and distinct chemical reactions that can be efficiently catalyzed by lipases, which make them quite attractive in the context of Green Chemistry. Thereby, this review describes different aspects of biolubricants by detailing their main applications and uses, as potential feedstock for instance, production routes, progress of the enzymatic catalysis and immobilization protocols for preparing heterogenous biocatalysts (immobilized lipases) as a promising route to obtain such an important class of compounds for the industrial sector and future prospects for enzymatic biolubricant production.

Keywords: Biolubricants production, Oleaginous feedstocks, Lipases, Heterogeneous biocatalysts.

1. Introduction

Lubricants are organic compounds capable of reducing friction between two surfaces in motion, in addition to reducing heating and wear of mechanical parts by forming a protective film [1–4]. The global lubricant sales reached $128.51 billion in 2018 and its production is estimated to be 43.87 million tons in 2022 at an annual growth rate of 2.4% [5]. China has the highest market volume of lubricants worldwide in 2019 (7.3 million tons), followed by United States of America (6.05 million tons), India (1.7 million tons), Japan (1.35 million tons), Russia (1.3 million tons), Brazil (1.1 million tons) and Germany (1.0 million tons) [6].

Currently, petroleum-based lubricants have been established as universal lubricanting agents for most industrial, commercial and personal applications, but this scenario has undergone severe changes, given that efficient techniques must be used for oil extraction as a result of scarcity of natural reserves, and elevated costs of transportation and refining processes [4, 7]. Concern and responsibility for environmental issues is also a critical issue for process industries and public agencies. With respect to lubricants, concern is caused by issues relating to a large amount of inappropriate disposal into the environment. It is estimated that 40–55% of all consumed lubricant is disposed of inadequately into aquatic environment, soil and atmosphere [2, 4]. Another alarming factor relating to this issue is that over 95% of lubricants that contaminate the environment are petroleum-based and their disposal causes damage to ecosystems due to high toxicity and low biodegradability [1]. In aquatic environments, they form an oil layer on their surface, which hinders the passage of sunlight and oxygen necessary for maintaining aquatic ecosystems; when disposed of into the soil, they decrease its filtration and ion exchange capacity, as well as fertility; in addition, they release toxic gases into the environment when incinerated [2, 7]. However, there are ways to reduce this negative impact caused by lubricants on the environment. In this context, recent research indicates that currently over 90% of petroleum-based lubricants produced can be replaced by biodegradable synthetic lubricants – biolubricants [1].

Biolubricants are a satisfactory alternative to replace lubricating oils from fossil sources [1, 3, 7–9]. When compared to mineral and semi-synthetic lubricants, they offer the following advantages such as lower toxicity, more effective lubrication, higher flash point (the lowest temperature at which a substance generates a sufficient amount of flammable vapor that can be ignited), lower volatility, higher viscosity index (a

dimensional parameter that represents viscosity variation of a hydraulic fluid at 40 °C and 100 °C) and excellent cold flow properties – pour point, the lowest temperature at which a liquid becomes semisolid and it loses its flowing property [4, 7, 9, 10]. Another advantage of biolubricants is the variety of renewable raw materials that can be used for their production, such as vegetable oils, animal fats, microbial oils from yeasts, filamentous fungus and microalgae, and waste oils [1, 4, 7, 9, 11–14].

These compounds can be produced by different types of chemical reactions such as esterification, transesterification, epoxidation and hydrosterification employing different types of chemical and/or enzymatic catalysts [9, 12, 15]. However, several limitations such as high temperature (high energy cost), long reaction times and generation of chemical residues in separation and purification processes have been reported for chemical routes [16–19]. In order to solve these problems, the application of enzymes (lipases) is a promising alternative for producing these esters due to a growing demand for environmentally correct products and processes [13, 20–25]. The application of lipases in soluble (liquid formulations) or aggregate (powder formulations) forms in industrial processes is limited due to difficult biocatalyst recovery from the reaction medium and subsequent reuse at the end of the process, high temperature sensitivity and organic solventes [26, 27]. These limitations can be mitigated or even overcome by using different immobilization protocols [26, 28–30].

Given the global importance of biolubricants, this review aims to present their overview by investigating their main industrial and everyday applications, used feedstock, production routes and the progress of heterogeneous enzymatic catalysis (immobilized lipases) as a promising route for obtaining them.

2. Applications, physicochemical and tribological characteristics of lubricants

Lubricants are an important class of materials used to facilitate relative motion of solid surfaces by minimizing friction and wear resulting from contact between moving surfaces, i.e. lubricating is necessary to reduce the coefficient of friction between very close surfaces that are in relative motion towards each other. In addition to reducing friction and wear, lubricants are also required to perform several other functions, such as cooling, corrosion prevention and energy transfer. Moreover, lubricants must liquidly seal moving surfaces and remove particles from wear, dust and moisture [1, 11, 15, 31].

There are multiple applications of lubricants in different industrial sectors. Furthermore, the choice of an appropriate product for each application assists in extending the lifespan of machines, parts, products, engines and their components, in addition to increasing the efficiency and reliability of processes [4]. The main uses of lubricants have been widely reported in literature, namely: *(i)* industrial oils: these are used for industrial applications, such as compressor oils, machine oils, hydraulic oils and metal molding fluids; *(ii)* automotive oils: oils used in the automotive and transportation sector, mainly in transmission and internal combustion engines, e.g. transmission fluids, engine oils, brake oil, as well as gearbox oils and hydraulic fluids; and *(iii)* special oils: these are used for specific purposes and operations, such as instrumental oils, process oils and biomedical applications in artificial joints; oils to be used in food processing machines [4, 7, 8, 10, 32, 33].

To perform these functions, lubricating oils must have specific physicochemical and tribological characteristics – characterizing lubrication [3, 9, 31]. As priorly reported, an effective lubricant is a substance that reduces friction and wear by providing a protective layer between two moving surfaces, and lubrication occurs when these two surfaces are separated by a lubricating film. One of the basic requirements is that the lubricant must remain in its liquid form in a wide temperature range so that limits can be determined by its pour point at low temperatures and by its flash point at high temperatures. Their pour point should be low so as to ensure that they are pumped when the equipment is started at extremely low temperatures and the flash point must be high in order to ensure safe operation and minimum volatilization at maximum operating temperature. For most demanding applications, such as aviation engine lubricants, an effective temperature range of liquids should be above 300 °C [7, 31, 33].

In addition, lubricant efficiency in reducing friction and wear is intrinsically related to a high viscosity index [3, 7]. The lubricant oils available on the market have viscosity index values ranging from 90 to 160 [34]. The higher the viscosity index, the lower the viscosity change of a fluid in a given temperature range becomes, and vice versa. Furthermore, their efficiency is also related to other features, such as thermal stability, ability to prevent corrosion and high oxidation resistance. Lubricants are available in liquid, solid and gaseous forms, among which liquids and solids are widely used in everyday life [7, 15, 33].

In open systems, lubricants such as chainsaw oils and drilling fluids are released directly into the environment, but they are not directly exposed to the environment in

closed systems. In practice, lubricants contaminate the environment by leaks, damaged pipes and human error. In literature, it is reported that over 40–50% of lubricants used worldwide contribute to environmental pollution due to total loss of lubrication, spillage and volatility [4, 35, 36]. Thereby, lubricants must be biodegradable and produced from renewable and sustainable sources, once biodegradability ensures safe reintegration of organic material into the natural carbon cycle, thus causing less impact to the environment [1, 4].

3. Biolubricants

There has been growing concern for pollution control and environmental preservation and it is gaining prominence by authorities worldwide, whose interest has been focused on safeguarding the environment and improving reliability, durability and energy efficiency of industries in general. Developing technological alternatives, such as the introduction of light materials, less harmful fuels and controlled combustion of fuels, are possible means to minimize environmental problems brought by vehicles and machinery. In the lubricant sector, it is focused on issues regarding a massive disposal of these products directly into the environment, thus contaminating water, soil and air [8, 36, 37].

It is estimated that 1 kg of fossil-based lubricants discarded into the environment is capable of polluting a million liters of water [38]. In addition, air is also affected by volatile substances present in these compounds, in addition to combustion products which emit traces of metals such as calcium, phosphorus, zinc, magnesium and iron nanoparticles and causing damage to the environment [7, 39]. Therefore, increasing environmental awareness becomes the decisive factor in developing new technologies, so that biodegradable synthetic lubricants (biolubricants) constitute an area that has been extensively explored in the field of Green Chemistry [1, 2, 4, 7, 9, 10, 36].

The simplest classification of biolubricants is in accordance with their synthesis process, namely biolubricants produced from natural oils and those produced from synthetic oils. Natural oils, also known as natural esters, are biolubricants produced from raw materials based on vegetable oils, microbes or animal fat [1, 3, 4, 9, 12, 15]. Synthetic oils are biolubricants that comprise polyalphaolefins, synthetic esters, polyalkylene glycols, alkylated aromatics, perfluoroalkylpolyethers or any other lubricant consisting of artificially synthesized chemical compounds [1].

Biolubricants are considered an alternative to mineral oils, due to the fact that they have natural properties and are biodegradable. When compared to mineral oils, which is an example of the most widely used lubricant in the automotive industry, biolubricants based on vegetable oil or esters usually have high lubrication performance, high viscosity index, high flash point and slight losses by volatilization, in addition to low pour point, which ensures excellent lubrication in cold starts [3, 9, 10]. For applications aimed at boundary lubrication, vegetable oil-based lubricants have an adequate chemical nature and polar groups in their carbon chain structure, which allows good adsorption of the lubricant on the metal surface to be protected and results in less wear and friction between parts [40]. Currently, there are several companies that produce biolubricants such as the Royal Dutch Shell, ExxonMobil Corporation, British Petroleum, Chevron Corporation, Castrol, Total S.A., United Bio Lube, Petrochina Company Limited, Fuchs Petrolub AG, Sinopec Limited, LUKOIL, Idemitsu Kosan Co. Ltd and Statoil Lubricants [41, 42]. These have specific product applications, such as grease, hydraulic fluids, gear and chainsaw oils, oils for bars and chains, two-stroke engine oils, vacuum pump oils and emollients in the cosmetics industry [4, 33]. These companies are mainly located in different countries of Europe (Netherlands, United Kindgom, France, Germany, and Russia), United States of America (USA), China, and Japan [4, 42].

4. Feedstocks for biolubricant production

The biolubricants available on the market are generally produced from several triacylglycerols (TAG), mainly vegetable oils [3, 15]. Their tribological properties depend essentially on the molecular structure and physical and chemical properties of its constituent compounds [3, 43, 44]. Biolubricants present polar and non-polar regions in their structures. The polar region is responsible for adsorption or adhesion to the sliding surface, while the non-polar region is responsible for the resistance and oiliness of the lubricating fluid [1, 4, 40].

The general structure of TAG and components (FFA and glycerol) is shown in Fig. 1 [4, 12]. The general capacity of TAG to adhere to metal surfaces, which is why they can be used as natural lubricants, is related to oxygen atoms in their structures. They establish a monolayer film on the metal surface, which allows reducing friction and wear of parts and structures, thus minimizing metal-to-metal contact [1, 3, 12].

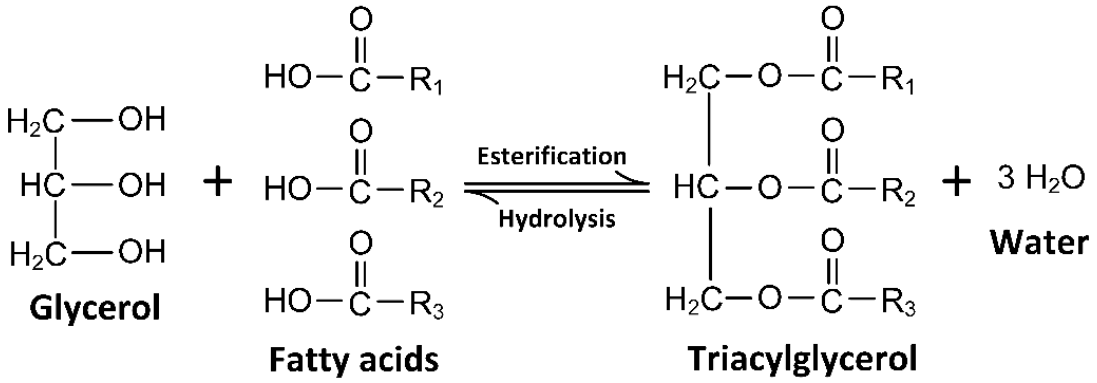

Fig. 1. General structure of TAG and components – FFA and glycerol.

A wide variety of vegetable oils has been used in the production of biolubricants, which differs from one region to another due to climate and geographical factors [4, 45]. For example, rapeseed and sunflower oils are often used in Europe, while palm and coconut oils are mainly used in Asia. On the other hand, the main raw material to produce biolubricants in the United States is soybean [4, 7, 46]. According to the National Supply Company (CONAB), Brazilian soybean crop in 2019/2020 has an estimated production of 122.2 million tons, which places Brazil as the second largest global producer and characterizes it as the raw material with the highest viability for biolubricant production in the country [47]. Table 1 shows the main oilseed raw materials [2, 4, 9, 15] and the world's largest producers in 2019/2020 [48, 49], as well as its fatty acid compositions [4, 50, 51].

Vegetable oils can be classified as edible and non-edible oils [33]. Thus, it is desirable to prioritize the use of non-edible oils and/or those used as raw materials for producing renewable lubricants, since they do not compete with the food industry and/or can be obtained from the use of edible oils or residues. Another factor that is worth mentioning is that this practice can reduce the total cost of renewable esters production, including biolubricants, by up to 80% if compared to that produced from raw edible oils [24, 52, 53].

However, the most commonly used oils in biolubricant production are edible oils reported in Table 1, such as palm oil [18, 54, 55], soybean oil [25, 40, 56–58], rapeseed oil [59–64], palm kernel oil [22, 32, 65], sunflower oil [40, 57], and coconut oil [44]. Among non-edible oils, jatropha oil [57, 63, 66, 67], and castor oil [16, 25, 64, 68–73] stand out. Other less commonly used raw materials that can be used for biolubricant

production are Tilapia oil [74], waste fish oil [75], microbial oils [13, 14], WCO [24, 57, 68], fat from chicken skin [76], rubber seed oil [77, 78], moringa oil [66, 79], passion fruit oil [79], etc.

Fatty acid profile is very important for producing lubricants from TAG. The carbon chain length in fatty acid moieties is a significant factor due to affecting some properties, such as coefficient of friction and wear [43, 44]. As for the latter, plants are manipulated so as to alter gene sequencing within organisms, and a gene becomes inoperative as a result, or by attaching a gene to an identified DNA region that starts transcribing a particular gene and acting as a source of gene expression. Genetically modified vegetable oils are generally focused on changing fatty acid profiles in their structures and consequently improving their thermal and oxidative stability. The purpose of these manipulations is to generate oils with higher degrees of saturation, thus they will be less susceptible to deterioration which improves cold flow properties of lubricants [1]. For instance, it is worth mentioning sunflower oil which is the fourth largest source of edible oils in the world, after palm, soybean, and rapeseed oils (see Table 1). It typically has approximately 66% linoleic acid (polyunsaturated fatty acid with two double bonds) and 22% of oleic acid (monounsaturated fatty acid) as shown in Table 1, which makes it highly susceptible to lipid oxidation when heated. In order to make it a good candidate for preparing biolubricants, studies report a genetic modification of sunflower so that the oil obtained contains over 87% oleic acid in its chemical composition (about 70% more than common oils) and decrease to 5.45% of linoleic acid, thence increasing its oxidative stability [80].

Table 1. Worldwide production of major vegetable oils widely used in the production of biolubricants, main producers and fatty acids composition.

Vegetable oils	Scientific name	World production 2019/2020 (million metric tons)	Main producers	Fatty acid composition (% wt.)
Palm	*Elaeis guineensis*	75.69	Indonesia, Malaysia and Thailand.	Myristic (1.2), palmitic (46.8), stearic (3.8), oleic (37.6), and linoleic (10.5).
Soybean	*Glycine max* L.	56.73	Brazil, USA, Argentina, China and Paraguay.	Palmitic (10.7), stearic (3.0), oleic (24.0), linoleic (56.7), and linolenic (5.4).
Rapeseed	*Brassica napus*	27.04	Canada, European Union (EU-27), China, India and Ukraine.	Palmitic (4.8), stearic (1.8), oleic (62.7), linoleic (19.5), and linolenic (8.6), arachidic (1.7), and erucic (1.0).
Sunflower seed	*Helianthus annuus* L.	20.65	Ukraine, Russia Federation, European Union (EU-27), and Argentina.	Palmitic (6.6), stearic (3.2), oleic (22.4), linoleic (66.2), and linolenic (1.0).
Palm kernel	*Elaeis guineensis*	8.79	Indonesia, Malaysia and Thailand.	Caprylic (4.3), capric (3.5), lauric (47.2), myristic (15.6), palmitic (8.6), stearic (2.0), oleic (15.9), and linoleic (2.8).
Peanut	*Arachis hypogaea* Linn	6.00	China, India, Nigeria and USA.	Palmitic (11.9), stearic (3.0), oleic (40.0), linoleic (40.7), linolenic (1.3), and behenic acid (3.2).

Table 1. *Continuation.*

Vegetable oils	Scientific name	World production 2019/2020 (million metric tons)	Main producers	Fatty acid composition (% wt.)
Cottonseed	*Gossypium hirsutum*	5.29	India, China, USA, Brazil and Pakistan.	Myristic (0.6), palmitic (23.3), stearic (2.1), oleic (16.9), linoleic (56.9), and linolenic (0.3).
Coconut	*Cocos nucifera*	3.58	Philippines, Indonesia, India, Viet Nam, and Mexico.	Caprylic (3.5), capric (4.5), lauric (44.7), myristic (17.5), palmitic (9.7), stearic (3.1), oleic (15.2), and linoleic (1.8).
Olive	*Olea europaea*	3.36	European Union (EU-27), Tunisia, Turkey and Morocco.	Palmitic (11.4), palmitoleic (0.6), stearic (2.6), oleic (80.6), linoleic (4.2), and linolenic (0.6).
Castor bean	*Ricinus communis* L.	NR	Brazil and India.	Palmitic (2.6), stearic (1.5), oleic (4.7), ricinoleic (82.8), and linoleic (8.4).
Jatropha	*Jatropha curcas* L.	NR	Mexico, Nicaragua, Thailand, India, Indonesia, Nepal, and Malaysia.	Palmitic (14.8), palmitoleic (0.8), stearic (4.2), oleic (41.0), linoleic (38.6), and linolenic (0.3).

NR: Not reported.

5. Routes for biolubricant production

Selecting the oleaginous raw material to be used to produce biolubricants is an extremely relevant process parameter to be studied, moreover, selecting the most appropriate production route for their synthesis is also of paramount importance [44, 46, 66]. Therefore, it is known that vegetable oils have some disadvantages, such as oxidative and hydrolytic instability, lubricating limitations at low temperatures and low compatibility with paints and sealants [7, 46, 81]. However, depending on the production route chosen to obtain biolubricants and the possibility of making chemical changes in vegetable oils, some of these undesirable aspects can be eliminated [1, 2, 4].

A direct application of vegetable oils as biolubricants is limited due to the chemical instability of unsaturations (double bonds) in fatty acids and the existence of β-hydrogen atoms in the chemical structure of glycerol that results in partial fragmentation of the molecule and production of oxidized compounds [2, 4]. In addition, vegetable oils have high viscosity, which hinders their lubricating action [3]. In this sense, a chemical modification of oils by different techniques has been successfully proposed in order to overcome the aforementioned limitations. Although there are several types of processes used in the chemical modification of TAG for biolubricant synthesis [9, 10], this study reports the most commonly used processes in literature, such as transesterification reactions of oils/fats and mono-alkyl esters, direct esterification of free fatty acids (FFA), sequential epoxidation and ring-opening processes and hydroesterification [1–4, 9].

5.1. Transesterification

Transesterification is a chemical reaction in which an ester is converted into another ester by exchanging the alkyl group of an alcohol. In this process, the glycerol molecule in the structure of TAG is usually replaced by different alcohols, mainly polyols such as trimethylolpropane (TMP), pentaerythritol (PE), and neopentilglycol (NPG) [4, 56, 61, 82, 83].

In literature, there are several examples of TAG used as raw materials for biolubricant synthesis by transesterification [11, 22, 46, 64, 83, 84]. These reactions are conducted in the presence of alkaline (homogeneous or heterogeneous) or biochemical (lipases) catalysts [2, 10, 11, 15]. In alkaline transesterification using homogeneous catalysts such as sodium and/or potassium hydroxides and alkoxides, TAG should have a

low concentration of FFA to avoid a saponification reaction, which hinders separation and purification processes of glycerol and esters [65, 85].

In this process, biolubricant production can be performed by a single reaction step between TAG and alcohols [22, 57, 86], or in two successive transesterification stages: in the first stage, there is the conversion of TAG into methyl or ethyl esters and a reaction of these esters with different alcohols occurs in the second stage [18, 25, 54, 57, 61, 62, 64, 83, 86], as shown in Fig. 2. It has been one of the main routes for biolubricant production by transesterification due to the high viscosity of TAG that interfere in mass transfer processes in the reaction and operational problems in the purification of esters [46].

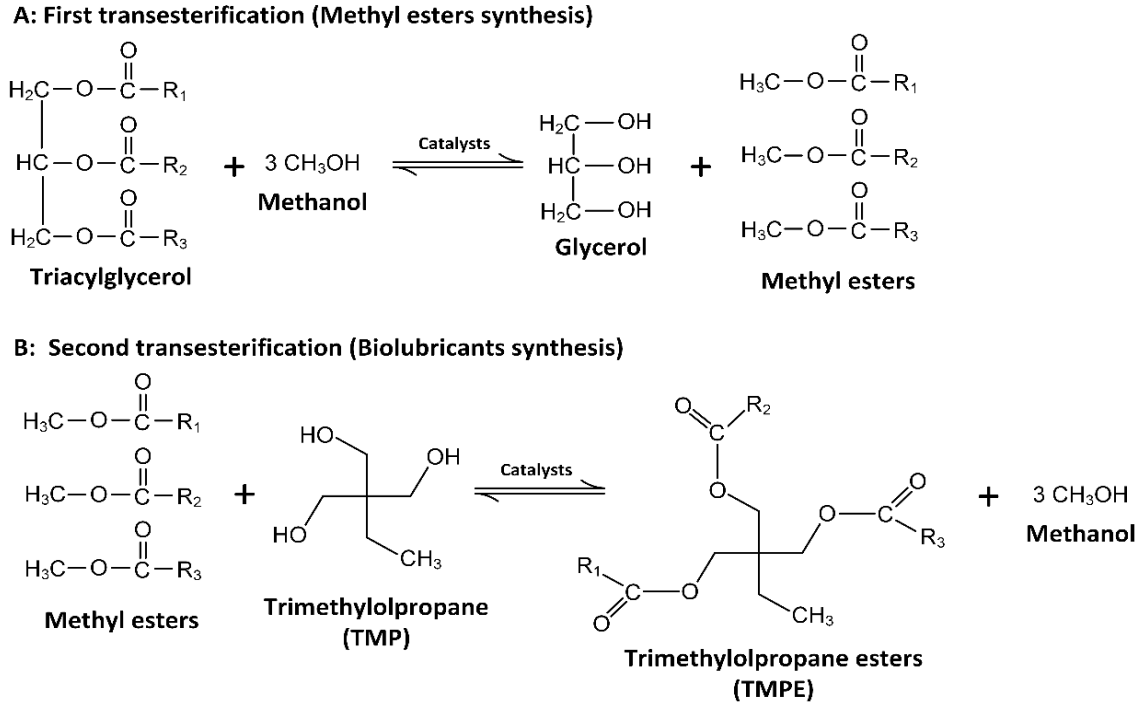

Fig. 2. Representative scheme of biolubricant synthesis via a two-step transesterification reaction.

In a study conducted by Encinar et al. [64], KOH was used in the first reaction stage as catalyst in the synthesis of methyl esters of rapeseed and castor oil. In the second stage, titanium isopropoxide was used as homogeneous catalyst in the synthesis of biolubricants by employing different alcohols such as 2-ethylhexanol (2-EH), 1-heptanol and 4-methyl-2-pentanol. In an another study, palm oil-based trimethylolpropane esters (PTMPE) were synthesized via successive transesterification of palm oil with methanol catalyzed by KOH to produce a mixture of palm methyl esters and PTMPE synthesis

using sodium methoxide (CH_3ONa) as catalyst [54]. The authors reported that the synthesized PTMPE can be succesfully used as boundary lubricants. A hybrid process to synthesize biolubricant esters by homogeneous base transesterification of rapeseed oil with methanol and enzymatic transesterification of methyl esters using different alcohols (2-EH, NPG and TMP) have been succesfully proposed [61]. In this study, an enzymatic step has been performed by using three different immobilized lipases from *Candida antarctica* type B – CALB (Novozym 435), *Pseudomonas cepacia* (or *Burkholderia cepacia* – BCL) and *Rhizomucor miehei* (RML). The authors observed that Novozym 435, a commercial biocatalyst prepared via ionic adsorption of CALB on poly(methyl methacrylate) cross-linked with divinylbenzene – Lewatit VP OC 1600 [87], was the most active biocatalyst used in this study. The physicochemical properties of synthesized esters showed their potential application as biolubricants due to improved properties if compared to rapeseed oil (better viscosisty, lower pour point and higher termo-oxidative stability). A mixture of fatty acid ethyl esters (FAEE) from waste fish oil was used as starting material for producing esters with lubricant properties via transesterification with three different branched alcohols (2-EH, 2-hexyldecanol – 2-HD, and isopropanol). In this study, three alkaline homogenous catalysts (KOH, C_2H_5ONa and 1,5,7-triazabyciclo[4.4.0]dec-5-ene – TBD) were used to synthesize esters and their performance were compared with heterogeneous biocatalysts (immobilized CALB – Novozym 435 and CalB immo Plus). Alkaline transesterification was more effective using isopropanol and enzymatic reaction with 2-HD. These biocatalysts could be recycled and reused without the loss of activity after consecutive eight batches. TMP esters by transesterification catalyzed by sodium ethoxide (C_2H_5ONa) [86]. The physicochemical properties of the synthesized esters suggest their satisfactory use as hydraulic liquids. In an interesting study, trimethylolpropane triesters (TMPTE) from palm oil methyl esters were synthesized using mixed oxides of calcium (CaO) and strontium (SrO) as catalysts [18]. Under optimal experimental conditions, TMPTE yield was 88.5% after 240 min of reaction at 180 °C and vacuum pressure of 2 mbar.

Biolubricant production by direct transesterification of vegetable oils and different alcohols has been preferably performed using immobilized lipases as catalysts [13, 22, 65]. In this field, an enzymatic synthesis of biolubricants via transesterification of palm kernel oil with simulated fusel oil, a byproduct obtained by distillation of alcohol fuel composed by isoamyl alcohol (>70%) and other short-chain alcohols, or isoamyl alcohol in batch (stirred-tank reactor) and continuous (packed bed reactor) mode has been

performed [22]. In this study, BCL immobilized on a hybrid support (epoxy silica-hydroxyethyl celulose) was used as heterogeneous biocatalyst. Under optimal conditions, high isoamyl esters formation (99 wt%) after 48 h of reaction in a batch mode and 8 h of space-time for continuous runs using both simulated fusel oil or isoamyl alcohol has been achieved. The esters produced obtained lower kinematic viscosity, viscosity index and oxidative stability than initial palm kernel oil.

5.2. Esterification

Biolubricant synthesis via an esterification reaction has been usually performed using several homogeneous and heterogeneous acid catalysts [35, 73, 78, 88–90] and immobilized lipases [20, 24, 35, 91]. This route is quite attractive for low-cost raw materials such as frying oil and effluents, and acid sludge refining vegetable oils. The use of these low-cost raw materials is very attractive from an industrial standpoint, as it reduces production costs and minimizes the environmental impact caused by inadequate disposal into the environment, since biorefineries can meet the zero waste goal [24].

Aravind et al. [78] synthesized a mixture of methy esters by esterifying FFA from rubber seed oil, a non-edible vegetable oil, with methanol using a homogeneous acid catalyst (H_2SO_4). The effect of a variety of natural or synthetic additives on viscosity, pour point, tribological properties and oxidative stability of produced methyl esters has been systematically studied. In another study, modified zeolite (Meso-HZ-5) was used as heterogeneous catalyst in order to synthesize octyl levulinate biolubricant, through which effects of catalyst loading, acid:alcohol molar ratio, and reaction temperature have been evaluated by a factorial design – Box-Behnken model [88]. Under optimal conditions, ester yield percentage of 95% after seven consecutive batches for 4 h of reaction has been observed. 2-ethylhexyl ricinoleate synthesis has been conducted using three commercial cation-exchange resins, such as Dowex 50W-X8, Amberlyst-15 and Purolite CT275DR [73]. Complete acid conversion was achived at 100 °C after 4 h of reaction using Amberlyst-15 as catalyst. After 5 reaction batches, the catalyst retained 60% of its initial activity. The physical properties of ester such as density at 20 °C (911.7 Kg/m^3), pour point (–39 °C), and viscosity index (132) have been determined. Oh et al. [89] synthesized different sulfated zirconia to be used as solid acid catalysts in biolubricant synthesis by esterification of FFA and several long-chain and polyols. High acid conversion percentage (>70%) was achieved after 4 h of reaction at 140 °C, except for a reaction using 3-octanol that converted only 31% of initial acid concentration. Kinematic viscosity

and viscosity index values of several alkyl oleate esters were similar to those of commercial lubricants. In this study, the prepared catalysts have also been employed in simultaneous transesterification/esterification of a mixture of oleic acid and soybean oil using several alcohols, and high esters yield were obtained. In a similar study, sulfonated activated carbon has been prepared, characterized and used in biolubricant synthesis via esterification of oleic acid with three alcohols – octanol, 2-EH and TMP. Under fixed experimental conditions (90 °C and 6 h of reaction), almost 100% acid conversion was achieved using both octanol and 2-EH, followed by TMP with 70% acid conversion [90]. Akerman et al. [35] tested silica–sulphuric acid, Amberlyst-15, and Novozym 435 as heterogeneous catalysts in biolubricant synthesis using TMP and carboxylic acids (valeric, caprylic and oleic acids). Silica–sulphuric acid was the most active catalyst tested in this study, although it was observed a dark coloration of products, which requires further purification steps such as bleaching and deodorization. The results indicate increased pour point (from –75 °C to –42 °C) and viscosity index (from 80 to 208) as the carbon chain of carboxylic acids increases from C5 to C18, although C18 (TMP-oleate) features desirable properties as hydraulic fluid at low temperatures. The authors used a tool for an environmental assessment of organic syntheses (EATOS), which confirms that the enzymatic route using Novozym 435 is the most eco-friendly alternative for chemical synthesis of lubricant esters.

An esterification reaction using homogeneous acid catalyst (H_2SO_4) has also been performed for starting materials whose acidity value was greater than 1% so as to avoid saponification in transesterification reactions performed using alkaline catalysts [19, 92]. This step was labelled as pre-transesterification treatment. Ghafar et al. [19] proposed a two-step process to produce biolubricants from WCO that consisted in a esterification reaction of FFA using methanol and transesterification involving TMP using CaO derived from waste cockle shell as catalyst. Another example lies in a study conducted by Amiril et al. [92], in which biolubricant was produced by a three-step process: an esterification reaction of FFA of Jatropha oil with methanol catalyzed by H_2SO_4, followed by a homogeneous alkaline transesterification reaction catalyzed by NaOH to produce methyl esters, and a second homogeneous alkaline transesterification reaction of methyl esters with TMP using sodium methoxide as catalyst. Biolubricant performance was assessed, which has revealed its satisfactory lubricating capacity, high corrosion inhibition and friction reduction, reduced surface area wear, excellent surface finish and higher efficiency of threading torque compared to conventional lubricants.

5.3. Epoxidation and ring-opening

Epoxidation of unsaturated fatty acids has received special attention due to possibilities of chemical modifications of vegetable oils for the synthesis of lubricating esters [1, 15, 68, 93]. This is due to the fact that epoxidation is one of the most important functionalization reactions that can occur in double bonds between carbons and convert them into oxirane rings (or epoxy groups) in order to improve oxidative stability, lubrication and low temperature behavior of biolubricants synthesized. Thus, a modification of vegetable oils via epoxidation allows subsequent reaction steps to occur in the synthesis of biolubricants under moderate conditions due to the high reactivity of oxirane groups formed in these oils [1, 15, 68]. Due to the high reactivity of oxirane groups introduced into the structure of the fatty acids, different groups such as hydroxyl, amino and thiols can easily perform nucleophilic attacks on the structure of oxirane ring, thus triggering its opening (also known as ring-opening stage) and promoting the formation of different compounds of added value and wide commercial use, including biolubricants [11, 36, 74, 94–96]. Fig. 3 shows a representative reaction of sequential epoxidation of fatty acids and an oxirane ring-opening reaction with an alcohol.

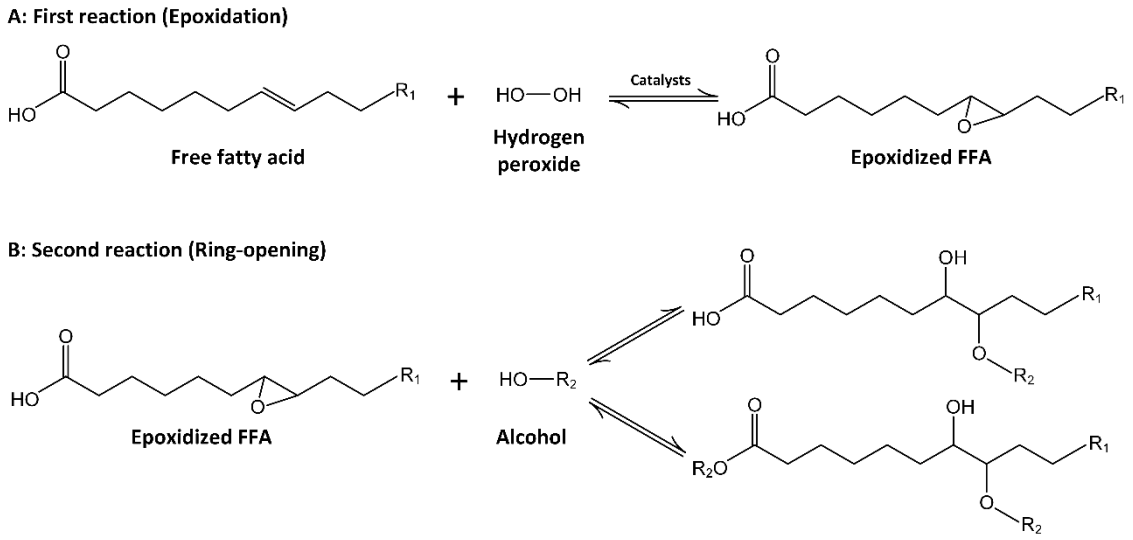

Fig. 3. Representative scheme of sequential epoxidation of unsaturated fatty acids and oxirane ring-opening reactions.

Epoxidized FFA, esters and TAG are usually produced from a reaction of double bonds in their structures with different peracids, which are usually produced through a hydrogen peroxide reaction and a carboxylic acid, either in separate steps or *in situ* [11].

Regardless of operation mode, H_2O_2 has been added slowly so as to avoid the formation of explosive mixtures [15]. In these processes, some reaction parameters have been evaluated for the formation of epoxidized raw materials, such as the molar ratio of reagents, reaction temperature, solvent nature, presence or absence of a catalyst (mineral acids/ion exchange resins/enzymes), type of peracid, mode and rate of H_2O_2 addition and reaction period [11, 36]. Other epoxidation methods consist in the use of dioxirane, alkyl hydroperoxides, and molecular oxygen [1, 97].

In a study conducted by Rios et al. [36], physicochemical properties and thermal degradation of different biolubricants synthesized from castor oil fatty acids have been evaluated, which occurred in three stages: esterification using 2-EH, epoxidation of double bonds in the obtained ester and, finally, oxirane ring-opening using different nucleophilic agentes such as 1-butanol (BIOBUT) and water (BIOWAT). The results indicated that BIOBUT exhibited better performance at low temperatures (pour point of –48 °C). In addition, BIOBUT obtained higher oxidative stability than BIOWAT, 4.22 h and 3.27 h, respectively. On the other hand, BIOWAT is more thermally stable than BIOBUT and it presents greater viscosity at 40 °C (> 470 mm^2/s).

In an another study, three different castor oil-based estolides, oligomeric fatty acid esters, such as castor-oleic triacylglycerol (cas-oleic TAG), castor-oleic 2-ethylhexyl ester (cas-oleic 2EH), and hydrogenated castor-oleic 2-ethylhexyl ester (H-cas-oleic 2EH) were firstly produced by esterification of castor oil and oleic acid [70]. These obtained esters were derivatized using hydrogen peroxide and formic acid to introduce oxirane groups in their estructures, followed by ring-opening through carbon dioxide in a supercritical medium using tetrabutylammonium bromide as catalyst. These new compounds showed high dynamic viscosity whose oxidation starting temperature was at around 200 °C, which indicates their promising use as industrial fluids and precursors in the synthesis of new polymers.

Epoxidized soybean oil was used by Turco et al. [96] for biolubricant production by ring-opening using different alcohols in the presence of polymeric fluorosulfonic acid Nafion™ on silica (SAC-13) as heterogeneous catalyst. In this study, different experimental trials were performed in a bench-scale reactor, through which the effect of alcohol (methanol, ethanol, 2 propanol, 2-butanol) on biolubricant physicochemical properties was assessed. The study confirmed that a ring-opening reaction heavily depends on alcohol branching and length. The biolubricants exhibited greater viscosity if compared to pure epoxidized soybean oil, particularly the one obtained from 2-butanol

due to the presence branched alcohol. According to the authors, the heterogeneous catalyst retained its initial activity after three successive reaction cycles.

Borugadda and Goud [68] produced epoxidized methyl esters from castor oil fatty acids by *in situ* epoxidation reaction with the aim of improving vegetable oil thermo-oxidative stability to be used as base for biolubricant production. During the reaction, acetic acid acted as active oxygen transporter and hydrogen peroxide as oxygen donor in the presence of a high-acidity ion-exchange resin as heterogeneous catalyst (Amberlite IR 120). The authors observed that the thermo-oxidative stability of castor oil has been significantly improved after a structural modification promoted by the epoxidation reaction according to the thermogravimetry analysis. The epoxidized product obtained in this study can be used as precursor for biolubricant production.

5.4. Hydroesterification

Hidroesterification has been used in the synthesis of biolubricants using feedstocks containing high percentages of FFA and water, such as animal fats, non-edible oils, and waste cooking oils [14, 71, 98, 99]. This is quite distinctive when compared to conventional homogeneous alkaline transesterification processes which require refined raw materials so as to minimize saponification drawbacks [100–103]. Another promising advantage of this process is that it produces glycerol with high purity, an important feature for pharmaceutical, cosmetic and food purposes [99]. Hydroesterification comprises a sequential hydrolysis step of TAGs to obtain FFA, followed by an esterification step using different alcohols [56, 101, 103, 104]. The representative scheme of biolubrican synthesis via hydroesterification is shown in Fig. 4.

Biolubricant production by hydrosterification has been conducted through different routes such as hydrolysis of TAG catalyzed by free lipases (liquid or powder extracts) from different sources and esterification catalyzed by heterogeneous acid catalysts such as an ion-exchange resin (Amberlyst-15) and niobic acid in pellets [105–107]. In another study, homogeneous catalysis in a two-stage process has been proposed [32]. Crude palm kernel oil was converted into FFA via alkaline hydrolysis reaction with KOH solution, followed by esterification reaction of purified FFA with TMP using a homogeneous acid catalyst (H_2SO_4). However, their vast majority are about lipases, both in hydrolysis and esterification stages [14, 71, 98, 104, 108, 109]. In some specific cases, hydrolysis occurs in subcritical water and esterification reaction catalyzed by immobilized lipases [11, 99, 103, 110].

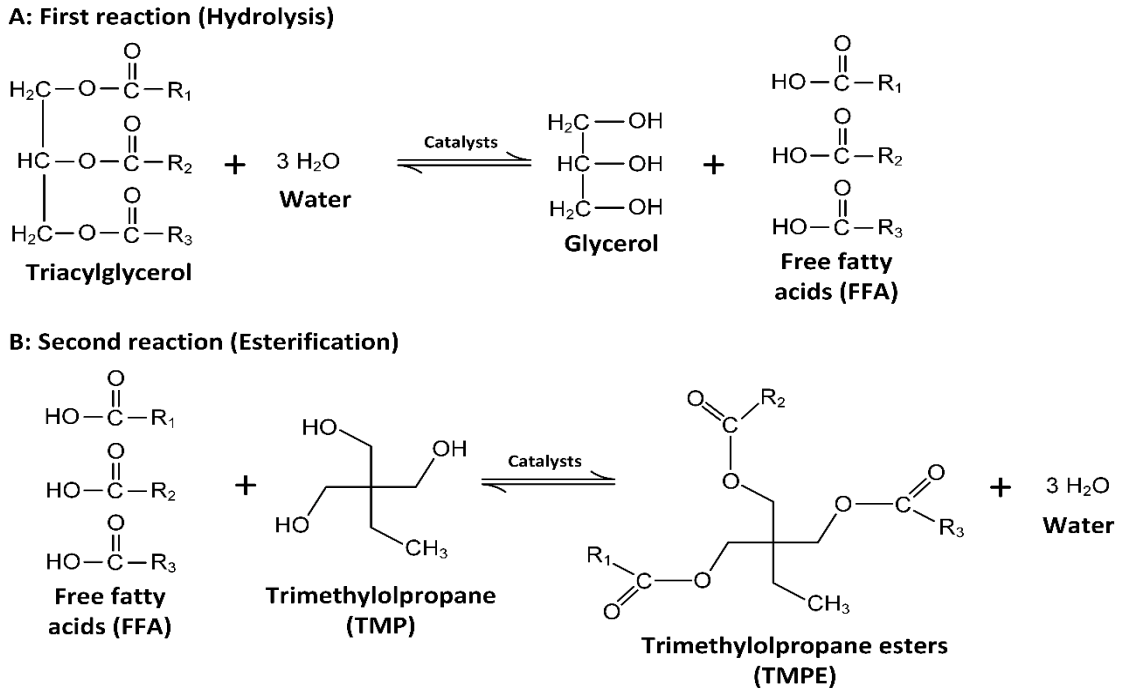

Fig. 4. Representative scheme of biolubricant synthesis via hydroesterification – sequential hydrolysis of TAG and esterification reactions.

Hydrosterification catalyzed by potassium hydroxide (hydrolysis) and sulfuric acid (esterification) was selected by Bahadi et al. [32] as route to produce biolubricant from palm oil and TMP. Effects of esterification process parameters, such as the molar ratio of fatty acids and TMP, reaction temperature, reaction time and amount of catalyst in reaction performance, have been examined using the response surface methodology. Under optimal conditions, high yield percentage (86.13 ± 0.7%) of TMP triester production was achieved, in addition to high viscosity index (154.8), low pour point (–10 °C), high flash point (320 °C) and good oxidative stability at 245 °C. The authors suggest that the biolubricant can be used as hydraulic fluid in food processing machines and are a potential match for commercial lubricants used for the same purpose according to their tribological properties.

Chowdhury et al. [107] produced biolubricant (a mixture of octyl esters) via hydrosterification using a chemoenzymatic approach – production of FFA by enzymatic hydrolysis of WCO catalyzed by CRL, and esterification with octanol using ion-exchange resin (Amberlyst-15) as catalyst. Maximum acid conversion of 98% after 3 h of reaction at 80 ºC was achieved in a batch mode.

Grego-Duarte et al. [71] used castor bean oil for producing biolubricants by enzymatic hydrosterification. In this study, hydrolysis reaction has been catalyzed by endogenous lipases from castor bean seeds, producing 93.13% of FFA in just 1 h at 30 °C. In the following stage (esterification), biolubricants were produced by reacting the obtained castor oil free fatty acids (COFFA) with different polyols (NPG, TMP and PE) catalyzed by powder enzymatic extract from CRL (Lipomod 34 MDP) and immobilized lipases – RML (Lipozyme RM-IM) and Novozym 435. Maximum acid conversion of over 75% was achieved for all polyols using powder lipase preparation from CRL as biocatalyst, the most active biocatalyst used in this study, after 96 h of reaction at 40 ºC. Analyses of the properties of biolubricants produced showed that home-made estolides from COFFA obtained satisfactory viscosity index (162) pour point (–42 ºC) and oxidative stability (51 min) when compared to estolides from commercial COFFA and esters produced by esterification with NPG and TMP, thus proving its succes.

Microbial oils from *Rhodosporidium toruloides* and *Cryptococcus curvatus* were used as feedstocks in the production of TMP and NPG esters (biolubricants) using powder enzymatic extract from CRL (Lipomod 34 MDP) as biocatalyst in both hidrolysis and esterification steps [14]. In this study, hydrolysis percentage above 96% was achieved after 24 h of reaction at pH 8,0 (100 mM Tris-HCl buffer), 30 °C and 2% (m/m, by oil) of biocatalyst. The esterification reaction of FFA and poliols (NPG and TMP) was performed in a solvente-free system under fixed conditions: 45 °C, stoichiometric molar ratio FFA:alcohols containing 1% (m/m) of distilled water and 4% (m/m) of biocatalyst. The highest acid conversion yields were obtained for NPG esters of *C. curvatus* (82.7%) and *R. toruloides* (88%) after 72 h of reaction in a batch mode.

In a recent study, Sarno et al. [24] produced a biolubricant from WCO and NPG by hydrosterification, in which lipase from *Thermomyces lanuginosus* (TLL) immobilized on magnetite nanoparticles modified with citric acid was used as catalyst in the two reaction steps of the process. Acid conversion of 88% after 24 h of esterification reaction at 45 ºC in a solvente-free system was observed. The biocatalyst prepared in this study maintained 75% of its initial activity after ten consectuive esterifcation bacthes. The addition of molecular sieves into the reaction medium to remove water molecules formed during the esterification reaction increased conversion by 94%. The physicochemical properties of biolubricants reached a viscosity index of 179.5 and a pour point of –8.3 °C, thus proving that the esters produced in this study can be used as biolubricant.

6. Homogeneous and heterogeneous catalysts used for biolubricant production

Biolubricant production from TAG has been performed in the presence of chemical and biochemical catalysts – homogeneous or heterogeneous ones [10, 19, 54, 57, 86]. The main chemical catalysts used in homogeneous catalysis are acids such as phosphoric, hydrochloric, p-toluenesulfonic and sulfuric acids and the most common bases are sodium and potassium hydroxides and alkoxides (sodium and/or potassium ethoxide), as reported above [10, 19, 54, 57, 86]. The main advantage of homogeneous catalysts is the low industrial cost when compared to other processes, such as enzymatic catalysis [111–113]. However, homogeneous catalysts poses some operational problems, such as difficult regeneration of reaction medium catalysts, separation of reaction products for subsequent reuse and high demand for water in the purification process of products [85, 114].

Currently, there has been a growing interest in the use of heterogeneous catalysts as an alternative to homogeneous catalysts, since it allows ease of separation of the obtained product, less equipment corrosion if compared to classic homogeneous catalysts, facilitates its subsequent reuse and reduces purification process expenses, which characterize them as environmentally friendly [85, 114].The main heterogeneous acid catalysts are WO_3-based catalysts supported on porous clay heterostructures with Si/Zr pillars – Zr15–20WO_3 [17], heteropolyacids immobilized on silica grafted with zirconium butoxide [115], ion-exchange resins [16, 68, 73], sulfonated actived carbons [90], sulfated zircônia [89], and silica–sulphuric acid [35]. The most commonly used heterogeneous base catalysts in biolubricant synthesis are magnesium methoxide, CaO, calcium alkoxide and barium hydroxide [116], calcium metoxide [117], CaO from waste cockle shells [19], and mixed oxides – CaO/SrO [18].

Heterogeneous chemical catalysts also has some disadvantages, once it requires more intense reaction conditions to achieve high conversion percentages, such as high temperatures in the range of 120 to 180 °C to reduce the medium viscosity and improve mass transfer processes [16–18, 89], and dark-colored products are generated due to a thermal degradation of fatty acids and/or TAG that requires posterior stages of purification of esters and leads to increased waste generation [35]. In this sense, it is necessary to seek milder and more technologically cleaner processes, such as the application of heterogeneous biocatalysts as immobilized lipases [118–123].

7. Immobilized lipases for biolubricant synthesis

In recent years, the search for technologically clean, efficient and highly productive processes has been a concern in the industrial sector. Thereby, the application of lipases to obtain these products is quite attractive, since reactions usually occur in mild conditions of temperature and pressure, present high specificity for natural and synthetic substrates, high degree of purity of products obtained due to high selectivity, which reduces the generation of residues and provides environmentally friendly processes [113, 124, 125].

Lipases (triacilglicerol acilhydlases - EC 3.1.1.3) are an important group of enzymes of industrial interest due to their capacity to cleave ester bonds of oils and fats at the water/oil interface, with subsequent release of FFA and glycerol [124, 126–129]. In addition, they can also catalyze esterification, transesterification, and interesterification reactions in non-aqueous media [26, 126, 127, 129]. From these reactions, it is possible to obtain products of high added value for chemical, pharmaceutical and food industries, among which biolubricants, biodiesel, emulsifiers, surfactants, fragrances and aromas, polymers and resolution of racemic mixtures stand out [126, 127, 129–132]. These enzymes are obtained from animal, plant and microbial sources, the latter being most commonly used by industries due to great biochemical diversity and simplicity in genetic manipulation and cultivation conditions [26, 129, 130, 132].

The application of lipases in their free forms (soluble or powder extracts) is not attractive to industries due to their high cost, difficult regeneration for subsequent reuse, formation of aggregates by adsorption of polar molecules produced in the reaction medium, such as water and alcohols. This leads to drastic catalytic activity reduction, active structure distortion from reaction temperature and organic solvents, and possible product contamination [30, 113, 128]. In order to overcome such limitations, different immobilization protocols have been proposed according to studies previously reported in literature [29, 113, 133–139]. Thus, immobilized lipases can be regenerated and reused in several reaction cycles, which can substantially reduce process cost and prevent final product contamination. In addition, enzyme stability can be improved after immobilization, thus making the biocatalyst more robust [20, 22, 56, 71, 91, 139–141]. The use of immobilized lipases as heterogeneous catalysts also enables the development of continuous processes, which is very attractive to oleochemical industries [30, 142].

Lipases can be immobilized by different protocols, as shown in Fig. 5, such as adsorption on hydrophobic or ionic supports, encapsulation, entrapment, covalent bonding and cross-linked enzyme aggregates (CLEAs). Selecting the most appropriate immobilization method should be based on several parameters, such as maximum activity of the immobilized enzyme, operational stability, immobilization cost, final toxicity of reagents and desired properties for the immobilized derivative [143].

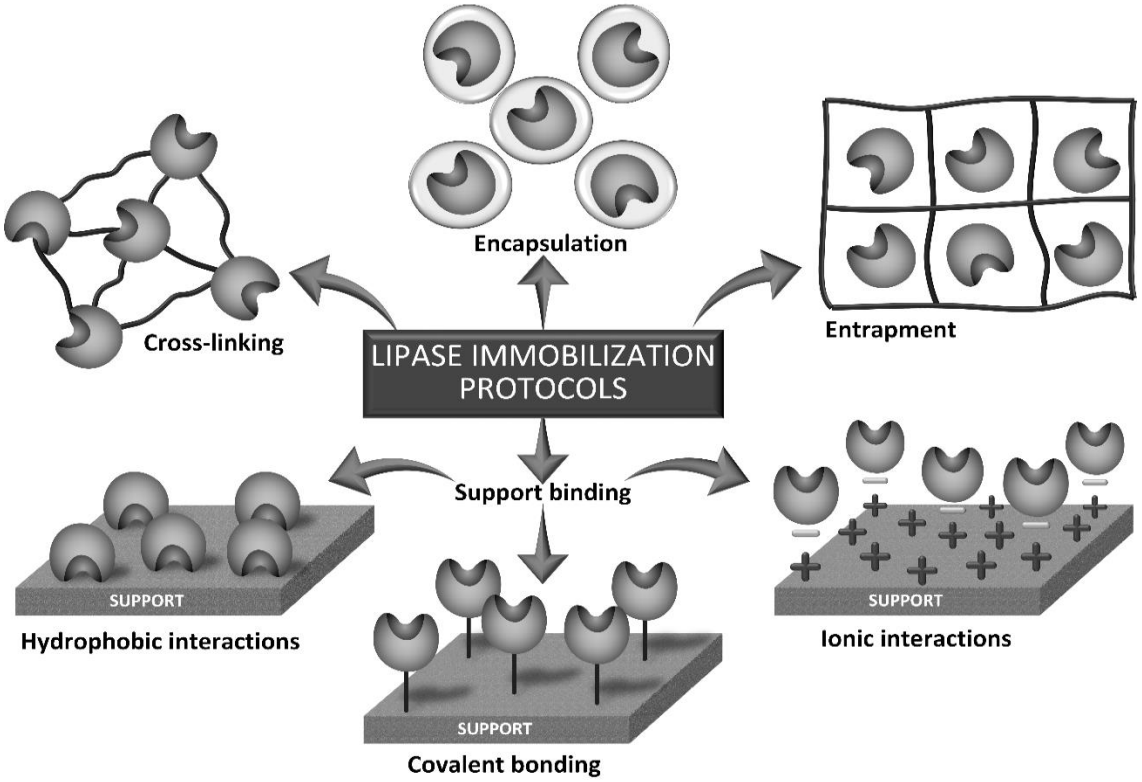

Fig. 5. Protocols of lipase immobilization.

The main characteristics to be observed while selecting a support for a given application are surface area, permeability, insolubility, regeneration capacity, morphology and composition, hydrophilic or hydrophobic nature, resistance to microbial attack, mechanical resistance, cost, etc. They can be featured as organic and inorganic supports and also by their morphology as porous, non-porous and gel-like materials [144–147]. Porous materials have a large internal surface area available for enzyme immobilization, where it is protected from the effects of vigorous agitation. Since most of the area available for immobilization is in its internal structure, it should be observed that pore diameter must be large enough to accommodate the enzyme and allow access for the substrate molecules [144, 148, 149]. Non-porous or low-porosity materials

eliminate internal mass resistance, but present low surface area available to enzyme binding [150].

These supports have been widely used in lipase immobilization and can be obtained from organic or inorganic materials – natural or synthetic sources [28, 145, 151–154]. Synthetic polymers exhibit several physical shapes and chemical structures that can be combined to prepare a variety of supports. Natural polymers are generally inexpensive, easily degradable and do not harm the environment. Among different organic supports used in lipase immobilization, agarose [153], chitosan hydrogels [154], acrylic resins [20, 21, 87, 155], poly(propylene) [156], poly(styrene-divinibenzeno) [140, 141, 151] stand out as supports. Inorganic materials such as silica, alumina, titania, zirconia, zeolites, magnetic particles, etc, also stand out as supports, since they have high surface area/pore size and excellent mechanical properties, high incubation stability in organic solvents, and can be chemically functionalized by a variety of compounds, such as alcoholsilan agents or ionic liquids [28, 145]. Silica-based inorganic supports have been the most widely used in lipase immobilization [28, 145, 152].

As it can be seen in Table 2, microbial lipases from *Candida antarctica* type B (CALB), *Thermomyces lanuginosus* (TLL), *Burkholderia cepacia* (BCL), *Candida rugosa* (CRL), *Candida* sp. 99–125, *Rhizomucor miehei* (RML) and *Rhizopus arrhizus* (RAL) immobilized by different protocols have been widely used as biocatalysts for biolubricant synthesis. These are preferably obtained by transesterification reactions employing methyl esters from vegetable oils or using vegetable oils directly as raw materials, direct esterification of commercially obtained fatty acids or low-cost raw materials as vegetable oil refining sludge, hydrosterification employing crude or processed vegetable oils and fatty acid epoxidization. These reactions have been preferentially conducted in stirred-tank reactors (STR) operating in batch mode due to smooth operation and maintenance (cleaning), and lower cost if compared to continuous-bed reactors such as packed-bed (PBR) and fluidized-bed (FBR) reactors [85, 142]. Representative schemes of the most typically used reactor configurations in enzymatic synthesis of biolubricants are shown in Fig. 6.

Continuous-bed reactors are more attractive from an industrial standpoint, since they can be conducted for long operating times interruptedly on a large scale. Reaction residence time can be set by the flow of equipment components as a function of the total volume available for the reaction to ocurr. Their operating parameters are more easily set, thus product quality fluctuates less often when compared to batch reactors. Thus, waste

and demand for interference by an operator can be minimized. The two most used continuous-bed reactor configurations in the oleochemical industry are packed-bed (PBR – Fig. 6B) and fluidized bed (FBR – Fig. 6C) reactors. They have been widely used in large-scale catalytic systems, due to their high efficiency and ease of construction and operation [142, 157].

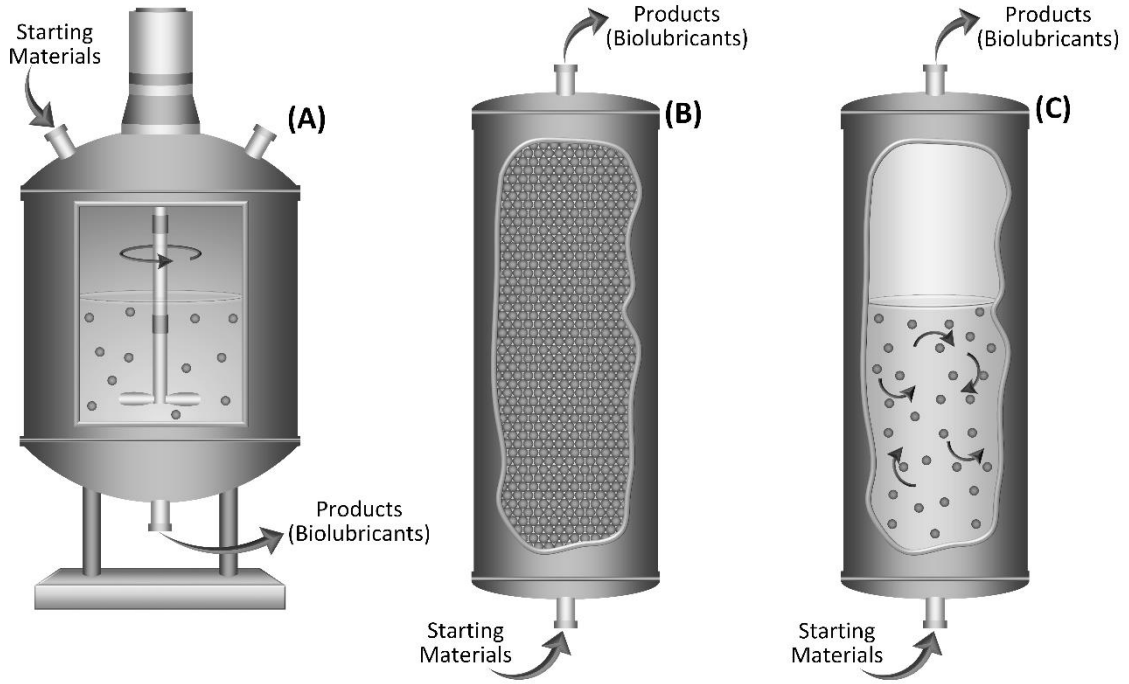

Fig. 6. Bioreactor configurations for enzymatic biolubricant production: STR (A), PBR (B) and FBR (C).

With regard to PBR, they require a minimum amount of auxiliary equipment and are very efficient, once there is high surface area for the reaction to occur per unit of volume and have been widely used for biotransformation of oils and fat catalyzed by immobilized lipases [85, 142, 157, 158]. PBR is kinetically more favorable than STR, once it does not have the disadvantage of high shear stresses from mechanical agitation [85, 142, 157, 158]. Compared to PBR, FBR exhibit reduced load loss in the reactor, more constant flow, and lower preferential channel formation [142]. Although continuous-bed reactors are widely used in the oleochemical industry, they are still seldom used for biolubricant synthesis [13, 22, 69].

7.1. Physical adsorption

Physical adsorption is a method for preparing heterogeneous biocatalysts that are widely used in lipase immobilization on account of its simplicity and inexpensiveness [27–29, 134]. It preserves the three-dimensional conformation of an enzyme and allows easy reuse of support by desorption of inactive enzymes [134, 139, 159, 160]. In addition, it promotes stabilization and improvement of catalytic activity [21, 26, 134, 141, 161–163]. This immobilization protocol is explained by different types of interactions such as Van der Waals forces, hydrogen bonding, ionic and hydrophobic interactions [28, 29, 134, 139, 149]. Such an interaction will depend on functional groups present on the support surface and the composition of amino acids present on the lipase surfaces [139, 161, 163, 164]. However, its main disadvantage is the possibility of enzyme desorption on the support surface caused by changes in temperature, pH, ionic strength or by the use of various chemicals such as surfactants, urea and co-solvents [30, 118, 139, 164].

In the field of lipases, adsorption via hydrophobic interactions is the most commonly selected immobilization protocol due to its peculiar mechanism of interfacial activation of the enzyme on hydrophobic surfaces – immobilization in open conformation [20, 140, 141, 155, 160, 163]. Interfacial activation is possible due to most lipases having a polypeptide chain (called lid) that covers their active site and makes them inaccessible to the reaction medium - closed conformation. Thus, in the presence of a hydrophobic surface, e.g. a hydrophobic support, the lid is displaced and the enzyme active site is exposed to the medium (open conformation) [155, 160]. The mechanism of interfacial activation of lipases on hydrophobic surfaces is shown in Fig. 7. Thus, lipase immobilization on hydrophobic supports has been performed at low ionic strength in order to change the closed/open balance towards open conformation and reduce the possibility of enzyme aggregates formation [155, 160, 163, 165].

Lipase adsorption via ionic interactions have also been widely reported in literature [161, 162, 164, 166, 167], as shown in Fig. 5. This immobilization method consists in an intense interaction between ionic groups of lipases and the support surfaces [26, 161, 162]. Functional groups present on the surface of supports can be natural or introduced by reversible (physical adsorption) or irreversible (covalent bonding) chemical modification using functionalized polymers, inorganic acids, amino acids, ionic liquids and alkoxy silane agentes [161, 162, 167–169]. These chemically modified supports create a more suitable microenvironment for enzyme immobilization and can protect the enzyme from possible unwanted interactions [161, 164, 166, 170].

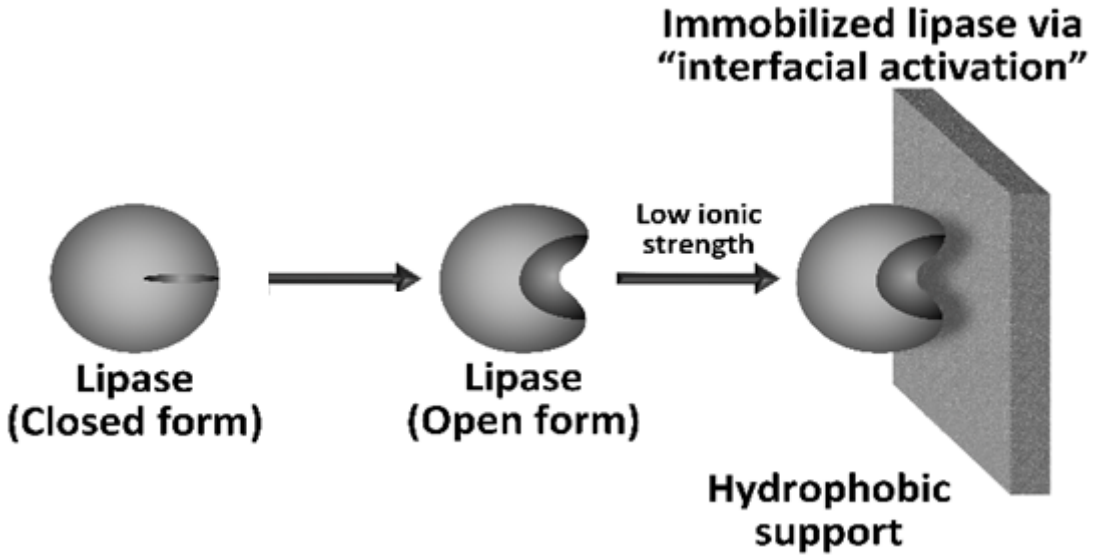

Fig. 7. Mechanism of interfacial activation of lipases on hydrophobic surfaces.

Novozym 435, a commercial immobilized CALB prepared via ionic adsorption on Lewatit VP OC 1600, has been the most used biocatalyst to synthesize biolubricants, as shown in Table 2. Enzymatic synthesis of TMP trioleate via esterification using Novozym 435 as biocatalyst has been reported by Akerman et al. [91]. The authors used a full factorial design to determine optimal experimental conditions. Under these conditions, the biocatalyst retained 20% of its initial activity after six successive batches of 24 h each. The synthesized triesters exhibited pour point at –42 °C. In a recent study, methyl esters with lubricant properties have been synthesized via transesterification of a by-product of palm oil processing (palm stearin) catalyzed by a commercial biocatalyst – Lipase B from *Candida antartica* immobilized on octadecyl methacrylate co-polymer via hydrophobic interations [65]. Under optimal experimental conditions, maximum yield of around 95.26% was achieved. Product formation has been confirmed by Fourier transform infrared spectroscopy (FT–IR) analysis. The product physicochemical properties such as viscosity, pour point, oxidation stability and friction properties suggest its great potential to be used as engine oil, especially in tropical regions.

Enzymatic synthesis using TLL immobilized via physical adsorption on a variety of supports has also been widely documented (see Table 2). In a study performed by Kleinaite et al. [62], methyl esters from rapeseed oil were used as starting material to produce biolubricant by transesterification with 2-EH in a solvent-free and water-free system using a 50 L stirred-tank reactor. For such a purpose, Lipozyme TL IM, a commercial biocatalyst from Novozymes prepared via ionic adsorption of TLL on silicate particles [171] was used as biocatalyst. A full conversion into ester was observed after 10

h of reaction. The purified product had kinematic viscosity at 40 °C of 8.063 mm^2/s, flash point of 226 °C, and density of 872.2 at 15 °C. These results reveal that its viscosity was 3 times lower whose flash point was higher than that of a standard lubricant, which suggests its use as potential biolubricant for specific applications. Lage et al. [21] immobilized TLL via hydrophobic adsorption on mesoporous poly-methacrylate (PMA) particles used as biocatalyst in the synthesis of isoamyl oleate, an ester with lubricant properties [172, 173]. In this study, the effect of initial protein loading on the immobilization parameters were evaluated and maximum immobilized protein loading of ≈100 mg/g was achieved. The influence of certain parameters on ester synthesis was evaluated, and maximum acid conversion of 85% after 30 min of reaction in a solvent-free system was obtained. The prepared biocatalyst retained 91.4% of its original activity after thirty successive cycles of reaction of 30 min each. In a subsequent study, five commercial lipases from porcine pancreas, *Mucor javanicus*, *Candida* sp., RML and TLL were immobilized via interfacial activation on PMA particles and used as biocatalysts in the synthesis of *n*-octyl oleate by esterification reaction. Under optimal conditions, the biocatalyst fully retained its original activity after twelve batches. Ester synthesis was confirmed by FT–IR spectroscopy analysis. The purified product was characterized according to ASTM standard methods (viscosity, acid value, specific gravity, pour point and flash point), and similar properties were observed if compared to commercial ester from two different companies [20]. In a recent study, Kim et al. [55] proposed an enzymatic synthesis of TMP triester via esterification of high oleic fatty acid (HOFA) from palm oil. In this study, TLL immobilized on Duolite A568 surface via hydrophobic interactions was used as heterogeneous biocatalyst. The effects of relevant factors on reactions have been systematically evaluated. Maximum acid conversion percentage of 95% was obtained after 9 h of reaction. The physical properties of esters synthesized in this study (viscosity index, pour point, cloud point, and color) were very similat to those of a commercial product (TMP ester) prepared by a chemical route.

In a recent study, Fernandes et al. [58] esterified FFA from soybean oil and NPG using CRL immobilized via interfacial activation on a poly(methyl-methacrylate) (PMMA) as catalyst. Ester synthesis reached 90% conversion after 6 h of reaction, in addition to the whole process occuring in batch mode using an open reactor with magnetic agitation and a coupled thermostat. Subsequently, a reuse test was performed and the biocatalyst maintained its original activity after eight consecutive cycles. Finally, lubricating properties of the final product were evaluated in accordance with the ASTM

standards, which aims to establish standards for specific physicochemical properties of lubricants, and the final product proved to be an environmentally friendly alternative to petroleum-based lubricants.

In an another study, ricinoleic acid estolides were synthesized via esterification catalyzed by a non-commercial biocatalyst prepared via physical adsorption of Lipase 2 from *Staphylococcus xylosus* (SXL2) on $CaCO_3$ [174]. In this study, an enzymatic synthesis of esters has been performed in the presence or absence of organic solvent. According to results, a maximum conversion of 65 ± 4% after 48 h of reaction was achieved in a solvent-free system. The structure of synthesised biolubricant has been confirmed by several techniques (LC–MS, FT–IR and ^{13}C NMR spectroscopy). Estolide esters had viscosity of 2.1 mPa.s, thus it could be used in several applications such as lubricant, coating agent, ink, cosmetic and surfactant.

7.2. Encapsulation

In encapsulation immobilization, enzymes are retained in porous polymeric network structures, which allow the diffusion of substrate and product molecules [27, 28, 113, 139]. Unlike adsorption, encapsulation protects the enzyme from direct contact with the reaction médium (Fig. 5), thus minimizing inactivation effects that may occur due to the nature of the solvent being used. In addition, the method allows enzymes to remain stable for a relatively long time [26, 29, 149, 175]. However, possible disadvantages of encapsulation are high resistance to mass transfer to the substrate and a possible release of enzymes from the internal structure of supports after successive reaction cycles [139, 176]. In addition, scientific articles using this immobilization protocol aimed to immobilize enzymes for biolubricant production are still scarce in literature.

In an interesting study performed by Zhang et al. [177], a lipase-entrapped membrane was prepared using immersion phase inversion and used to synthesize lauryl stearate, an important ester with lubricant and plasticizer properties, by esterification in a pervaporation membrane reactor. The biocatalyst consisted of three flat layers: the top layer is a porous catalytic layer immobilized with CRL using glutaraldehyde as cross-linker agent, the middle layer is a dense polyvinyl alcohol (PVA) selective layer and the bottom layer is composed of polyethersulfone (PES). The effect of relevant factors (PVA, lipase and cross-linker agent concentrations) on the biocatalyst catalytic properties and stability (pH, temperature and reusability) using olive oil emulsion as substrate was studied. The effect of reaction time and temperature on lauryl stearate synthesis was also

examined and maximum acid conversion percentage of 83% was observed. In this study, it was proved that excess water removal by the membrane improves the biocatalyst activity and stability.

7.3. Covalent bonding

Enzyme immobilization protocol by covalent bonding is one of the most important techniques nowadays, since irreversible bonds are formed between the enzyme and the support, thereby avoiding enzyme desorption, even in intense reactional conditions [26, 30, 113, 133, 178]. The chemical bonds formed between the enzyme and the support take place between functional groups of protein side chains, such as carboxyl, hydroxyl, thiol and amino groups, and reactive groups previously introduced onto the surface of supports. Other advantages of this method are increased mechanical and thermal stability of the enzyme and extended half-life, thus producing a more robust, resistant and suitable biocatalyst to be used in processes involving continuous processes [30, 142].

In this immobilization process, the use of activating agents, usually bifunctional ones, for introducing functional groups on the surface of supports that are capable of reacting with nucleophilic groups of lipases has been widely used [30, 119, 178, 179]. Among which, one of the most commonly used to activate supports is glutaraldehyde due to its low cost, high reactivity with nucleophilic groups in the enzyme structure, e.g. amino terminal and ε-amino groups of lysine residues, in addition to allowing biocatalyst preparation with improved enzymatic activity retention [119, 179]. It has been widely used to prepare heterogeneous biocatalysts via covalent bonding of enzymes (e.g., glucose isomerase, pectolytic enzymes, naringinase and lipases) on different supports for producing industrial-scale compounds [120]. The preparation of heterogeneous biocatalysts by covalent immobilization of lipases on glutaraldehyde-activated supports has been reported in literature, as described below. Hajar and Vahabzadeh [69] studied biolubricant production from castor oil and methanol by transesterification catalyzed by CRL covalently immobilized on Fe_3O_4 nanoparticles functionalized with (3-aminopropyl)triethoxysilane (APTES), and activated with glutaraldehyde. A representative scheme of sequential functionalization of the support with APTES, activation with glutaraldehyde and covalent lipase immobilization is shown in Fig. 8. An enzymatic conversion of castor oil into biolubricant was performed in a magnetically-stabilized FBR. A maximum ester yield of 96.9% yield was achieved in lubricating ester

production after 24 h of reaction. In addition, in reuse tests, 87% of initial activity was retained after eight continuous cycles of 24 h each. In a recent study, Da Silva et al. [13] studied an enzymatic synthesis of fatty acid esters with potential application as biolubricants from *Dunaliella salina* microbial oil and fusel alcohol using BCL immobilized via covalent bonding on functionalized hydrous niobium oxide (Nb_2O_5) particles. The support was been prepared via a sequential pre-treatment with nitric acid, silanized with APTES and activated with glutaraldehyde. The authors observed that the biocatalyst produced was very efficient at converting microbial oil into lubricating esters, whose yield was 82.1% in 120 h of reaction. Kinematic viscosities of esters produced confirmed the transesterification reaction high yields, given that microbial oil viscosity decreased from 60.1 m^2/s to a range of 8.9–11.3 m^2/s at 40 °C.

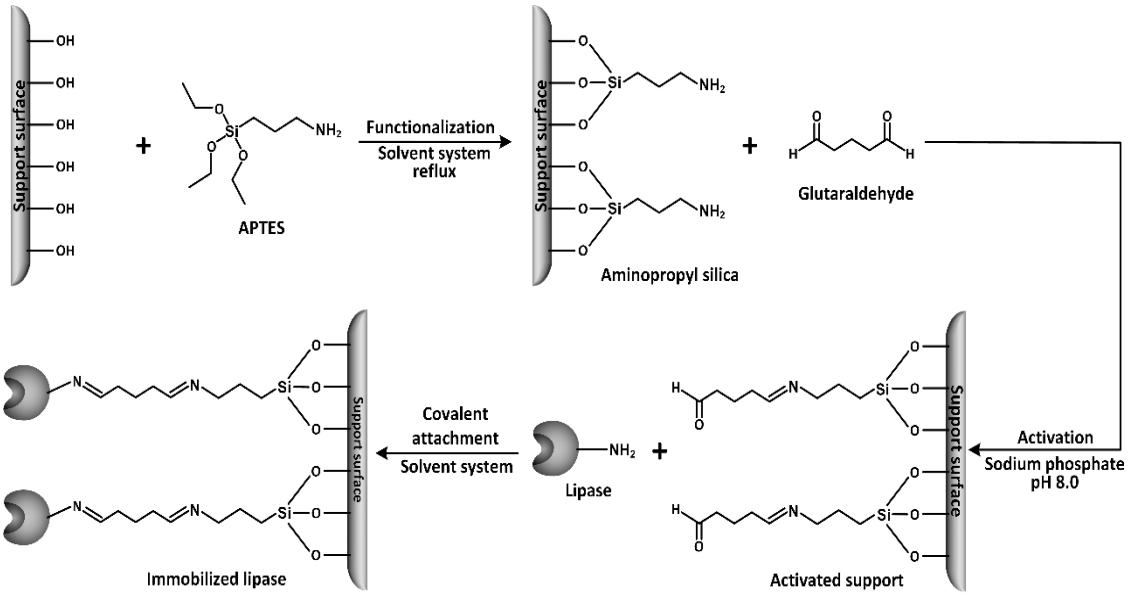

Fig. 8. Representative scheme of enzyme immobilization via covalent bonding on supports prepared via sequential functionalization with APTES and activation with glutaraldehyde.

Enzyme immobilization protocols involving epoxy supports are widely described in literature for the prepation of active and stable biocatalysts. These matrices are very stable at neutral pH, even in wet conditions, and can be stored for long periods. In addition, these supports has been used to stabilize industrial enzymes. Furthermore, while other immobilization protocols effect major changes in protein surface,

immobilization on epoxy supports allows slight chemical modifications in the protein. Epoxy groups undergo nucleophilic attack by different enzyme surface groups, such as amino, thiol and hydroxyl, thus allowing intense interactions between the enzyme and the support [121, 178, 180]. Fig. 9 illustrates the process of epoxy-support preparation and covalent immobilization of lipases. Céron et al. [22] used BCL immobilized by covalent bonding in epoxy silica-hydroxyethyl celulose as biocatalyst to synthesize lubricating esters in a PBR using palm kernel oil as raw material, as aforementioned. In this study, physicochemical properties were also determined according to ASTM standards, and kinematic viscosities at 40 °C confirmed a high conversion of esters, which modified the initial viscosity of palm oil from 30.13 mm^2/s to values ranging from 3 to 6 mm^2/s. Moreover, the biocatalyst was stable, thus revealing a half-life of about 38 days.

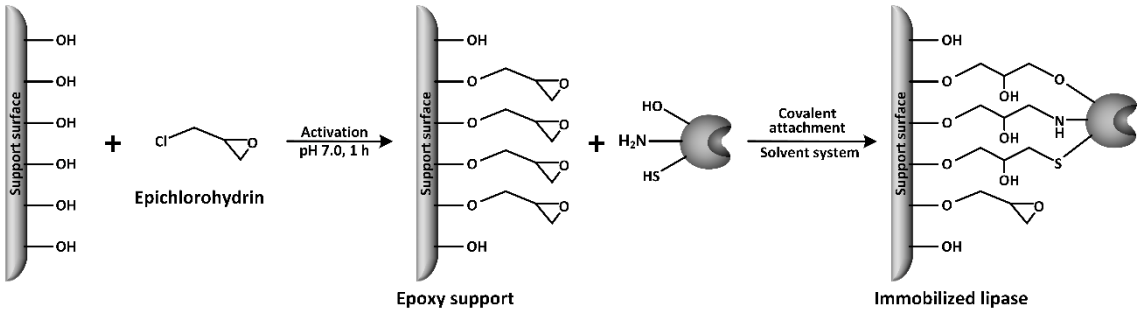

Fig. 9. Representative scheme of enzyme immobilization via covalent bonding on epoxy-supports.

7.4. Cross-linking enzyme aggregates (CLEAs)

This is an immobilization method that requires no support. In this process, the enzyme precipitation has been promoted by organic solvents (acetone, ethanol, tert-butanol, etc.) or salts, e.g. ammonium sulfate, or polymers such as polyethylene glycol, dextrans and polyethyleneimine, etc. [181, 182]. Then, a cross-linking agent, usually glutaraldehyde, is added, which form intramolecular and intermolecular networks with specific groups of amino acids present on the enzyme surface, resulting in the formation of enzyme aggregates [122, 181, 182], as shown in Fig. 10. Cross-linking agent concentration is always a parameter to be optimized, once excess can cause enzyme inactivation and promote reduced inter(cross-linking) at low concentrations [122, 182]. Its main advantages are high catalytic activity and stability, and supports are not required, which can incur expenditures on biocatalysts. Thereby, CLEAs is an important strategy

for preparing biocatalysts to be employed in industrial processes [122, 123, 139, 181–183].

In a recent study, a novel micro-reactor with rotating magnetic field (RMF) operating in a continuous mode has been proposed for an enzymatic synthesis of butyl oleate, i.e. an ester used as diesel additive, plasticizer and water resisting agent in hydraulic fluids [183]. Magnetic CLEAs (M-CLEAs) have been prepared by adding nano-Fe_3O_4 particles to a solution of lipase from *Rhizpous arrhizus*, followed by protein precipitation with acetone at 4 °C, and cross-linking with a glutaraldehyde solution to prepare a robust and active biocatalyst. The esterification reaction was conducted in a rotating magneto-micro-reactor with three layers of immobilized lipase and maximum acid conversion of 84% was achieved in a RMF. The ester synthesis was influenced by amount and distribution of M-CLEAs. The characteristics of M-CLEAs before and after 4 operative cycles have been evaluated by determining kinetic parameters (K_M and V_{max}), scanning electronic microscopy (SEM), conformation changes in lipase strcture by FT–IR analysis, and three-dimensional fluorescence spectroscopy. In this study, the authors provided a new concept in the synthesis of esters of industrial interest using magnetic CLEAs as biocatalysts.

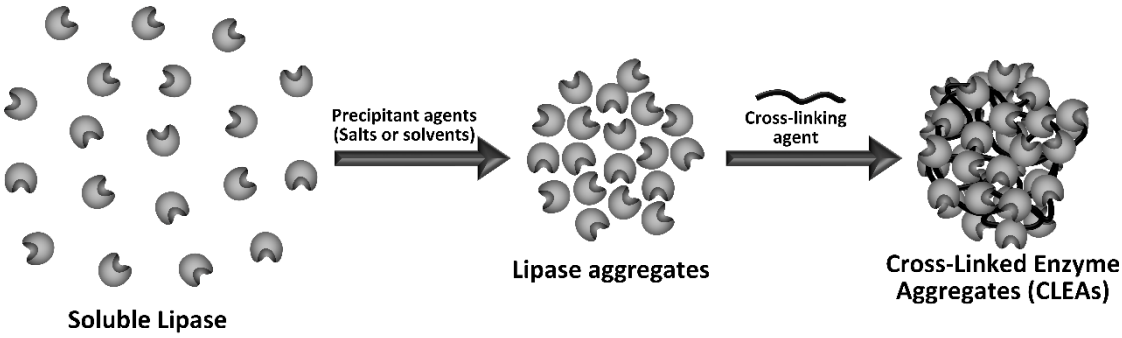

Fig. 10. Representation scheme of CLEAs preparation.

8. Future perspectives

Biolubricant synthesis using immobilized lipases has proved to be a very promising route due to high selectivity and catalytic activity of enzymes and requirement of mild reaction conditions, as reported above. However, it poses some challenges that must be faced so that it can be used for biolubricant production on an industrial scale. Despite the great interest and demand for lipases, especially those of microbial origin,

production costs are still quite high if compared to typical chemical catalysts (homogeneous and heterogeneous catalysts), which compromises their use as a biocatalysts and hinders their industrial production. Therefore, the development of more economical and environmentally friendly bioprocesses on a large scale for lipase production from agro-industrial waste generated from oleochemical industries, such as residual vegetable oil cakes and other lignocellulosic materials can be a very attractive strategy to reduce production costs in the industrial sector, since means of cultivation generally represent up to 50% of total production costs [130]. The search and selection of lipase-producing microorganisms and the use of genetic engineering can also lead to the development of new industrial enzymes with improved stability and specificity. In recent years, recombinant DNA technology advances have made it possible to supply industrial enzymes, including microbial lipases, adapted to new process conditions due to the production of enzymes with modified structures that confer greater activity or thermal stability or the ability to act on new types of substrates [184, 185]. In addition, it allows producing enzymes with greater productivity and ease of separation and purification processes [130, 185].

It is also worth mentioning the search for immobilization protocols and preparation of supports in order to obtain immobilized lipases with high catalytic activity and stabilization. As previously reported, immobilization by interfacial activation on hydrophobic supports has been the most widely used protocol due to high stabilization of its structures in open conformation [133, 151, 155, 160]. It is a reversible process and may result in desorbing some superficial lipase molecules on the supports after successive reaction cycles by the influence of substrates and/or reaction products [165]. However, there are other protocols that also allow preparing highly active and stable biocatalysts that are still seldom used in the oleochemical sector, such as immobilization on heterofunctional supports, supports with different groups introduced via activation and/or functionalization on different supports capable of changing lipase orientation on the surfaces of supports. The preparation of immobilized lipases on heterofunctional supports with improved properties when compared to monofunctional supports has been widely reported in literature [119, 135, 167, 178]. Usually, some functional groups have been introduced on the surface of supports for lipase immobilization by covalent bonding and other groups for immobilziation via adsorption by hydrophobic (interfacial activation of lipases) and/or ionic interactions [121, 135, 167, 178]. In a few cases, lipases are

immobilized via physical adsorption followed by post-immobilization with bifunctional agents and/or polymers in order to reduce lipase desorption [169, 186, 187].

Enzymatic production of biolubricants has been carried out preferably in STR in batch mode (Table 2). Continuous production of biolubricants is not yet extensively explored in literature. Thereby, the development of continuous processes is more attractive from an industrial standpoint, since it reduces operational costs and increases the gross profit of the process [85, 142]. In addition, there are other types of reactors that operate in continuous mode with high performance in biodiesel production, which have not yet been used for biolubricant synthesis by employing immobilized lipases as heterogeneous catalysts, e.g. a continuous stirred-tank reactor (CSTR), bubble column reactor, microchannel reactor, reactive distillation column, plug flow reactor, etc. [85].

Thus, further studies are expected in upcoming years on biolubricant synthesis employing immobilized lipases as catalysts with the aim of developing new technologies in the oleochemical sector, which is of paramount interest to countries with great agricultural diversity, such as Brazil.

Table 2. Literature survey for biolubricants synthesis catalyzed by immobilized lipases.

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
RML/Duolite ES-561 (Lipozyme IM 20)	Physical adsorption (ionic interactions)	Rapeseed oil methyl esters and TMP[a] (Transesterification)	58 °C, vacuum level at 5.3 kPa, no added water and 40% wt. of biocatalyst (STR[b] – batch)	Triester yield of 75% (24 h)	NP[c]	[59]
		Rapeseed oil and TMP[a] (Transesterification)	47 °C, vacuum level at 2 kPa, 15% of added water and 40% wt. of biocatalyst (STR[b] – batch)	Triester yield of 73% (68 h)	NP[c]	[60]
RML/ Duolite ES 562 (Lipozyme RM IM)	Physical adsorption (ionic interactions)	Methyl esters from soybean and castor oils with NPG[d] (Transesterification)	Larger outlet reactor (20 mL scale), alcohol:methyl esters molar ratio of 1:3.75, 4% wt. of biocatalyst, water addition at 1% wt., and 40 °C (STR[b] – batch)	Hydroxyl groups conversion >90% (24 h) for soybean methyl esters and ≈100% for castor methyl esters (72 h)	6 cycles of 24 h (70% for soybean methyl esters and 77% for castor methyl esters)	[25]
CALB/Lewatit VP OC 1600 (Novozym 435)				Hydroxyl groups conversion >90% (24 h) for soybean methyl esters and ≈100% for castor methyl esters (48 h)	6 cycles of 24 h (48% for soybean methyl esters and 98% for castor methyl esters)	
Staphylococcus xylosus 2/$CaCO_3$	Physical adsorption	Ricinoleic acid (Esterification)	3 g of ricinoleic acid in a solvente-free system, 25% wt. of molecular sieve, 400 units (U) of biocatalyst, 55 °C, and 220 rpm (STR[b] – batch)	Acid conversion of 65 ± 4% (48 h)	15 cycles (50%)	[174]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
CALB/Lewatit VP OC 1600 (Novozym 435)	Physical adsorption (ionic interactions)	Isoamyl alcohol and oleic acid (Esterification)	Acid:alcohol molar ratio of 1:2, 0.5% of biocatalyst, 60 °C, 150 rpm and water removal system by pervaporation (STR[b] – batch)	Acid conversion of 99.8% (6 h)	NP[c]	[172]
			Reactor X-Cube™ 45 °C, isoamyl alcohol/oleic acid molar ratio: 6.5, residence time of 2.43 min, mass of biocatalyst of 105 mg and zeolite as dehydrating agent (PBR[e] – continuous)	Acid conversion of 98% (6 h)	6 cycles of 24 h each (≈100%)	[173]
		Oleic acid and TMP[a] (Esterification)	Acid:alcohol molar ratio of 3.3:1, 2% of biocatalyst, 70 °C, and 700 rpm (STR[b] – batch)	Acid conversion of 98% (6 h)	6 cycles of 24 h each (20%)	[91]
		Linoleic acid and H_2O_2 (Epoxidation)	1.4 g of acid in 10 mL toluene, 15 μL of H_2O_2 at 30% v/v, and 120 mg of biocatalyst (STR[b] – batch)	Monoepoxyde yield of 82.14% (7 h)	NP[c]	[188]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
CALB/Lewatit VP OC 1600 (Novozym 435)	Physical adsorption (ionic interactions)	Rapeseed oil methyl esters and three alcohols (2-EH, NPG[d] and TMP[a]) (Transesterification)	35 °C, 250 rpm, stoichiometric methyl esters:alcohol molar ratio, and 2% wt. of biocatalyst (STR[b] – batch)	Conversion of 98% (50 h for 2-EH, 150 h for NP, and 200 h for TMP[a])	NP[c]	[61]
		10(*S*)-hydroxy-8(E)-octadecenoic – (10*S*)-HOME (Esterification)	0.5 g of biocatalyst, 0.5 g of (10S)-HOME, 20 ml of *n*-hexane, 50 °C (STR[b] – batch)	Stolide yield of 30% (48 h)	10 cycles (46.7%)	[189]
		Sebacic acid and and octanol (Esterification)	Acid:alcohol molar ratio of 1:5, 5% wt. of biocatalyst, and 100 °C (STR[b] – batch)	Acid conversion of 100% and diester yield > 90% (150 min)	3 cycles (22.2-26.4%)	[190]
		Castor oil and glycerol (Transesterification)	90 °C, 300 rpm, oil:alcohol molar ratio of 1:6, and 5% wt. of biocatalyst (STR[b] – batch)	Yields of 54.47 ± 0.58% for monoricinolein and 38.01 ± 0.50% for diricinolein (3 h)	NP[c]	[72]
CALB/Octadecyl methacrylate co-polymer	Physical adsorption (hydrophobic interactions)	Palm stearin and methanol (Transesterification)	Oil:alcohol molar ratio of 4:1, 6% wt. of biocatalyst, 60 °C, and 700 rpm (STR[b] – batch)	Yield of 95.26% (8 h)	NP[c]	[65]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
CALB/Indion®PA 500 resin	Physical adsorption (hydrophobic interactions)	Ricinoleic acid and PE[f] (Esterification)	Acid:alcohol molar ratio of 1:4, 2% wt. of biocatalyst, 200 rpm, 200 g/L molecular sieves in the presence of *tert*-butanol and 60 °C (STR[b] – batch)	Acid conversion of 93% (24 h)	NP[c]	[191]
Candida sp. 99–125/diatomaceous earth	Physical adsorption	FFA[g] from *Sapindus mukurossi* oil and H_2O_2 (Epoxidation)	20 g of FFA, 15% wt. of biocatalyst, 350 rpm, 690 μL of H_2O_2 at 30% v/v, and 30 °C for 6 h + 35 °C for 2 h of reaction in a solvente-free system (STR[b] – batch)	Yield of 83% (8 h)	NP[c]	[192]
Candida sp. 99–125/Polyurethane foam	Physical adsorption	Caprylic acid and TMP[a] (Esterification)	16% wt. of biocatalyst, alcohol:acid molar ratio of 1:10, 190 rpm, 40 °C and water amount of 0.8% wt. (STR[b] – batch)	Acid convesion of 96% and triester yield of 93% (60 h)	NP[c]	[193]
Candida sp. 99–125/Surfactant modified cotton membrane			30% wt. biocatalyst (based on acid amount), alcohol:acid molar ratio of 1:8.4, 190 rpm, and 45 °C (STR[b] – batch)	Acid convesion of 97.3% and triester yield of 95.5% (47 h)		[194]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
TLL/Silicate particles (Lipozym TL IM)	Physical adsorption (ionic interactions)	Rapeseed oil methyl esters and 2-EH (Transesterification)	10% wt. biocatalyst (based on acid amount), alcohol:methyl esters mass ratio of 1:2.4, 60 °C under vacuum (100-150 mmHg) (STR[b] – batch)	Convesion of 100% in a 500 mL reactor after 5 h of reaction and 10 h in a 50 L reactor	10 cycles of 5 h each (≈95%)	[62]
TLL/Duolite A568	Physical adsorption	High oleic fatty acid from palm oil and TMP[a] (Esterification)	60 °C, 15% wt. of biocatalyst, acid:alcohol molar ratio of 3:1, vacuum level at 6.7 kPa, and water activity of 0.5-0.9 (STR[b] – batch)	Conversion of 95% (9 h)	NP[c]	[55]
TLL/Poly-methacrylate	Physical adsorption (hydrophobic interactions)	Isoamyl alcohol and oleic acid (Esterification)	45 °C, 20% m/v of biocatalyst, 200 rpm, stoichiometric acid:alcohol molar ratio in a solvent-free system (STR[b] – batch)	Acid conversion of 85% (30 min)	30 cycles (91.4%)	[21]
		n-octanol and oleic acid (Esterification)	45 °C, 10% m/v of biocatalyst, 240 rpm, acid:alcohol molar ratio of 1:1.5 in a solvent-free system (STR[b] – batch)	Acid conversion of 95.1 ± 1.3% (60 min)	12 cycles (100%)	[20]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
TLL/Citric acid modified Fe_3O_4 nanoparticles (MNPs)	Physical adsorption	FFA[g] from WCO[h] and NPG[d] (Hydroesterification)	45 °C; 5% wt. of biocatalyst (based on FFA amount), acid:alcohol molar ratio of 4:1, and 250 rpm (STR[b] – batch)	Acid conversion of 94% (24 h)	12 cycles (75%)	[24]
TLL/chitosan-glutaraldehyde-glycine hydrogel (Chit–GA–Glu)	Physical adsorption and covalent bonding	Isoamyl alcohol and palmitic acid	50 °C; 10% m/v of biocatalyst, stoichiometric acid:alcohol molar ratio in iso-octane, and 240 rpm (STR[b] – batch)	Acid conversion of 85% (90 min)	9 cycles (40%)	[164]
CRL/glutaraldehyde-activated amino-Fe_3O_4 nanoparticles (MNPs)	Covalent bonding	Castor oil and methanol (Transesterification)	13.3% wt. of biocatalyst (based on oil mass), oil:alcohol molar ratio of 1:5 in iso-octane medium, 40 °C, flow rate of 15 mL/min, and magnetic field strength of 10 mT (FBR[i] – continuous)	Ester yield of 96.9% (24 h)	8 cycles (87%)	[69]
CRL/"sandwich-like" membrane (PVA/PES)[j]	Entrapment	Lauryl alcohol and stearic acid (Esterification)	Effective membrane area of 19.85 cm^2 (biocatalyst), stoichiometric acid:alcohol molar ratio (3 mmol of each reactant) in 25 mL cyclohexane, 40 °C under vacuum (4–5 kPa) (STR[b] – batch)	Acid conversion of 83% (16 h)	NP[c]	[177]

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
CRL/Microporous polypropylene (Accurel MP1000)	Physical adsorption (hydrophobic interactions)	FFA[g] from soybean oil and polyols (NPG[d], and TMP[a]) (Hydroesterification)	6.5% wt. of biocatalyst, 40 °C, and acid:alcohol molar ratio of 3.75:1 (STR[b] – batch)	Hydroxyl groups conversion of 99% for NPG[d] and 92% for TMP[a] (24 h)	6 cycles of reaction (≈100%)	[56]
CRL/Poly(methyl methacrylate)	Physical adsorption (hydrophobic interactions)	Soybean fatty acid distillate and polyols (NPG[d], and TMP[a]) (Esterification)	4% wt. of biocatalyst, 45 °C, and acid:alcohol molar ratio of 3.75:1 (STR[b] – batch)	Hydroxyl groups conversion of 90% for NPG[d] (8 h) and ≈15% for TMP[a] (24 h)	8 cycles of reaction (≈100%)	[58]
CRL/Microporous polypropylene (Accurel MP1000)				Hydroxyl groups conversion of 90% for NPG[d] (8 h) and 94% for TMP[a] (24 h)		
BCL/epoxy matrix silica-hydroxyethyl cellulose	Covalent bonding	Palm kernel oil and simulated fusel oil or isoamyl alcohol (Transesterification)	Biocatalyst concentration of 500 U per g of oil, 45 °C, 150 rpm and oil:alcohol molar ratio of 1:4 (STR[b] – batch)	Ester yield >99% for both isoamyl alcohol and simulated fusel oil (48 h)	NP[c]	[22]
			Glass column with 21 g of biocatalyst (work volume of 20.9 cm^3), 45 °C, oil:alcohol molar ratio of 1:4 and fixed flow rate correspondent to a space-time of 8 h for 22 days (PBR[e] – continuous)	Ester yield >98% for both isoamyl alcohol and simulated fusel oil (8 h of space-time)	Average half-life time of 38 days for isoamyl alcohol and 45 days for simulated fusel oil	

Table 2. *Continuation.*

Lipase/Support	Immobilization protocol	Starting materials (Reaction type)	Experimental conditions (Reactor design/mode)	Conversion/Yield (Reaction time)	Reuse (Residual activity)	References
BCL/Glutaraldehyde-activated amino-hydrous niobium oxide	Covalent bonding	Microbial oil from *Dunaliella salina* and fusel oil (Transesterification)	Biocatalyst concentration of 500 U per g of oil, 45 °C, 150 rpm and oil:alcohol molar ratio of 1:8 in iso-octane medium (STR[b] – batch)	Ester yield of 90.0 ± 0.9% for simulated fusel oil and 89.0 ± 1.2% for fusel oil (120 h)	NP[c]	[13]
BCL/ceramic (PSCI-Amano Lipase)	Physical adsorption	Oleic acid and H_2O_2 (Epoxidation)	2 mmol of acid in ethyl acetate (5 mL), 55 °C, 10% of biocatalyst, 0.2% of H_2O_2 at 30% v/v and 150 rpm (STR[b] – batch)	Yield of 88% (3 h)	NP[c]	[195]
RAL/glutaraldehyde-functionalized Fe_3O_4 nanoparticles (MNPs)	CLEAs	Oleic acid and butanol (Esterification)	Rotating magneto-micro-reactor with three layers containing 3 mg of biocatalyst each, 25 °C, stoichiometric acid:alcohol molar ratio, substrate concentration of 0.12 mol/L and fluid flow rate at 60 mL/min (PBR[e] – continuous).	Acid conversion of 84% (15 h)	4 cycles of 18 h each (≈100%)	[183]

a – Trimethylolpropane
b – Stirred tank reactor
c – Not performed
d – Neopentyl glycol
e – Packed bed reactor
f – Pentaerythritol
g – Free fatty acids
h – Waste cooking oil
i – Fluidized bed reactor
j – Polyvinyl alcohol/polyethersulfone

9. Conclusion

Replacing petroleum-based lubricants with biodegradable products is one of the ways to reduce the adverse effects caused by fossil derivatives on the environment. Physicochemical and tribological characteristics of biolubricants from TAG satisfy environmental, economic and technical performance challenges altogether, thus proving the great potential of biological-based lubricants to replace conventional lubricants. The use of TAG as raw materials may bring socioeconomic and environmental benefits to Brazil, given that the country is one of the world's largest producers of edible and non-edible vegetable oils, as shown in Table 1. The selection of lipid raw material for the production of lubricating esters is an extremely relevant process parameter to be studied, in addition to an appropriate production route to be selected for their synthesis which is also of paramount importance. However, a direct application of TAG as lubricants has some disadvantages, as aforementioned, which can be overcome by employing different chemical reactions discussed in this review, such as transesterification, esterification, epoxidation/ring-opening and hydroesterification catalyzed by immobilized lipases, a promising class of heterogeneous catalysts. The application of immobilized lipases in the biotransformation of TAG for biolubricant synthesis has been widely researched in recent years, as previously reported, due to the requirement of mild conditions in the process, high specificity of substrates, high degree of purity of obtained products and possibility of biocatalyst reuse. The application of immobilized lipases fosters the development of technologically clean processes for the production of such an important class of oleochemical compounds on an industrial scale as biolubricants.

Author contributions

Iara C. A. Bolina, Raphael A. B. Gomes and Adriano A. Mendes performed the paper writing and final editing of the manuscript. Adriano A. Mendes took care of the conceptualization, supervision, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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CAPÍTULO 3

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Abstract

Ion-exchange supports have been prepared via sequential functionalization of silica-based materials with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) (Epx-SiO_2) and activation with glycine (Gly-Epx-SiO_2) in order to immobilize lipase from *Themomyces lanuginosus* (TLL) via adsorption. Rice husk silica (RHS) was selected as support with the aim of comparing its performance with commercial silica (Immobead S60S). Sequential functionalization/activation of SiO_2-based supports has been confirmed by AFM, SEM and N_2 adsorption-desorption analyses. Maximum TLL adsorption capacities of 14.8 ± 0.1 mg/g and 16.1 ± 0.6 mg/g using RHS and Immobead S60S as supports, respectively, have been reached. The Sips isotherm model has been used, which was well fitted to experimental data on TLL adsorption. Catalytic activities of immobilized TLL were assayed by olive oil emulsion hydrolysis and butyl stearate synthesis via an esterification reaction. Hydrolytic activity of the biocatalyst prepared with a commercial support (357.6 ± 11.2 IU/g) was slightly higher than that of Gly-Epx-SiO_2 prepared with RHS (307.4 ± 7.2 IU/g). On the other hand, both biocatalysts presented similar activity (around 90% conversion within 9-10 h of reaction) and operational stability after 6 consecutive cycles of butyl stearate synthesis in batch systems.

Keywords: Silica materials, Functionalization, Ion-exchanger supports, Lipase immobilization, Catalytic properties.

1. Introduction

In recent decades, the use of enzymes in industrial processes (e.g. food-manufacturing processes, detergent additives, paper and pulp processing, textile manufacture, biofuel generation, wastewater treatment, pharmaceutical and fine chemical production) has received great attention due to reduced energy consumption, non-toxicity, eco-friendly characteristics and high selectivity (stereoselectivity, regioselectivity and enantioselectivity) [1–3]. However, the main drawbacks of using free enzymes (in powder or soluble forms) on an industrial scale are their difficult recovery and recyclability, elevated extraction costs, and strong inactivation induced by reaction temperature, organic solvents and mechanical shear [2–5]. In this context, enzyme immobilization has demonstrated to be a promising strategy to overcome such limitations, since heterogeneous biocatalysts can be recovered and reused at the end of the process. Moreover, this strategy promotes enzyme stabilization, facilitates product purification, avoids enzyme aggregation and proteolysis and improves reactor design flexibility [3–5].

Several techniques have been successfully developed to immobilize industrial enzymes such as: *(i)* covalent attachment on activated-supports [3–6], *(ii)* physical adsorption [7], *(iii)* entrapment [3–5], *(iv)* hybrid processes – physical adsorption followed by cross-linking of adsorbed enzymes via ionic interaction or covalent attachment [8,9], simultaneous physical adsorption/covalent attachment by using heterofunctional supports [10], and *(v)* cross-linking – cross-linked enzyme crystals (CLECs) and cross-linked enzyme aggregates (CLEAs) [11]. Among them, physical adsorption is still the most extensively used immobilization technique to prepare heterogeneous biocatalysts due to its simplicity, low-cost and possibility of support recyclability by desorption of inactive enzyme molecules [3]. Moreover, it promotes stabilization and improvement of catalytic activity [3,7,12–14]. On the other hand, its main disadvantage is enzyme desorption from the support surface by changes in temperature, pH and ionic strength or by using several different chemicals (surfactants, urea, cosolvents, etc.) [12,13]. Several industrial enzymes, including lipases, have been commonly used to catalyze reactions in non-aqueous media (solvent or solvent-free systems) [15,16]. However, enzymes are insoluble in these media and physical adsorption may be a suitable immobilization technique for such a purpose [17].

Physical adsorption of enzymes, such as lipases, on supports can occur via hydrogen bounds or ionic and hydrophobic interactions [2,3,7]. With respect to lipases,

hydrophobic interaction is the most widely used immobilization protocol due to its peculiar mechanism of interfacial activation on hydrophobic surfaces – immobilization by open conformation [2,3,7,14,18]. However, preparation of stable and active heterogeneous biocatalysts via reversible immobilization of lipase on ion-exchange supports has been broadly reported in previous literature [19–22]. This is due to an intense interaction between carboxylate (from C-terminal and aspartate/glutamate residues) or amino (from N-terminal and arginine/histidine/lysine residues) groups in enzyme structures and ionic groups on the support surface. These groups have been commonly introduced via reversible (physical adsorption) or irreversible (covalent attachment) chemical modification using functionalized polymers, amino acids, ionic liquids, alkoxysilane agents, etc. [19–26]. In certain cases, ionic groups have been preferentially bonded via covalent attachment of nucleophilic groups of functional molecules and electrophilic groups on the support surface, e.g. epoxy groups that were previously introduced via functionalization of inorganic supports with (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) [27–29], or organic supports with epichlorohydrin [30–32]. These ion-exchange supports have been prepared so as to protect enzymes from undesired interactions with the support surface, which can lead to their inactivation (insertion of spacer chains) [28] and create a more appropriate microenvironment for enzyme immobilization [33]. Moreover, ion-exchange supports have been used as promising supports in enzyme purification via specific adsorption techniques [34,35].

Different supports have been used for enzyme immobilization, such as natural or organic and inorganic/synthetic materials [36]. Inorganic materials, such as metal oxides and silica-based compounds, have been extensively assessed due to their high thermal, mechanical and chemical stabilities towards several organic solvents, commonly used as reaction media, and microbial resistance [37]. Among different inorganic materials, silica-based supports are the most widely used to immobilize industrial enzymes on account of their high surface area/pore volume and reactivity concerning several functionalizing agents [6]. These supports can be commercially obtained from several companies, prepared via sol-gel using several alkoxysilane precursors [38], or through hydrothermal processes of several residual biomass, including rice husks [39,40].

In the present study, ion-exchange supports have been prepared by sequential functionalization of silica particles with GPTMS to introduce epoxy groups (Epx-SiO_2) and covalent attachment of glycine to obtain high-density ionic surface groups (Gly-Epx-

SiO_2). Rice husk SiO_2 (RHS) was tested as potential support and its performance was compared with commercial SiO_2 particles (Immobead S60S). Preparation of ion-exchange supports by activation of epoxy-SiO_2-based supports with amino acids was reported in a previous study conducted by Bolivar and Nidetzky [28]. These authors prepared two different cationic supports by introducing high-density carboxylate surface groups via sequential functionalization of porous glass with: *(i)* GPTMS (epoxy-support) and covalent attachment of L-aspartate and, *(ii)* (3-aminopropyl)triethoxysilane (3-APTES) followed by derivatization with glutaraldehyde and covalent attachment of glycine. These functionalized supports were used to immobilize D-Amino acid oxidase from *Trigonopsis variabilis* (*Tv*DAO), i.e. an industrial enzyme used in the synthesis of pharmaceutical intermediates and fine chemicals. Glycine-activated epoxy-SiO_2 (Gly-Epx-SiO_2) has been tested as support to adsorb lipase from *Thermomyces lanuginosus* (TLL) herein. It was selected as enzyme model due to its high catalytic activity in hydrolysis reactions and ester syntheses by esterification, transesterification and interesterification reactions performed in either solvent or solvent-free systems [14,41–44]. The novelty value of this study is the use of these ion-exchange supports for TLL immobilization as heterogeneous biocatalysts in biotransformation reactions in aqueous (hydrolysis of olive oil emulsion) and non-aqueous (butyl stearate synthesis via esterification reaction) media. These supports were characterized by several techniques to confirm their functionalization. The effect of relevant factors (incubation pH and initial enzyme loading) on immobilization parameters has been systematically assessed. Moreover, isotherm adsorption studies have also been undertaken in order to elucidate adsorption processes.

2. Materials and methods

2.1. Materials

TLL (Lipolase® 100L) was acquired from Sigma-Aldrich (St. Louis, MO, USA). It is a lipase solution with protein concentration of 18 mg/g (Lot # SLBK0930V). Rice husks were acquired from Arroz Rei Ouro Ltda. (Itajubá, MG, Brazil). Rice husk SiO_2 (RHS) was prepared via a hydrothermal process reported in a previous study [40]. This support has particle size distribution in the range of 78.5–453.1 μm and average particle size of 269.1 μm. Commercial SiO_2 (Immobead S60S), a support with particle size of 60-200 μm, was acquired from Chiral Vision (Leiden, The Netherlands). (3-

Glycidyloxypropyl)trimethoxysilane (GPTMS) was purchased from Sigma-Aldrich. Butyl alcohol, stearic acid and glycine were purchased from Synth® (São Paulo, SP, Brazil). Bovine serum albumin (BSA) was acquired from Bio-Rad Laboratories Inc. (Hercules, CA, USA). Olive oil (low acidity – Carbonell) was purchased at a local market (Alfenas, MG, Brazil). All other chemical reagents and organic solvents were of analytical grade acquired from Synth® and Vetec Química Ltd. (São Paulo, SP, Brazil).

2.2. Preparation of the ion-exchanger supports

Epoxy-SiO_2 supports (Epx-SiO_2) were prepared according methodology described in a previous study [10]. A suspension containing 1 g of dry SiO_2 and 20 mL of a mixture of alkoxysilane precursor (GPTMS) in toluene (volume ratio alkoxysilane:solvent of 1:10) was kept under reflux within 4 h at 120 °C. Functionalized supports were filtered in a Buchner funnel under vacuum using Whatman no. 41 filter paper and thoroughly washed with acetone, ethanol and distilled water, and dried at 40 °C for 24 h.

A modified method was used to prepare ion-exchanger supports (Gly-Epx-SiO_2) [32]. Epx-SiO_2 (1 g) was incubated in 10 mL of 1 M freshly glycine solution at pH 8.0. The suspension was kept under continuous stirring (200 rpm) within 48 h of incubation at room temperature. Subsequently, Gly-Epx-SiO_2 supports were filtered in a Buchner funnel under vacuum using Whatman no. 41 filter paper, washed with distilled water in excess and stored overnight at 4 °C.

2.3. Characterization of non- and functionalized SiO_2 particles

AFM images were acquired using a Cypher ES microscope (Asylum Research) in tapping mode configuration with scanned surface area of 1 × 1 μm. In this study, the effect of functionalization on the support surfaces was evaluated in terms of average roughness (R_a), root mean square roughness (R_{rms}), and average height (Z). SEM images were acquired on a Quanta 200 FEI equipment under vacuum with an acceleration voltage of 5 kV. All surfaces were coated with a thin layer of gold (10 nm) to increase the conductivity before to measurement. Specific surface area (S_A) was determined by N_2 adsorption-desorption isotherms at 77 K using the Brunauer-Emmett-Teller (B.E.T.) method in a LMAsorp instrument with degassing at 250 °C within 4 h before the analysis.

Barrett–Joyner–Halenda (BJH) model was used to determine pore volume (V_p) and average pore size (D_p).

2.4. Lipase immobilization procedure

TLL immobilization consisted of the preparation of suspensions containing 19 mL of enzyme solution prepared in a buffer solution (ionic strength fixed at 5 mM) and 1 g of support [14,42,43]. The suspensions were kept under continuous stirring in an orbital shaker (200 rpm) at room temperature. Heterogeneous biocatalysts were recovered by filtration in a Buchner funnel under vacuum using Whatman n° 41 filter paper and thoroughly washed with distilled water. The adsorption process was monitored by measuring the residual protein concentration in the supernatant solution according Bradford's method by using BSA as the protein standard [45]. Finally, the biocatalysts were stored in a refrigerator at 4 °C.

2.4.1. Effect of pH on the immobilization parameters

To investigate the effect of pH value on the immobilization parameters, the initial values of pH solution varied from 3.0 to 7.0 (buffer sodium citrate pH 3.0, buffer sodium acetate pH 4.0–5.0, buffer sodium phosphate pH 6.0–7.0). These tests were performed by using initial protein loading of 5 mg/g and contact time of 15 h.

2.4.2. Effect of initial protein loading on the immobilization parameters

The effect of initial protein loading on the immobilization parameters was investigated in the range of 5–40 mg/g of support. Adsorption tests were conducted at pH 4.0 buffer sodium phosphate and contact time of 15 h.

In this set of experiments, non-linear Langmuir (Eq. (1)), Sips (Eq. (2)) and Redlich–Peterson (Eq. (3)) isotherm models were fitted to the experimental data to explain how TLL molecules interact with both ion-exchanger support surfaces [46].

$$q_e = \frac{q_{\max} \times C_e}{K_L + C_e} \quad (1)$$

$$q_e = \frac{K_S \times C_e^{\beta_S}}{1 + a_S \times C_e^{\beta_S}} \quad (2)$$

$$q_e = \frac{K_R \times C_e}{1 + a_R \times C_e^{\,g}} \quad (3)$$

where q_e is the adsorption capacity at equilibrium (mg protein/g support), C_e is defined as the residual mass of protein in unit volume of lipase solution (mg protein/mL or mg protein/g of solution), q_{max} is the maximum adsorption capacity (mg protein/g support), K_L is the Langmuir constant related to the energy of adsorption (mL/mg protein), K_S is the Sips isotherm model constant (mL/mg), β_S is the Sips exponent (dimensionless), a_S is the Sips model constant (mL/mg), K_R is the Redlich–Peterson isotherm constant (mL/mg), a_R is the Redlich–Peterson isotherm constant (1/mg) and g is the Redlich–Peterson exponent (dimensionless).

The affinity between TLL and ion-exchanger supports was also predicted in terms of Langmuir dimensionless constant separation factor (R_L), as shown in Eq. (4) [14,46]:

$$R_L = \frac{1}{1 + K_L \times C_0} \quad (4)$$

where C_0 is the initial protein concentration in the immobilization supernatant (mg/L).

The experimental data from the TLL adsorption process (isotherm and kinetic studies) were represented as mean ± standard deviation of three replications. These data were analyzed by using software OriginPro version 8.0 (OriginLab Corporation, Northampton, MA, USA).

2.4.3. Error analysis

In this study, a relatively high correlation coefficient (R^2) and low root-mean-square error (RMSE) values were used as criteria to select the better fit for isotherm adsorption studies [47]. These parameters were obtained by using Solver® of Microsoft Excel.

R^2 values were calculated by determining the residual sum of squares (SS_{res}) and total sum of squares (SS_{tot}) – Eq. (5), (6) and (7), respectively. To calculate SS_{tot}, mean of experimental adsorbed protein at certain time t ($\overline{q_t}$) has been determined (Eq. (8)).

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \quad (5)$$

$$SS_{res} = \sum_{i=1}^{N} \left(q_t^{\exp} - q_t^{theor} \right)^2 \quad (6)$$

$$SS_{tot} = \sum_{i=1}^{N} \left(q_t^{\exp} - \overline{q_t} \right)^2 \quad (7)$$

$$\overline{q_t} = \frac{1}{N} \sum_{i=1}^{N} q_t^{\exp} \quad (8)$$

RMSE has been calculated as follows (Eq. (9)):

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left(q_t^{\exp} - q_t^{theor} \right)^2} \quad 9)$$

where: q_t^{exp} and q_t^{theor} are experimental and theoretical adsorbed lipase concentration at certain time *t*, respectively, and *N* is the number of samples.

2.5. Determination of immobilization parameters

Immobilized protein concentration at equilibrium (q_e – mg/g of support) was determined according to Eq. (10) [14,43]:

$$q_e = \frac{V \times (C_0 - C_e)}{m} \quad 10)$$

where *V* is the volume of enzyme solution (mL), C_0 is the initial protein concentration in the immobilization supernatant (mg/mL), C_e is the residual mass of protein in unit volume of lipase solution (mg protein/mL) and *m* is the mass of support (g).

The method for determining the catalytic activity of immobilized TLL was based on the hydrolysis of olive oil emulsion [14,42,43]. The emulsion was prepared by mixing 1.25 g of olive oil with 3.75 g of Arabic Gum solution at 3% m/v and 5 mL of 100 mM buffer sodium phosphate pH 8.0. The mixture was placed in a 125 mL conical flask and

incubated in a conventional water bath shaker with controlled temperature (37 °C) under continuous stirring (200 rpm). The hydrolysis reaction was started by adding 0.1 g of immobilized TLL. Subsequently, 10 mL of ethanol solution at 95% m/m was added to the reaction mixture and titrated with a 30 mM NaOH solution using phenolphthalein as indicator. One international unit (IU) of activity was defined as being the mass of enzyme required to release 1 µmol of free fatty acid per minute of reaction.

Specific activity (SA) was determined as follows (Eq. (11)) [14,43]:

$$SA = \frac{HA}{q_e} \quad (11)$$

where *HA* is the hydrolytic activity of the prepared biocatalysts (IU/g of support) and q_e is the immobilized protein concentration at equilibrium (mg/g of support).

2.6. Butyl stearate synthesis and reusability tests

Butyl stearate was synthesized via direct esterification reaction. The reactions were performed in 100 mL closed screw-capped glass containing reaction mixtures (6 mL) composed of stoichiometric concentrations of stearic acid and butyl alcohol (1 M of each reactant) in isooctane medium containing 20 mg of immobilized protein – 1.24 g and 1.35 g of adsorbed TLL on ion-exchanger support prepared by using Immobead S60S and RHS, respectively. The suspensions were kept under continuous stirring (240 rpm) in an orbital shaker at 50 °C. Samples were periodically withdrawn from the reaction mixtures (100 µL), diluted in 10 mL of ethanol solution (95% m/m) and titrated against a standard 30 mM NaOH solution using phenolphthalein as indicator to determine the residual stearic acid concentration and, thus, the ester conversion percentage [14,42–44]. In this study, all the samples were titrated thrice for accuracy.

Reusability tests were performed after 6 consecutive cycles of ester synthesis of 9 and 10 h each in a batch system by using ion-exchanger support prepared with Immobead S60S and RHS, respectively. At the end of each cycle, the biocatalysts were removed by filtration, washed with cold hexane in order to remove any substrate or product molecules that were eventually retained in their microenvironments and re-suspended in a freshly substrate to start new runs. Ester conversion percentage was estimated at the end of each run.

3. Results and discussion

Ion-exchange supports have been prepared via sequential functionalization of silica-based materials (Immobead S60S and RHS) with GPTMS (Epx-SiO_2 supports) followed by covalent attachment of glycine (Gly-Epx-SiO_2). In this study, glycine was chosen as amino acid model due to being successful at blocking residual epoxy groups after multipoint covalent attachment of enzymes on several epoxy-supports at alkaline values (pH above 8). This strategy has been used so as to stop additional interactions between the enzyme and the support, which can drastically reduce their catalytic activity/stability and enable the creation of a more hydrophilic microenvironment in order to maintain the essential hydration layer bound to the 3D structure of enzymes, thus preserving their catalytic activity [48,49]. Moreover, the use of activated epoxy-SiO_2 with glycine as ion-exchange support to immobilize lipase has not been reported in literature yet. TLL adsorption was performed at fixed ionic strength (5 mM buffer solutions) so as to avoid possible competition between ions in the solution and ionic groups from the enzyme structure to interact with the support surface. A schematic showing the preparation of both ion-exchange supports is in Fig. 1. Sequential functionalization of SiO_2-based supports was confirmed by several techniques, such as AFM, SEM, and N_2 adsorption-desorption analyses.

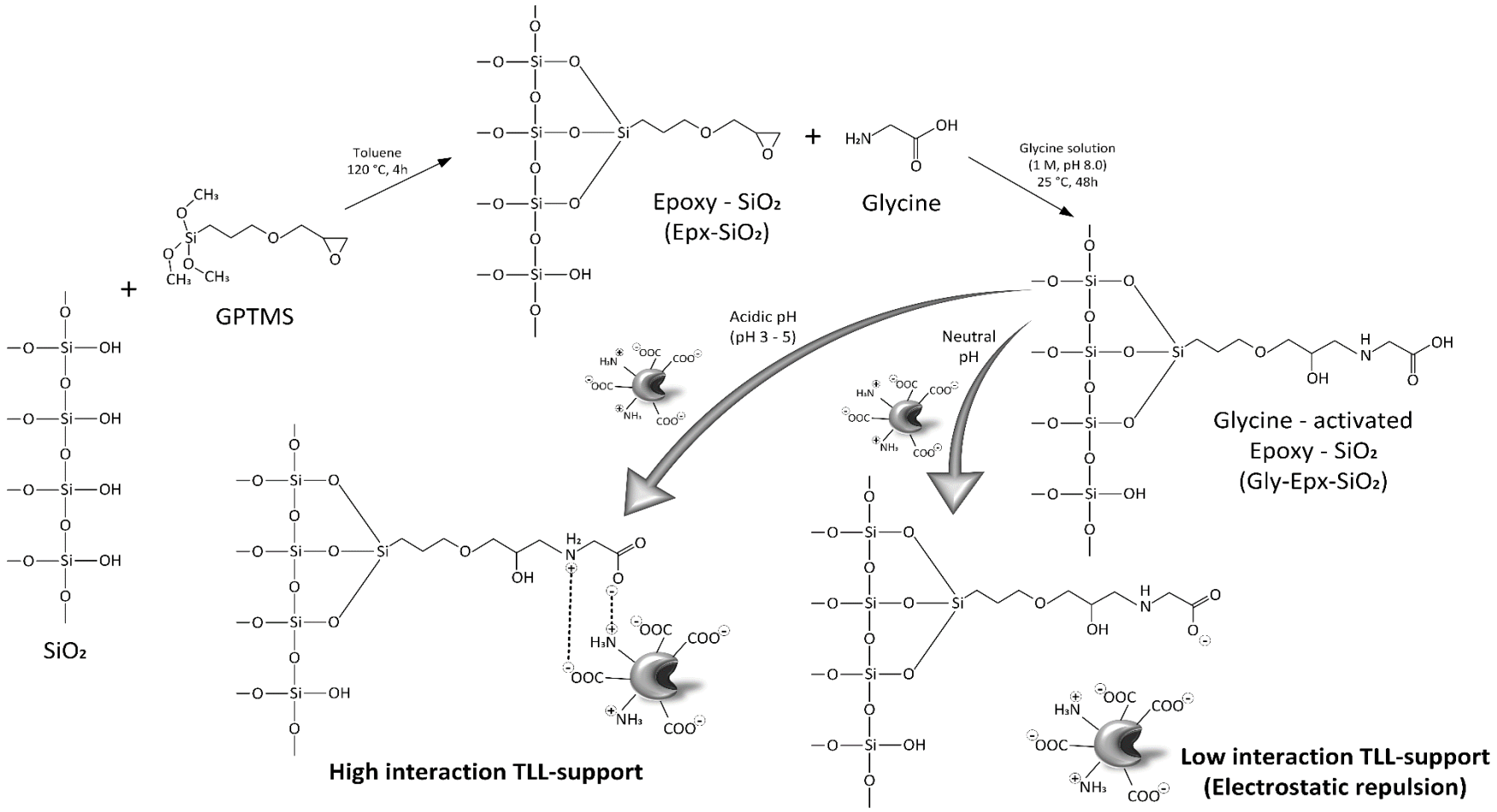

Figure 1. Representative scheme of preparation of ion exchange support Gly-Epx-SiO_2 and influence of pH of incubation on the physical adsorption of TLL via ionic interactions.

3.1. Characterization of ion-exchanger supports

AFM and SEM imaging have been extensively used to investigate surface topography and morphology [50,51]. 3D AFM images of non-functionalized, functionalized supports with GPTMS (Epx-SiO_2) and glycine-activated supports (Gly-Epx-SiO_2) are shown in Fig. 2. The values of roughness concerning R_a and R_{rms} and height (Z) were also determined and reported in Table 1. AFM images revealed the formation of more uniform (smooth) surfaces after a sequential functionalization with GPTMS (Fig. 2B,E) and activation with glycine (Fig. 2C,F). As expected, R_a, R_{rms} and Z values drastically decreased after functionalization/activation due to steric effects of larger moieties introduced onto both support surfaces. The highest reduction of roughness (R_a and R_{rms}) and Z values observed for functionalized RHS particles could be possibly due to their better geometric congruence than Immobead S60S, which resulted in a more intense functionalization/activation of its external surface.

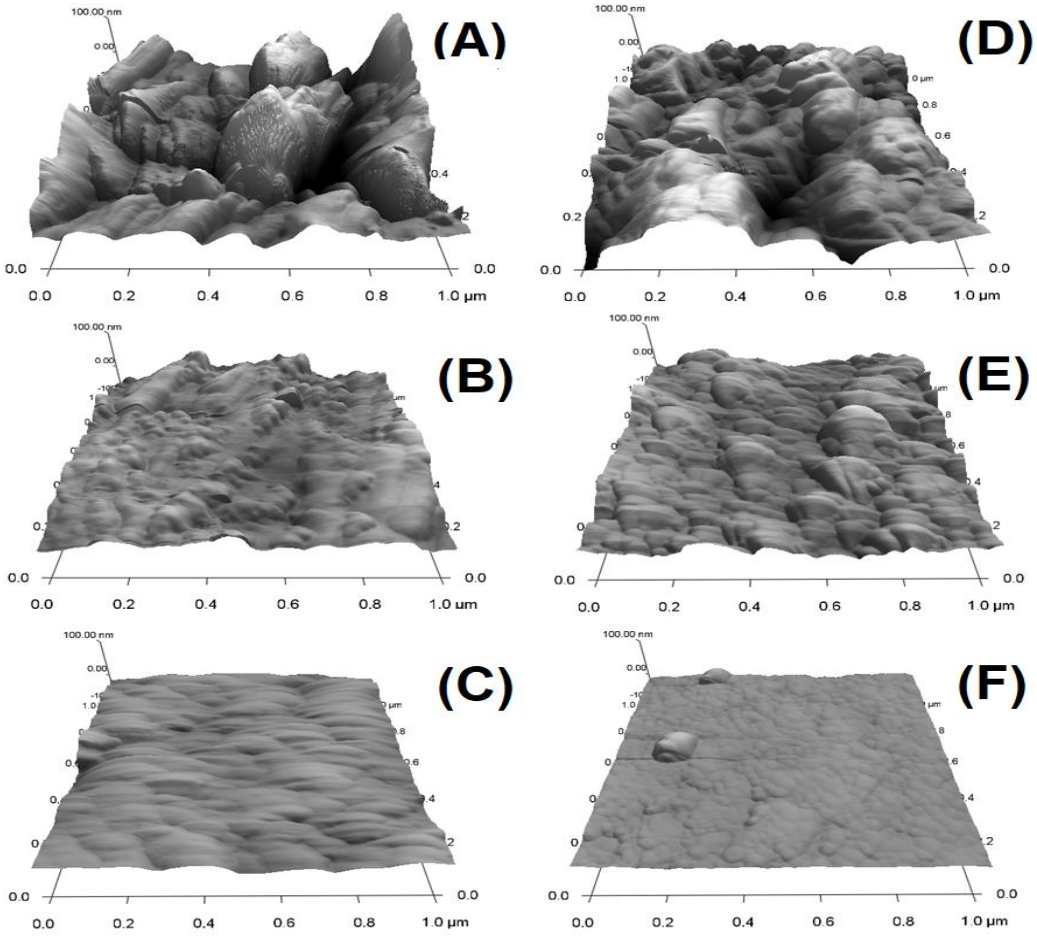

Figure 2. 3D AFM images of Immobead S60S [SiO_2 (A), Epx-SiO_2 (B) and Gly-Epx-SiO_2 (C)] and RHS [SiO_2 (D), Epx-SiO_2 (E) and Gly-Epx-SiO_2 (F)].

Table 1. Properties of non- and functionalized SiO_2-based materials

Parameters	Immobead S160S			Rice husk		
	SiO_2	Epx-SiO_2	Gly-Epx-SiO_2	SiO_2	Epx-SiO_2	Gly-Epx-SiO_2
R_a (nm)	17.9	11.6	5.7	25.4	11.5	2.6
R_{rms} (nm)	22.9	14.7	7.1	32.0	14.2	4.2
Z (nm)	19.2	3.3	2.7	77.7	2.9	0.1
S_A (m^2/g)	244.1	190.1	178.2	222.1	39.8	33.6
V_p (cm^3/g)	1.13	0.89	0.65	0.383	0.108	0.101
D_p (Å)	159.1	138.0	117.2	36.3	36.1	35.7

SEM images of non-functionalized supports with 500× magnification clearly show that particle size distribution of commercial SiO_2 (Fig. 3A) is more uniform than that of RHS (Fig. 3D). Moreover, it should also be noted that RHS surface roughness is greater than that of Immobead S60S surface according to the AFM analysis. SEM images with 20000× magnification indicate that non-functionalized Immobead S60S (Fig. 3B) has a more porous surface than RHS (Fig. 3E). However, a drastic decrease in pore diameter, thus the formation of more homogeneous surfaces, was observed for both Gly-Epx-SiO_2 supports due to carbon chain introduction onto both support surfaces according to the AFM analysis.

Analyses of N_2 adsorption-desorption isotherms reveal a significant decrease in S_A and V_p after a sequential functionalization/activation of SiO_2-based supports due to organic moieties insertion onto their surfaces, as expected. A drastic decrease in S_A for RHS of around 85% can be observed (from 222.1 to 33.6 m^2/g). As for Immobead S60S, S_A values ranged from 244.1 to 178.2 m^2/g (S_A reduction of 27% only). These results could be due to a more efficient functionalization of RHS than Immobead S60S, as aforementioned in accordance with surface topography analyses (SEM and AFM). In addition, V_p for RHS was also drastically reduced from 0.383 cm^3/g to 0.101 cm^3/g. On the other hand, similar D_p values (between 36.3 and 35.7 Å) were observed, which suggests preferential functionalization of its external surface. With respect to Immobead S60S, a decrease in D_p of 26.3% (from 159.1 to 117.2 Å) and 42.5% (from 1.13 to 0.65 cm^3/g) for V_p was observed, which can be explained by possible changes in pore size distribution to smaller diameters due to blockage of a portion with larger pores [52].

Similar results were observed in a previous study on the functionalization of mesoporous cellular siliceous foams with several alkoxytriethoxysilane and aminoalkoxytriethoxysilane agents used in the physical adsorption of 6-phospogluconate dehydrogenase (6PGDH) from *Geobacillus stearothermophilus* [53].

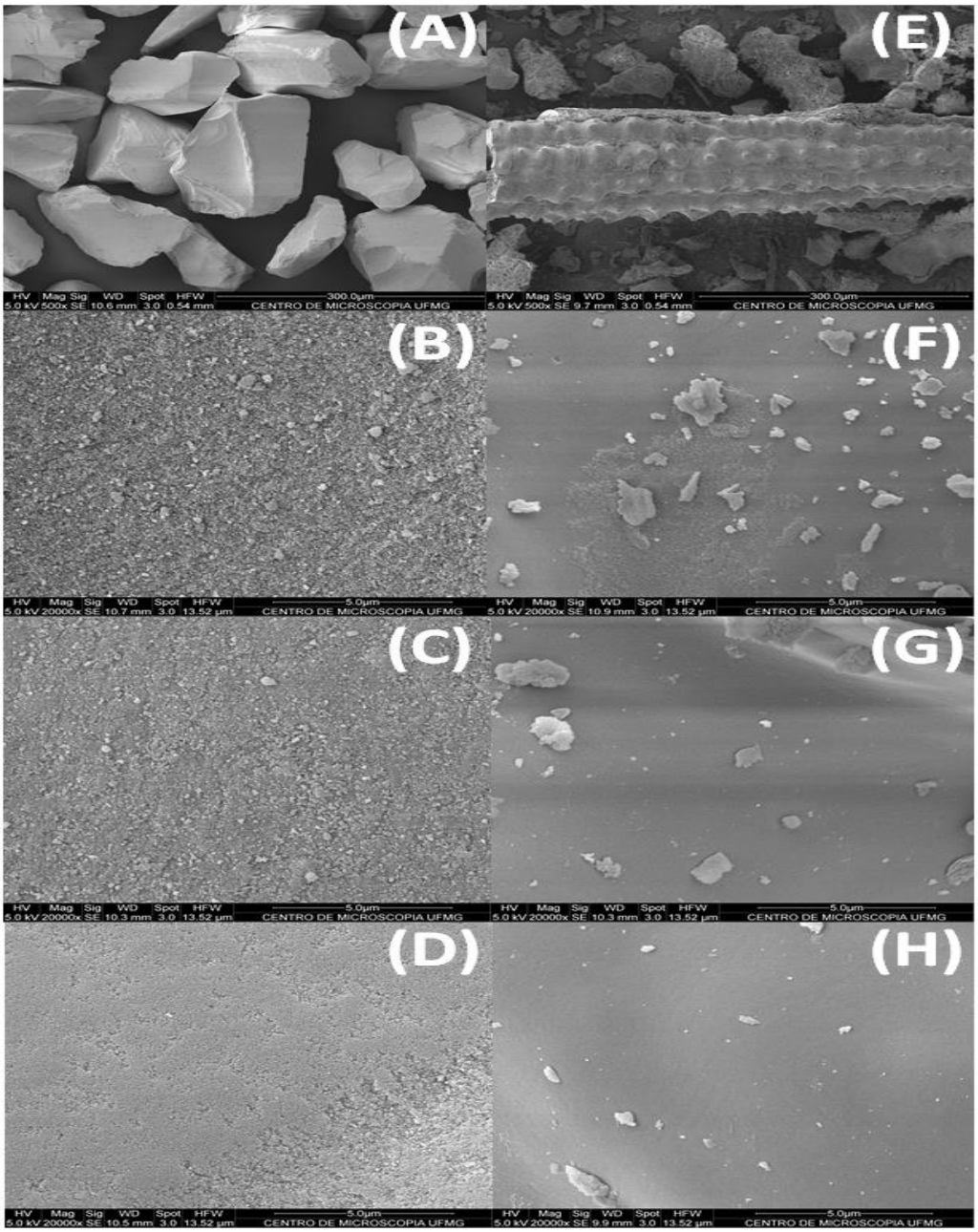

Figure 3. SEM images of Immobead S60S [SiO_2 (magnification of 500× – A, and 20000× – B), Epx-SiO_2 (magnification of 20000× – C) and Gly-Epx-SiO_2 (magnification of 20000× – D)] and RHS [SiO_2 (magnification of 500× – E, and 20000× – F), Epx-SiO_2 (magnification of 20000× – G) and Gly-Epx-SiO_2 (magnification of 20000× – H)].

According to IUPAC classification, both ion-exchange supports prepared in this study were characterized as mesoporous materials (2–50 nm). However, D_p value of ion-exchange support prepared with commercial SiO_2 (Immobead S60S) is 2.2 times higher than the molecular diameter of TLL – 117.2 and 53.2 Å [14], respectively. Thus, TLL can be adsorbed on both internal and external surfaces. On the other hand, preferential adsorption on the external surface of Gly-Epx-SiO_2 prepared with RHS is expected due to its lower D_p value (35.7 Å).

3.2. Effect of the functionalization step

The effect of a sequential functionalization and activation of both SiO_2 particles on TLL adsorption and catalytic activity of prepared heterogeneous biocatalysts has been assessed. In this study, non-functionalized SiO_2, Epx-SiO_2 and Gly-Epx-SiO_2 were tested as potential supports. Isoelectric points (pI) of non-functionalized SiO_2 and TLL are at pH ≈1.0 [54,55] and 4.4 [41], respectively. At pH 5.0, both TLL and SiO_2 are negatively charged. Thus, their adsorption process could be due to van der Waals' forces, hydrogen bonds between silanol groups of SiO_2 surfaces, and hydroxyl or thiol groups of TLL molecules. On the other hand, TLL immobilization on Epx-SiO_2 could proceed by covalent attachment via nucleophilic attacks by the amino terminal group of the enzyme (pKa≈6.5) on the support epoxy groups. Functionalization of several organic or inorganic supports with epoxy agents promotes the formation of more hydrophobic surfaces [10,49,56]. In this case, TLL adsorption on Epx-SiO_2 can also occur via hydrophobic interactions due to its high affinity towards hydrophobic support surfaces [2,312–14]. Epx-SiO_2 activation with glycine promotes the formation of ion-exchange supports which were used to immobilize TLL via ionic adsorption. A schematic diagram showing the preparation and ionic adsorption procedure of TLL on Gly-Epx-SiO_2 is in Fig. 1. The insertion of different organic moieties onto both silica surfaces promotes different orientations of enzymes and modulation of their catalytic properties due to different interactions between them, thus providing versatile biocatalysts to be used in several reactions of industrial interest [10,30,57].

From experimental data summarized in Table 2, the lower adsorption capacity of non-functionalized SiO_2 is due to possible repulsions between negative charges of supports and TLL. RHS retained a larger amount of TLL (3.2 ± 0.1 mg/g) than commercial SiO_2 (2.6 ± 0.1 mg/g) due to its excellent textural morphology (better geometric congruence), which allows adsorbing a greater number of enzyme molecules

per surface area unit (see comments in Section 3.1 about AFM analysis). Hydrolytic and specific activity values for the biocatalyst prepared using non-functionalized RHS were lower than for Immobead S60S due to better dispersion of TLL molecules on its internal/external surfaces, which facilitates diffusion of oil droplets in the enzyme active sites. A gradual decrease in S_A and V_p for both supports after functionalization with GPTMS (Epx-SiO_2) and activation with glycine (Gly-Epx-SiO_2) was observed (see Table 1). However, adsorption capacity was greater by increasing the carbon bond lengths on the support surfaces due to the creation of microenvironments that are more favorable to interact with TLL molecules. However, the accessibility of substrate molecules (droplets of olive oil) from the bulk reaction to their microenvironments was drastically reduced by increased TLL adsorption capacity due to severe mass transfer limitations. In fact, a drastic decrease in hydrolytic activity and specific activity values was observed when compared to non-functionalized supports (Table 2). Although there was a decrease in the catalytic activity of biocatalysts by using olive oil emulsion as substrate model, further studies were performed with ion-exchange supports (Gly-Epx-SiO_2). The criterion used to select such supports was their high adsorption capacities to immobilize TLL, thus providing heterogeneous biocatalysts with high catalytic activity per reactor volume to be used in cosmetic ester synthesis (butyl stearate) via an esterification reaction.

Table 2. Effect of functionalization of SiO_2-based materials on the immobilization parameters of TLL.

SiO_2 source	Support	q_e[a] (mg/g)	Ce[b] (mg/g)	HA[c] (IU/g)	SA[d] (IU/mg_{IP})
Immobead S60S	SiO_2	2.6 ± 0.1	3.7×10^{-2}	554.7 ± 30.5	213.3 ± 1.6
	Epx-SiO_2	4.3 ± 0.1	1.3×10^{-1}	103.5 ± 6.1	24.1 ± 1.2
	Gly-Epx-SiO_2	4.8 ± 0.1	1.1×10^{-2}	136.1 ± 6.7	28.8 ± 0.8
RHS	SiO_2	3.2 ± 0.1	3.7×10^{-2}	453.2 ± 20.2	141.3 ± 1.5
	Epx-SiO_2	4.3 ± 0.1	9.5×10^{-2}	121.8 ± 6.3	28.4 ± 0.6
	Gly-Epx-SiO_2	4.9 ± 0.1	5.3×10^{-3}	134.7 ± 6.0	27.5 ± 0.7

a – Immobilized protein concentration at equilibrium. b – Residual protein concentration in solution at equilibrium. c – Hydrolytic activity. d – Specific activity

3.3. Effect of pH on the immobilization parameters

Incubation pH affects surface charges of supports, as well as the ionization degree of enzymes. In this study, the effect of incubation pH on immobilization parameters (TLL adsorption and catalytic activity) was assessed in the range of 3.0–7.0. Biocatalysts were prepared by using the lowest initial protein loading (5 mg/g of support) to prevent possible interferences promoted by diffusional limitations. Adsorption process rate decreased by increasing pH and maximum TLL adsorption capacity was achieved in the range of 3.0–5.0 for Immobead S60S and 3.0–4.0 for RHS, respectively (Table 3). This decrease in the amount of TLL adsorption by higher pH is due to an increase in negative charges on the enzyme surface, thus promoting electrostatic repulsion between the enzyme and support surfaces. TLL has higher density of carboxylate groups (1 C-terminal group + 13 from glutamate residues + 19 from aspartate residues) than protonated amino groups (1 N-terminal group + 14 from arginine residues + 7 from lysine residues), as shown by its 3D structure [58]. Thus, immobilization occurs via preferential ionic interactions between carboxylate groups from aspartate/glutamate residues or TLL C-terminal and protonated secondary amino groups bounded to support surfaces. Thereby, this increase in pH could lead to deprotonation of these secondary amine groups, thus reducing the number of ionic interactions between the enzyme and supports. Moreover, it promotes deprotonation of N-terminal group of TLL structure (pKa ≈ 6.5) that can interact with carboxylate groups of supports, thus reducing their adsorption capacity at neutral pH (see Fig. 1).

With respect to hydrolytic activity, maxima values were observed at pH 4.0 and 3.0–4.0 for TLL adsorbed on commercial and RHS supports, respectively. In contrast, physical adsorption at neutral pH exhibited the highest specific activity values due to good dispersion of TLL molecules on the support surfaces, which allowed better access of olive oil molecules to the immobilized TLL active sites. Although high specific activity values were observed at pH 7.0, subsequent tests were performed at pH 4.0 due to highest hydrolytic activity values and maximum TLL adsorption capacity on both ion-exchange supports.

Table 3. Influence of pH of incubation on the immobilization parameters of adsorbed TLL via ionic interaction on Gly-Epx-SiO_2.

SiO_2 source	pH	q_e[a] (mg/g)	Ce[b] (mg/g)	HA[c] (IU/g)	SA[d] (IU/mg_{IP})
Immobead S60S	3.0	4.9 ± 0.1	5.3×10^{-3}	167.1 ± 10.5	31.4 ± 1.0
	4.0	4.9 ± 0.1	5.2×10^{-3}	204.6 ± 8.7	41.7 ± 1.3
	5.0	4.8 ± 0.1	1.1×10^{-2}	136.1 ± 6.7	28.8 ± 0.8
	6.0	3.4 ± 0.2	8.4×10^{-2}	119.5 ± 0.7	30.7 ± 3.8
	7.0	0.8 ± 0.1	2.2×10^{-1}	93.1 ± 0.6	98.9 ± 18.7
RHS	3.0	4.4 ± 0.1	3.2×10^{-2}	157.8 ± 5.4	34.2 ± 1.9
	4.0	4.9 ± 0.1	5.3×10^{-3}	152.1 ± 6.6	36.9 ± 1.8
	5.0	4.9 ± 0.1	5.3×10^{-3}	134.7 ± 6.0	27.5 ± 0.7
	6.0	2.3 ± 0.1	1.4×10^{-1}	79.4 ± 5.7	30.8 ± 3.5
	7.0	0.4 ± 0.1	2.4×10^{-1}	38.3 ± 3.3	102.9 ± 5.3

a – Immobilized protein concentration at equilibrium. b – Residual protein concentration in solution at equilibrium. c – Hydrolytic activity. d – Specific activity

3.4. Effect of initial protein loading on the immobilization parameters and adsorption isotherm studies

The effect of initial protein loading on the adsorption process and catalytic properties of biocatalysts (hydrolytic activity and specific activity values) has also been assessed in order to determine maximum adsorption capacity of each ion-exchange support and catalytic activity through olive oil emulsion hydrolysis. Increased initial protein loading from 5 to 20 mg/g raised adsorption capacity, and maximum immobilized protein concentration of 16.1 ± 0.6 mg/g and 14.8 ± 0.1 mg/g for ion-exchange supports prepared with commercial SiO_2 and RHS was observed, respectively (Table 4). These results show that both ion-exchange supports exhibited similar capacity to adsorb TLL. Further increase of initial protein loading has not improved adsorption efficiency and catalytic properties of biocatalysts prepared due to support saturation, as it had been previously reported in previous studies [14,42,43]. These ion-exchange supports displayed higher enzyme adsorption capacity than those obtained by immobilizing lipase from *Burkholderia ambifaria* YCJ01 on functionalized mesoporous TiO_2 with 3-(phenylamino)propyltrimethoxysilane (Ph–TiO_2) – 12.8 ± 0.7 mg/g [57], lipase B from *Candida antarctica* (CALB) on metakaolin – 12.77 mg/g [59], lipase from *Aspergillus*

sp. (Resinase A 2X) on pyrolyzed sugar industry waste product – 8.3 ± 0.65 mg/g [60], porcine pancreatic lipase (PPL) on eggshell – 10.63 mg/g [61], and *Candida antarctica* lipase B expressed in *Pichia pastoris* (LIPB) on calcined and non-calcined pore-expanded SBA-15 particles – 0.37 ± 0.1 mg/g and 0.12 ± 0.1 mg/g, respectively [62].

Table 4. Influence of initial protein loading on the immobilization parameters of adsorbed TLL via ionic interaction on Gly-Epx-SiO_2.

SiO_2 source	Initial protein loading (mg/g)	q_e[a] (mg/g)	Ce[b] (mg/g)	HA[c] (IU/g)	SA[d] (IU/mg_{IP})
Immobead S60S	5	4.9 ± 0.1	5.2×10^{-3}	204.6 ± 8.7	41.7 ± 1.3
	10	9.2 ± 0.3	4.2×10^{-2}	237.0 ± 25.6	25.7 ± 2.7
	15	13.9± 0.1	5.8×10^{-2}	303.3 ± 22.8	21.8 ± 2.0
	20	16.1± 0.6	2.1×10^{-1}	322.0 ± 3.1	20.0 ± 1.2
	25	15.9± 0.3	4.8×10^{-1}	342.8 ± 7.2	21.6 ± 0.1
	30	16.1± 0.4	7.3×10^{-1}	357.6 ± 11.2	22.1 ± 0.1
RHS	5	4.9 ± 0.1	5.3×10^{-3}	152.1 ± 6.6	36.9 ± 1.8
	10	9.3 ± 0.2	3.7×10^{-2}	192.8 ± 5.4	20.8 ± 0.2
	15	14.0± 0.2	5.3×10^{-2}	231.6 ± 16.3	16.5 ± 1.3
	20	14.8± 0.1	2.7×10^{-1}	279.2 ± 10.0	18.9 ± 0.8
	25	14.8 ± 0.3	5.4×10^{-1}	307.4 ± 7.2	20.8 ± 0.1
	30	15.0 ± 0.4	7.9×10^{-1}	299.3 ± 11.2	20.0 ± 0.3

a – Immobilized protein concentration at equilibrium. b – Residual protein concentration in solution at equilibrium. c – Hydrolytic activity. d – Specific activity

Generally, in order to illustrate the interaction between TLL molecules and ion-exchange supports, the obtained equilibrium adsorption data were fitted to three classical isotherm models – Langmuir, Sips and Redlich-Peterson [46]. Graphical representations of these non-linear models are presented in Fig. 4A,B. Isotherm constants, correlation coefficients (R^2) and root-mean-square errors (RMSE) are listed in Table 5. According to Fig. 4A,B, both adsorption processes are characterized by high adsorption capacity at low TLL concentration, followed by a plateau at high lipase concentration ($C_e \geq 0.2$ mg/mL that corresponds to an initial protein loading of 20 mg/g of support – see Table 4). Similar R^2 and RMSE values were observed for all three isotherm models (Table 5), thus

suggesting that these models can be adequately used to describe TLL adsorption process on both ion-exchange supports. However, the Sips isotherm model presented the highest R^2 and the lowest RMSE values when compared to Redlich-Peterson and Langmuir models. Similar values of theoretical and experimental maximum adsorption capacity obtained for an initial protein loading of 20 mg/g of ion-exchange supports by using Immobead S60S (theoretical q_e = 15.1 mg/g and experimental q_e = 16.1 ± 0.6 mg/g) and RHS (theoretical q_e = 14.6 mg/g and experimental q_e = 14.8 ± 0.1 mg/g) were also observed. This model is a combination of Langmuir and Freundlich isotherm models. At low adsorbate concentration, it reduces to the Freundlich model, thus not obeying Henry's law. At high adsorbate concentration, it achieves a monolayer adsorption capacity that is characteristic of the Langmuir isotherm model [43,46]. This model has also been used for describing TLL adsorption on mesoporous poly-(styrene-divinylbenzene) particles [43], and cellulase from *Hypocrea jecorina* (Celluclast 1.5L) on bagasse sulfite pulp [63].

R_L values represent the adsorption process nature which is unfavorable ($R_L > 1$), linear ($R_L = 1$), irreversible ($R_L = 0$) and favorable ($0 < R_L < 1$) [14,46]. According to Fig. 5, R_L values for ion-exchange supports prepared by using Immobead S60S and RHS ranged from 0.226 to 0.048 and from 0.167 to 0.032, respectively. These values show that TLL adsorption was a favorable process. Moreover, a decrease in R_L values by increasing initial protein loading was observed, thus demonstrating that the adsorption process is less favorable at high enzyme concentration.

The effect of initial protein loading on hydrolytic activity and specific activity has also been studied. According to Table 4, there was greater hydrolytic activity by increased initial protein loading. Hydrolytic activity of TLL immobilized on the ion-exchange support prepared with Immobead S60S ranged from 204.6 ± 8.7 (5 mg/g) to 357.6 ± 11.2 IU/g (30 mg/g). Once RHS was used as SiO_2 source, hydrolytic activity values ranged from 152.1 ± 6.6 to 307.4 ± 7.2 IU/g by 5-fold increase in initial TLL loading (from 5 to 25 mg/g of support). These results clearly reveal that increased hydrolytic activity does not mean an increase in initial enzyme loading due to effects of strong diffusional limitation. In fact, the highest specific activity values were obtained at the lowest initial protein loading (5 mg/g) – 41.7 ± 1.3 and 36.9 ± 1.8 IU/mg of immobilized protein on ion-exchange supports prepared with Immobead S60S and RHS, respectively. Afterwards, similar values (around 20 IU/mg of immobilized protein) were observed due to limited access of oil droplets to the active sites of immobilized TLL, as aforementioned. Thus, subsequent tests were conducted at 20 mg/g of support.

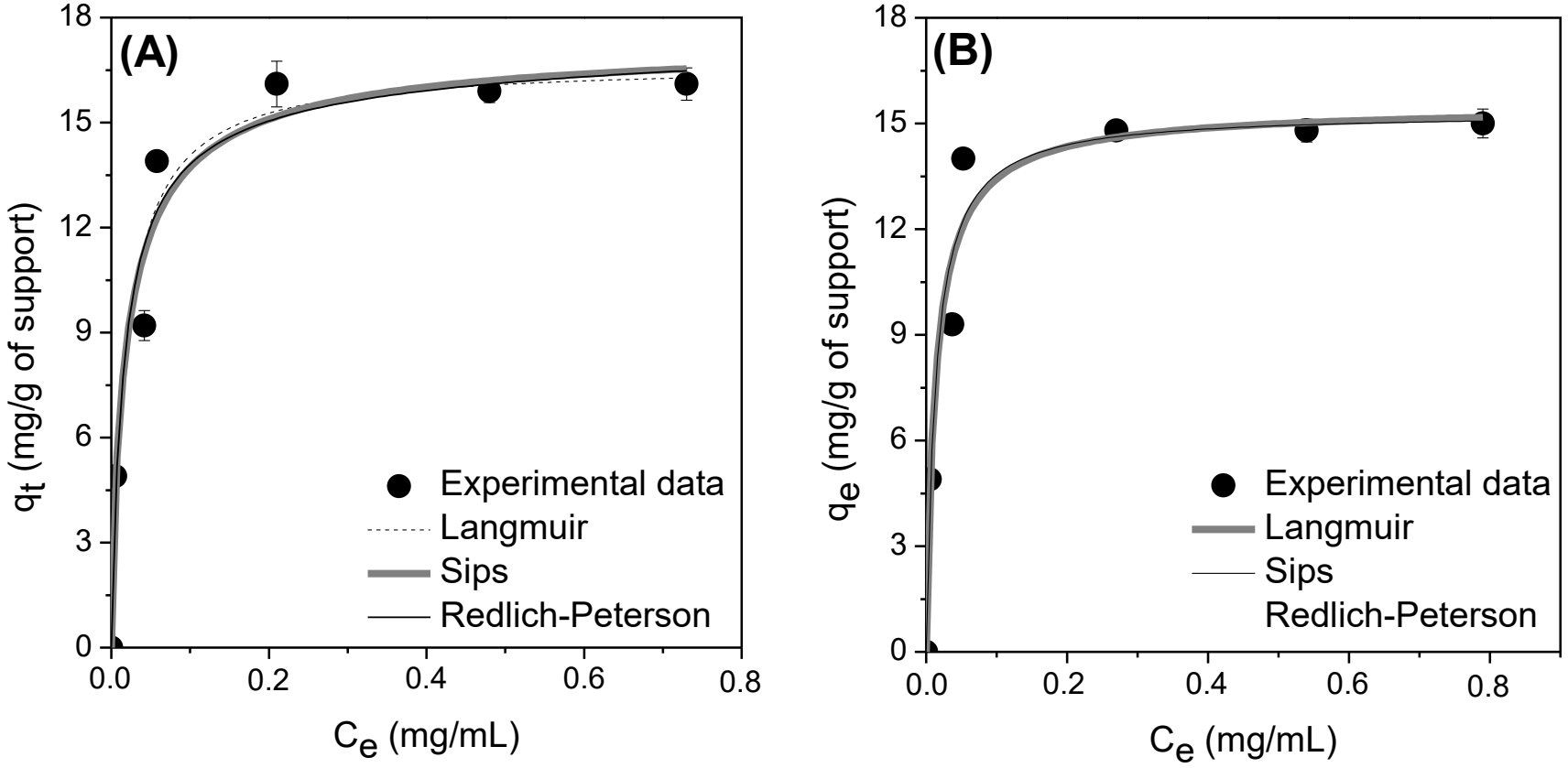

Figure 4. Isotherm models of TLL adsorption on Gly-Epx-SiO_2 prepared by using Immobead S60S and RHS as supports, respectively. The adsorption processes were performed under continuous stirring (200 rpm) at pH 4.0 (5 mM buffer sodium acetate) and room

Table 5. Determination of isotherm parameters for the adsorption of TLL on both Gly-Epx-SiO_2 supports.

Isotherm model	Support	Equation	Parameters	Values
Langmuir	Immobead S60S	$q_e = \frac{16.7 \times C_e}{0.019 + C_e}$	q_{max} (mg/g)	16.7
			K_L (L/mg)	0.019
			R^2	0.913
			RMSE	1.253
	RHS	$q_e = \frac{15.3 \times C_e}{0.013 + C_e}$	q_{max} (mg/g)	15.3
			K_L (L/mg)	0.013
			R^2	0.915
			RMSE	1.104
Sips	Immobead S60S	$q_e = \frac{405.7 \times C_e^{0.80}}{1 + 23.3 \times C_e^{0.80}}$	q_e (mg/g)[a]	15.1
			K_S (mL/mg)	405.7
			β_S	0.80
			a_S	23.3
			R^2	0.923
			RMSE	1.178
	RHS	$q_e = \frac{835.1 \times C_e^{0.92}}{1 + 53.8 \times C_e^{0.92}}$	q_e (mg/g)[b]	14.6
			K_S (mL/mg)	835.1
			β_S	0.92
			a_S	53.8
			R^2	0.917
			RMSE	1.094
Redlich-Peterson	Immobead S60S	$q_e = \frac{1088.3 \times C_e}{1 + 64.0 \times C_e^{0.97}}$	q_e (mg/g)[a]	15.2
			K_R (mL/mg)	1088.3
			a_R (1/mg)	64.0
			g	0.97
			R^2	0.914
			RMSE	1.241
	RHS	$q_e = \frac{1143.3 \times C_e}{1 + 74.9 \times C_e^{1.02}}$	q_e (mg/g)[b]	14.5
			K_R (mL/mg)	1143.3
			a_R (1/mg)	74.8
			g	1.02
			R^2	0.915
			RMSE	1.105

a: q_e values determined for initial protein loading of 20 mg/g of support – Immobead S60S (C_e = 0.21 mg/g of TLL solution).
b: q_e values determined for initial protein loading of 20 mg/g of support – RHS (C_e = 0.27 mg/g of TLL solution).

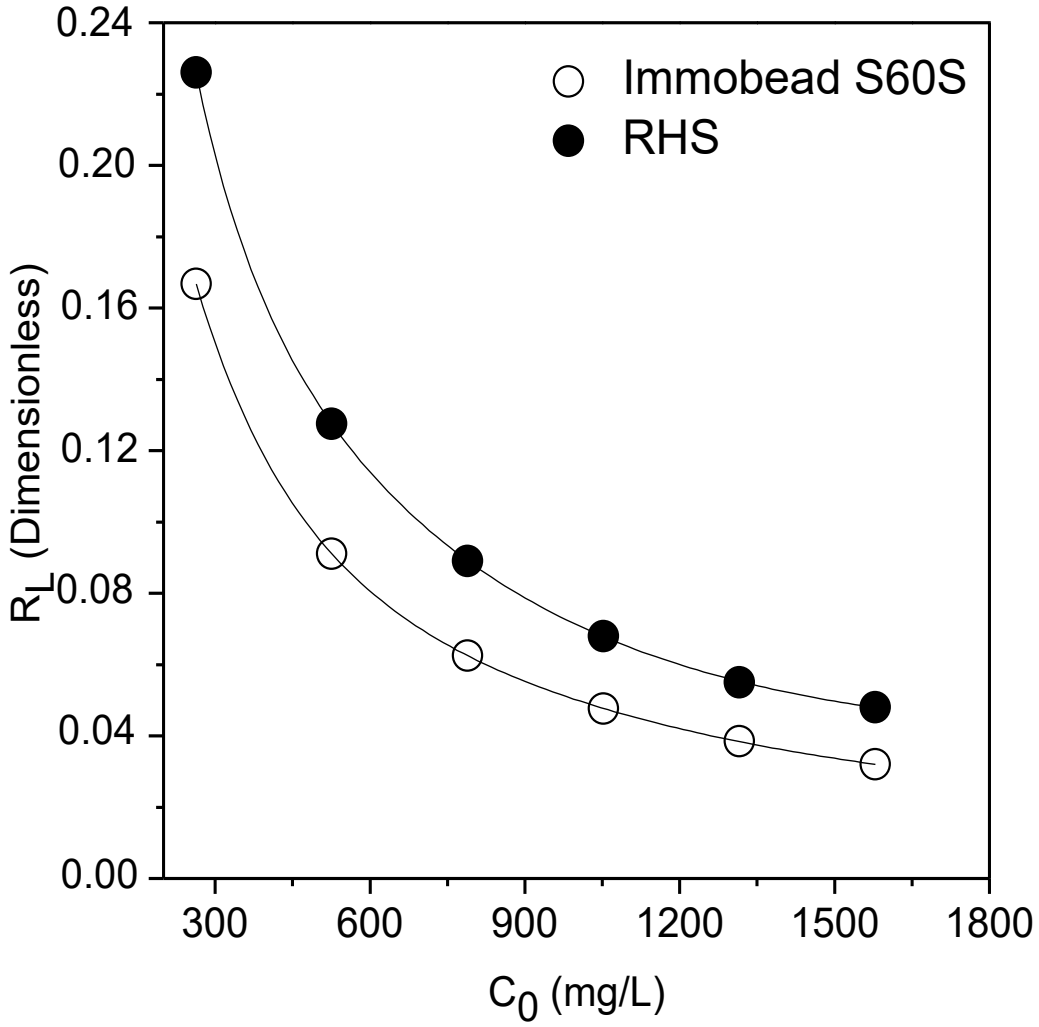

Figure 5. R_L values for the ionic adsorption of TLL on both ion-exchanger supports at different initial protein loadings.

3.5. Enzymatic synthesis of butyl stearate via esterification reaction

The prepared biocatalysts were tested through butyl stearate synthesis via an esterification reaction so as to determine their catalytic activities in a non-aqueous system. Butyl stearate was selected as reaction system model due to its successful use in cosmetic formulations. It acts as an emollient and skin care conditioning agent in personal care formulations, plasticizer in nail varnishes and masking agent in fragrances [64]. Esterification reactions were conducted in a solvent system in order to improve solubility of stearic acid (melting point = 69 °C), miscibility between reactant molecules and

viscosity reduction in the bulk solution to intensify diffusion of reactant molecules to biocatalyst microenvironments [44]. In this study, isooctane was used as hydrophobic organic solvent. It has been commonly used as reaction medium in enzymatic synthesis of cosmetic esters due to its high hydrophobicity (Log P = 4.5) and due to reducing possible distorting effects on the 3D structure of lipase [65–67]. Free TLL (crude enzymatic extract) was not used as biocatalyst due to protein aggregation, which drastically reduces diffusion of substrate molecules to its active sites and distorting effects by mechanical shear and temperature [14,49,62].

The effect of reaction time on butyl stearate synthesis is presented in Fig. 6A. Similar ester synthesis profiles can be observed by using both heterogeneous biocatalysts and maximum conversion around of 90% within 9 to 10 h of reaction was obtained for ester synthesis catalyzed by immobilized TLL on Gly-Epx-SiO_2 prepared with Immobead S60S and RHS, respectively. Operational stability tests were also performed, whose results are presented in Fig. 6B. After 6 consecutive reaction cycles, the heterogeneous biocatalyst prepared with Immobead S60S exhibited high stability due to adsorption of TLL molecules in its internal microenvironment, which avoided deleterious effects on its 3D structure. TLL immobilized on the ion-exchange support prepared with RHS also exhibited satisfactory stability on account of retaining 90% of its original activity. This slight decrease in its original activity could be explained by the preferential adsorption of TLL on its external surface, thus resulting in thermal inactivation of some TLL molecules.

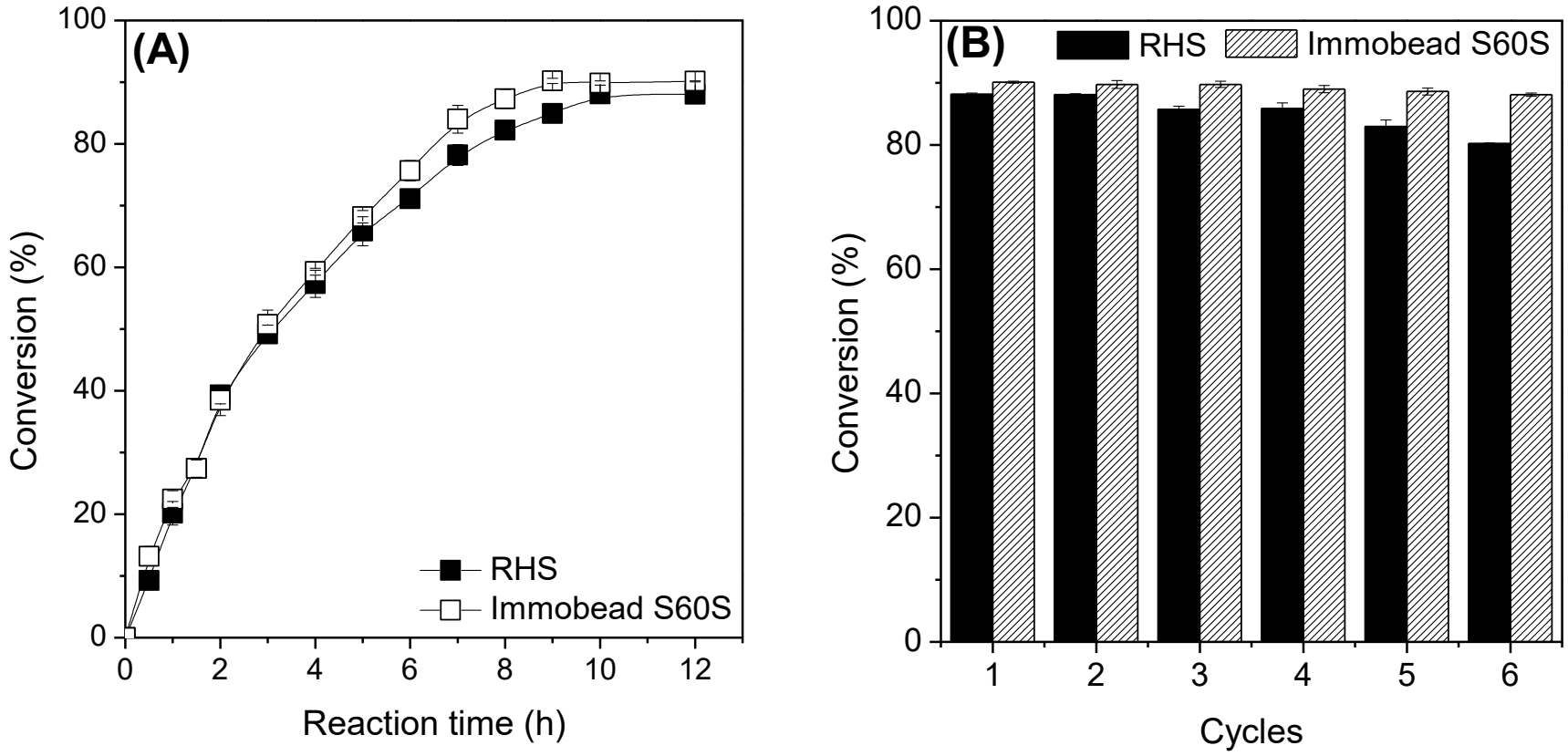

Figure 6. Effect of reaction time on the enzymatic synthesis of butyl stearate (A) and operational stability tests (B) for ester synthesis catalyzed by immobilized TLL on ion-exchanger supports prepared with Immobead S60S and RHS. The reactions were conducted at stoichiometric concentrations of stearic acid and butyl alcohol (1 M of each reactant) in isooctane medium, 20 mg of immobilized TLL, 240 rpm and 50 °C.

Conclusion

Ion-exchange supports were prepared via sequential functionalization of silica-based supports with alkoxysilane agent (GPTMS), followed by activation with glycine to be used as TLL immobilization supports. Rice husk silica (RHS) and commercial SiO_2 particles (Immobead S60S) were tested as potential supports. Several analyses (AFM, MEV and N_2 adsorption-desorption) were used to confirm their functionalization/activation. Similar immobilized protein concentration (between 14.8 and 16.1 mg/g) was observed. Moreover, these biocatalysts also displayed similar catalytic activity in olive oil emulsion hydrolysis and butyl stearate synthesis via esterification. After 6 consecutive cycles of ester synthesis, a slight decrease of around 10% in the original activity was observed for the biocatalyst prepared with RHS. This study shows that RHS has enormous potential to be used as silica source to prepare ion-exchange supports due to its easy availability (from biomass waste), lower price in comparison with commercial support (Immobead S60S) and the possibility of preparing active and stable biocatalysts in both aqueous and non-aqueous media.

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ANEXO I

Trabalhos com os resultados adicionais e parciais obtidos durante o projeto de tese que foram divulgados em eventos científicos**.**

1. BOLINA, I. C. A. ; CREN, E. C. ; AGUIAR MENDES, ADRIANO. Preparation of ion-exchange supports via functionalization of SiO2-based materials and their use in lipase adsorption. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

2. BOLINA, I. C. A. ; CREN, E. C. ; AGUIAR MENDES, ADRIANO. Enzymatic synthesis of a cosmetic ester catalyzed by lipase immobilized on functionalized SiO2-based supports via adsorption. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

3. MENDOZA-ORTIZ, P. A. ; BOLINA, I. C. A. ; CREN, E. C. ; AGUIAR MENDES, ADRIANO. Covalent attachment of lipase on glutaraldehyde-activated amino-SiO2 particles: effect of crosslinking agent on the immobilization parameters. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

4. BOLINA, I. C. A. ; MENDOZA-ORTIZ, P. A. ; CREN, E. C. ; AGUIAR MENDES, ADRIANO. Isotherm studies of adsorption of a lipase on ion-exchange supports prepared by functionalization of SiO2-based matrices. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

5. MIGUEZ, J. P. ; GAMA, R. S. ; BOLINA, I. C. A. ; AGUIAR MENDES, ADRIANO. Characterization of amino-functionalized rice husk silica and its use as support to immobilize *Thermomyces lanuginosus* lipase. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

6. GAMA, R. S. ; BOLINA, I. C. A. ; CREN, E. C. ; AGUIAR MENDES, ADRIANO. Preparation of phenyl-functionalized rice husk silica to immobilize lipase via hydrophobic adsorption. In: XXII Simpósio Nacional de Bioprocessos (XXII SINAFERM) e XII Simpósio de Hidrólise Enzimática de Biomassas (XII SHEB), 2019, Uberlândia. Book of Abstracts, 2019.

7. BOLINA, I. C. A.; CREN, E. C. ; MENDES, A.A. Preparation of ion exchange matrices via functionalization of silica-based particles and application as supports in lipase immobilization. In: XIII Seminário Brasileiro de Tecnologia Enzimática, 2018, Florianópolis. Livro de anais, 2018.

8. MIGUEZ, JOÃO P. ; MACHADO, N. B. ; BOLINA, I. C. A. ; MENDES, A.A. Mass transfer studies on the adsorption of a microbial lipase on functionalized rice husk silica particles. In: XIII Seminário Brasileiro de Tecnologia Enzimática, 2018, Florianópolis. Livros de resumos, 2018.

9. DE LARA, A. B. B ; SABI, G. J ; BOLINA, I. C. A ; MENDES, A. A . Preparação e caracterização de um adsorvente obtido a partir do epicarpo de macaúba para a remoção de azul de metileno - Estudos termodinâmicos. In: XXII Congresso Brasileiro de Engenharia Química, 2018, São Paulo. Blucher Chemical Engineering Proceedings. São Paulo: Editora Blucher, 2018. p. 3893.

10. MACHADO, N. B; MIGUEZ, J. P.; BOLINA, I. C. A.; MENDES, A. A. PREPARATION AND CHARACTERIZATION OF FUNCTIONALIZED RICE HUSK SILICA FOR LIPASE ADSORPTION. In: XXII Congresso Brasileiro de Engenharia Química, 2018, São Paulo. Blucher Chemical Engineering Proceedings. São Paulo: Editora Blucher, 2018. p. 4614.

11. ALVES, M. D.; BOLINA, I. C. A.; CREN, E. C.; MENDES, A. A. MECHANISM OF ADSORPTION OF LIPASE FROM *Thermomyces lanuginosus* ON A MESOPOROUS AND HYDROPHOBIC SUPPORT. In: XXI Simpósio Nacional de Bioprocessos e XII Simpósio de Hidrólise Enzimática de Biomassas, 2017, Aracaju. Anais do XXI SINAFERM e XII SHEB, 2017.

ANEXO II

Artigos científicos publicados em periódicos indexados em coautoria.

1. MIGUEZ, J. P.; GAMA, R. S.; BOLINA, I. C. A.; MELO, C. C.; CORDEIRO, M. R.; HIRATA, D. B.; MENDES, A. A. Enzymatic synthesis optimization of a cosmetic ester catalyzed by a homemade biocatalyst prepared via physical adsorption of lipase on amino-functionalized rice husk silica. CHEMICAL ENGINEERING RESEARCH & DESIGN, v. 139, p. 296-308, 2018. https://doi.org/10.1016/j.cherd.2018.09.037

2. GAMA, R. S.; BOLINA, I. C. A.; CREN, E. C.; MENDES, A. A. A novel functionalized SiO2-based support prepared from biomass waste for lipase adsorption. MATERIALS CHEMISTRY AND PHYSICS, v. 234, p. 146-150, 2019. https://doi.org/10.1016/j.matchemphys.2019.06.002

3. MACHADO, N. B.; MIGUEZ, J. P.; BOLINA, I. C. A.; SALVIANO, A. B.; GOMES, R. A. B.; TAVANO, O. L.; BARBOZA, J. C. S.; Tardioli, P. W.; CREN, E. C.; MENDES, A. A. Preparation, functionalization and characterization of rice husk silica for lipase immobilization via adsorption. ENZYME AND MICROBIAL TECHNOLOGY, v. 128, p. 9-21, 2019. https://doi.org/10.1016/j.enzmictec.2019.05.001