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APLICAÇÃO DE EXTRATOS ENZIMÁTICOS CONTENDO LACASES FÚNGICAS PARA A BIORREMEDIAÇÃO DE FÁRMACOS

Gabriela Gambato

CAXIAS DO SUL

2025

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Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, como parte dos requisitos para a obtenção de grau de Doutora em Biotecnologia.

Orientadora: Prof. Dra. Marli Camassola

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Dedico este trabalho à minha filha, Antonella.
Se um dia você foi o motivo que me fez pensar em desistir, hoje é, com certeza,
a razão pela qual cheguei até aqui.
Você é o fator de impacto de maior magnitude na minha felicidade, e não há
métrica capaz de expressar a dádiva que você representa na minha vida.
Você é o propósito que cultivo diariamente.
Te amo eternamente.

"Jamais considere seus estudos como uma obrigação, mas como uma oportunidade invejável para aprender a conhecer a beleza libertadora do intelecto para seu próprio prazer pessoal e para proveito da comunidade à qual seu futuro trabalho pertencer."

Albert Einstein

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2,6-DMP	2,6-dimetoxifenol
ABTS	2,2-azinobis-(3-etilbenzotiazolina-6-sulfonato)
AINEs	Anti-inflamatórios não esteroides
ATCC	American type culture collection
BSA	Bovine serum albumin
EPH	Eletrodo Padrão de Hidrogênio
HBT	1-hidroxibenzotriazol
LCEs	Profiling of laccase crude extracts
NSAIDs	Non-steroidal anti-inflammatory drugs
PDA	Potato dextrose agar
POAs	Processos de oxidação avançada
RNA	Ácido ribonucleico
SDGs	United Nations Sustainable Development Goals
SDS-PAGE	Eletroforese em gel de poliacrilamida com dodecil sulfato de sódio
TEMPO	N-oxil-2,2,6,6-tetrametilpiperidina

RESUMO

A presença de contaminantes emergentes, especialmente fármacos e produtos farmacêuticos, no meio ambiente tem se consolidado como uma preocupação global. Essas substâncias, devido à sua persistência e baixa biodegradabilidade, apresentam desafios significativos para os sistemas de tratamento convencionais, o que resulta em sua acumulação nos ecossistemas. Nesse contexto, as lacases emergem como ferramentas promissoras de biorremediação por catalisarem reações sob condições brandas, com alta eficiência e baixo impacto ambiental. Assim, esta tese teve como objetivo analisar o potencial das lacases fúngicas na biorremediação de fármacos por meio de duas abordagens complementares: (i) um estudo cienciométrico, para mapear tendências de pesquisa, redes de colaboração e lacunas temáticas; e (ii) uma investigação experimental, focada na produção e aplicação dos extratos brutos contendo lacases fúngicas na biorremediação de fármacos. O estudo cienciométrico revelou que a pesquisa sobre lacases aplicadas à biorremediação de fármacos encontra-se em expansão, concentrada principalmente em Ciências Ambientais, Engenharia Ambiental e Microbiologia Aplicada à Biotecnologia. Foram identificados grupos de pesquisa coesos, porém com conectividade limitada entre os *clusters*, reforçando a necessidade de maior internacionalização e cooperação interdisciplinar. Os periódicos *Chemosphere*, *Bioresource Technology* e *Environmental Science and Pollution Research* destacaram-se como veículos centrais de publicação dos estudos. O mapeamento de palavras-chave evidenciou foco em imobilização, águas residuais e degradação, com destaque para fármacos como diclofenaco, estrogênios, tetraciclina e carbamazepina, ao passo que outros compostos como antitumorais e hormônios tireoidianos permanecem pouco explorados. Na etapa experimental, foram cultivadas as espécies *Agaricus blazei*, *Marasmiellus palmivorus* VE111, *Pycnoporus sanguineus* 14G e *Trametes* sp. 50/90, as quais foram avaliadas quanto à cinética de desenvolvimento micelial e aos parâmetros bioquímicos, tais como pH, consumo de carboidratos, proteínas e produção de lacases. Os extratos enzimáticos obtidos foram posteriormente avaliados quanto à estabilidade de armazenamento e à capacidade de biorremediação dos fármacos paracetamol e diclofenaco. Foram observados perfis distintos de produção enzimática, biomassa e metabolismo para cada cultivo. A estabilização com o glicerol e a liofilização viabilizaram a manutenção da atividade das lacases no período de armazenamento. Na biorremediação, *M. palmivorus* VE111

apresentou o maior percentual de degradação do paracetamol (96%), enquanto o *A. blazei* para o diclofenaco (82%). Em conjunto, os resultados indicam que as lacases fúngicas constituem uma alternativa promissora, econômica e sustentável para a biorremediação de fármacos. A integração entre os achados cienciométricos e experimentais fornece subsídios para o avanço científico e tecnológico na área, orientando pesquisas futuras, políticas públicas e desenvolvimento de estratégias de inovação voltadas ao enfrentamento da contaminação ambiental por fármacos.

Palavras-chave: oxidases multicobre, fungos, remediação biológica, insumos farmacêuticos, cienciometria, estabilidade.

ABSTRACT

The presence of emerging contaminants, especially pharmaceuticals, in the environment has become a global concern. Due to their persistence and low biodegradability, these substances present significant challenges to conventional treatment systems, resulting in their accumulation in ecosystems. In this context, laccases emerge as promising bioremediation tools because they catalyze reactions under mild conditions, with high efficiency and low environmental impact. Thus, this thesis aimed to analyze the potential of fungal laccases in the bioremediation of pharmaceuticals through two complementary approaches: (i) a scientometric study to map research trends, collaboration networks, and thematic gaps; and (ii) an experimental investigation focused on the production and application of crude extracts containing fungal laccases in the bioremediation of pharmaceuticals. The scientometric study revealed that research on laccases applied to the bioremediation of pharmaceuticals is expanding, mainly concentrated in Environmental Sciences, Environmental Engineering, and Microbiology Applied to Biotechnology. Cohesive research groups were identified, but with limited connectivity between clusters, reinforcing the need for greater internationalization and interdisciplinary cooperation. The journals *Chemosphere*, *Bioresource Technology*, and *Environmental Science and Pollution Research* stood out as central vehicles for the publication of studies. Keyword mapping showed a focus on immobilization, wastewater, and degradation, highlighting pharmaceuticals such as diclofenac, estrogens, tetracycline, and carbamazepine, while other compounds such as antitumor drugs and thyroid hormones remain underexplored. In the experimental stage, the species *Agaricus blazei*, *Marasmiellus palmivorus* VE111, *Pycnoporus sanguineus* 14G, and *Trametes* sp. 50/90 were cultivated, which were evaluated for mycelial development kinetics and biochemical parameters such as pH, carbohydrate and protein consumption, and laccase production. The enzymatic extracts obtained were subsequently evaluated for storage stability and bioremediation capacity of the pharmaceuticals acetaminophen and diclofenac. Distinct profiles of enzymatic production, biomass, and metabolism were observed for each culture. Stabilization with glycerol and lyophilization enabled the maintenance of laccase activity during the storage period. In bioremediation, *M. palmivorus* VE111 showed the highest percentage of acetaminophen degradation (96%), while *A. blazei* showed the

highest degradation of diclofenac (82%). Taken together, the results indicate that fungal laccases constitute a promising, economical, and sustainable alternative for the bioremediation of drugs. The integration of scientometric and experimental findings provides support for scientific and technological advancement in the field, guiding future research, public policies, and the development of innovation strategies aimed at tackling environmental contamination by pharmaceuticals.

Keywords: oxidases multicopper, fungi, biological remediation, pharmaceuticals, scientometrics, stability.

1. INTRODUÇÃO

O crescimento da população mundial, os avanços em pesquisa e desenvolvimento e o envelhecimento demográfico têm contribuído para o aumento significativo na demanda por serviços de saúde e pelo consumo de medicamentos (Estrada-Almeida et al., 2024). Este cenário tem contribuído para alterações ambientais, uma vez que a produção e o uso desses produtos em larga escala repercutem na liberação de poluentes no meio ambiente (Ortúzar et al., 2022).

Poluentes são substâncias lançadas na natureza em concentrações capazes de causar desequilíbrios ecológicos ou representar riscos à saúde humana e ao meio ambiente. Os micropoluentes correspondem a contaminantes presentes em baixas concentrações (ng/L a μg/L), mas que podem gerar impactos ambientais relevantes, como toxicidade crônica, desregulação hormonal, bioacumulação, resistência microbiana e efeitos ecossistêmicos de longo prazo, especialmente em ambientes aquáticos (Silori et al., 2022). Dentre esses, os contaminantes emergentes são compostos químicos ou agentes biológicos recentemente detectados no meio ambiente, muitas vezes ainda pouco regulamentados e com potencial nocivo (Wang et al., 2024). Assim, os fármacos classificam-se como microcontaminantes emergentes.

Fármacos comumente prescritos para uso humano e veterinário, como antibióticos, antifúngicos, anticonvulsivantes, anti-inflamatórios, substâncias psicoativas, hormônios, β-bloqueadores, antirretrovirais e agentes antitumorais, já foram detectados em águas superficiais, solos e lodo (Estrada-Almeida et al., 2024; Guerra et al., 2014; Ortúzar et al., 2022; Silori et al., 2022; Tarcomnicu et al., 2011). Muitos destes compostos são projetados para serem química e biologicamente estáveis, afetando sua taxa de remoção por meio de tratamento convencional de água, por exemplo. Os anti-inflamatórios não esteroidais e os analgésicos estão entre os fármacos que mais preocupam, pois a estrutura química implica em um perfil recalcitrante. Além disso, há o elevado consumo destes, tendo uma produção anual estimada de centenas de toneladas (Ortúzar et al., 2022).

A crescente preocupação com a contaminação ambiental por micropoluentes emergentes, como os fármacos, tem intensificado a busca por soluções inovadoras e eficazes para minimizar seus impactos ambientais (Inamuddin, 2023). Diferentes alternativas já foram exploradas, incluindo processos avançados de oxidação, biosorção e tecnologias de membranas. Contudo, a biorremediação destaca-se como uma

estratégia particularmente promissora, por aliar eficiência na remoção de diversos micropoluentes à sustentabilidade ambiental e econômica (Estrada-Almeida et al., 2024).

A biorremediação utiliza organismos vivos, enzimas ou consórcios microbianos para degradar, transformar ou remover contaminantes, configurando-se como uma abordagem sustentável, de baixo custo e alinhada aos princípios da bioeconomia circular (Inamuddin, 2023). Diante disso, as lacases fúngicas emergem como ferramentas propícias devido à sua capacidade de catalisar reações de oxidação. Estas enzimas oxidorredutases pertencentes à superfamília das oxidases multicobre podem biotransformar distintas moléculas fenólicas e diversos princípios ativos utilizados na formulação de medicamentos (Bilal et al., 2019a).

Desde sua descoberta nos exsudatos da árvore laca oriental, as lacases têm sido identificadas em diferentes origens biológicas, especialmente em fungos, nos quais desempenham papéis essenciasi em processos fisiológicos, como a degradação da lignina e a proteção contra o estresse oxidativo (Arregui et al., 2019). Muitas espécies de fungos de degradação branca, reconhecidas por sua habilidade excepcional de degradar lignina, produzem lacases em quantidades elevadas, o que as torna particularmente abundantes em ambientes naturais (Gianfreda et al., 1999).

As reações catalisadas por lacases apresentam a vantagem de gerar apenas água como subproduto, caracterizando-se como processos limpos e ambientalmente adequados. Além disso, ocorrem sem a necessidade de reagentes agressivos ou condições reacionais extremas, atributos que reforçam seu potencial como ferramentas ecológicas. Nesse contexto, as lacases fúngicas configuram-se como alternativas promissoras e sustentáveis para a biorremediação de fármacos em matrizes ambientais (Naghdi et al., 2018).

A partir desse contexto, o presente estudo teve como objetivo elucidar as principais tendências e desafios da pesquisa científica relacionados à aplicação de lacases fúngicas na biorremediação de fármacos. Paralelamente, foram produzidos extratos brutos enzimáticos contendo lacases e avaliado seu desempenho na degradação de contaminantes emergentes, como diclofenaco e paracetamol, amplamente detectados em matrizes aquáticas e reconhecidos por seus efeitos adversos ao meio ambiente.

2. REVISÃO DA LITERATURA

2.1 Aspectos gerais dos fármacos

Os fármacos são substâncias de origem natural ou sintética capazes de alterar o funcionamento celular ou tecidual dos organismos. Podem ser descritos como sinônimos de substância ativa, droga, matéria-prima ativa ou insumo farmacêutico ativo (ANVISA, 2019). Quando submetidos às operações farmacêuticas, com a adição ou não de excipientes e adjuvantes, originam os medicamentos, disponíveis nas mais diversas formas farmacêuticas (Nirmal e Jain, 2018). De acordo com seu efeito farmacológico, os fármacos podem ser classificados em diferentes grupos terapêuticos, como anti-inflamatórios, analgésicos, antivirais, anticancerígenos, ansiolíticos, antidepressivos, antipsicóticos, anti-hipertensivos, antibacterianos, antiarrítmicos e diuréticos, entre outros (Bruton, 2013).

O uso global de medicamentos tem aumentado de forma expressiva nas últimas décadas. Estimativas apontam que, em 2028, mais de 3,8 trilhões de doses diárias definidas sejam consumidas em todo o mundo, um aumento de 400 milhões em relação ao nível de 2023 (IQVIA, 2024). Esse crescimento está associado ao envelhecimento populacional, à expansão dos sistemas de saúde, especialmente em países de baixa e média renda, e ao aumento da disponibilidade de terapias inovadoras. Além disso, o mercado farmacêutico movimentou aproximadamente US$ 1,5 trilhão em 2023, refletindo não apenas a elevada demanda global, mas também a ampla diversidade de moléculas em circulação (IQVIA, 2024).

No processo de desenvolvimento, as moléculas candidatas a fármacos precisam apresentar propriedades químicas e físicas adequadas para garantir estabilidade, biodisponibilidade, segurança e eficácia terapêutica (Wong e Datla, 2005). A estabilidade frente a condições de estresse, estabelecidas em compêndios e regulamentações, busca avaliar a tendência de degradação, assegurando a manutenção da atividade farmacológica durante o maior tempo de prateleira possível (Silva et al., 2009). Entre os mecanismos mais comuns de degradação química estão a hidrólise, oxidação, racemização e fotólise, embora processos de dimerização ou polimerização também possam ocorrer (Aulton e Taylor, 2016).

A biodisponibilidade oral é um dos critérios centrais no desenvolvimento de fármacos. A regra de Lipinski descreve as propriedades desejáveis para que uma

molécula apresente boa absorção por esta via: até cinco doadores de ligações de hidrogênio e no máximo dez aceptores, massa molecular inferior a 500 Da e coeficiente de partição (logP) não superior a 5 (Lipinski et al., 2012). Em sua maioria, os fármacos são compostos orgânicos que contêm diferentes funções químicas, como álcoois, aminas, cetonas, ácidos carboxílicos e ésteres (Figura 1). Estas funções são fundamentais para a interação com alvos biológicos e determinantes de propriedades como solubilidade, reatividade e seletividade (Barreiro e Fraga, 2015).

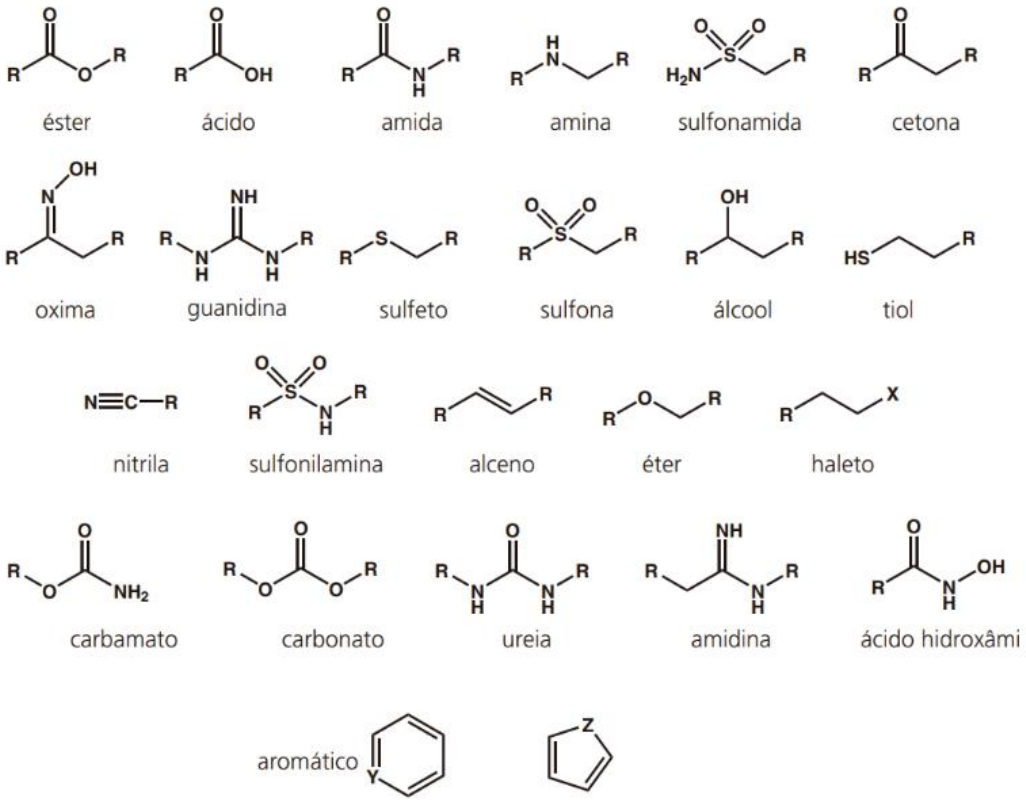

Figura 1: Diversidade de grupos funcionais comumente presentes nas moléculas dos fármacos. Imagem retirada de Barreiro e Fraga (2015).

Após a administração, os fármacos sofrem processos de absorção, distribuição, metabolismo e excreção. As reações de biotransformação visam aumentar a solubilidade dos compostos, favorecendo sua eliminação. Na fase I, predominam reações de oxidação, redução e hidrólise, catalisadas principalmente por enzimas do complexo citocromo P450, localizadas no fígado e no trato gastrointestinal. Já na fase II, ocorrem reações de conjugação, como glicuronidação, sulfatação e acetilação, que aumentam a polaridade dos metabólitos, facilitando sua excreção. A eliminação ocorre

por diferentes vias, sendo a renal a mais importante, por meio da excreção urinária (Bruton, 2013).

Apesar dos mecanismos de bitransformação, uma fração significativa dos fármacos é excretada inalterada (Barreiro e Fraga, 2015). Assim, os fármacos íntegros, bem como seus metabólitos, chegam ao esgoto, que poderá ser tratado antes de chegar ao ecossistema. Entretanto, estudos têm verificado que muitos fármacos e seus metabólitos não são completamente eliminados pelas estações de tratamento de águas e esgoto, transformando-os em poluentes ambientais (Estrada-Almeida et al., 2024; Fram e Belitz, 2011; Vaudin et al., 2022).

Nas condições ambientais, as moléculas dos fármacos podem apresentar diferentes graus de degradação, variando de acordo com fatores físico-químicos e biológicos presentes nos ecossistemas (Quadra et al., 2017). Desde o momento em que são liberadas no ambiente até sua completa transformação, esses compostos podem sofrer alterações estruturais diversas, cujos efeitos têm sido amplamente discutidos em estudos ecotoxicológicos (dos Santos et al., 2024). Nesse contexto, torna-se necessária a adoção de estratégias que acelerem e otimizem a degradação desses contaminantes, tanto em estações de tratamento de esgoto quanto em processos de obtenção de água potável.

2.2 Fármacos como micropoluentes ambientais

Os setores industrial, urbano e agrícola utilizam uma ampla variedade de produtos químicos em suas atividades, muitos alcançam o ecossistema, podendo provocar sérios impactos à saúde humana e ambiental (Wang et al., 2024). Essas substâncias são denominadas poluentes (Aquino et al., 2013; Lima et al., 2017). Quando presentes em concentrações muito baixas (ng/L ou μg/L), recebem a designação de micropoluentes ou contaminantes emergentes, que incluem pesticidas, poluentes orgânicos persistentes, hormônios, surfactantes, produtos de cuidados pessoais, microplásticos e fármacos, muitas dessas substâncias também podem ser classificadas como desreguladores endócrinos (dos Santos et al., 2024; Ebele et al., 2017).

O avanço tecnológico e o envelhecimento populacional impulsionaram a indústria farmacêutica, aumentando a demanda por insumos de saúde. Dentre eles, os medicamentos representam a principal tecnologia empregada na detecção, prevenção e tratamento de doenças (IQVIA, 2024; Lubick, 2010; Wang et al., 2024). Nesse

contexto, é necessário considerar todo o ciclo de vida desses produtos, desde a síntese e produção até o consumo e descarte, uma vez que cada etapa pode contribuir para a liberação de poluentes no ambiente (Figura 2).

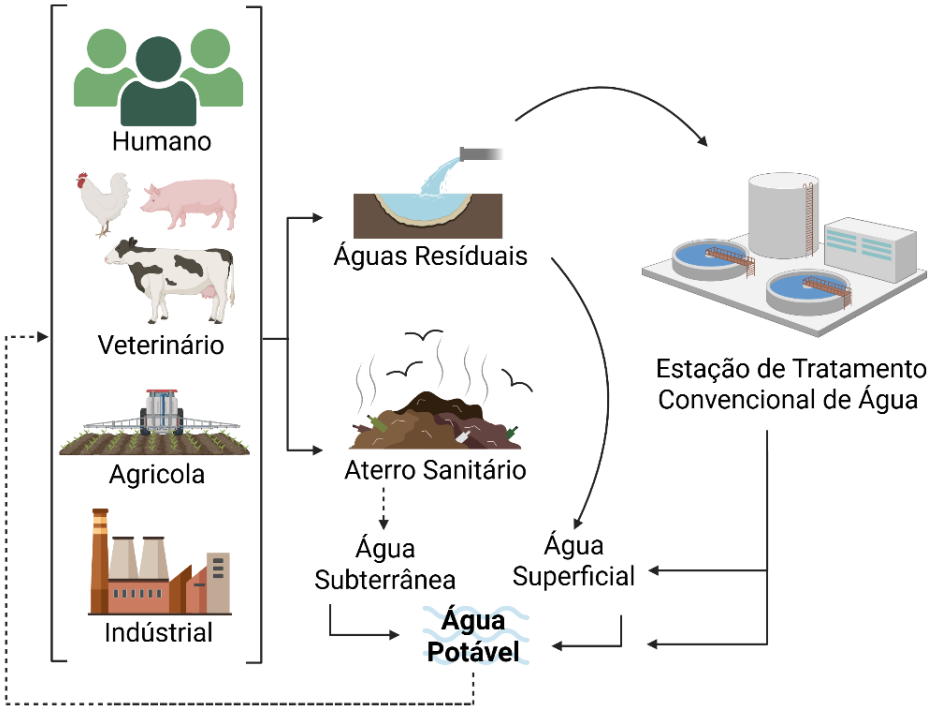

Figura 2: Principais rotas de introdução de fármacos e outros contaminantes emergentes no meio ambiente, provenientes de atividades humanas, veterinárias, agrícolas e industriais. As setas indicam o percurso desses compostos até os corpos d'água superficiais e subterrâneos, bem como sua reentrada no ciclo da água potável. Imagem adaptada de Ortúzar et al. (2022).

As propriedades físico-químicas dos fármacos não possibilitam generalizações sobre seu comportamento em diferentes matrizes ambientais (Estrada-Almeida et al., 2024). Enquanto o ibuprofeno pode levar mais de 400 dias para se degradar por fotólise, o ácido clofíbrico e o diazepam apresentam tempos de biodegradação de cerca de 119 e 300 dias, respectivamente, em ambientes aquáticos (Quadra et al., 2017). A baixa ou moderada biodegradabilidade favorece sua persistência e ampla ocorrência ambiental, já documentada em 71 países de todos os continentes (Figura 3) (aus der Beek et al., 2016; Suleiman et al., 2024).

Na maioria dos países, ainda não há regulamentação específica que estabeleça limites para a presença de fármacos em matrizes ambientais, o que evidencia uma

lacuna importante frente ao acúmulo crescente desses contaminantes (Estrada-Almeida et al., 2024; Patel et al., 2019). Algumas iniciativas, contudo, já estão em andamento. A União Europeia incluiu compostos como diclofenaco e hormônios (17α-etinilestradiol e 17β-estradiol) na lista de substâncias prioritárias para monitoramento em águas superficiais, sinalizando a preocupação com riscos ecotoxicológicos (EEA, 2010). Da mesma forma, países como Suécia e Alemanha avançaram na formulação de diretrizes para avaliação e controle de resíduos farmacêuticos em efluentes (Patel et al., 2019). A Austrália, por sua vez, exerceu papel pioneiro no estabelecimento de diretrizes específicas para fármacos em água potável (Pomati, 2007). Esses exemplos demonstram que, embora incipiente, o processo regulatório internacional vem ganhando espaço e pode servir de modelo para outras nações.

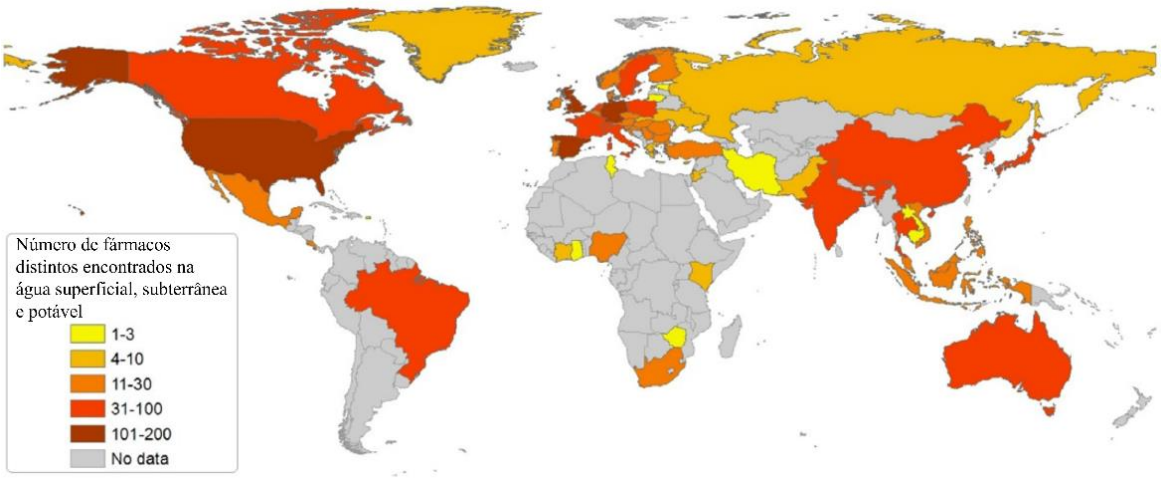

Figura 3: Distribuição global do número de substâncias farmacêuticas detectadas em águas superficiais, subterrâneas e potáveis. O mapa evidencia a variação no número de compostos identificados em diferentes países, refletindo desigualdades no monitoramento e na ocorrência de fármacos no ambiente. Imagem adaptada de aus der Beek et al. (2016).

No Brasil, já foram detectados em sistemas de abastecimento de água fármacos como ácido acetilsalicílico, ibuprofeno, paracetamol, diclofenaco, genfibrozila, naproxeno, sulfametoxazol, trimetoprima, benzafibrato, estrona, estradiol, etinilestradiol e estriol (Dias, 2014). No Rio Dilúvio, em Porto Alegre-RS, oito antibióticos, entre eles azitromicina, ciprofloxacino e sulfametoxazol, foram encontrados em todas as amostras coletadas ao longo de dois anos (Arsand et al., 2020).

Na Baía de Guanabara (RJ), a presença de *Escherichia coli* com genes de resistência a antibióticos reflete contaminação por esgotos domésticos e industriais (Gonçalves et al., 2019), enquanto bactérias resistentes já foram detectadas também em estações de tratamento de esgoto (Wang et al., 2020).

Situação semelhante tem sido relatada em diversos países. Na Inglaterra, sete drogas psicoativas foram detectadas em água potável (Peng et al., 2019), e a risperidona foi encontrada em concentrações variando de 0,0014 a 0,0034 μg/L em diferentes ambientes aquáticos (Kalichak et al., 2017). Na África do Sul, o antifúngico fluconazol apresentou frequência de 96% em amostras de água potável, com concentrações superiores a 9959 ng/L (Assress et al., 2020). Além disso, hospitais constituem fontes relevantes de contaminação, já que seus efluentes apresentam ampla diversidade de fármacos. No Brasil, enalapril, metformina, paracetamol e tetraciclina foram detectados em amostras de efluentes hospitalares (Chiarello et al., 2016), enquanto na Espanha foram identificados outros 17 fármacos em condições semelhantes (Souza et al., 2017).

Entre os fármacos mais consumidos globalmente, destacam-se os analgésicos (ex.: paracetamol, oxicodona) e anti-inflamatórios (ex.: diclofenaco, ibuprofeno, naproxeno), que, devido ao seu uso recorrente e em larga escala, constam entre os contaminantes mais frequentes em diferentes matrizes ambientais (Estrada-Almeida et al., 2024; Ortúzar et al., 2022; Placova et al., 2023). O diclofenaco, por exemplo, apresenta elevada persistência no meio aquático, com baixa taxa de biodegradação, o que favorece sua bioacumulação e o estabelecimento de efeitos adversos bem documentados como lesões renais e hepáticas em peixes, comprometimento do desenvolvimento embrionário, alterações comportamentais e redução do sucesso reprodutivo em organismos aquáticos não-alvo (Duarte et al., 2024; Lonappan et al., 2016). Já o paracetamol, embora mais suscetível à degradação, também tem sua toxicidade já evidenciada, sendo associado a efeitos hepatotóxicos e nefrotóxicos em organismos aquáticos expostos, além de potenciais riscos para a saúde humana em exposição crônica (Phong Vo et al., 2019; Vieira et al., 2024).

Os fármacos raramente ocorrem isoladamente no ambiente, estando geralmente presentes em misturas complexas com outros compostos (Suleiman et al., 2024). Nessas condições, suas interações podem gerar efeitos somativos, resultando em respostas mais severas (Białk et al., 2022). Embora alguns efeitos antagônicos tenham sido observados, estudos indicam que a tendência predominante é o aumento da toxicidade quanto maior a quantidade de fármacos combinados, como demonstrado por dos Santos et al. (2024).

O tratamento de efluentes contendo fármacos é desafiador, pois as matrizes de águas residuais comumente apresentam composição complexa, caracterizada por elevada carga orgânica, presença de sais e toxicidade microbiana, fatores que dificultam sua biodegradação (Guo et al., 2017). O tratamento convencional de água e esgoto combina processos físicos, químicos e biológicos, como filtração, sedimentação, flotação, hidrólise, oxidação e decomposição microbiana porém, sua eficiência é questionável (Mutegoa, 2024; Patel et al., 2019). Gracia-Lor et al. (2012) constataram que, de 50 fármacos analisados antes e depois do tratamento em uma estação de esgoto, 17 ainda foram detectados no efluente final. Outro estudo concluiu que a eficiência de remoção de contaminantes depende fortemente da tecnologia adotada nas estações de tratamento de águas residuais. Estações com leitos filtrantes percoladores alcançaram menos de 70% de remoção dos 55 produtos farmacêuticos analisados, enquanto aquelas baseadas em lodo ativado apresentaram eficiência significativamente maior, acima de 85% (Kasprzyk-Hordern et al., 2009).

A remoção de anti-inflamatórios não esteroides (AINEs) em estações de tratamento de água e esgoto apresenta grande variabilidade e, na maioria dos casos, eficiência insuficiente, com compostos como diclofenaco, naproxeno e cetoprofeno frequentemente persistindo mesmo após o tratamento (Archer et al., 2017; Guerra et al., 2014). As tecnologias convencionais raramente ultrapassam 50-70% de remoção para esses fármacos, sendo influenciadas por fatores sazonais, temperatura e limites analíticos de detecção (Golovko et al., 2014; Petrie et al., 2015). Mesmo processos avançados, como fotocatálise, ozonização e reações de oxidação avançada (POAs), embora capazes de atingir altas taxas de degradação, envolvem custos elevados, complexidade operacional e risco de formação de subprodutos tóxicos (Souza et al., 2017). Alternativas como biorreatores com membranas e adsorventes (carvão ativado, zeólitas, argilas e biossorventes) têm demonstrado melhores resultados, mas ainda enfrentam desafios de escalabilidade, regeneração e viabilidade econômica (Clara et al., 2005; Nguyen et al., 2016; Patel et al., 2019).

Assim, apesar do avanço tecnológico, nenhum método isolado se mostra plenamente eficaz, evidenciando a urgência por soluções integradas e sustentáveis capazes de garantir a remoção segura desses contaminantes emergentes (Patel et al., 2019). Entre as alternativas promissoras, a biorremediação enzimática se destaca por ser ecológica e economicamente viável em comparação a técnicas físico-químicas convencionais e de oxidação avançada (Inamuddin, 2023).

2.3 Conceitos fundamentais sobre enzimas

A palavra *enzima* deriva dos termos gregos *en* (“dentro”) e *zume* (“levedura”). Foi utilizada pela primeira vez pelo fisiologista alemão Frederick Wilhelm Kühne, em 1878, ao descrever a capacidade da levedura de produzir álcool a partir de açúcares (Gutfreud, 1976; Robinson, 2015).

Com exceção de poucos RNAs catalíticos, todas as enzimas conhecidas são proteínas. Elas funcionam como catalisadores, acelerando a transformação de substratos em produtos (Nelson e Cox, 2011). As enzimas estão no centro de cada processo bioquímico, possuindo papel fundamental no metabolismo celular (Junqueira e Carneiro, 2023). Dentro das células, costumam se associar a outras biomoléculas, como proteínas, ácidos nucleicos, polissacarídeos e lipídios. Já as enzimas extracelulares, também chamadas de exoenzimas, são produzidas na célula e depois liberadas para o ambiente externo (Copeland, 2023).

Quando as enzimas são extraídas de sua origem biológica, podem ser empregadas na catálise de processos biotecnológicos comercialmente importantes, como na fermentação, no cultivo de células e na produção de fármacos (Porter, 2016). Geralmente são necessárias em baixas concentrações e exibem especificidade considerável (Farhan et al., 2025). Algumas enzimas são altamente específicas, como a glicose oxidase, enquanto outras apresentam especificidade por grupos funcionais peculiares, como a fosfatase alcalina, que atua nos grupos fosfato de diversas moléculas (Robinson, 2015). Essa especificidade decorre das características únicas do sítio ativo, sendo determinante em ensaios analíticos e dispositivos como biossensores, empregados na detecção de analitos em misturas complexas (Sodhi et al., 2024) .

Duas teorias clássicas explicam a interação entre enzima e substrato: a teoria da chave-fechadura e a teoria do encaixe induzido (Nelson e Cox, 2011). Proposta por Fischer, no início do século XX, a teoria da chave-fechadura sugere que o substrato (a chave) se encaixa perfeitamente no sítio ativo da enzima (a fechadura), dependendo da complementaridade estrutural, polaridade e tamanho. Já a teoria do encaixe induzido, proposta por Koshland na década de 1960, defende que o sítio ativo é flexível, sofrendo pequenas alterações conformacionais quando o substrato se aproxima, o que facilita a interação (Robinson, 2015).

Estruturalmente, a macromolécula enzimática pode ser dividida em duas regiões: o microambiente do sítio ativo e o restante da proteína. O sítio ativo, por sua vez, possui duas sub-regiões: o sítio de ligação, responsável pelo reconhecimento e encaixe do substrato, e o sítio catalítico, onde ocorre a transformação química (Copeland, 2023). A complementaridade entre enzima e substrato envolve o ajuste estereoquímico, a compatibilidade eletrostática, as interações hidrofóbicas e as ligações de hidrogênio. Para o reconhecimento adequado, é necessário, no mínimo, três pontos de contato entre as estruturas (Robinson, 2015).

A atividade ótima das enzimas está intrinsecamente relacionada à manutenção de suas conformações nativas, uma vez que a desnaturação ou dissociação das subunidades geralmente resulta na perda da função catalítica (Nelson e Cox, 2011). Diversos fatores podem afetá-la, incluindo aspectos físico-químicos (pH, temperatura, força iônica, atividade de água), químicos (ativadores, inibidores, estabilizadores) e físicos (pressão, forças de cisalhamento) (Klibanov, 2001). Tais variáveis podem induzir alterações reversíveis ou irreversíveis na estrutura proteica, impactando a taxa da reação catalisada (Copeland, 2023). No entanto, quando a degradação da enzima atinge o nível dos aminoácidos constituintes, a atividade catalítica é irreversivelmente perdida (Gianfreda e Scarfi, 1991). Assim, a integridade das estruturas primária, secundária, terciária e quaternária é indispensável para a manutenção da atividade enzimática (Vitolo et al., 2015).

Quanto à nomenclatura, a maioria das enzimas recebe nomes terminados em -*ase*, geralmente associados à reação catalisada ou ao substrato envolvido (ex.: glicose oxidase, álcool desidrogenase, piruvato descarboxilase) (Nelson e Cox, 2011). Enzimas proteolíticas, contudo, tradicionalmente possuem o sufixo -*ina* (ex.: tripsina, quimotripsina, papaína). Alguns nomes históricos, como invertase e catalase, não fornecem informações claras sobre o substrato ou reação (Robinson, 2015; Vitolo et al., 2015). Para reduzir ambiguidades, a União Internacional de Bioquímica estabeleceu a Comissão de Enzimas, responsável pela padronização da nomenclatura. Nesse sistema, cada enzima recebe um número de quatro dígitos (EC), em que o primeiro indica a classe de reação catalisada, enquanto os demais detalham o tipo de substrato e o mecanismo envolvido (Robinson, 2015). O Banco de Dados de Nomenclatura de Enzimas (http://enzyme.expasy.org) constitui uma fonte confiável para consulta.

O estudo das enzimas transcende os limites da biologia básica, consolidando-se como um pilar essencial tanto na medicina moderna quanto em diversas áreas da

biotecnologia. No contexto médico, sua relevância é evidente, uma vez que doenças genéticas hereditárias podem resultar da deficiência ou ausência completa de determinadas enzimas, enquanto outras enfermidades estão associadas à atividade exacerbada dessas biomoléculas (Nelson e Cox, 2011). Além disso, inúmeros fármacos atuam por meio da modulação da atividade enzimática, reforçando sua importância terapêutica (Bruton, 2013). Para além da medicina, as enzimas têm papel estratégico na biotecnologia industrial e ambiental, visto que o desenvolvimento de tecnologias inovadoras demanda catalisadores altamente eficientes, seletivos e ambientalmente sustentáveis, características que tornam essas biomoléculas insubstituíveis em diferentes aplicações (Couto e Herrera, 2006; Farhan et al., 2025). Entre elas, destacam-se as lacases, oxidases multicobre com grande potencial em processos de biorremediação e em outras aplicações de interesse biotecnológico (Steinbüchel, 2020).

2.3.1 Características gerais das lacases

As lacases (EC 1.10.3.2) são enzimas oxidorredutases com atividade polifenol oxidase, pertencentes à superfamília das oxidases multicobre, que também inclui ascorbato oxidases (EC 1.10.3.3) e ferroxidases (EC 1.16.3.1), entre outras. A atividade catalítica dessas enzimas depende de íons cobre, os quais participam da oxidação de diferentes substratos, acoplada à redução de O_2 a H_2O, gerada como subproduto da reação (Hakulinen e Rouvinen, 2015).

O primeiro registro científico sobre lacase remonta a Yoshida (1883), que descreveu a composição química e as reações de oxidação promovidas pelo exsudato da árvore popularmente conhecida como laca oriental (*Toxicodendron vernicifluum*, anteriormente *Rhus vernicifera*) (Yoshida, 1883). No entanto, Bertrand, em 1894, foi responsável por nomear a enzima, após observar a oxidação de moléculas orgânicas com função fenólica em seus experimentos (Wisniak, 2014). Mais tarde, Nakamura (1960) e Omura (1961) realizaram sua purificação e elucidação do mecanismo de reação (Nakamura, 1960; Omura, 1961).

Além das plantas, as lacases foram identificadas em fungos, bactérias e insetos, o que as torna uma das enzimas amplamente distribuídas na natureza, desempenhando múltiplos papéis fisiológicos (Figura 4) (Aza e Camarero, 2023; Wisniak, 2014). Em bactérias, contribuem para a síntese de pigmentos, esporulação e proteção contra estresse oxidativo e radiação ultravioleta. Nas plantas, estão associadas à lignificação

da parede celular, além de processos de resposta a danos e ao estresse ambiental (Polaina e MacCabe, 2007). Nos insetos, participam da esclerotização da cutícula e da pigmentação (Steinbüchel, 2020). Já nas espécies fúngicas, além de funções relacionadas à proteção, virulência e formação de pigmentos, as lacases têm como papel mais estudado a biodegradação da lignina (Edens et al., 1999; Steinbüchel, 2020).

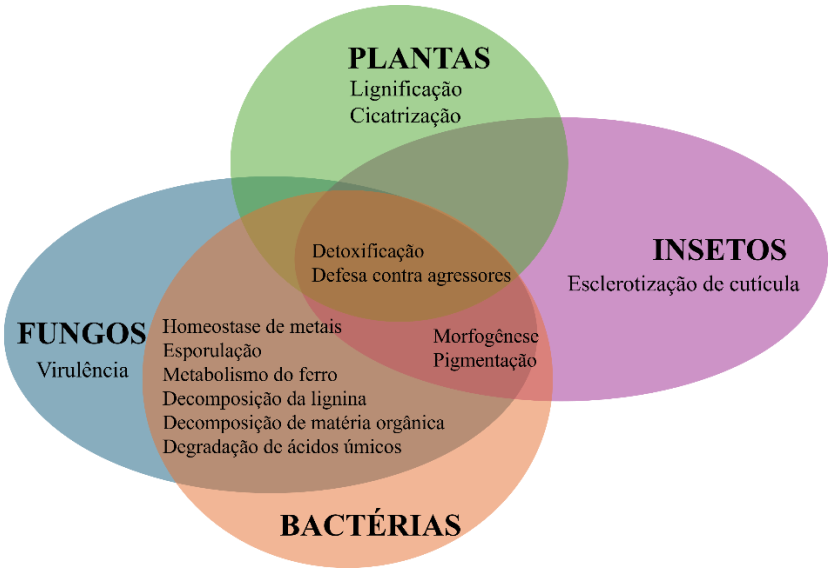

Figura 4: Funções biológicas atribuídas às lacases em diferentes grupos de organismos. O diagrama de Venn destaca os papéis específicos dessas enzimas em plantas, fungos, bactérias e insetos, bem como suas funções compartilhadas, como detoxificação e defesa contra agressores. Imagem adaptada de Janusz et al. (2020).

2.3.1.1 Lacases fúngicas

Os fungos filamentosos desempenham papel essencial na decomposição da lignocelulose, sendo indispensáveis ao ciclo do carbono na Terra, à formação de matéria húmica no solo e à manutenção da estrutura fina do solo. Entre eles, os decompositores de madeira pertencem principalmente aos filos Basidiomycota e Ascomycota, especializados na degradação dos componentes da parede celular do xilema, como celulose, hemiceluloses, ligninas e extrativos (Janusz et al., 2020).

A capacidade de decompor os polímeros aromáticos da lignina é particularmente evidente nos basidiomicetos de degradação branca. Essa atividade é mediada pela secreção de ácidos orgânicos, metabólitos secundários e enzimas oxidorredutoras, como heme peroxidases e lacases, codificadas por famílias de genes divergentes nesses fungos (Dwivedi et al., 2011; Peralta et al., 2017). Por outro lado, os basidiomicetos de

degradação parda dependem mais de mecanismos não enzimáticos para degradar a celulose e modificar a lignina (Lundell et al., 2010).

As lacases estão amplamente distribuídas entre as classes dos ascomicetos, deuteromicetos e basidiomicetos (Dwivedi et al., 2011). Estudos indicam que o número de genes de lacase em basidiomicetos varia significativamente conforme o ambiente: organismos provenientes de solos florestais ricos em lignina apresentam de 5 a 10 vezes mais genes de lacase do que aqueles de ambientes com baixo teor de lignina, sugerindo uma adaptação funcional à disponibilidade de substratos lignocelulósicos (Janusz et al., 2020). Além disso, observa-se uma considerável diversidade de isoenzimas produzidas. Por exemplo, na espécie *Pleurotus ostreatus*, oito diferentes isoformas de lacase foram sintetizadas, das quais seis foram caracterizadas (Torres-Farradá et al., 2024). A presença de famílias gênicas tão complexas e a variedade de isoenzimas provavelmente refletem os distintos papéis fisiológicos desempenhados pela lacase ao longo do ciclo de vida dos fungos (Lundell et al., 2010).

A secreção de lacases é uma resposta adaptativa dos fungos a condições ambientais desfavoráveis, incluindo a presença de microrganismos competidores, metais, toxinas, xenobióticos e outros compostos biologicamente ativos (Eggert, 1997; Janusz et al., 2020). Essas enzimas oxidam não apenas compostos fenólicos, mas também moléculas não fenólicas, como aminas aromáticas, hidrocarbonetos policíclicos aromáticos, corantes sintéticos, antibióticos e outros substratos atípicos (Mikolasch e Schauer, 2009). Consequentemente, as lacases funcionam como ferramentas enzimáticas versáteis na detoxificação de compostos naturais e sintéticos, desempenhando um papel central nos mecanismos de defesa ativa dos fungos (Janusz et al., 2020).

Devido à sua ampla versatilidade catalítica, as lacases fúngicas despertam crescente interesse industrial (Couto e Herrera, 2006; Mate e Alcalde, 2015). Na indústria têxtil, as lacases de *Trametes versicolor* e *Myceliophthora thermophila*, disponíveis em formulações comerciais, são utilizadas na estonagem de tecidos e na descoloração de corantes sintéticos em efluentes, contribuindo para processos mais eficientes e ambientalmente sustentáveis (Patel et al., 2018). Na indústria de papel e celulose, essas enzimas auxiliam no branqueamento e na remoção de lignina residual das polpas, reduzindo a necessidade de reagentes químicos agressivos, como o cloro e seus derivados (Debnath e Saha, 2020). No setor alimentício, as lacases promovem reações de homo- e heteropolimerização, sendo empregadas na estabilização de vinhos

e cervejas, no processamento de sucos de frutas, na panificação, na melhoria de parâmetros sensoriais de alimentos e na gelificação de pectina (Lettera et al., 2016; Mate e Alcalde, 2017).

Além dessas aplicações industriais consolidadas, os pesquisadores têm investigado o potencial das lacases fúngicas em campos emergentes, como a biorremediação de fármacos e a biossíntese de moléculas de interesse terapêutico (Mate e Alcalde, 2015; Mogharabi e Faramarzi, 2014). Nesse contexto, as enzimas atuam como catalisadores em reações de acoplamento oxidativo e na modificação estrutural de compostos aromáticos, possibilitando a síntese de intermediários farmacêuticos, alcaloides e derivados fenólicos com propriedades antioxidantes, antimicrobianas e antitumorais (Hahn, 2023; Mikolasch e Schauer, 2009; Mogharabi e Faramarzi, 2014). Adicionalmente, as lacases têm se mostrado promissoras na degradação de contaminantes emergentes, incluindo fármacos recalcitrantes como analgésicos, anti-inflamatórios e antibióticos, contribuindo para a redução do risco ecotoxicológico e promovendo processos de tratamento ambiental mais sustentáveis (Arregui et al., 2019; Yang et al., 2017).

2.3.1.1.1 Aspectos estruturais e funcionais das lacases fúngicas

As lacases desempenham múltiplas funções fisiológicas nos fungos, estando envolvidas em processos essenciais como pigmentação associada ao desenvolvimento (Langfelder et al., 2003), formação de corpos de frutificação, morfogênese, esporulação (Janusz et al., 2020), desintoxicação de compostos xenobióticos (Torres-Farradá et al., 2024) e patogênese (Eggert, 1997). Essas funções contribuem diretamente para a sobrevivência, adaptação ambiental e sucesso ecológico dos fungos em diferentes habitats, especialmente em ambientes ricos em matéria orgânica complexa (Dwivedi et al., 2011; Polaina e MacCabe, 2007).

No contexto do desenvolvimento fúngico, as lacases participam da síntese e polimerização de pigmentos fenólicos, como melaninas, os quais estão associados à proteção contra radiação UV, estresse oxidativo e ataque microbiano, além de desempenharem papel estrutural durante a diferenciação celular e a formação de estruturas reprodutivas (Langfelder et al., 2003; Polaina e MacCabe, 2007). Essas enzimas também têm sido implicadas na formação de corpos de frutificação e na esporulação, atuando na remodelação da parede celular e na regulação de processos

morfogenéticos dependentes de oxidação fenólica (Janusz et al., 2020; Lundell et al., 2010).

Um exemplo claro da contribuição das lacases para a defesa química e sobrevivência fúngica é sua participação na biossíntese do ácido cínabarico em *Pycnoporus cinnabarinus*, um metabólito secundário com atividade antibacteriana que confere proteção contra a competição microbiana e predação (Eggert, 1997). Além disso, as lacases desempenham papel relevante na desintoxicação de compostos fenólicos tóxicos, permitindo que os fungos tolerem ambientes contaminados e metabolizem substâncias potencialmente nocivas (Dwivedi et al., 2011; Torres-Farradá et al., 2024).

Do ponto de vista ecológico e biogeoquímico, as lacases destacam-se pela sua atuação na degradação da lignina, processo central para o apodrecimento da madeira e para a ciclagem do carbono em ecossistemas terrestres (Torres-Farradá et al., 2024). Nessa função, as lacases atuam de forma sinérgica com outras enzimas oxidativas, como peroxidases ligninolíticas, bem como com enzimas hidrolíticas e mediadores redox de baixo peso molecular, ampliando o espectro de substratos oxidados e promovendo a despolimerização da lignina (Christopher et al., 2014).

A localização celular dessas enzimas está intimamente associada à sua função fisiológica e à disponibilidade de substratos (Polaina e MacCabe, 2007). As lacases intracelulares participam da transformação de compostos fenólicos de baixo peso molecular, enquanto aquelas ancoradas na parede celular estão frequentemente relacionadas à formação de melaninas e outros polímeros protetores da parede fúngica (Baldrian, 2006).

Estruturalmente, as lacases apresentam uma conformação tridimensional altamente conservada, com quatro sítios de ligação ao substrato e quatro sequências de aminoácidos que atuam como ligantes de cobre (Hakulinen e Rouvinen, 2015). A lacase fúngica típica é uma glicoproteína com massa molecular aproximada de 50-130 kDa e ponto isoelétrico ácido (pI) entre 2,6 e 4,5. Observa-se considerável heterogeneidade nas propriedades das lacases isoladas de fungos, especialmente em relação a massa molecular (Ba et al., 2013; Baldrian, 2006; Torres-Farradá et al., 2024). Geralmente são monoméricas, embora formas diméricas ou tetraméricas também tenham sido relatadas (Baldrian, 2006; Dwivedi et al., 2011). Cada cadeia polipeptídica se dobra em três domínios do tipo cupredoxina (D1, D2 e D3), formados por folhas β dispostas em uma

estrutura clássica, contendo no mínimo quatro átomos de cobre por monômero (Figura 5).

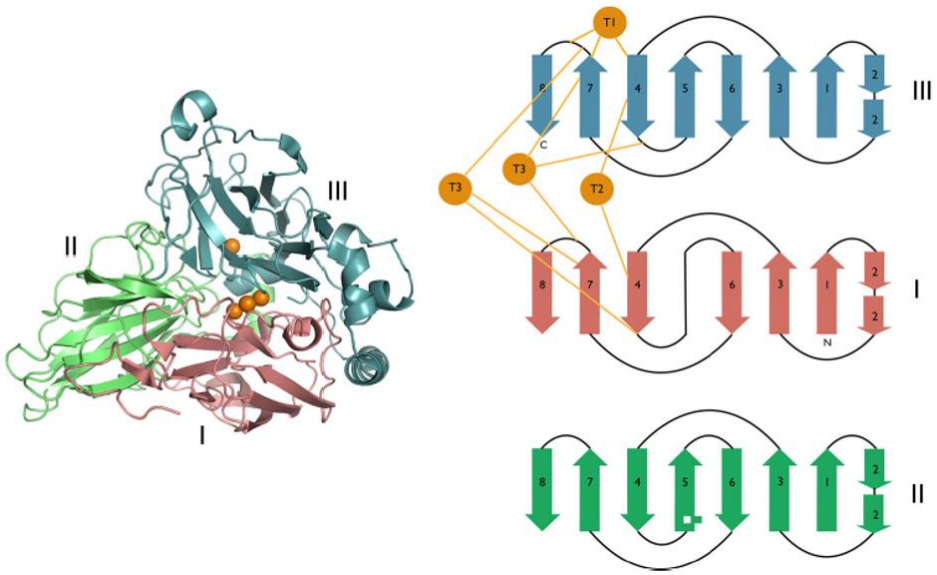

Figura 5: Estrutura tridimensional de uma lacase fúngica monomérica. As cores distintas evidenciam os três domínios estruturais da enzima, enquanto os íons de cobre, representados por esferas laranja, indicam os centros catalíticos responsáveis pela atividade oxidativa. Imagem adaptada de Hakulinen e Rouvinen (2015).

Os íons de cobre são classificados quanto às propriedades espectroscópicas: T1 (cobre tipo 1 ou azul, absorção ~600 nm), T2 (cobre tipo 2 ou normal, sem absorção visível) e T3 (cobre binuclear tipo 3, absorção ~330 nm) (Mehra et al., 2018). Os cobres T2 e T3 formam o *cluster* trinuclear, conectado ao sítio do cobre T1 por meio da tríade conservada His-Cys-His, composta por dois ligantes de histidina dos cobres T3 e um ligante de cisteína do cobre T1 (Polaina e MacCabe, 2007).

O sítio de ligação, localizado próximo ao centro catalítico T1, é formado por *loops* flexíveis que variam em tamanho, conformação e composição de resíduos de aminoácidos entre diferentes lacases. Essa diversidade estrutural modula as interações enzima-substrato e contribui para a ampla especificidade e capacidade oxidativa dessas enzimas (Dwivedi et al., 2011). As lacases são frequentemente descritas como “enzimas azuis”, devido à absorção em 600 nm; contudo, condições específicas de cultivo podem favorecer a produção de variantes com absorção na faixa amarela (Leontievsky et al., 1997).

O potencial redox (E°), definido como a energia necessária para a remoção de um elétron de um substrato redutor com consequente formação de um radical cátion, constitui uma das características relevantes das lacases fúngicas (Polaina e MacCabe, 2007). Conforme a estrutura e o potencial redox de seus centros de cobre, em especial do cobre T1, essas enzimas são classificadas em baixo, médio ou alto potencial redox (Mate e Alcalde, 2015). As lacases fúngicas se enquadram nas classes de potencial redox médio e alto. O grupo de lacases de potencial redox médio inclui enzimas de ascomicetos e basidiomicetos, com um $E°'_{T1}$ variando de +460 a +710 mV *vs*. Eletrodo Padrão de Hidrogênio (EPH). As lacases de alto potencial redox são produzidas principalmente por fungos basidiomicetos de degradação branca, com um $E°'_{T1}$ entre +730 a +790 mV *vs*. EPH, o que lhes permite extrair elétrons de uma ampla gama de substratos (Mate e Alcalde, 2015; Torres-Farradá et al., 2024). Em contraste, as lacases de origem bacteriana e vegetal são classificadas como enzimas de baixo potencial redox ($E° < +460$ mV *vs*. EPH) (Dwivedi et al., 2011; Mate e Alcalde, 2015). Essa propriedade justifica o destaque das lacases fúngicas na biorremediação e na degradação de compostos aromáticos recalcitrantes, tornando-as especialmente atrativas para aplicações biotecnológicas (Couto e Herrera, 2006; Yang et al., 2017).

2.3.1.1.2 Produção de lacases fúngicas

A produção de lacases em larga escala é um requisito essencial para sua aplicação em processos industriais e ambientais (Polaina e MacCabe, 2007; Yang et al., 2017). Diversas estratégias têm sido investigadas para viabilizar esse objetivo (Figura 6) (Brijwani et al., 2010; Debnath e Saha, 2020). Como enzimas típicas do metabolismo secundário, as lacases apresentam sua síntese fortemente modulada por fatores externos. Assim, parâmetros como pH, aeração, agitação, temperatura, umidade e disponibilidade de nutrientes afetam diretamente o rendimento enzimático (Brijwani et al., 2010; Schneider et al., 2018; Yang et al., 2017).

As formulações dos meios de cultivo dos fungos são desenvolvidas visando a viabilidade econômica, a escalabilidade e a reprodutibilidade (Brijwani et al., 2010; Gianfreda et al., 1999). Nesse contexto, resíduos agroindustriais, tanto sólidos quanto líquidos, têm sido amplamente utilizados como fontes de nutrientes. Exemplos incluem bagaço de maçã, celulose e papel (Lonappan et al., 2017), casca de melancia (Çaloglu e Binay, 2023), cascas de tucumã e pupunha (Lotas et al., 2024), casca de café e polpa

de cítricos (Almeida et al., 2018), além de efluentes como águas residuais de moinhos de azeite (Lakhtar et al., 2010). O aproveitamento desses resíduos não apenas reduz custos, mas também agrega valor à materiais de baixo aproveitamento e contribui para a redução do impacto ambiental associado ao seu descarte inadequado (Debnath e Saha, 2020).

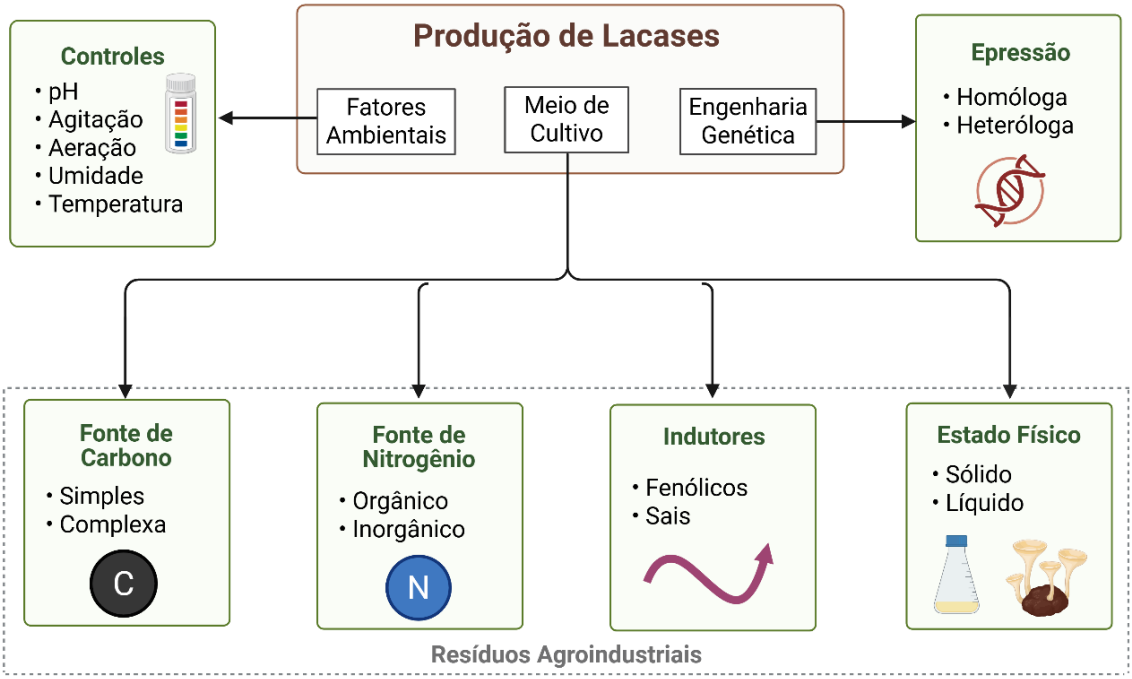

Figura 6: Principais fatores que influenciam a produção de lacases, incluindo condições ambientais, composição do meio de cultivo e estratégias de expressão. Elaborada pela autora.

A disponibilidade de carbono e nitrogênio no meio de cultivo exerce um impacto decisivo sobre a síntese e a expressão de lacases por fungos filamentosos, influenciando diretamente os níveis de atividade enzimática observados (Schneider et al., 2018). De modo geral, os fungos apresentam elevada versatilidade metabólica no aproveitamento de diferentes fontes de carbono, demonstrando preferência por carboidratos prontamente assimiláveis em detrimento de proteínas ou polímeros mais complexos (He et al., 2021; Vahidi et al., 2006). Em condições laboratoriais, a glicose é amplamente empregada como fonte de carbono primária e tende a ser consumida de forma prioritária quando disponibilizada em combinação com outros substratos carbonados, em razão de sua fácil assimilação e do efeito de repressão catabólica

associado (Chang e Miles, 2004). Entretanto, esse comportamento não é universal, uma vez que determinadas espécies fúngicas apresentam maior eficiência metabólica e produtiva quando cultivadas em meios contendo fontes alternativas ou combinações específicas de carbono (Ullrich et al., 2005). Nesse contexto, substratos carbonados complexos, como resíduos da poda de videira e casca de tangerina, têm demonstrado maior capacidade de induzir a produção de lacases e aumentar a atividade enzimática em *Pleurotus ostreatus* quando comparados a açúcares simples, possivelmente devido à presença de compostos fenólicos e lignocelulósicos que atuam como indutores da expressão gênica dessas enzimas (Mikiashvili et al., 2006).

A concentração e a origem do nitrogênio também influenciam significativamente a produção enzimática (Valle et al., 2015). O aumento da disponibilidade de nitrogênio tende a elevar os rendimentos, como evidenciado nos cultivos de *Lentinula edodes* e *Rigidoporus lignosus*, que em concentrações de 24–26 mM promoveram produção de lacases significativamente maior do que aquelas observadas em condições restritas (1,2–2,5 mM) (Dwivedi et al., 2011; Fu et al., 1997). Em contrapartida, para *Pycnoporus cinnabarinus* e *Phlebia radiata*, a limitação de nitrogênio (≈2,4 mM) promoveu maior síntese de lacases (Dwivedi et al., 2011; Eggert et al., 1996). No cultivo de *Pleurotus ostreatus*, sais inorgânicos como nitrato de amônio (NH_4NO_3) e fosfato de amônio ($NH_4H_2PO_4$) reduziram a produção enzimática, enquanto fontes orgânicas, como peptona e caseína, aumentaram, mesmo em concentrações relativamente altas (≈30 mM) (Mikiashvili et al., 2006). Já em *Marasmiellus palmivorus* VE111, a caseína promoveu rendimentos superiores à peptona (Schneider et al., 2018).

Além dos nutrientes, substâncias indutoras têm desempenhado papel relevante na regulação da síntese de lacases. Compostos fenólicos (ex.: álcool veratrílico, ácido ferúlico) e sais metálicos (ex.: $CuSO_4$, $MnSO_4$) têm sido amplamente empregados como indutores enzimáticos (Gianfreda et al., 1999; Schneider et al., 2019). Esses compostos podem atuar mimetizando substratos naturais, modulando fatores transcricionais associados ao estresse oxidativo ou estimulando diretamente a expressão de genes relacionados à síntese enzimática (Yang et al., 2013). No caso específico do cobre, sua adição ao meio de cultivo, além de fornecer cofator essencial ao centro catalítico multicobre da lacase, desencadeia respostas adaptativas que frequentemente resultam em maior atividade da enzima secretada (Yang et al., 2017).

O estado físico do meio de cultivo dos fungos pode se apresentar na forma sólida ou líquida, influenciando a produção e na recuperação das lacases (Brijwani et al., 2010; Couto e Toca-Herrera, 2007; Debnath e Saha, 2020). O cultivo em meio sólido mimetiza o ambiente natural dos fungos, favorecendo a síntese de enzimas ligninolíticas, e os rendimentos geralmente são proporcionais à presença de indutores (Brijwani et al., 2010; Viniegra-González et al., 2003). Nesse tipo de cultivo, caracterizado pela ausência ou quase ausência de líquido livre, o desenvolvimento do fungo ocorre sobre um suporte sólido, que pode ser constituído por substratos inertes (materiais sintéticos) ou naturais (materiais orgânicos) (Couto e Toca-Herrera, 2007). A principal limitação desse sistema é a ausência de biorreatores plenamente estabelecidos, embora diversos projetos abordem restrições à transferência de calor e massa (Brijwani et al., 2010). Outro fator relevante é a concentração de CO_2 durante a fermentação, que pode interferir na síntese enzimática (Fenice et al., 2003; White e Boddy, 1992).

O cultivo submerso consiste no crescimento de microrganismos em um meio líquido rico em nutrientes e com alta concentração de oxigênio (condições aeróbicas) (Couto e Toca-Herrera, 2007; Nguyen et al., 2020). A produção industrial de enzimas é realizada principalmente por este tipo de cultivo, pois permite maior controle das variáveis ambientais (pH, aeração, agitação, nutrientes) e facilita a recuperação da enzima (Brijwani et al., 2010; Couto e Toca-Herrera, 2007). Entretanto, sua homogeneidade pode reduzir a indução da atividade enzimática, além de possuir problemas associados à viscosidade do meio causada pelo crescimento micelial, que limita a transferência de oxigênio e a difusão de indutores (Brijwani et al., 2010; Lakhtar et al., 2010). Dessa forma, a escolha entre cultivo sólido ou submerso deve equilibrar rendimento, facilidade de recuperação enzimática e viabilidade de escalonamento (Nguyen et al., 2020).

Para além da otimização dos cultivos, estratégias de engenharia genética vêm sendo cada vez mais exploradas (Sodhi et al., 2024). A expressão homóloga e heteróloga de lacases em bactérias, leveduras, fungos filamentosos e plantas tem permitido aumentar a produção e a estabilidade enzimática, possibilitando a expansão de suas aplicações (Debnath e Saha, 2020). Um dos primeiros relatos foi a expressão heteróloga do gene de *Phlebia radiata* em *Trichoderma reesei* (Saloheimo e Niku-Paavola, 1991). Desde então, as lacases fúngicas têm sido expressas em diversos hospedeiros, incluindo leveduras (*Pichia pastoris*, *Saccharomyces cerevisiae*), fungos

filamentosos (*Aspergillus niger*) e plantas (arroz e tabaco) (de Wilde et al., 2008; Sonoki et al., 2005; Wang et al., 2004). Contudo, em hospedeiros vegetais, a atividade observada é geralmente inferior à de fungos, devido a diferenças de glicosilação e dobramento proteico (Nakagawa et al., 2010; Sakamoto et al., 2008).

Atualmente, diversas preparações comerciais de lacases estão disponíveis. Entre os basidiomicetos produtores destacam-se espécies de degradação branca, como *Cerrena unicolor* (Jena Bioscience, Alemanha), *Agaricus bisporus* e *Trametes versicolor* (ASA Spezialenzyme GmbH, Alemanha). As lacases de ascomicetos termofílicos, como *Myceliophthora thermophila*, são comercializadas pela Novonesis (Dinamarca), enquanto preparações de *Aspergillus* sp. estão disponíveis pela Sigma-Aldrich (Alemanha). Esse mercado reflete não apenas a relevância biotecnológica das lacases, mas também a contínua necessidade de processos sustentáveis para ampliar sua produção e aplicação.

2.3.1.2 Atividade catalítica das lacases

A lacase catalisa a redução de quatro elétrons do oxigênio molecular (O_2) a água (H_2O), concomitante à oxidação de um elétron do substrato redutor (Figura 7A), sem a formação de subprodutos tóxicos como o peróxido de hidrogênio. Essa característica, aliada à sua ampla versatilidade catalítica, contribui para o interesse industrial e ambiental nessas enzimas (Polaina e MacCabe, 2007). Fenóis e aminas constituem seus substratos naturais, embora algumas isoformas sejam capazes de oxidar uma variedade mais ampla de compostos orgânicos e inorgânicos (Jones e Solomon, 2015).

A oxidação inicial do substrato ocorre no centro catalítico T1 (Figura 7B), de onde os elétrons são transferidos por aproximadamente 13 Å através da via Cys–His até o centro trinuclear de cobre (T2/T3), onde ocorre a redução do oxigênio molecular (Mehra et al., 2018).

O potencial eletroquímico do cobre T1 (400 a 800 mV *vs.* EPH) é um dos principais determinantes da eficiência catalítica da lacase. Apesar de relativamente baixos quando comparados às peroxidases ligninolíticas, esses valores definem a gama de substratos passíveis de oxidação e, consequentemente, influenciam diretamente sua aplicabilidade em biorremediação e transformações biotecnológicas (Cañas e Camarero, 2010).

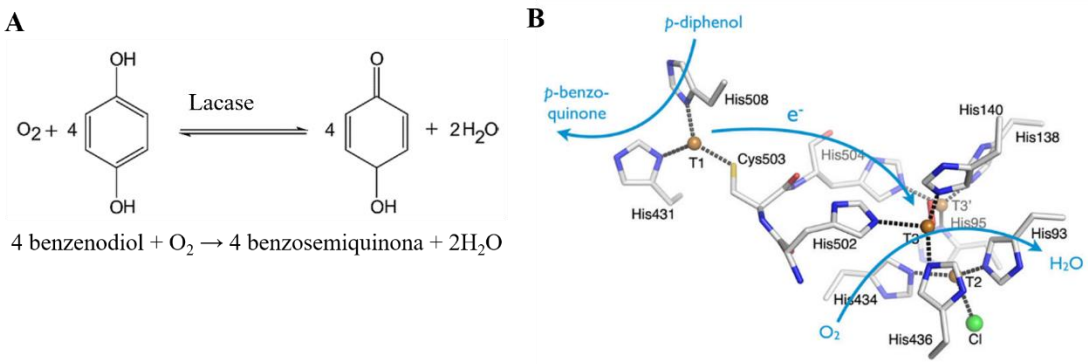

Figura 7: (A) Reação geral catalisada por lacases, envolvendo a oxidação de substratos fenólicos com a redução concomitante do oxigênio molecular a água. (B) Representação esquemática do mecanismo catalítico das lacases, destacando o transporte de elétrons entre os centros cúpricos T1, T2 e T3. Imagem adaptada de Hakulinen e Rouvinen (2015).

Uma estratégia para expandir o espectro de ação das lacases é o uso de mediadores redox, pequenas moléculas que funcionam como transportadores de elétrons (Zhang et al., 2023). Após serem oxidados pela enzima, esses compostos formam radicais relativamente estáveis (Figura 8) que se difundem no meio e promovem a oxidação de moléculas de maior complexidade estrutural ou elevado potencial redox, as quais não seriam diretamente oxidadas pela lacase (Bourbonnais et al., 1998; Camarero et al., 2007). O mediador ideal deve ser estável em sua forma oxidada, apresentar elevado poder oxidante, não inibir a enzima e ser capaz de realizar múltiplos ciclos catalíticos sem degradação ou formação de subprodutos indesejados (Steinbüchel, 2020). Esses mediadores podem ser classificados como naturais (ex.: siringaldeído, vanilina) ou sintéticos (ex.: ABTS, HBT) (Zhang et al., 2023).

Embora a adição de mediadores possa aumentar significativamente a eficiência da lacase na oxidação de substratos, o seu uso em larga escala enfrenta limitações devido à toxicidade, instabilidade e alto custo, podendo causar contaminação secundária (Aghaee et al., 2024). Apesar da identificação de novos mediadores, ainda persistem desafios quanto à solubilidade em água, estabilidade operacional e reutilização da enzima. Assim, torna-se essencial desenvolver sistemas de mediadores mais sustentáveis, eficientes e ambientalmente seguros (Zhang et al., 2023).

A atividade das lacases pode ser afetada por diversos inibidores. Pequenos ânions, como azida, cianeto, haletos, tiocianato, fluoreto e hidróxido, ligam-se aos

centros T2/T3, bloqueando a transferência interna de elétrons. Além disso, íons metálicos (Hg^{2+}, Mg^{2+}, Ca^{2+}, Sn^{2+}, Ba^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}), ácidos graxos, reagentes sulfidrila, hidroxiglicina e detergentes catiônicos podem inibir a enzima por diferentes mecanismos, incluindo quelação do cobre, modificações de resíduos de aminoácidos ou alterações conformacionais (Baldrian, 2006; Gianfreda et al., 1999).

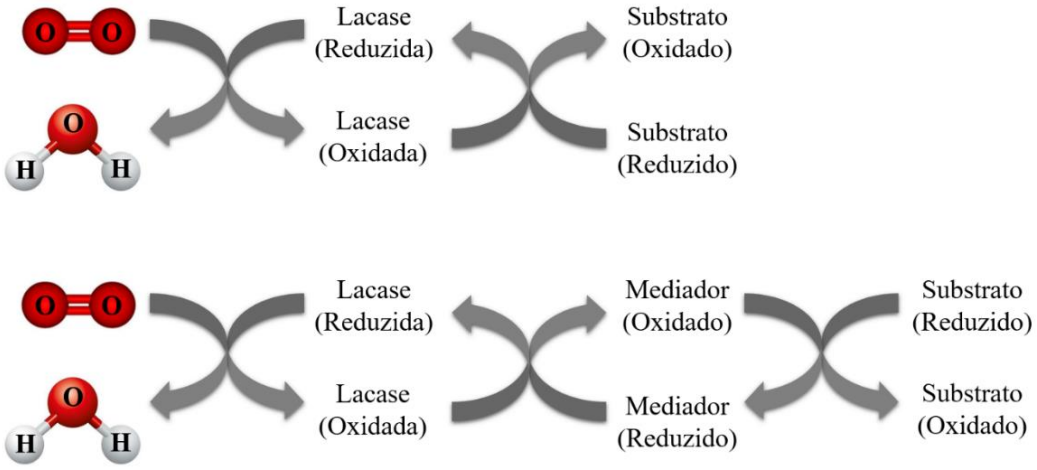

Figura 8: Representação esquemática dos ciclos catalíticos da lacase durante a oxidação de substratos na ausência (superior) e na presença (inferior) de um mediador redox. Imagem adaptada de Mogharabi e Faramarzi (2014).

A atividade das lacases pode ser determinada através da reação entre a enzima e substratos cromogênicos ou fenólicos, cujo consumo ou formação de produtos é monitorado espectrofotometricamente (Johannes e Majcherczyk, 2000). Nesses ensaios, é fundamental assegurar que nenhum componente proveniente do meio de cultivo, associado à enzima, interfira na reação, seja reagindo com o substrato, seja com o produto final (Johannes e Majcherczyk, 2000).

Substratos comumente usados para a determinação da atividade da lacase (Figura 9) são guaiacol (Ullrich et al., 2005), ABTS [2,2-azinobis-(3-etilbenzotiazolina-6-sulfonato)] (Robert Bourbonnais e Paice, 1990), 2,6-dimetoxifenol (2,6-DMP) (Edens et al., 1999), siringaldazina (Harkin et al., 1974) e catecol (Cañas e Camarero, 2010).

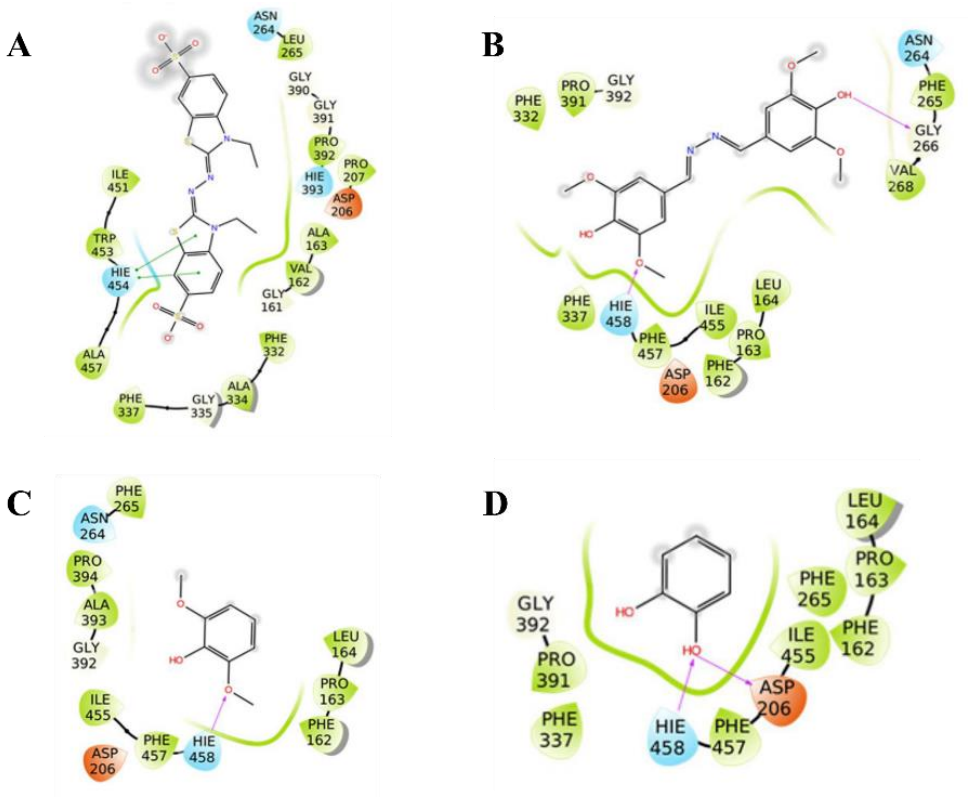

Figura 9: Modelos ilustrativos das interações moleculares entre a lacase e substratos comumente utilizados na determinação de sua atividade catalítica. (A) ABTS [2,2'-azinobis-(3-etilbenzotiazolina-6-sulfonato)], (B) siringaldazina, (C) 2,6-dimetoxifenol (2,6-DMP) e (D) catecol. Imagem adaptada de Mehra et al., (2018).

2.3.2 Estratégias de estabilização enzimática

Em meios aquosos, as enzimas apresentam a mobilidade conformacional necessária para desempenhar sua atividade catalítica de forma eficiente. No entanto, esse mesmo ambiente favorece processos que podem comprometer sua estabilidade estrutural e funcional (Klibanov, 2001; Zaks e Klibanov, 1988). A estrutura nativa de uma enzima corresponde à conformação adotada em ambiente celular ou quando isolada, apresentando sua máxima atividade biológica. A desnaturação proteica consiste em modificações da sua estrutura tridimensional sem alterações na sequência de aminoácidos (Figura 10A). Consequentemente, esse processo compromete o arranjo correto do sítio ativo e resulta na inativação da enzima. Assim, a estabilização

enzimática visa prevenir tais modificações e preservar a conformação nativa (Gianfreda e Scarfi, 1991).

A estabilidade enzimática constitui um dos principais parâmetros determinantes para a aplicação de enzimas em processos biotecnológicos, uma vez que condiciona diretamente a eficiência operacional, a reprodutibilidade e a viabilidade econômica de sistemas baseados em catalisadores biológicos (Iyer e Ananthanarayan, 2008). Enzimas estáveis mantêm sua atividade catalítica por períodos prolongados, característica essencial em aplicações industriais, como nas indústrias de alimentos, farmacêutica, de bioenergia e no tratamento de resíduos (Copeland, 2023). Mesmo enzimas que apresentam elevada atividade específica tornam-se inviáveis do ponto de vista tecnológico se não preservarem sua funcionalidade ao longo do período de armazenamento ou durante a condução do bioprocesso (Linde et al., 2018).

Para viabilizar a utilização de enzimas como biocatalisadores industriais, é necessário obter preparações com elevada estabilidade operacional. Isso pode ser alcançado de duas maneiras principais: (i) utilizando enzimas com estabilidade natural extrema ou (ii) estabilizando enzimas naturalmente instáveis (Janeček, 1993). Entre as estratégias empregadas para estabilização enzimática, destacam-se a imobilização, que também permite a reutilização das enzimas, a engenharia de proteínas para aprimorar a resistência estrutural e a adição de estabilizantes químicos capazes de proteger a conformação enzimática (Iyer e Ananthanarayan, 2008). Mais recentemente, têm ganhado destaque alternativas tecnológicas de estabilização como a liofilização e a secagem por aspersão, que possibilitam a obtenção de preparações enzimáticas em pó, facilitando o armazenamento a longo prazo, o transporte e a aplicação em diferentes setores (Liu et al., 2016; Rey e May, 2023). Essas abordagens são fundamentais não apenas para ampliar a aplicação das enzimas em processos biotecnológicos, mas também para atender às demandas de sustentabilidade e redução de custos operacionais.

2.3.2.1 Estabilização com aditivos químicos

Moléculas como açúcares, polióis, aminoácidos, sais neutros, polímeros hidrofílicos e surfactantes leves podem interagir com a superfície proteica ou modificar o microambiente ao redor da enzima, reduzindo a exposição de regiões hidrofóbicas e restringindo movimentos estruturais que levam à desnaturação (Iyer e Ananthanarayan, 2008). A escolha do estabilizante ideal depende das características estruturais da

enzima, do ambiente de aplicação e do tipo de estresse a ser prevenido. Estudos comparativos demonstram que variações na concentração, na combinação de compostos ou na sequência da sua adição podem influenciar significativamente a estabilidade enzimática (Costa et al., 2002; Haque et al., 2005).

Entre os estabilizantes mais utilizados, os polióis se destacam pela capacidade de proteger a estrutura tridimensional das enzimas em condições adversas (Haque et al., 2005). Compostos como glicerol, sorbitol, manitol, etilenoglicol e propilenoglicol atuam principalmente por mecanismos relacionados à exclusão preferencial e à modulação da atividade da água (Vagenende et al., 2009). Ao interagirem indiretamente com a superfície proteica, os polióis favorecem a manutenção de interações intramoleculares e reduzem a flexibilidade excessiva que pode levar ao desdobramento parcial da enzima (Dashnau e Vanderkooi, 2007). Além disso, contribuem para estabilizar a camada de hidratação ao redor da proteína, diminuindo a exposição de regiões hidrofóbicas e prevenindo a agregação (Vagenende et al., 2009).

A eficácia do poliol varia conforme sua estrutura química, concentração e compatibilidade com a enzima, sendo frequentemente necessária a otimização experimental para definir as condições mais favoráveis à atividade e estabilidade catalítica (Priev et al., 1996). Para lipase, por exemplo, verificaram que o efeito protetor aumentou com a elevação da concentração do poliol e com o uso de moléculas de maior cadeia carbônica. Nesse estudo, o sorbitol apresentou desempenho superior ao observado para xilitol, eritritol, glicerol e etilenoglicol (Matsumoto et al., 1997).

O glicerol é uma das menores e mais simples moléculas da classe dos polióis, além de ser um dos agentes mais utilizados na estabilização de enzimas, devido à sua alta compatibilidade osmótica, baixa toxicidade e capacidade de modular o microambiente proteico (Haque et al., 2005). Essa molécula induz a compactação de proteínas, reduz sua flexibilidade, estabiliza intermediários parcialmente desdobrados e interfere na agregação de proteínas nativas e não nativas (Back et al., 1979; Vagenende et al., 2009).

Sua ação estabilizante está relacionada principalmente ao efeito de exclusão preferencial, pelo qual as moléculas de glicerol são repelidas da superfície proteica, favorecendo a manutenção da conformação nativa (Figura 10B) (Vagenende et al., 2009). Esse comportamento contribui para a redução da entropia conformacional e dificulta o desenovelamento parcial da enzima. Além disso, o glicerol interage com a

água do meio, diminuindo a atividade do solvente e prevenindo a exposição de regiões hidrofóbicas e a formação de agregados (Matsumoto et al., 1997).

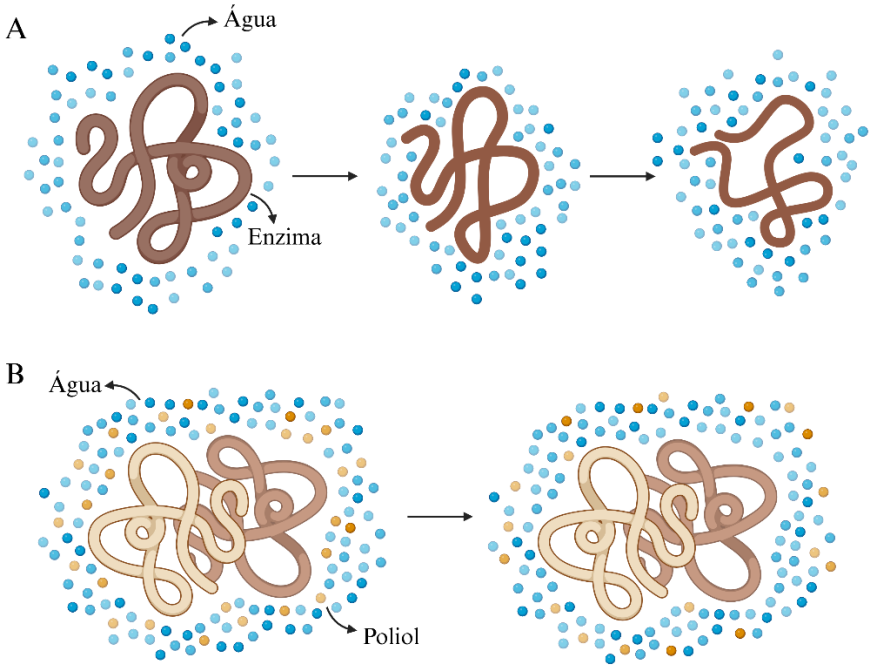

Figura 10: Representação da estabilidade enzimática em diferentes ambientes. (A) Desnaturação de uma enzima em solução aquosa, resultando na perda de sua estrutura nativa. (B) Efeito estabilizador promovido pela presença de um poliol, que auxilia na manutenção da conformação enzimática. Imagem adaptada de Matsumoto et al. (1997).

A exposição da lacase a temperaturas elevadas pode promover a liberação de íons cobre presentes nos centros catalíticos, resultando não apenas na perda de atividade enzimática, mas também no provável desacoplamento estrutural entre domínios proteicos dependentes desse metal (Koroleva et al., 2001). Essa depleção, entretanto, pode ser reversível, desde que haja reposição adequada dos íons ou condições favoráveis à reincorporação (Koroleva et al., 2001; Stepanova et al., 2003). Nesse contexto, o uso de agentes termoprotetores como o glicerol pode atenuar esse processo, uma vez que esse poliol contribui para a manutenção da conformação nativa da proteína e reduz a desnaturação térmica (Vagenende et al., 2009). Dessa forma, preserva indiretamente a estabilidade dos centros metálicos e favorece a recuperação funcional da enzima após o estresse térmico (Back et al., 1979; Haque et al., 2005).

As lacases obtidas de *Fomes sclerodermeus* apresentaram maior estabilidade em baixas concentrações de glicerol (0,2%) e, quando esse poliol foi associado ao

sulfato de cobre ($CuSO_4$), o tempo de meia-vida a 40 ºC aumentou aproximadamente três vezes (Papinutti et al., 2008). De forma semelhante, a combinação de glicerol e betaína quadruplicou a estabilidade térmica (70 ºC) da lacase POXA1b de *Pleurotus ostreatus* e de outras cinco variantes mutantes (Varriale et al., 2022). Estratégias análogas de estabilização também mostraram melhorar significativamente a termoestabilidade de lacases de *Trametes versicolor*, reforçando o potencial dessas abordagens para aplicações em condições térmicas desafiadoras (Delorme et al., 2020).

Esse comportamento protetor foi igualmente observado em outras enzimas. Costa et al. (2002) observaram que, ao submeter catalase a 60 °C, a adição de glicerol resultou em um aumento de 31% na atividade enzimática em comparação ao controle, reforçando sua eficácia contra a inativação térmica. Back et al. (1979) demonstraram que a temperatura de perda da atividade da lisozima aumentou de 66,5 ºC para 75 ºC quando se utilizou 50% de glicerol como estabilizante. Para essa mesma enzima, Chen et al. (2019) verificaram que concentrações crescentes de glicerol (0–80%) promoveram aumento proporcional da termoestabilidade.

O glicerol também vem sendo avaliado como aditivo para estabilização de enzimas imobilizadas, com resultados heterogêneos entre diferentes biocatalisadores e condições reacionais (Braham et al., 2021; Cheetham, 1984). Estudo com cinco lipases, três proteases, uma lacase, uma catalase e uma penicilina acilase demonstou que o glicerol não apresenta efeito protetor universal. Em determinadas condições, inclusive certo grau de desestabilização da enzima imobilizada foi observado. Além disso, o impacto do glicerol foi altamente dependente da concentração, e nem sempre as maiores concentrações resultaram nas melhores respostas, bem como das condições de inativação, especialmente o pH. Em pH 9, por exemplo, registraram-se os efeitos desestabilizantes mais pronunciados, evidenciando que sua aplicação requer otimização caso a caso (Braham et al., 2021).

Dessa forma, a aplicação do glicerol como estabilizante químico em lacases é promissora, mas não absoluta (Chen et al., 2019). A literatura evidencia uma ampla variação nas concentrações testadas e forte dependência da origem enzimática e das condições reacionais (Braham et al., 2021). Além disso, fatores como pH e temperatura de armazenamento podem modular significativamente os efeitos observados (Varriale et al., 2022). Isso reforça a necessidade de delineamentos experimentais que integrem múltiplas variáveis para definir combinações otimizadas e tecnicamente viáveis para cada isoforma ou extrato enzimático.

2.3.2.2 Estabilização por imobilização enzimática

A imobilização enzimática possui uma trajetória centenária. A primeira tentativa documentada ocorreu em 1916, quando Nelson e Griffin demonstraram que a enzima invertase podia ser adsorvida em carvão, mantendo sua atividade mesmo após a liberação do adsorvente (Griffin e Nelson, 1916). Esse marco inicial impulsionou o desenvolvimento contínuo de diferentes métodos ao longo das décadas (Vitolo et al., 2015). A imobilização é reconhecida como uma das estratégias mais promissoras para aplicação de enzimas em escala industrial, uma vez que permite sua recuperação e reutilização, favorecendo a sustentabilidade e a viabilidade econômica dos processos biotecnológicos (Sheldon e van Pelt, 2013).

A imobilização das lacases consiste em reter a enzima em uma superfície ou suporte sólido, de forma que ela permaneça cataliticamente ativa e possa ser facilmente recuperada do meio reacional (Gonçalves et al., 2019). Comparadas às lacases livres, as lacases imobilizadas frequentemente apresentam maior estabilidade operacional, melhor atividade catalítica em condições adversas de pH e temperatura e maior tolerância à presença de inibidores, como sais e solventes orgânicos, além de resistirem melhor a longos períodos de armazenamento(Yang et al., 2017). Outro aspecto relevante é a possibilidade de reutilização do biocatalisador em múltiplos ciclos reacionais, o que pode contribuir para a redução dos custos globais (Gonçalves et al., 2019).

As lacases imobilizadas têm sido amplamente estudadas para aplicações em diferentes setores industriais, com destaque para os setores alimentício, farmacêutico e ambiental (Ren et al., 2020). O uso de lacase não é permitido como aditivo alimentar, portanto, é usada na forma imobilizada para remover fenólicos e polifenóis específicos, garantindo sua reutilização e eliminação (Lettera et al., 2016; Servili et al., 2000). Além disso, a imobilização é fundamental em tecnologias de alta especificidade, como no desenvolvimento de biossensores à base de lacase para a detecção de compostos fenólicos, azidas, alcaloides e flavonoides (Patel et al., 2018). Por outro lado, no campo da biorremediação, a imobilização pode representar tanto vantagens quanto limitações, dependendo da matriz ambiental envolvida (ex.: água, solo) e da estratégia

complementar adotada (ex.: acoplamento a tratamentos convencionais de águas residuárias) (Zhou et al., 2021).

Os solos contaminados e os efluentes possuem composição complexa e podem apresentar características físico-químicas adversas à catálise por lacases na forma livre (Datta et al., 2021). A imobilização enzimática em diferentes suportes surge como uma estratégia promissora para contornar essas limitações, proporcionando biocatalisadores mais estáveis e adequados para aplicações de longo prazo (Gianfreda et al., 1999). Diversos métodos de imobilização têm sido empregados com as lacases (Figura 11), destacando-se a adsorção, ligação covalente, aprisionamento em polímeros, reticulação e encapsulação (Aghaee et al., 2024).

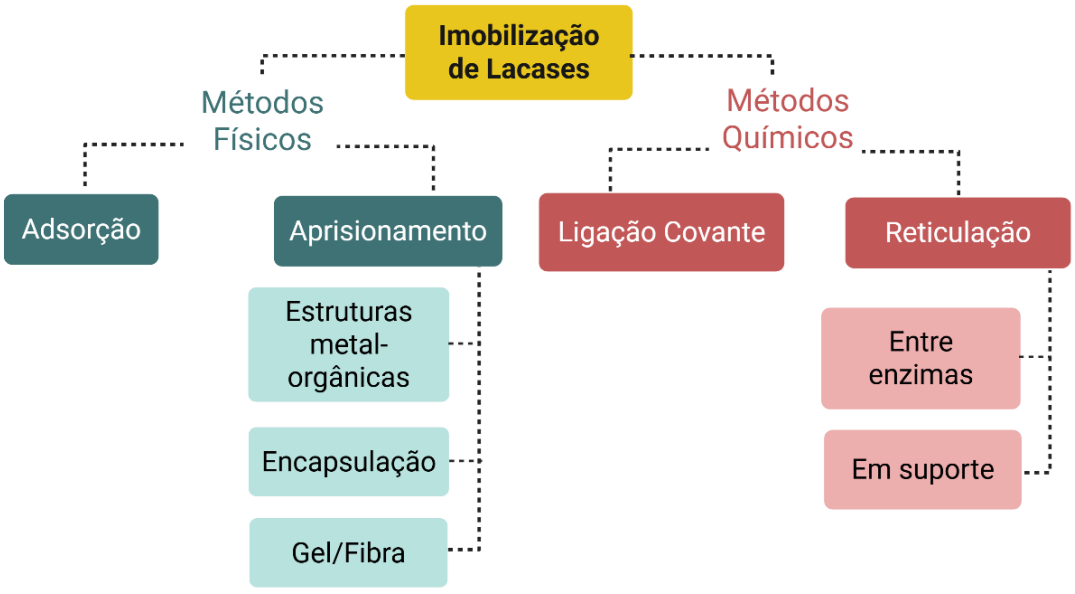

Figura 11: Estratégias comumente empregadas para imobilização de lacases. Imagem adaptada de Daronch et al. (2020).

A imobilização enzimática pode ser realizada por métodos físicos ou químicos, cada um com vantagens e limitações específicas (Aghaee et al., 2024). Nos métodos físicos ocorrem interações não específicas, por meio de ligações de hidrogênio, interações iônicas e hidrofóbicas. Os métodos físicos incluem aprisionamento e adsorção, e não há necessidade de funcionalização do suporte (Lonappan et al., 2018a). A adsorção é uma técnica simples e de baixo custo, porém nela a enzima está sujeita à dessorção em condições adversas (Jesionowski et al., 2014). O aprisionamento envolve

a retenção da enzima em uma matriz polimérica porosa ou o confinamento da enzima em microcápsulas, permitindo o acesso do substrato ao sítio ativo e protegendo a biomolécula contra desnaturação, mas pode apresentar difusão limitada (Aghaee et al., 2024). Os métodos de imobilização química incluem a ligação da enzima à matriz por ligações covalentes. A criação de fortes ligações químicas resulta em maior resistência à lixiviação, embora possa comprometer parcialmente a atividade catalítica devido a modificações estruturais (Lonappan et al., 2018b). As enzimas também podem ser reticuladas (*cross-linking*) entre si ou em um suporte criando um agregado enzimático reticulado, ou criando um cristal enzimático reticulado (Datta et al., 2013). A seleção da técnica mais adequada depende, portanto, das características da enzima, do suporte disponível, da aplicação pretendida e das condições operacionais do processo (Daronch et al., 2020; Gonçalves et al., 2019).

Embora diversos métodos de imobilização enzimática tenham sido amplamente descritos em patentes e publicações científicas, relativamente poucos processos utilizando enzimas imobilizadas alcançaram aplicação comercial bem-sucedida (Aghaee et al., 2024). Em muitos casos, o custo das enzimas industriais representa apenas uma fração secundária na economia global de um processo, de modo que os gastos adicionais associados à imobilização nem sempre se justificam. Na prática, a adoção dessa estratégia costuma estar mais relacionada às vantagens operacionais proporcionadas pelo biocatalisador imobilizado, tais como a viabilização de sistemas de produção contínua, o aumento da estabilidade operacional e a prevenção da presença da enzima livre no produto final (Di Cosimo et al., 2013). Dessa forma, a investigação contínua nesta área é importante para superar limitações atuais e ampliar o potencial de aplicação das lacases imobilizadas.

2.3.2.3 Estabilização por liofilização

A liofilização, também chamada de secagem por congelamento, é uma técnica de desidratação baseada na remoção de água por sublimação sob pressão reduzida. O processo envolve o congelamento da amostra, seguido da sublimação do gelo diretamente para a fase gasosa, sem passar pelo estado líquido, o que evita danos estruturais às moléculas sensíveis (Rey e May, 2023). A técnica compreende três estágios principais: o congelamento (solidificação), a secagem primária (sublimação do gelo) e a secagem secundária, na qual ocorre a dessorção da umidade remanescente

(Bjelošević et al., 2018). Esse método é especialmente indicado para preservar compostos instáveis em meio aquoso, como microrganismos, sistemas nanoparticulados, ácidos nucleicos e proteínas (Abla e Mehanna, 2022).

Entre as principais vantagens do método estão o aumento da vida útil, a manutenção da atividade biológica e a facilidade de armazenamento e transporte em temperatura ambiente (Ó'Fágáin e Colliton, 2023). Essas características fazem da liofilização uma das principais estratégias de estabilização de produtos farmacêuticos que exigem condições específicas de transporte e cadeia de frio (Abla e Mehanna, 2022). Na última década, aproximadamente 50% dos produtos biofarmacêuticos aprovados foram disponibilizados em formas farmacêuticas liofilizadas e, com o desenvolvimento de moléculas terapêuticas complexas baseadas em estruturas proteicas (Bjelošević et al., 2018).

A remoção da água por este método minimiza processos de degradação química, oxidação e autólise, além de permitir a reconstituição rápida por adição de solvente (Carpenter et al., 1992). Esse efeito é particularmente relevante para enzimas como as lacases, que geralmente apresentam estabilidade limitada em soluções aquosas e podem sofrer inativação progressiva por hidrólise, ação de proteases residuais ou oxidação dos centros metálicos (Koroleva et al., 2001).

No entanto, a liofilização também apresenta limitações importantes. A formação de cristais de gelo pode causar desnaturação, colapso estrutural ou agregação proteica, e alterações no microambiente durante a sublimação podem comprometer a atividade final (Ó'Fágáin e Colliton, 2023). Além dos danos mecânicos e conformacionais decorrentes do congelamento, há impactos significativos na estabilidade eletrostática e na organização interna das biomoléculas. Proteínas em solução são altamente hidratadas e dependem da interação com moléculas de água para manter seu dobramento nativo e a distribuição de cargas na superfície (Nelson e Cox, 2011). Durante a remoção da água, ocorre a neutralização progressiva de grupos ionizados, uma vez que, em ambiente pobre em água, há transferência de prótons para grupos carboxila desprotonados, reduzindo as cargas negativas disponíveis e favorecendo rearranjos estruturais indesejáveis (Wang, 2000). Para minimizar esses efeitos, é comum o uso de excipientes ou aditivos, como açúcares (trealose, sacarose), polióis (glicerol, manitol), aminoácidos e polímeros, que atuam como agentes vitrificantes e estabilizantes estruturais (Carpenter et al., 1992; Milosavić et al., 2017; Wang, 2000).

A liofilização tem sido utilizada como estratégia para a preservação da atividade de lacases fúngicas durante o armazenamento (Cheute et al., 2025; Cordi et al., 2007; Rodriguez-Rodriguez et al., 2011; Varriale et al., 2022). As lacases de *Agaricus bisporus* e *Trametes versicolor*, por exemplo, são comercializadas na forma de pó liofilizado (Sigma-Aldrich). Entretanto, os estudos disponíveis apresentam resultados divergentes, o que evidencia a necessidade de delineamentos experimentais específicos para avaliar cada caso.

Cantele et al. (2017) verificaram que os extratos enzimáticos de *Marasmiellus palmivorus* mantiveram sua atividade após a liofilização, indicando a viabilidade de utilização desta técnica para fins comerciais do extrato. Bou-Mitri e Kermasha (2018) observaram que a estabilidade da lacase no extrato enzimático de *Coriolus hirsutus* foi significativamente aumentada na presença de manitol durante a liofilização, apresentando 96,2%, 38,9% e 24,7% de atividade residual após quatro semanas de armazenamento a -80 °C, 4 °C e 25 °C, respectivamente. Stepanova et al. (2003) verificaram que as lacases dos fungos *C. hirsutus* e *Coriolus zonatus* apresentaram redução de 44% e 48% na atividade enzimática quando submetidas à liofilização. Entretanto, o uso de aditivos proporcionou até 95% de preservação da atividade para *C. hirsutus* com dextrana a 0,5% e até 102% da atividade inicial para a lacase de *C. zonatus* liofilizada na presença de ácido poliacrílico a 1%. Okazaki et al. (2000) relataram que o complexo lacase–surfactante catalisou efetivamente reações de oxidação em diversos solventes orgânicos, enquanto as lacases liofilizadas a partir de solução tampão aquosa não apresentaram atividade catalítica. De forma semelhante, Shleev et al. (2007) investigaram os padrões eletroquímicos e cinéticos de lacases liofilizadas de *Cerrena unicolor* e demonstraram que a forma liofilizada apresentou menor atividade específica e estabilidade a longo prazo, concluindo que as lacases não liofilizadas constituem uma fonte enzimática superior às suas versões liofilizadas.

Portanto, a liofilização representa uma alternativa viável para a estabilização de enzimas como as lacases, desde que associada à seleção adequada das condições de congelamento e dos agentes protetores (Bou-Mitri e Kermasha, 2018). O uso apropriado dessa técnica pode superar limitações típicas de enzimas em meio aquoso e favorecer o armazenamento prolongado, além de contribuir para o desenvolvimento de formulações promissoras para fins industriais, biotecnológicos e ambientais (Varriale et al., 2022).

2.4 Biorremediação de fármacos

O conceito de biorremediação surgiu na década de 1930, quando Tausz e Donath demonstraram o uso de microrganismos no tratamento de solos contaminados com derivados de petróleo (Tausz e Donath, 1930). Desde então, a biorremediação tem se expandido, sendo cada vez mais aplicada como uma tecnologia sustentável e ecologicamente adequada para a descontaminação de diversos poluentes orgânicos e inorgânicos (Bhatt et al., 2023). Consiste em uma ampla gama de processos, utilizando plantas, microrganismos e/ou enzimas específicas (Figura 12) para reduzir ou eliminar poluentes em solos, sedimentos e águas, transformando-os em substâncias de baixa toxicidade ou inofensivas (Inamuddin, 2023).

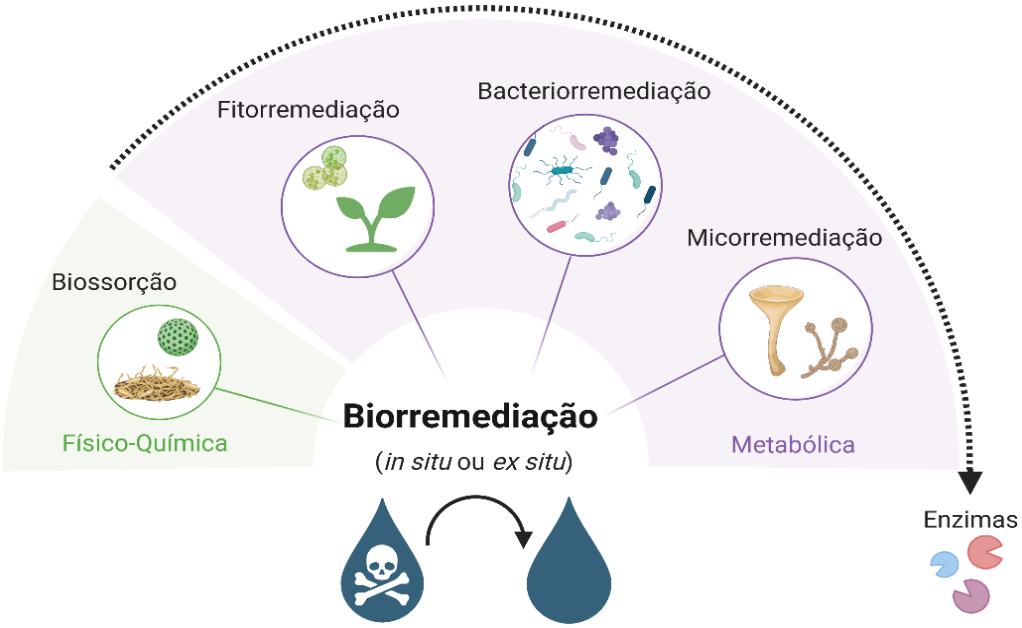

Figura 12: Visão geral das principais estratégias de biorremediação, que podem ocorrer *in situ* ou *ex situ*. A biossorção representa um processo físico-químico baseado na adsorção de contaminantes por biomassa inativa, enquanto as abordagens metabólicas incluem a fitorremediação (uso de plantas), a bacteriorremediação (uso de bactérias) e a micorremediação (uso de fungos). Nestas últimas, enzimas extracelulares desempenham papel fundamental na degradação e/ou transformação de poluentes. Imagem elaborada pela autora.

A biorremediação pode ser aplicada *in situ*, isto é, diretamente no local contaminado, onde os agentes selecionados — como fungos e plantas — são introduzidos com o objetivo de degradar os poluentes no próprio ambiente afetado. Alternativamente, pode-se empregar a técnica *ex situ*, que consiste na remoção do material contaminado para um ambiente controlado, no qual será realizado o processo de tratamento (Dinakarkumar et al., 2024).

Nas últimas duas décadas, o foco da pesquisa em biorremediação tem gradualmente sido direcionado dos métodos microbianos tradicionais para a biorremediação enzimática, considerada mais rápida e eficaz (Singh et al., 2024). A biorremediação que utiliza extratos enzimáticos, sejam eles brutos ou parcialmente purificados, não requer que o microrganismo se desenvolva diretamente no local contaminado. Em vez disso, ela se baseia na ação catalítica das enzimas que esses microrganismos produzem, o que representa uma vantagem, especialmente em solos com poucos nutrientes. Outro ponto positivo é que a biotransformação mediada por enzimas pode resultar na formação de subprodutos atóxicos, diferentemente do que pode ocorrer em processos microbianos, tornando essa abordagem potencialmente mais segura para o meio ambiente (Peralta, 2022). Entre as dez enzimas mais investigadas na biodegradação enzimática, a lacase ocupa posição de destaque, liderando o ranking em relação às peroxidases e dioxigenases (Singh et al., 2024).

Diversos estudos têm demonstrado a atividade das lacases na oxidação de contaminantes emergentes presentes na água (Bilal et al., 2019b; Singh et al., 2025). No caso de fármacos antidepressivos contendo grupos fenólicos ou anéis benzênicos, como paroxetina, fluoxetina e sertralina, foi observada elevada taxa de remoção, cerca de 80% após 4h de reação, utilizando as lacases produzidas em meio de cultivo de *Pleurotus ostreatus* (Kózka et al., 2020). Embora este fungo seja raramente empregado na degradação de fármacos em meio líquido, ele tem sido utilizado com sucesso na remoção de desreguladores endócrinos, como 17α-etinilestradiol, p-n-nonilfenol, bisfenol A, estrona e triclosan (Nguyen et al., 2013).

O gênero *Trametes* destaca-se por sua elevada capacidade de secreção de lacases, conferindo-lhe um papel relevante nos processos de degradação da lignina e de compostos fenólicos. Essa característica torna-o promissor para diversas aplicações biotecnológicas, como biorremediação, branqueamento de papel e síntese de compostos bioativos (Sultan K. Alharbi et al., 2019; Brugnari et al., 2021). As lacases isoladas do cultivo de *Trametes hirsuta* demonstraram capacidade significativa na degradação de

compostos farmacêuticos, alcançando até 80% de remoção de 17α-etinilestradiol na primeira hora de reação e aproximadamente 40% de degradação de diclofenaco após 20 horas de tratamento (El Yagoubi et al., 2023). O cloranfenicol, antibiótico inibidor da síntese proteica bacteriana comumente detectado em resíduos farmacêuticos, foi degradado por lacases de *T. hirsuta*, atingindo 100% de remoção após sete dias. Esse desempenho foi significativamente acelerado com a adição de mediadores redox, como vanilina e siringaldeído, permitindo obter o mesmo percentual de degradação em apenas 48 horas de reação (Navada e Kulal, 2019).

As lacases obtidas do cultivo de *Trametes versicolor* (ATCC 20869) demonstraram capacidade de degradar o fármaco carbamazepina, sendo que a imobilização da enzima em aerogéis de poliimida modificada ampliou sua estabilidade térmica. Nesse sistema, a enzima foi capaz de biotransformar 65% da carbamazepina mesmo após sete ciclos de reutilização (Simón-Herrero et al., 2019). Além disso, as lacases desta espécie mostraram-se eficientes na remoção de betabloqueadores, como labetalol e atenolol, especialmente quando associadas a mediadores (ABTS e TEMPO) ou a nanomateriais, como compósitos de lacase-grafeno. Esses sistemas aceleram a taxa de transformação, alcançando a remoção completa em menos de 24h de reação, além de contribuir para a redução da toxicidade dos produtos degradados (Dong et al., 2019; Feng et al., 2019).

A biorremediação por lacases aplica-se também a matrizes sólidas, como o lodo proveniente de estações de tratamento de esgoto. Em estudo conduzido por Rodriguez-Rodriguez et al. (2011), amostras de lodo submetidas à biorremediação por *T. versicolor* (ATCC 4253) demonstraram que as enzimas extracelulares produzidas pelo fungo degradaram 43% da concentração inicial de carbamazepina e diazepam, atingindo 100% de remoção para fenofibrato, benzofibrato, cimetidina, claritromicina e atenolol.

As lacases de *Pycnoporus sanguineus* também apresentam potencial significativo para a biorremediação de fármacos persistentes e tóxicos em ambientes aquáticos (Cheute et al., 2024). Efluentes provenientes da indústria farmacêutica tratados com *P. sanguineus* apresentaram redução significativa no conteúdo de compostos fenólicos totais e redução da genotoxicidade (Maroneze et al., 2012). Outro estudo demonstrou alta eficiência na remoção de antibióticos amplamente utilizados, como ciprofloxacino, norfloxacino e sulfametoxazol, com taxas de remoção de 98,5%, 96,4% e 100%, respectivamente, após 48 horas de reação. Quando aplicados

concomitantemente, os três fármacos foram eliminados completamente no mesmo período (Gao et al., 2018a).

Ainda há muito a ser explorado sobre o potencial biotecnológico de diferentes espécies fúngicas, como *Agaricus blazei* e *Marasmiellus palmivorus*, reconhecidas produtoras de lacases (Schneider et al., 2018; Ullrich et al., 2005). Cantele et al. (2017) demostraram que quanto maior for a atividade enzimática de lacases obtida de *M. palmivorus* utilizada na descoloração dos corantes Azul Reativo 220 e Verde Ácido 28, menor o tempo necessário para remover 90% e 75% da cor, respectivamente. Silveira et al. (2020) verificaram que a reação de descoloração do corante laranja de metila utilizando ABTS como mediador para as lacases de *M. palmivorus* permitiu 30 ciclos de reutilização com um percentual de descoloração acima de 60%.

Observa-se, portanto, que as pesquisas envolvendo as lacases e suas aplicações na biorremediação têm apresentado diversos exemplos de sucesso (Chmelová et al., 2024). Nesse contexto, o investimento contínuo em recursos para o desenvolvimento de tecnologias baseadas nessa enzima revela-se essencial para o enfrentamento dos desafios causados pelos poluentes emergentes.

3. OBJETIVOS

3.1 Objetivo geral

Mapear as tendências globais de pesquisa sobre as lacases fúngicas aplicadas à biorremediação de fármacos e avaliar o potencial de extratos enzimáticos contendo lacases fúngicas na biorremediação de paracetamol e diclofenaco.

3.2 Objetivos específicos

- Analisar o panorama da produção científica relacionada à aplicação das lacases fúngicas na biorremediação de fármacos;
- Caracterizar o cultivo submerso dos fungos *Agaricus blazei*, *Marasmiellus palmivorus* VE111, *Pycnoporus sanguineus* 14G e *Trametes* sp. 50/90 em relação à produção de lacases, pH, desenvolvimento micelial, concentração de proteínas e consumo de carboidratos;
- Determinar a atividade de lacases nos extratos enzimáticos obtidos e verificar a influencia da liofilização, do glicerol e das condições de armazenamento na estabilidade enzimática;
- Avaliar a eficiência de biorremediação dos fármacos diclofenaco e paracetamol promovida pelos extratos enzimáticos em diferentes condições de concentração e tempo de reação.

4. RESULTADOS E DISCUSSÃO

Os resultados e discussão desta tese são apresentados em dois capítulos, redigidos na forma de artigos científicos:

Capítulo 1: Global research trends and future perspectives on fungal laccases for pharmaceutical bioremediation

Este capítulo apresenta um estudo cienciométrico que mapeia as tendências globais da pesquisa envolvendo as lacases fúngicas, com ênfase em sua aplicação na biorremediação de fármacos. São discutidos os avanços científicos, as redes de colaboração entre autores e instituições, além das principais temáticas emergentes que conectam a biotecnologia de lacases aos desafios ambientais atuais.

Capítulo 2: Fungal laccase crude extracts: production and pharmaceutical bioremediation

Este capítulo reúne os resultados experimentais obtidos a partir do cultivo submerso de diferentes espécies fúngicas e da caracterização dos extratos enzimáticos produzidos. São apresentadas as análises relacionadas à produção de lacases, estabilidade enzimática e capacidade de biotransformação de fármacos selecionados, destacando o potencial desses extratos brutos como estratégia para a biorremediação de contaminantes farmacêuticos. Este conjunto de resultados foi aceito para publicação como artigo científico na revista *Environmental Technology* (https://doi.org/10.1080/09593330.2025.2604793).

4.1 Capítulo 1: Global research trends and future perspectives on fungal laccases for pharmaceutical bioremediation

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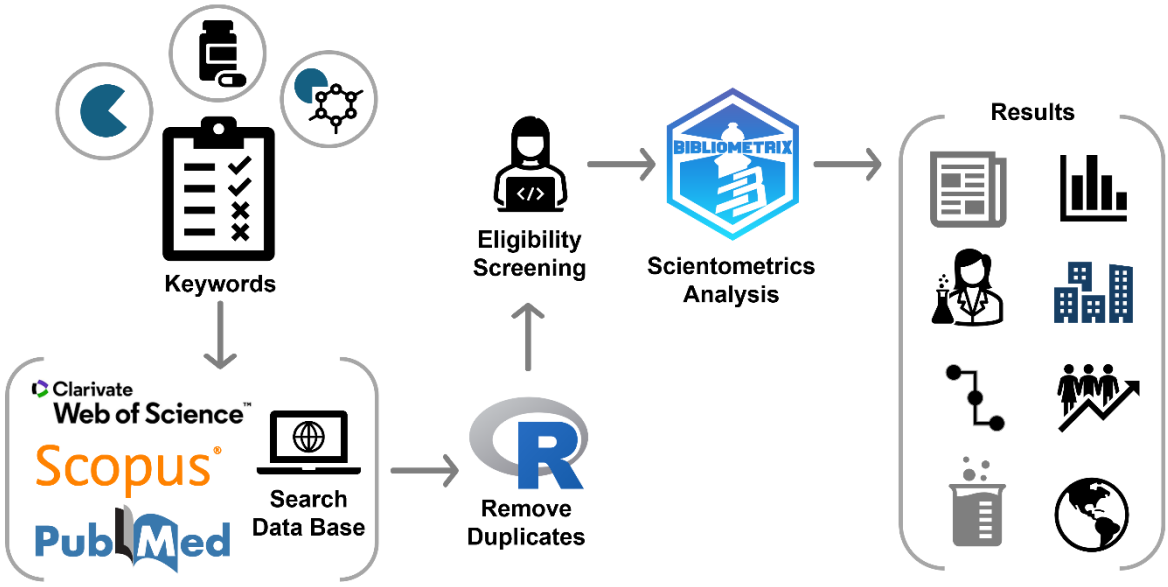
Graphical Abstract



Abstract

The benefits that pharmaceuticals provide to human and animal health are undeniable, as is their widespread presence in the environment. This concern has prompted the scientific community to pursue sustainable strategies for bioremediation, with fungal laccases emerging as promising biocatalysts. This study presents a scientometric analysis of global research trends on the application of fungal laccases in pharmaceutical bioremediation. A comprehensive search was conducted in PubMed, Scopus, and Web of Science databases, covering the period from 2008 to 2024, yielding 182 eligible publications. The findings reveal an expanding research field, with an annual growth rate of 18.92%. The main subject categories were Environmental

Sciences (29%) and Environmental Engineering (17%). The most prolific authors, Hai F., Nghiem L., and Price W., are affiliated with the University of Wollongong, which leads in academic productivity with 26% of publications. Bradford's Law analysis identified Chemosphere, Bioresource Technology, and Environmental Science and Pollution Research as the core journals, with Bioresource Technology being the most cited (960 citations). Keyword mapping highlighted research hotspots, including immobilization, wastewater, and degradation. Hotspot related to pharmaceuticals such as diclofenac, estrogens, tetracycline, and carbamazepine also were distinguished. No keywords related to solid environmental matrices were identified. Australia, Canada, and China lead in publication output and collaboration, although international networking can be expanded. These results identify emerging trends and research gaps, providing valuable insights for future investigations and the development of laccase-based, sustainable solutions for pharmaceutical pollution mitigation.

Keywords: enzymes; bioresource; sustainability; pollutants; scientometric; wastewater

1. Introduction

The continuous discharge of pharmaceutical compounds into natural environments has become a critical global concern. This issue has far-reaching ecological and public health implications (Patel et al. 2019; Sharma et al. 2021; Vaudin et al. 2022).

Commonly prescribed pharmaceuticals for both humans and animals such as antibiotics, antifungals, anticonvulsants, anti-inflammatories, psychoactive substances, hormones, β-blockers, antiretrovirals, and anticancer agents are often found in various environmental matrices, including surface waters, soils, and sludge (Tarcomnicu et al. 2011; Guerra et al. 2014; Ortúzar et al. 2022; Silori et al. 2022; Estrada-Almeida et al. 2024). These substances can reach the environment from homes, hospital effluents, and pharmaceutical manufacturing (Patel et al. 2019; Bouabadi et al. 2024).

Pharmaceuticals have physicochemical characteristics (e.g., molecular weight, pKa, logP) that contribute to recalcitrant behavior and resistance to conventional methods for addressing pollution management (Wang et al. 2020; Ortúzar et al. 2022). As a result, their accumulation in aquatic and terrestrial ecosystems has been strongly associated with ecotoxicological effects such as microbial resistance, endocrine

disruption, and trophic imbalances (Ortúzar et al. 2022; Vaudin et al. 2022; dos Santos et al. 2024; Estrada-Almeida et al. 2024).

Ecologically sustainable and economically viable remediation strategies are currently being actively researched (Patel et al. 2019). Among the most promising are biocatalytic systems based on oxidative enzymes (e.g., laccase, peroxidase, dioxygenase), which offer effective and environmentally friendly alternatives to conventional physicochemical treatments (Estrada-Almeida et al. 2024; Singh et al. 2024; Aghaee et al. 2024).

Fungal laccases (EC 1.10.3.2), a group of multicopper oxidases secreted by white-rot fungi, have emerged as versatile biocatalysts due to their ability to oxidize a wide range of substrates, including ortho- and para-diphenols, phenolic acids, aromatic amines, and other electron-rich compounds (Bourbonnais and Paice 1990; Brugnari et al. 2021; Kyomuhimbo and Brink 2023). Laccases utilize molecular oxygen as a terminal electron acceptor, producing water as the only byproduct. Their operation under mild conditions, without the need for harsh reagents, further increases their appeal for environmental and industrial applications (Brugnari et al. 2021; Khan 2025).

Fungal laccases have been successfully applied in diverse industries, including textile dye decolorization, food processing, biofuel production, lignin degradation in the pulp and paper industry, biosensors, and the synthesis of medical and cosmetic compounds (Couto and Herrera 2006; Upadhyay et al. 2016; Kyomuhimbo and Brink 2023). In environmental biotechnology, they have demonstrated significant potential for the degradation of persistent organic pollutants, such as pharmaceuticals, dyes, pesticides, and endocrine-disrupting chemicals (Couto and Herrera 2006; Nguyen et al. 2014; Thathola et al. 2024). Integration into enzymatic reactors, biofiltration systems, and hybrid technologies reflects a broader shift toward circular economy principles and green waste management solutions (Abejón et al. 2015; Puspita et al. 2023). Recent advances in immobilization techniques and carrier materials (e.g., activated carbon, silica, polymeric matrices) have further enhanced catalytic performance and operational stability (Sheldon and van Pelt 2013; Al-Sareji et al. 2023; Sodhi et al. 2024).

Despite their recognized potential, comprehensive knowledge mapping of fungal laccases specifically for pharmaceutical bioremediation is lacking. While Puspita et al. (2023) analyzed laccase applications in wastewater treatment over four decades, their study did not focus on specific pollutant classes (e.g., pharmaceuticals, pesticides) or differentiate laccases by biological origin (fungal, bacterial, or plant). To

date, no study has systematically mapped global research on fungal laccases applied to pharmaceutical bioremediation.

Given the growing importance of fungal laccases in environmental biotechnology and the need for a clearer understanding of the scientific landscape, the present study provides a comprehensive scientometric analysis of global trends in this field. This analysis examines publication trends, subject categories, authorship patterns and collaboration networks, institutional and geographic distribution, journal dissemination, highly cited documents, and keyword mapping with hotspot clustering. By addressing these dimensions, the study identifies key research trends, knowledge gaps, and emerging opportunities, advancing scientific knowledge and guiding future research toward scalable, efficient, and sustainable fungal laccase-based biocatalytic systems for pharmaceutical pollution mitigation, in alignment with the United Nations Sustainable Development Goals (SDGs) (ONU 2025) and the One Health framework (Wang et al. 2024).

2. Methodology

2.1 Data sources and retrieval strategy

Scientific publications were systematically retrieved from PubMed, Scopus, and Web of Science (WoS), covering the period from the earliest available record to December 31, 2024. The search was performed using the following keyword combination applied across all searchable fields: (Laccase* OR “Fung* Laccase* enzyme*” OR “immobilized laccase” OR “Laccase* enzyme*”) AND (Pharmaceut* OR “organic contaminant*” OR micropollutant*) AND (Bioremediation OR Biotransformation OR Degradation OR Treatment OR Removal OR Transformation OR “Environmental applications”) NOT (Dye* OR Colorant OR oil* OR Pesticid*). Duplicates were removed in R (v4.4.2) (R Core 2023). In accordance with PRISMA guidelines (Page et al. 2021), all retrieved records were screened and assessed for relevance. Review articles, conference papers, brief surveys, book chapters, and publications falling outside the study scope were excluded. The final dataset consisted of 182 peer-reviewed articles, as illustrated in Figure 1.

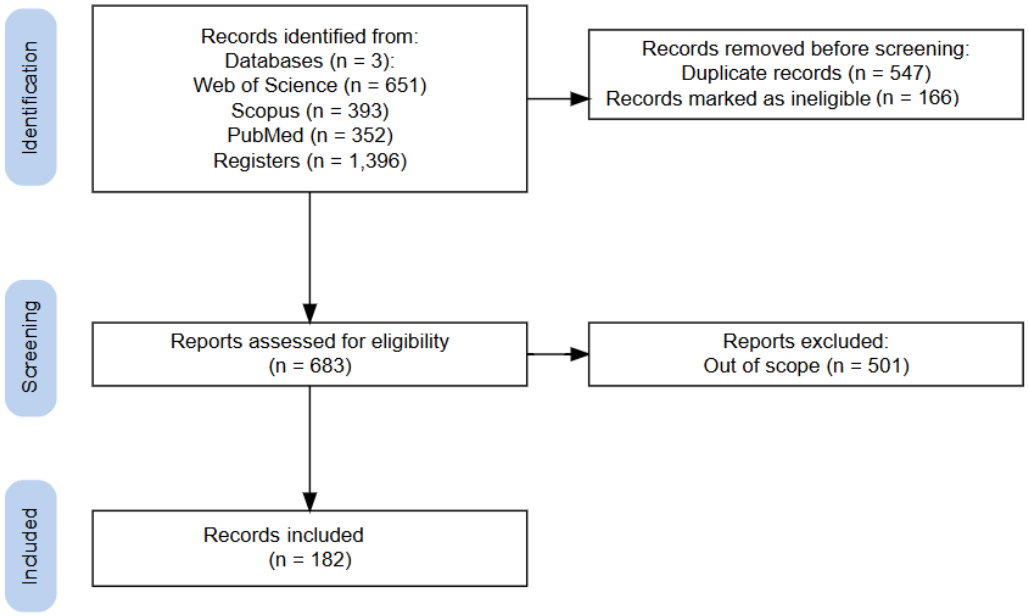


Figure 1. Flowchart used in the scientometric analysis, adapted from the PRISMA 2021 guidelines.

2.2 Scientometric analysis

Descriptive and mapping analyses were conducted using the *Bibliometrix* package (v4.1.3) (Aria and Cuccurullo 2017) within the R environment (v4.4.2) (R Core 2023). The analysis included the number of publications, average annual citation rates, and subject classifications based on WoS categories. Global publication trends were examined with emphasis on the most prolific authors, highly cited documents, influential journals, and leading institutions and countries.

Scientific productivity was assessed using Lotka's Law (Lotka 1926), based on the frequency distribution of authorship. Additionally, the temporal publication performance of the top 10 most productive authors was analyzed. Institutional output was evaluated based on author affiliations.

To identify journal citation patterns, Bradford's Law of Scattering (Bradford 1934) was applied. A Sankey diagram was also constructed to visualize the interconnections among authors, journals, and keywords visualize the knowledge structure of the field and highlight the interactions between authors, core journals, and emerging thematic areas.

For the keyword mapping and thematic focus analyses, no terms were excluded from the dataset. However, a controlled vocabulary was created by grouping synonyms under unified labels to ensure clarity and consistency (Supplementary Table 1). The

methodological parameters for the co-occurrence network included automatic layout generation, the Walktrap clustering algorithm, association strength normalization, a maximum of 50 nodes, exclusion of isolated nodes, a repulsion strength of 0.1, and a minimum of two co-occurrence links per edge.

A global collaboration map complemented analyses of annual publication trends and country productivity, based on article counts and citation metrics, providing insights into the structure, strength, and geographical distribution of international research collaborations.

Graphical representations were generated based on outputs obtained from the *Bibliometrix* package (v4.1.3) and Microsoft *Excel* (v2511).

3. Results and discussion

3.1 Publication output and subject categories

The initial search retrieved 1,396 documents (Figure 1), from which 547 duplicates were removed. An additional 166 documents were excluded (Supplementary Table 2) due to their publication type, including book chapters (n = 20), conference papers (n = 3), retracted publications (n = 1), review articles (n = 140), and short surveys (n = 2). The remaining 683 documents were assessed for matching the scope of the study. Articles focusing exclusively on computational docking protocols, laccases of non-fungal origin or produced via recombinant technologies, fungal bioreactors, or the bioremediation of non-pharmaceutical compounds were considered out of scope and therefore excluded. As a result, 182 publications (Supplementary Table 3) were deemed eligible and included in the scientometric analysis focused on fungal laccase applications in pharmaceutical bioremediation.

The earliest publication in this field dates back to 2008 (Bialk and Pedersen 2008), defining the study period as spanning from 2008 to December 31, 2024. As shown in Figure 2(a), there has been a consistent rise in the number of publications over this period, with an estimated annual growth rate of 18.92%, resulting in an average output of 10.71 articles per year. This output trend reflects the growing recognition of fungal laccases as a valuable bioresource in pharmaceutical bioremediation. Publication activity has intensified since 2013, reaching its highest levels in 2022 and 2023. This shift suggests the emergence and consolidation of specialized research related to the domain that bridges environmental biotechnology, enzymology, and pharmaceutical waste management (Sybuia et al. 2024; Wysokowski et al. 2024).

Selected articles exhibited an average citation count of 35.14 per publication. In 2011, only one study was published within the field; nevertheless, it received 218 citations (Prieto et al. 2011). This high citation count, despite the low publication volume, underscores the pivotal role of seminal studies in influencing subsequent research and driving innovation.

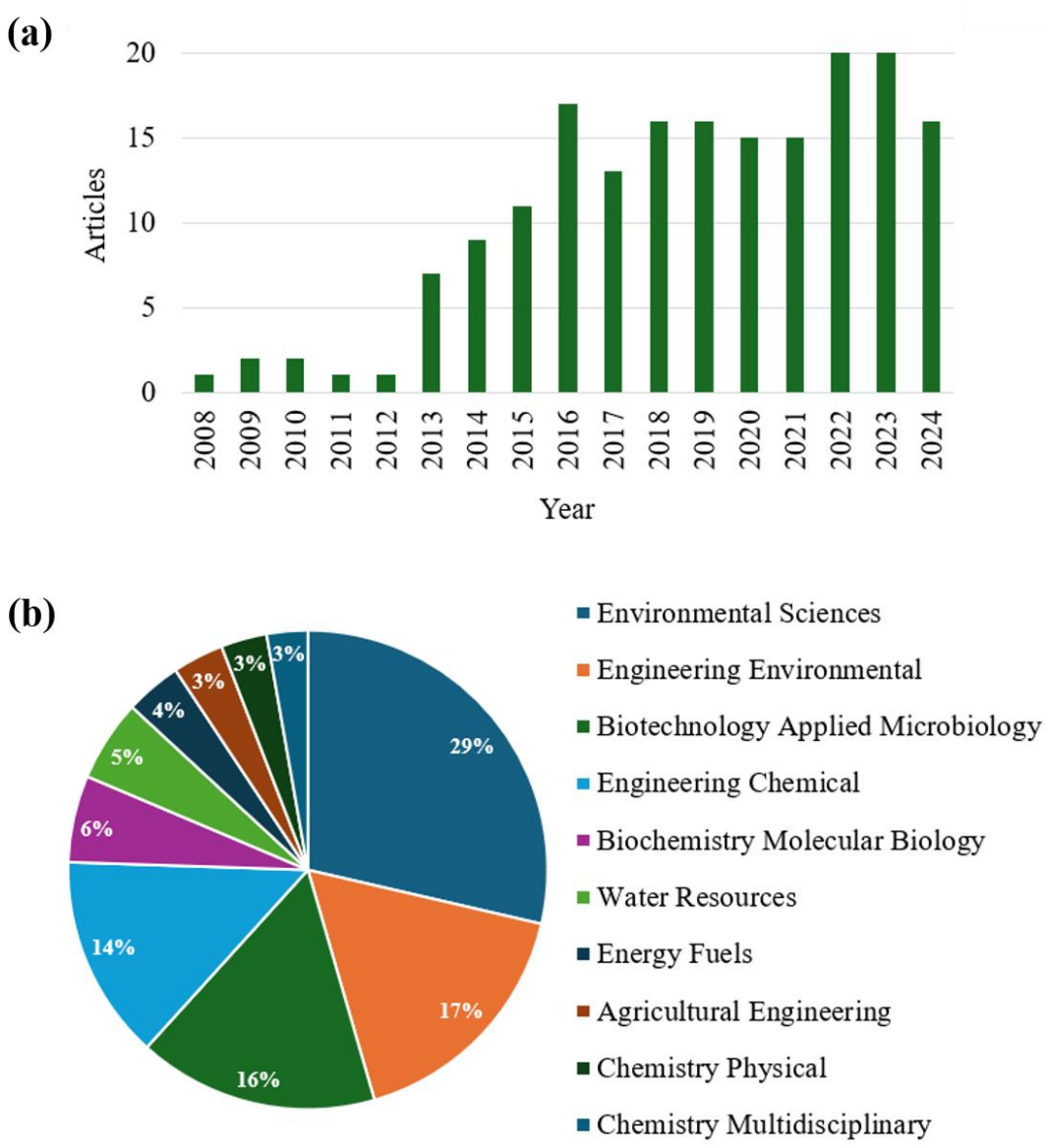


Figure 2. Publications trends on fungal laccases for pharmaceutical bioremediation (2008–2024). **(a)** Annual number of articles (right axis, green bars) and average total citations per article (left axis, orange line). **(b)** Proportional distribution of the top 10 Web of Science subject categories assigned to the retrieved publications.

Based on the WoS categories (Figure 2b), the 182 articles were classified, resulting in 290 category assignments, as many publications were indexed across multiple domains. This finding underscores the highly interdisciplinary nature of research on fungal laccases for pharmaceutical bioremediation. For instance, studies developing crude laccase-based approaches in real wastewater (Maryskova et al. 2022) and groundwater matrices (Rodríguez-delgado et al. 2016) were classified under both "Environmental Sciences" and "Biotechnology & Applied Microbiology" categories. Similarly, articles involving technologies based on immobilized laccase systems, such as biochar (García-Delgado et al. 2018) and PET bottle waste (Kijeńska-Gawrońska et al. 2024) were categorized under "Environmental Engineering" and "Biotechnology & Applied Microbiology".

The subject categories "Environmental Sciences" (29%) and "Environmental Engineering" (17%) emerged as the most represented, reflecting both the ecological drivers of the field and the demand for technological innovations to mitigate pharmaceutical pollutants. While the prominence of the "Biotechnology & Applied Microbiology" category (16%) highlights the practical orientation of research on fungal laccases towards scalable and sustainable solutions for real-world applications.

These findings confirm the recognition of fungal laccases as a promising bioresource for waste bioremediation and ecosystem restoration, in agreement with previous reports (Puspita et al. 2023; Singh et al. 2024). The thematic alignment with pressing environmental challenges reinforces the relevance of this research for advancing multiple SDGs, particularly SDG 6 (Clean Water and Sanitation), SDG 9 (Industry, Innovation, and Infrastructure), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land).

3.2 Authorship trends and collaboration networks

The analyzed literature comprises contributions from 730 authors, with 39.01% of the publications involving international co-authorship. The average number of authors per article was 6.19, and only one article was published by a single author.

Author productivity (Figure 3a) follows Lotka's law, indicating that a small subset of researchers contributes the majority of scientific knowledge in the field of fungal laccases for pharmaceutical bioremediation. Specifically, 78.4%, 11.9%, and 4.0% of authors contributed to one, two, and three articles, respectively, while only 5.7% authored four or more papers (Supplementary Table 4). In fact, the top 10 authors

produced together 119 articles, 65% of all records, showing a high dominance of some authors in the field.

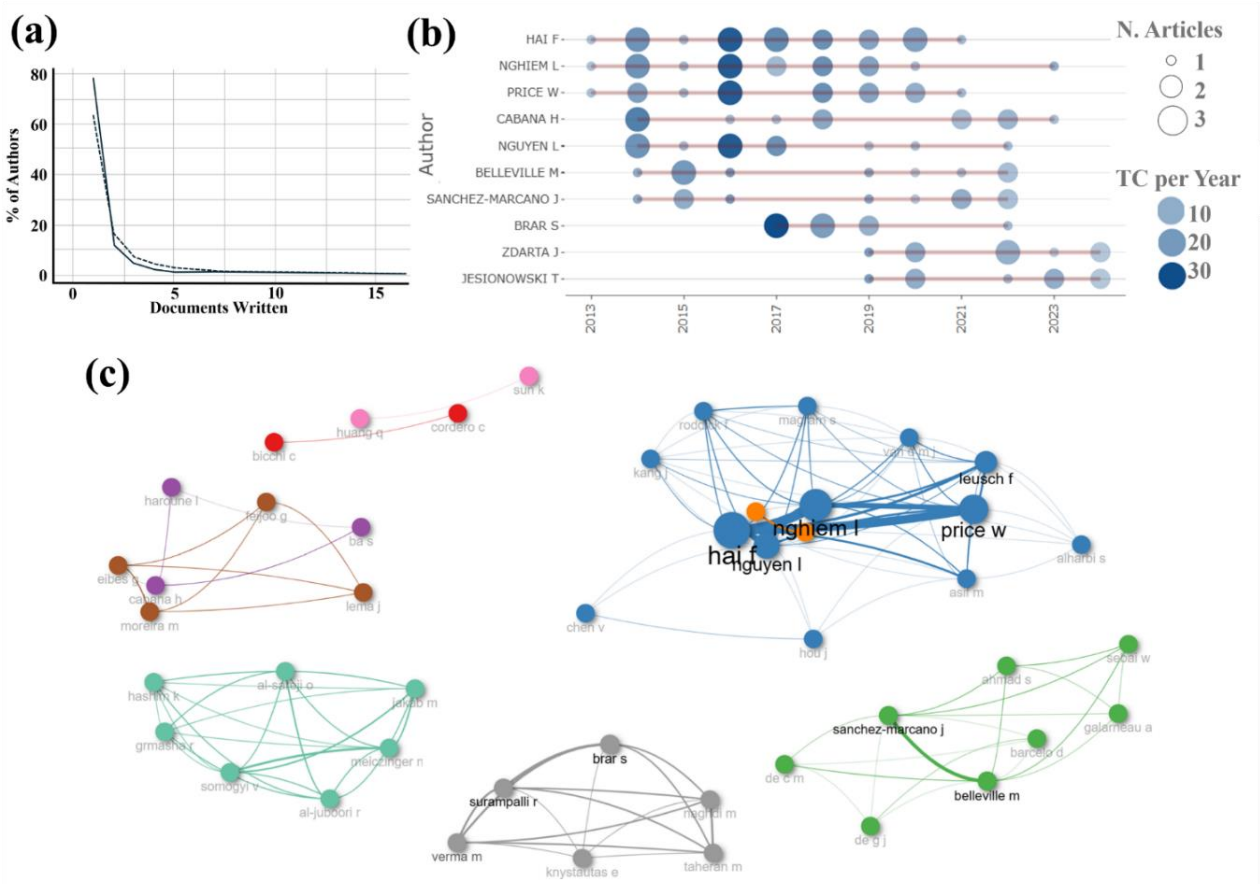


Figure 3. Authorship trends and collaboration networks in research on fungal laccases for pharmaceutical bioremediation. (a) Author productivity distribution based on Lotka's Law; the x-axis represents the number of articles published per author, and the y-axis shows the corresponding percentage of authors. (b) Timeline of the top 10 most prolific authors; bubble size indicates the number of articles published, and color intensity reflects total citations per year. (c) Co-authorship network of the 50 most productive authors. Each node represents an author, with size proportional to publication count and color indicating the cluster. The distance and the thickness of the line connecting nodes indicate the relatedness and strength between authors. The network is divided into nine distinct clusters, each distinguished by a color.

Among the top 10 authors (Figure 3b), Hai F was the most prolific, with 19 articles, followed by Nghiem L with 16 and Price W with 14, all affiliated with the University of Wollongong (Australia). These patterns suggest strong institutional support or access to substantial research funding. Nghiem L has the longest continuous

publication record, whereas Zdarta J and Jesionowski T are more recent contributors, entering the field in 2019 and publishing 9 and 8 articles, respectively, an indicator of the field's ability to attract new, high-output researchers.

To complement the analysis of author productivity, Supplementary Table 5 reports detailed performance metrics, publication count, h-index, g-index, and m-index, providing a broader perspective on scientific impact and influence in the field (Egghe 2006; Hirsch and Buela-Casal 2014). Across all assessed metrics, Hai F consistently ranks highest, underscoring both prolific output and scholarly impact. In contrast, although Nguyen L and Cabana H have each published 12 articles, Nguyen L's work has received approximately 66% more citations, indicating greater scientific resonance and influence.

The co-authorship network analysis among the 50 most productive authors is presented in Figure 3(c). Nodes represent individual authors, while lines denote co-authorship links. The network reveals nine distinct collaboration clusters, highlighting the formation of cohesive research communities with limited cluster connectivity. This pattern suggests that collaborations in the field remain relatively regionalized or confined to specific disciplinary boundaries.

The blue cluster is the largest, comprising 13 authors with centrality scores highlighting Hai F (18,909), Nguyen L (10,669), and Nghiem L (7,335), as pivotal figures fostering connectivity and knowledge dissemination. The orange cluster, formed by Zdarta J and Jesionowski T, acts as a bridge to the main network, indicating emerging collaborations between early-career and established researchers. The green cluster represents the second most prominent group, consisting of 8 authors. It is characterized by strong co-authorship links between Belleville M and Sanchez-Marcano J, with centrality scores of 4,714 and 4,286, respectively. This cluster, along with the third and fourth most productive groups, remains disconnected from the broader network, suggesting the presence of well-structured yet isolated research teams dedicated to specific subfields within the broader domain of fungal laccase research for pharmaceutical bioremediation.

These patterns underscore the need for strategic investment in collaborative scientific networks, particularly in a research field with strong global applicability such as fungal laccase–based bioremediation. Although laccases represent versatile and environmentally benign biocatalysts capable of addressing pharmaceutical contamination across diverse environmental and socio-economic contexts, the observed

fragmentation among leading authors suggests that knowledge generation remains largely compartmentalized (Thathola et al. 2024). Initiatives such as joint research programs, international researcher mobility, and transnational funding schemes could promote stronger interconnections among research groups, fostering more cohesive and resilient scientific communities (Isfandyari-Moghaddam et al. 2023; Borger 2024). Strengthening collaborative networks would not only enhance the global visibility and transferability of fungal laccase research but also facilitate methodological harmonization, cross-validation of results, and the integration of complementary expertise (Borger 2024). Ultimately, such cooperation is essential to accelerate the translation of laboratory-scale findings into scalable, sustainable biotechnological solutions for mitigating pharmaceutical pollution across different environmental matrices.

3.3 Institutional distribution of scientific output

Institutional contributions and collaboration patterns in fungal laccase research for pharmaceutical bioremediation are presented in Figure 4. This analysis reveals that universities from diverse regions are actively contributing to this field, including those in Australia (University of Wollongong), Canada (University of Sherbrooke and University of Quebec), Europe (University of Pannonia, Poznan University of Technology, University of Montpellier, University of Santiago de Compostela), and Asia (University of Tehran Medical Sciences, Nanjing University, University of Babylon).

The University of Wollongong emerges as the leading institution, accounting for ~26% of total publications (Figure 4a) and demonstrating the most consistent and rapid growth, particularly after 2015, remaining significantly ahead through 2024 (Figure 4b). The University of Sherbrooke, initially growing more slowly, showed a marked increase in output from 2017, ranking as the second most productive institution. These trends suggest that top institutions have benefited from increased research funding for environmental restoration, particularly following the formal adoption of the SDGs in 2015.

A collaboration network was constructed to assess interactions among the top 10 most productive institutions (Figure 4c). The resulting map revealed the formation of six distinct clusters. The blue cluster, led by the University of Sherbrooke, includes the University of Santiago de Compostela and Nanjing University, indicating a clear

tendency for international collaboration. The red cluster, which features the University of Wollongong as the most productive institution in the dataset, demonstrates limited international collaboration, with moderate connections to Poznan University of Technology. Similarly, the green cluster highlights strong bilateral collaboration between the University of Pannonia and the University of Babylon. Other institutions appear in isolated clusters, suggesting a lack of direct cooperation with the other top institutions.

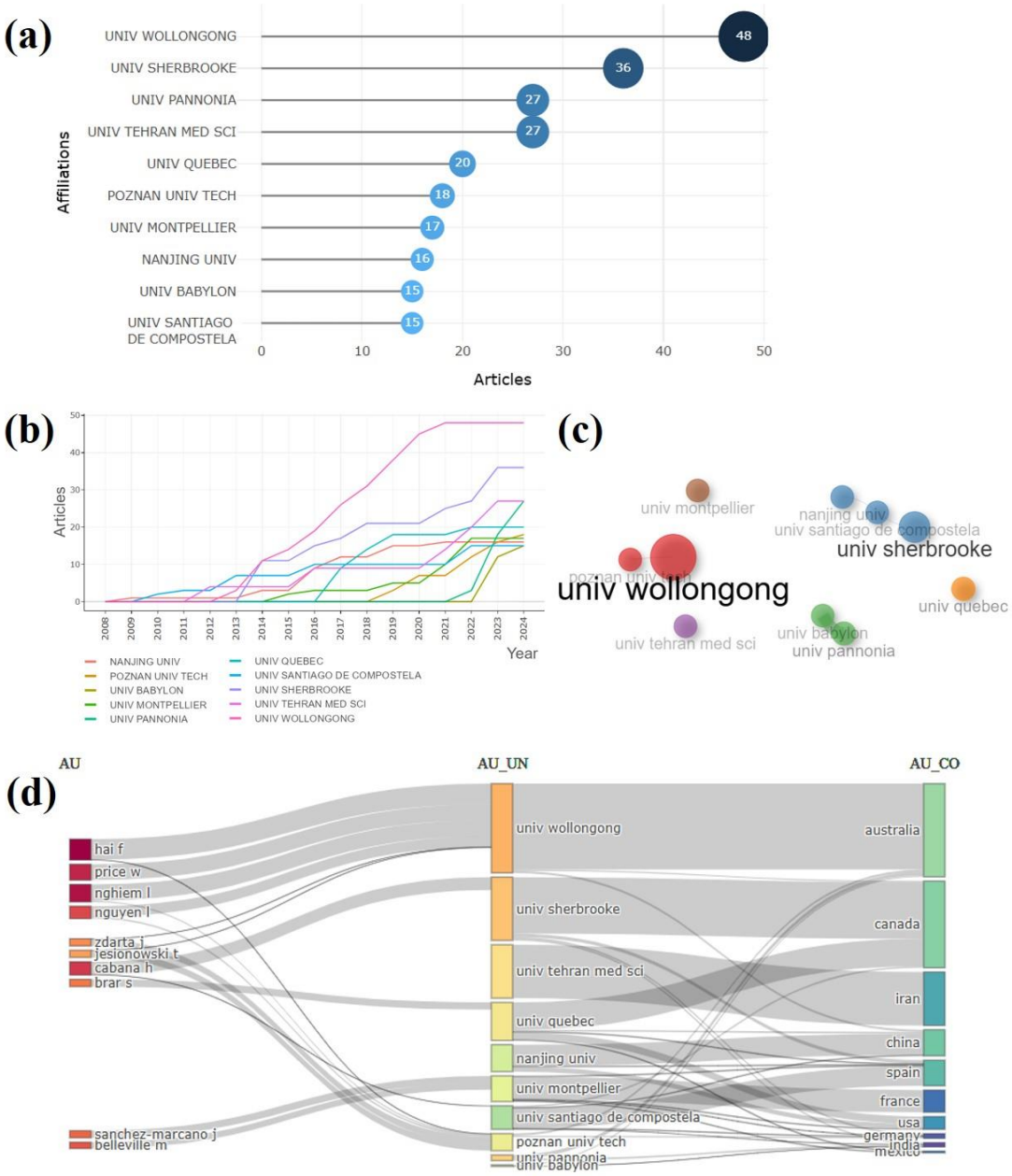


Figure 4. Institutional contributions and collaboration networks in fungal laccase research for pharmaceutical bioremediation. **(a)** Top 10 most productive institutions by number of published articles; bubble size represents publication count. **(b)** Cumulative

article output (2008–2024) by the same institutions, differentiated by color. **(c)** Institutional collaboration network. Each node represents an institution, with size proportional to publication count and color indicating the cluster. The distance and the thickness of the line connecting nodes indicate the relatedness and strength between the institutions. The network is divided into six distinct clusters, each distinguished by a color. **(d)** Sankey diagram linking prolific authors (AU) to their institutions (AU_UN) and countries (AU_CO).

The presence of weakly connected or isolated clusters indicates that, despite the global relevance of pharmaceutical bioremediation, international partnerships remain underdeveloped. This observation aligns with the author–institution–country flows in the Sankey diagram (Figure 4d), which show limited inter-institutional mobility and scarce cross-country collaborations. Most authors remain affiliated with their home institutions, highlighting the prevalence of locally consolidated research groups. The University of Wollongong hosts a concentration of leading authors (e.g., Hai F., Price W., Nghiem L., Nguyen L.), reflecting a strong national research infrastructure. Similarly, the University of Sherbrooke, represented by Cabana H. and Brar S., has made a significant contribution to Canada's output. Other institutions, such as the University of Tehran Medical Sciences, Nanjing University, and the University of Santiago de Compostela, contribute valuable but more isolated research efforts.

These patterns underscore both strengths and missed opportunities in global collaboration, suggesting that targeted initiatives to foster international networking and inter-institutional partnerships could enhance scientific impact, promote knowledge exchange, and accelerate innovation in fungal laccase applications for pharmaceutical bioremediation (Borger 2024).

3.4 Global research output by country

Australia stands out among the top ten most productive countries in fungal laccase research applied to pharmaceutical bioremediation, with 90 published articles, representing approximately 49.5% of total publications in this field, and accumulating 1,098 citations, averaging 49.9 citations per article (Figure 5a). Canada and China follow closely, with 69 and 68 publications, respectively. In terms of citation impact, Spain ranks second after Australia, with 916 citations across 40 articles. Although the

earliest study originated in the United States, its overall output remains modest compared to the leading five countries (Figure 5b).

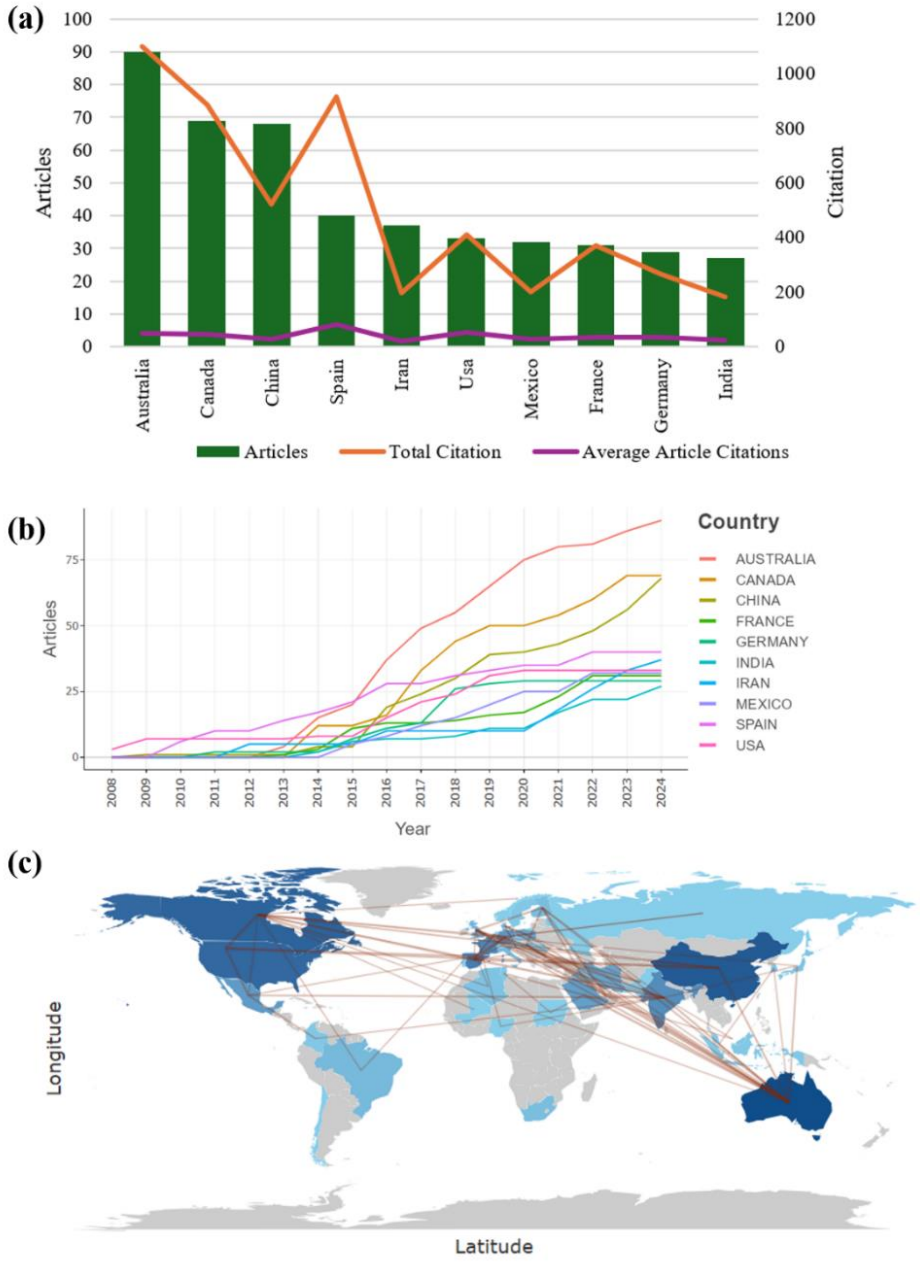


Figure 5. Global research output and international collaboration by country in fungal laccase research for pharmaceutical bioremediation. **(a)** Article count (green bars), total citations (orange line), and average citations per article (purple line) for the top 10 contributing countries. **(b)** Cumulative article output by country (2008–2024), with color-coded representation. **(c)** Global collaboration map; node shading indicates publication volume, and connecting lines represent co-authorship links between countries.

These findings are consistent with previous scientometric studies identifying these countries as key contributors to research on pharmaceutical effluent treatment (Davarazar et al. 2020) and laccase-based wastewater treatment (Puspita et al. 2023). China, in particular, leads global scientific output in broader areas of environmental biotechnology, including enzymatic bioremediation (Singh et al. 2024), enzyme immobilization techniques (Gonçalves et al. 2019) and biochar applications for antibiotic removal (Liu et al. 2024). However, as evidenced by the results of this study, Australia maintains a distinct leadership role specifically in fungal laccase applications targeting pharmaceutical pollutants, as reflected by both publication volume and citation performance.

The temporal analysis reveals a sharp growth in scientific output related to fungal laccase application for pharmaceutical bioremediation from 2015 onwards (Figure 5b). This trend coincides with the implementation of policy frameworks and targeted funding initiatives aimed at mitigating the environmental impact of emerging contaminants while promoting innovation in sustainable technologies (Wang et al. 2024; ONU 2025). It also reflects growing global awareness of the ecotoxicological risks posed by pharmaceutical micropollutants and the potential of enzymatic bioremediation as a green and effective alternative (Puspita et al. 2023; Thathola et al. 2024).

Multiple factors contribute to addressing pharmaceutical pollution, including individual practices (e.g., proper disposal of unused medications) (Ehrhart et al. 2020), technological progress supported by robust research infrastructure (Wang et al. 2024), increased scientific engagement (Garduño-Jiménez et al. 2025), and the implementation of science-based policies aligned with the SDGs and the One Health framework (Wang et al. 2024; Garduño-Jiménez et al. 2025). Given that our dataset is based on peer-reviewed literature, the results underscore the essential role of universities and research institutions in enhancing national capacities for developing and deploying biotechnological tools aimed at mitigating pharmaceutical pollution.

The international collaboration network (Figure 5c) further emphasizes Australia's leadership, with 32 documented collaborations involving 15 countries, the highest global cooperation within this domain. Key partners include Canada, China, and several European nations, illustrating a robust, interdisciplinary network that fosters knowledge exchange and resource sharing. In contrast, countries such as

Austria, Brazil, South Korea, and Tunisia registered only a single international collaboration, highlighting disparities in global scientific engagement.

Persistent inequities in international collaboration risk reinforcing structural barriers to developing fungal laccase–based bioremediation strategies. Underrepresentation of Global South countries, particularly across Africa and parts of Latin America, limits access to advanced analytical tools, funding, and high-impact publication avenues. Conversely, countries with strong collaborative networks leverage diverse expertise and shared infrastructure to accelerate technological innovation and practical application (Isfandyari-Moghaddam et al. 2023; Borger 2024). Without targeted initiatives promoting equitable partnerships, capacity building, and inclusive knowledge exchange, the global scientific community risks entrenching a two-tier system in which sustainable bioremediation solutions are developed primarily in resource-rich contexts. This imbalance not only delays context-specific application of bioresource technologies but also compromises the achievement of equitable environmental restoration and shared sustainability goals.

In summary, sustained scientific leadership and robust international collaboration, as exemplified by Australia, are critical to translating fungal laccase research into scalable bioremediation technologies. Strengthening participation from underrepresented regions through equitable partnerships and technology transfer is essential to ensure that pharmaceutical pollution mitigation strategies are globally relevant, technically feasible, and aligned with shared sustainability targets.

3.5 Journal distribution analysis

The eligible articles were published across 84 journals (Supplementary Table 6). Applying Bradford's law of scattering, eight journals were identified in the core zone (Zone 1, shaded in Figure 6a), which comprised 61 articles, followed by 22 journals in Zone 2 (62 articles) and 54 in Zone 3 (59 articles). This distribution reflects a classical Bradford pattern, characterized by a few highly productive sources, a broader group of journals with moderate productivity, and a large set of sources with progressively lower output (Bradford 1934).

Among the core journals, *Chemosphere* emerges as the most prolific source, publishing 12 articles. *Bioresource Technology* follows it with 10 publications and *Environmental Science and Pollution Research* with 8. *Journal of Environmental Chemical Engineering*, *Journal of Hazardous Materials*, and *Science of the Total*

Environment each contributed 7 articles. Additionally, *Biocatalysis and Biotransformation* and *Environmental Research* published 5 articles each. Journals in Zones 2 and 3 published an average of approximately 2 and 1 article(s), respectively, indicating a marked drop in publication frequency beyond the core.

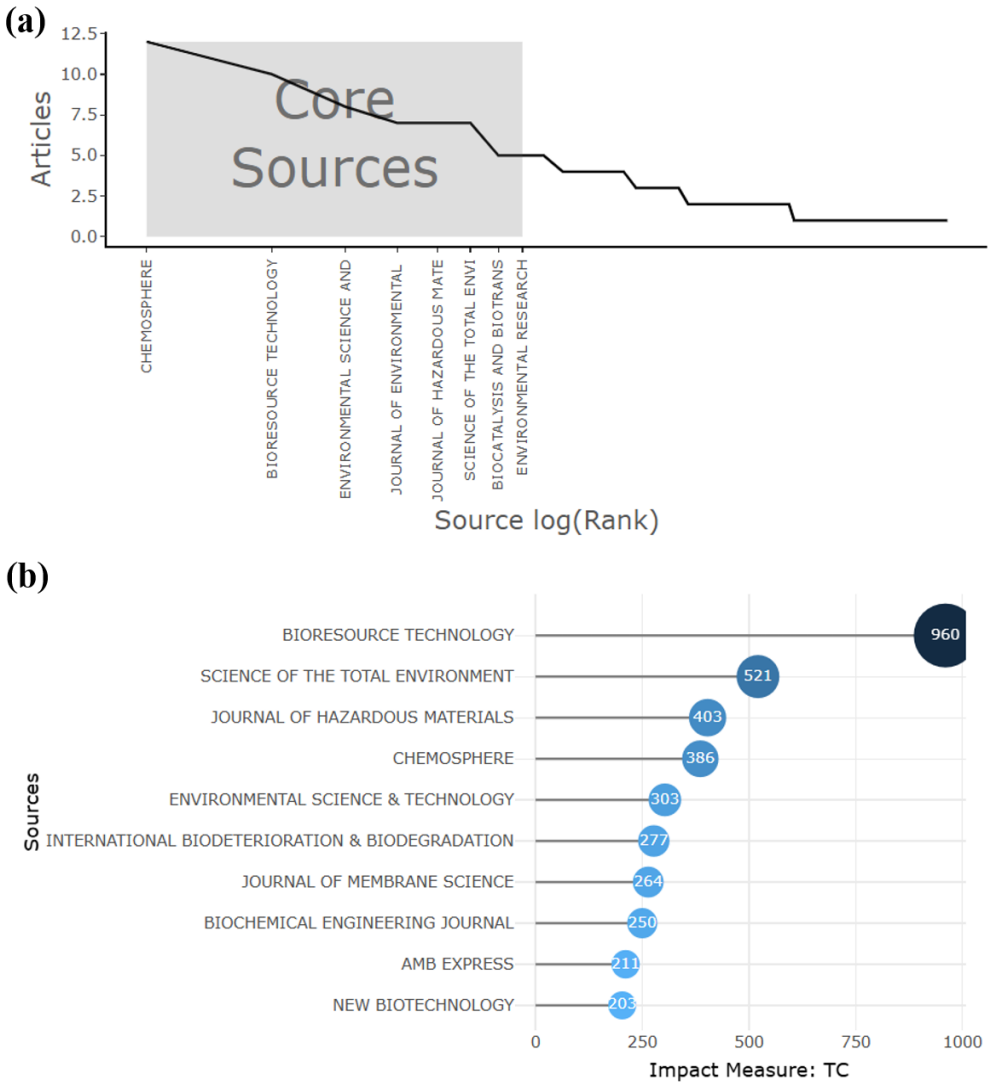


Figure 6. Core journals and citation impact in fungal laccase research for pharmaceutical bioremediation. **(a)** Core sources identified using Bradford's Law; number of articles plotted against the logarithmic rank of each journal. The shaded area represents the core zone of most productive journals. **(b)** Total citation (TC) count per journal, used to assess local impact within the dataset.

This distribution is consistent with the alignment between the thematic scopes of core journals and the interdisciplinary nature of fungal laccase research in pharmaceutical bioremediation. Core journals predominantly focus on environmental

biotechnology, pollutant degradation, sustainable technologies, and enzymatic processes, which directly correspond to the research objectives of the analyzed studies.

To complement productivity analysis, local impact was evaluated using total citations (TC) per journal (Figure 6b). *Bioresource Technology* emerged as the most impactful journal, with 960 citations, approximately three times more than *Chemosphere*, despite having published fewer articles. This suggests that while *Chemosphere* is an important volume publication, *Bioresource Technology* publishes articles of higher citation density. A similar trend was noted in a previous bibliometric analysis covering four decades of laccase-assisted wastewater treatment identified *Bioresource Technology* as the leading journal in number of publications, followed by the *Journal of Hazardous Materials* and *Chemosphere*, which ranked second and third, respectively (Puspita et al. 2023).

Journals exhibiting both high productivity and citation impact play a central role in consolidating scientific knowledge, as citation frequency often reflects research relevance, methodological robustness, and practical applicability (Garfield 2006; Bornmann et al. 2008). By fostering visibility and recognition, these journals act as strategic platforms for disseminating innovations in fungal laccase applications, extending their influence beyond academia into industrial, social, and environmental domains (Bornmann and Mutz 2015).

Overall, this analysis highlights the strategic importance of core journals in guiding researchers toward high-impact sources, consolidating the scientific landscape of fungal laccase–based pharmaceutical bioremediation, and supporting the advancement of this emerging field.

3.6 Highly cited documents

The ten most cited documents in the field are summarized in Table 1. Four were published in *Bioresource Technology* and two in *Science of the Total Environment*, highlighting the central role of these journals in disseminating advances in environmental biotechnology and pollutant degradation. The remaining publications appeared in diverse journals, reflecting the interdisciplinary and rapidly evolving nature of the field.

The average total citation count for these studies was 154, with the top-ranked article by Prieto et al. (2011) receiving 218 citations during the analyzed period. This pioneering work compared *T. versicolor* and its isolated laccase in the

biotransformation of the antibiotics ciprofloxacin and norfloxacin. Beyond identifying environmentally relevant transformation products, the study suggested the involvement of additional enzymatic systems, such as cytochrome P450, in the degradation pathway. Its innovative experimental design, coupled with robust analytical methods, has established this study as a benchmark for pharmaceutical bioremediation research.

Table 1. List of most cited documents on fungal laccase for pharmaceutical bioremediation

Rank	Title	Author	Year	Journal	TC	TCY	DOI
1	Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products	Prieto A	2011	Bioresour Technol	218	15,57	10.1016/j.biortech.2011.08.055
2	Bacterial versus fungal laccase: potential for micropollutant degradation	Margot J	2013	Amb Express	195	16,25	10.1186/2191-0855-3-63
3	Simultaneous removal and degradation characteristics of sulfonamide, tetracycline, and quinolone antibiotics by laccase-mediated oxidation coupled with soil adsorption	Ding H	2016	J Hazard Mater	175	19,44	10.1016/j.jhazmat.2015.12.062
4	Laccase-catalyzed degradation of anti-inflammatories and estrogens	Lloret L	2010	Biochem Eng J	174	11,60	10.1016/j.bej.2010.06.005
5	Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR	Marco-Urrea E	2010	Bioresour Technol	150	10,00	10.1016/j.biortech.2009.11.019
6	Continuous adsorption and biotransformation of micropollutants by granular activated carbon-bound laccase in a packed-bed enzyme reactor	Nguyen L	2016	Bioresour Technol	137	15,22	10.1016/j.biortech.2016.01.014
7	Removal of antibiotics in wastewater by enzymatic treatment with fungal laccase - Degradation of compounds does not always eliminate toxicity	Becker D	2016	Bioresour Technol	135	15,00	10.1016/j.biortech.2016.08.004
8	Immobilized laccase on oxygen functionalized nanobiochars through mineral acids treatment for removal of carbamazepine	Naghdi M	2017	Sci Total Environ	118	14,75	10.1016/j.scitotenv.2017.01.021
9	Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals	Touahar I	2014	Sci Total Environ	118	10,73	10.1016/j.scitotenv.2014.01.132
10	Direct immobilization of laccase on titania nanoparticles from crude enzyme extracts of *P. ostreatus* culture for micro-pollutant degradation	Ji C	2017	Sep Purif Technol	117	14,63	10.1016/j.seppur.2017.01.043

TC: Total Citation – TCY: Total Citation Per Year

A closer examination of Table 1 reveals several important trends. First, a substantial portion of highly cited studies focuses on antibiotic degradation, including ciprofloxacin and norfloxacin (Prieto, 2011), sulfonamides, tetracyclines, and quinolones (Ding, 2016), highlighting the environmental concern regarding these persistent micropollutants. Analgesics and anti-inflammatory drugs, such as naproxen (Marco-Urrea, 2010) and estrogens (Lloret, 2010), are also addressed, reflecting the broad applicability of fungal laccases across pharmaceutical classes.

Second, the data show a strong emphasis on technological innovation. Several studies explore enzyme immobilization and continuous-flow systems, including granular activated carbon-bound laccase (Nguyen, 2016) and direct immobilization on titania nanoparticles (Ji, 2017). The combination of multiple enzymes in cross-linked aggregates (Touahar, 2014) and functionalized nanobiochars (Naghdi, 2017) further illustrates the drive toward scalable and efficient biocatalytic platforms. Margot (2013) compares bacterial and fungal laccases, emphasizing the potential of fungi in micropollutant degradation relative to other enzymatic systems.

Another important trend is the integration of sustainability considerations. Several top-cited works incorporate green chemistry principles, cost-effective enzyme production, and environmentally benign treatment alternatives, such as minimizing toxic by-products (Becker, 2016). These strategies underscore the alignment of the field with sustainable wastewater treatment and broader environmental safety goals (Bilal et al. 2019; Garduño-Jiménez et al. 2025).

Finally, the diversity of journals represented in the top ten indicates the interdisciplinary nature of pharmaceutical bioremediation, bridging microbiology, enzymology, chemical engineering, and environmental science. Collectively, these studies show that impactful research in this area combines fundamental enzymology with application-oriented innovation and sustainability considerations.

Future work aiming for high scientific and technological impact should continue integrating novel biocatalysis strategies with scalable, environmentally responsible approaches, consolidating fungal laccases as a key tool for pharmaceutical bioremediation.

3.7 Keyword mapping and thematic focus

To explore the conceptual structure of research on fungal laccases for pharmaceutical bioremediation, the thematic relationships among key terms and their

temporal dynamics were examined (Figure 7). This integrated perspective reveals the evolutionary patterns of the field, highlighting both well-established research domains and persistent thematic gaps.

The Sankey diagram (Figure 7a) illustrates the relationship between the most productive authors (AU), journals (SO), and keywords (DE) identified in the analyzed studies. Prominent researchers such as Nghiem L., Hai F., and Price W., on the left, show strong associations with high-impact journals including *Bioresource Technology* and *Chemosphere*. The diagram aligns with Bradford's Law, identifying the same core journals highlighted in Figs. 6a and 6b. These journals, in turn, are strongly linked to descriptors such as "laccase," "immobilization," "biodegradation," and "pharmaceuticals," which reflect the core themes in fungal laccase research. Additional terms such as "diclofenac," "biotransformation," and "enzyme immobilization", indicate global trends in addressing pharmaceutical pollutants and advancing sustainable remediation technologies. The high density of connections across all three fields suggests a thematically cohesive research landscape, supporting depth, coherence, and practical relevance.

The co-occurrence network of the 50 most frequent author keywords (Figure 7b) revealed 10 distinct clusters, with the red, blue, and green clusters emerging as the most prominent according to the node density and thematic cohesion.

The red cluster, the largest, centers on the keyword "laccase" and comprises 27 nodes. It aggregates terms with high betweenness centrality scores, such as "immobilization" (17,569), "degradation" (10,861), and "micropollutants" (6,393), underscoring the central role of fungal laccase applications in environmental bioremediation.

Several keywords in the red cluster refer to specific pharmaceutical compounds, including "diclofenac", "estrogens", "tetracycline", "carbamazepine", "antibiotics", "ciprofloxacin", "ibuprofen", "levofloxacin", and "sulfamethoxazole", listed in order of their PageRank scores. These are well known for their persistence and ecotoxicity in aquatic and terrestrial ecosystems (Gao et al. 2018; Estrada-Almeida et al. 2024; Singh et al. 2025). This pattern indicates that fungal laccase research is actively targeting real-world contaminants. However, pharmaceuticals such as thyroid hormones, corticosteroids, psychoactive substances, antiretrovirals, and anticancer agents, although present in environmental matrices and recognized for their high biological

activity even at trace levels (Gong et al., 2019; Singh et al., 2025), remain underexplored, signaling important opportunities for future studies.

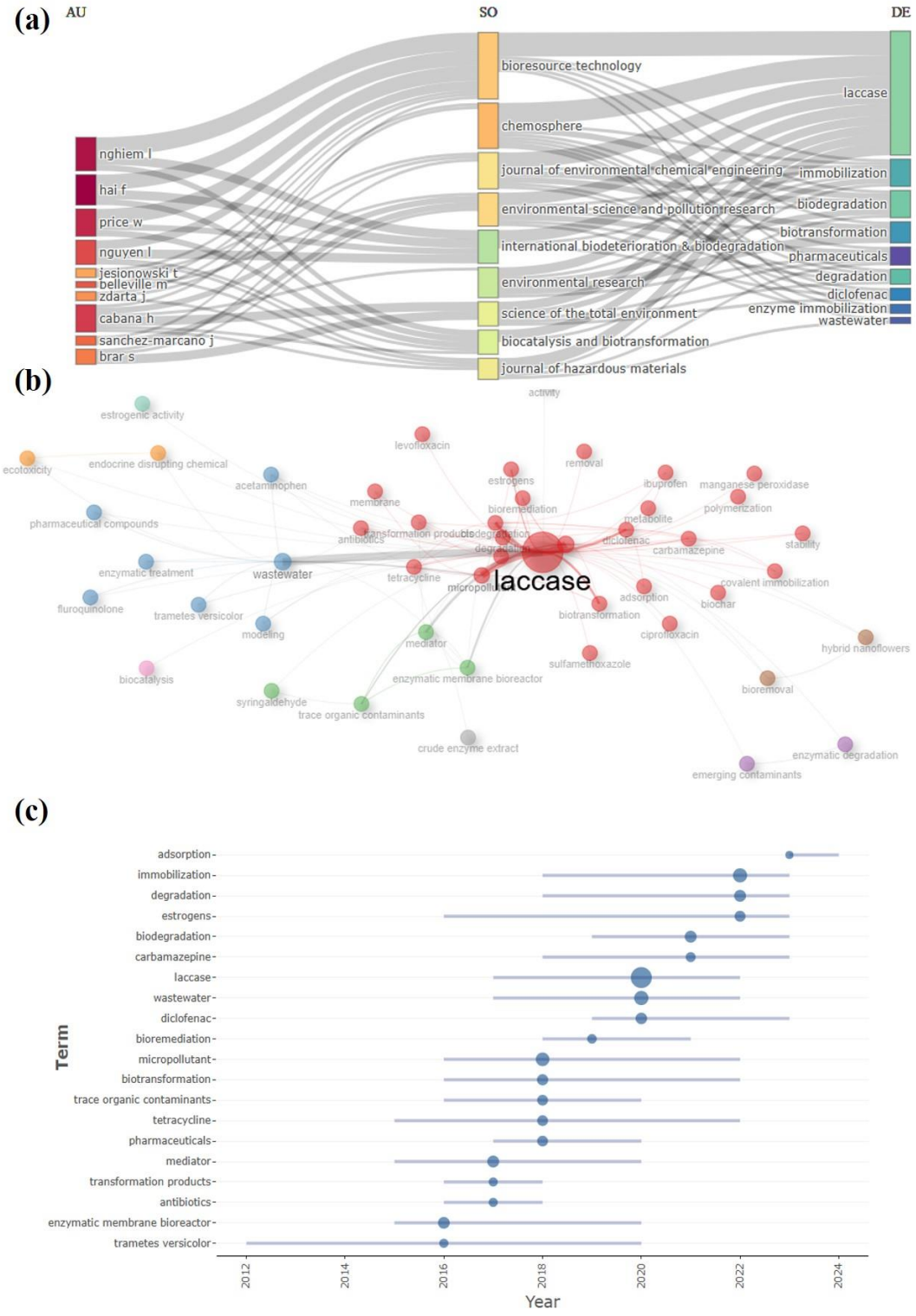


Figure 7. Key terms and emerging topics in fungal laccase applications for pharmaceutical bioremediation. **(a)** Sankey diagram linking the top 10 contributing

authors (AU), journals (SO), and author keywords (DE), depicting the structural composition of the literature. **(b)** Keyword co-occurrence network of the 50 most frequent terms. Each node represents a keyword, with size proportional to publication count and color indicating the cluster. The distance and the thickness of the line connecting nodes indicate the relatedness and strength between keywords. **(c)** Temporal evolution of trending topics based on author keywords (2008–2024); bubble size denotes annual frequency of occurrence.

The blue cluster, composed of seven nodes, is centered on the term "wastewater" and is connected to the red cluster through the keyword "laccase," indicating a strong thematic association between these areas. It also contains "enzymatic treatment," "pharmaceutical compounds," and drug-related terms such as "acetaminophen" and "fluoroquinolone." These links highlight the focus on wastewater as a critical pathway for pharmaceutical pollutant dissemination (Ortúzar et al. 2022; Wang et al. 2024).

The green cluster, composed of four nodes, is linked to the red cluster through the terms "mediator" and "laccase," reflecting a strategic approach to broadening the substrate range of fungal laccases. Mediators such as syringaldehyde, detected in this cluster, are small molecules that act as electron shuttles, enabling the oxidation of complex substrates otherwise inaccessible to the enzyme's active site due to steric hindrance or high redox potential (Bourbonnais and Paice 1990; Singh et al. 2025). Once oxidized, these mediators form relatively stable radicals that diffuse from the active site and oxidize target compounds via non-enzymatic pathways (Cañas and Camarero 2010). The absence of other mediators in the co-occurrence network suggests that compounds commonly used in laccase-based treatments of colorants or pesticides — such as ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid), HBT (1-hydroxy benzotriazole), TEMPO (2,2,6,6-tetramethylpi-peridine oxide), and violuric acid — have not yet been fully explored for their potential in pharmaceutical bioremediation. This research gap highlights the need to investigate structure-activity relationships and environmentally friendly alternatives, particularly for complex drug molecules or those lacking phenolic structures.

Pharmaceuticals can enter the environment through multiple pathways, originating from their production or use across diverse sectors, including industry, agriculture, households, healthcare, and even wastewater treatment plants, ultimately reaching environmental compartments such as air, soil, and water (Ortúzar et al. 2022;

Vaudin et al. 2022; Wang et al. 2024). Among these, water serves as the primary medium for the dissemination of pharmaceutical pollutants due to its global connectivity and transport capacity (Ortúzar et al. 2022; Wang et al. 2024).

To mitigate these impacts, various wastewater treatment strategies, such as advanced oxidation processes (AOPs), membrane filtration, and activated carbon adsorption, have been implemented (Guo et al. 2017; Patel et al. 2019; Mutegoa 2024). Nevertheless, these approaches have often proven insufficient, as numerous pharmaceutical residues are still detected in treated effluents, surface, and even drinking water sources (Heberer 2002; Gracia-Lor et al. 2012; Tran et al. 2018; Freitas and Radis-Baptista 2021). In response, alternative strategies such as enzymatic bioremediation have garnered increasing attention (Chmelová et al. 2024; Thathola et al. 2024). As demonstrated by Singh et al. (2024), laccases are among the most extensively studied enzymes for such applications. However, despite the growing interest in fungal laccases for pharmaceutical bioremediation, current research remains largely focused on aqueous matrices, for instance, see Singh et al. (2025), Naghdi et al. (2018) and Puspita et al. (2023).

The absence of keywords related to solid matrices, such as soil and sludge, was noted. Soil and sediment texture and chemical composition play a critical role in the fixation and deposition of pharmaceuticals, and the presence of these compounds in such environmental matrices has been documented (Beretta et al. 2014; Tran et al. 2018). Similarly, sludge can act as a concentrating medium for certain chemical contaminants during wastewater treatment. Nevertheless, most treated sludge is directly applied to agricultural land without prior screening for emerging contaminants, posing potential environmental and health risks (Petrie et al. 2015; Tran et al. 2018). Therefore, the application of fungal laccases in solid environmental matrices represents a research gap. Addressing this gap could also contribute to SDG 15, which emphasizes the protection, restoration, and sustainable use of terrestrial ecosystems.

The temporal evolution of the author keywords was analyzed, and the results are presented in Figure 7c. Up to five terms were included per year, each appearing in a minimum of five articles. Among pharmaceutical-related terms, “estrogens” and “tetracycline” exhibited the longest temporal persistence, appearing consecutively for seven years, with 13 and 12 occurrences, respectively. As expected, “laccase” was the most frequently cited author keyword overall, with 136 occurrences. While this

prevalence was anticipated, the term was retained in the analysis to assess its longitudinal relevance and co-occurrence dynamics with other keywords.

Trametes versicolor showed a steady rise in frequency from 2012 to 2020, reinforcing its status as a model organism for laccase production (Viswanath et al. 2014; Yang et al. 2017; Chmelová et al. 2024). Its enzymes are widely studied for bioremediation of micropollutants such as dyes (Puspita et al. 2023), pharmaceuticals (Prieto et al. 2011; Alharbi et al. 2019), pesticides (Beltrán-Flores et al. 2021), and other xenobiotic compounds (Torres-Farradá et al. 2024). Although the keyword analysis parameters did not capture additional fungal species within the dataset, several others, such as *Ganoderma lucidum*, *Lentinula edodes*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Pycnoporus sanguineus,* have been reported in the literature for their promising laccase activity in pharmaceutical bioremediation (Gao et al. 2018; Cheute et al. 2024; Flórez-Restrepo et al. 2025).

Recently, the keywords “adsorption” and “immobilization” have become prominent topics. “Adsorption” first appeared in 2023 and quickly reached a frequency of six. In contrast, “immobilization” entered the network in 2018 and reached a frequency of 36, making it the third most cited keyword overall. Studies have demonstrated that immobilized enzymes offer significant advantages over free enzymes (Gonçalves et al. 2019; Kyomuhimbo and Brink 2023). Among various physical immobilization strategies, adsorption stands out as a key method for attaching laccases to solid support matrices (Jesionowski et al. 2014). This approach enhances the enzymatic activity and stability, extends the enzyme’s half-life, and enables its reuse over multiple catalytic cycles, ultimately reducing the cost of the biocatalytic process (Gonçalves et al. 2019; Sodhi et al. 2024).

The growing emphasis on immobilization reflects a broader research interest in designing robust biocatalytic systems suited for real-world applications, including continuous-flow reactors and wastewater treatment infrastructures (Ba et al. 2013; Singh et al. 2025). The choice of support materials, such as biochar, silica, and polymeric matrices, is crucial in determining the catalytic performance and environmental sustainability of immobilized laccase systems (Di Cosimo et al. 2013; Gonçalves et al. 2019). Moreover, designing and functionalizing supports for specific enzymes can be complex and costly (Di Cosimo et al. 2013; Aghaee et al. 2024). Therefore, novel immobilization strategies should be guided by systematic economic feasibility assessments to ensure scalability and practical applicability in

bioremediation. Moreover, alternative stabilization approaches, such as lyophilization or chemical stabilizers, remain underexplored. Addressing this gap is essential, as effective enzyme stabilization extends shelf life and facilitates transport, particularly for laboratories lacking immobilization expertise, thereby supporting the broader commercialization of laccases for environmental applications (Iyer and Ananthanarayan 2008; Sheldon and van Pelt 2013).

4. Conclusions and future perspectives

This study presents the first comprehensive mapping of global research trends on fungal laccases applied to pharmaceutical bioremediation. The increasing number of publications, driven by a relatively small group of prolific authors, institutions, and countries, reflects the growing scientific and technological interest in enzymatic biocatalysis as a sustainable strategy to mitigate pharmaceutical pollution.

Keyword mapping and thematic clustering revealed dominant research directions centered on enzymatic degradation mechanisms, immobilization strategies, and applications in water and wastewater matrices. Nevertheless, the analysis also highlights persistent knowledge gaps, notably the limited attention given to soil and sludge bioremediation, the underrepresentation of several pharmaceutical classes, such as thyroid hormones, antiretrovirals, and anticancer agents, and the scarcity of mechanistic studies addressing oxidation–reduction pathways and enzyme stability. Addressing these gaps would broaden the field's scope, strengthen its scientific foundations, and enhance the practical applicability of fungal laccases across diverse environmental contexts.

While the scientometric approach provides a structured and comprehensive overview of the research landscape, some limitations should be acknowledged. These include the limited ability to capture regional research nuances, the delayed citation impact of recent publications, and the exclusive focus on fungal laccases, which excludes other promising enzymatic systems, such as bacterial or recombinant laccases, that may offer complementary or superior performance under specific operational conditions.

To advance the field, future research should prioritize comparative evaluations of laccases from diverse biological origins to identify enzymes with optimal catalytic efficiency and operational stability, alongside systematic reviews aimed at reducing methodological heterogeneity and establishing standardized performance benchmarks.

In addition, greater emphasis should be placed on underexplored pharmaceutical classes and solid environmental matrices, as well as on comprehensive toxicological assessments of biotransformation products to ensure environmental safety and regulatory compliance. From an applied perspective, the development of immobilization strategies that enhance enzyme stability should be coupled with economic feasibility analyses to support industrial-scale implementation. Furthermore, alternative stabilization approaches beyond immobilization, such as lyophilization and the use of chemical stabilizers, remain insufficiently explored and represent promising avenues for future investigation.

Aligning fungal laccase research with the Sustainable Development Goals - particularly SDG 6 (Clean Water and Sanitation), SDG 9 (Industry, Innovation, and Infrastructure), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land) - within a One Health framework underscores its potential to deliver environmentally sound, scalable, and innovative solutions. Overall, this integrated analysis not only delineates the intellectual structure of the field but also provides a foundation for targeted interdisciplinary collaboration among academia, industry, and policymakers.

5. References

Abejón R, De Cazes M, Belleville MP, Sanchez-Marcano J (2015) Large-scale enzymatic membrane reactors for tetracycline degradation in WWTP effluents. Water Res 73:118–131. https://doi.org/10.1016/j.watres.2015.01.012

Aghaee M, Salehipour M, Rezaei S, Mogharabi-Manzari M (2024) Bioremediation of organic pollutants by laccase-metal-organic framework composites: A review of current knowledge and future perspective. Bioresour Technol 406:131072. https://doi.org/10.1016/j.biortech.2024.131072

Al-Sareji OJ, Meiczinger M, Al-Juboori RA, et al (2023) Efficient removal of pharmaceutical contaminants from water and wastewater using immobilized laccase on activated carbon derived from pomegranate peels. Sci Rep 13:11933. https://doi.org/10.1038/s41598-023-38821-3

Alharbi SK, Nghiem LD, van de Merwe JP, et al (2019) Degradation of diclofenac, trimethoprim, carbamazepine, andsulfamethoxazole by laccase from *Trametes versicolor*:Transformation products and toxicity of treated effluent. Biocatal Biotransformation 37:399–408. https://doi.org/10.1080/10242422.2019.1580268

Aria M, Cuccurullo C (2017) Bibliometrix: An R-tool for comprehensive science mapping analysis. J Informetr 11:959–975. https://doi.org/10.1016/j.joi.2017.08.007

Ba S, Arsenault A, Hassani T, et al (2013) Laccase immobilization and insolubilization: From fundamentals to applications for the elimination of emerging contaminants in wastewater treatment. Crit Rev Biotechnol 33:404–418. https://doi.org/10.3109/07388551.2012.725390

Beltrán-Flores E, Sarrà M, Blánquez P (2021) Pesticide bioremediation by Trametes versicolor: Application in a fixed-bed reactor, sorption contribution and bioregeneration. Sci Total Environ 794:148386. https://doi.org/10.1016/J.SCITOTENV.2021.148386

Beretta M, Britto V, Tavares TM, et al (2014) Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J Soils Sediments 14:1278–1286. https://doi.org/10.1007/s11368-014-0884-6

Bialk HM, Pedersen JA (2008) NMR investigation of enzymatic coupling of sulfonamide antimicrobials with humic substances. Environ Sci Technol 42:106–112. https://doi.org/10.1021/es070779d

Bilal M, Adeel M, Rasheed T, et al (2019) Emerging contaminants of high concern and their enzyme-assisted biodegradation – A review. Environ Int 124:336–353. https://doi.org/10.1016/j.envint.2019.01.011

Borger JG (2024) The power of networking in science and academia. Immunol Cell Biol 102:871–877. https://doi.org/10.1111/imcb.12832

Bornmann L, Daniel HHD, Bornmann L, Daniel HHD (2008) What do citation counts measure? A review of studies on citing behavior. J Doc 64:45–80. https://doi.org/10.1108/00220410810844150

Bornmann L, Mutz R (2015) Growth rates of modern science: A bibliometric analysis based on the number of publications and cited references. J Assoc Inf Sci Technol 66:2215–2222. https://doi.org/10.1002/asi.23329

Bouabadi I, Miyah Y, Benjelloun M, et al (2024) Advanced strategies for the innovative treatment of hospital liquid effluents: A comprehensive review. Bioresour Technol Reports 28:101990. https://doi.org/10.1016/j.biteb.2024.101990

Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. FEBS Lett 267:99–102.

https://doi.org/10.1016/0014-5793(90)80298-W

Bradford SC (1934) Sources of information on specific subjects. J Inf Sci 137:85–86. https://doi.org/doi.org/10.1177/016555158501000407

Brugnari T, Braga DM, dos Santos CSA, et al (2021) Laccases as green and versatile biocatalysts: from lab to enzyme market—an overview. Bioresour Bioprocess 8:1–29. https://doi.org/10.1186/s40643-021-00484-1

Cañas AI, Camarero S (2010) Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol Adv 28:694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002

Cheute VM, Uber TM, dos Santos LF, et al (2024) Biotransformation of pollutants by Pycnoporus spp. in submerged and solid-state fermentation: mechanisms, achievements, and perspectives. Biomass 4:313–328

Chmelová D, Ondrejovič M, Miertuš S (2024) Laccases as effective tools in the removal of pharmaceutical products from aquatic systems. Life 14:. https://doi.org/10.3390/life14020230

Couto SR, Herrera JLT (2006) Industrial and biotechnological applications of laccases: A review. Biotechnol Adv 24:500–513. https://doi.org/10.1016/j.biotechadv.2006.04.003

Davarazar M, Mostafaie A, Jahanianfard D, et al (2020) Treatment technologies for pharmaceutical effluents-A scientometric study. J Environ Manage 254:109800. https://doi.org/10.1016/j.jenvman.2019.109800

Di Cosimo R, Mc Auliffe J, Poulose AJ, Bohlmann G (2013) Industrial use of immobilized enzymes. Chem Soc Rev 42:6437–6474. https://doi.org/10.1039/c3cs35506c

dos Santos CR, Rosa e Silva GO, Valias C de F, et al (2024) Ecotoxicological study of seven pharmaceutically active compounds: Mixture effects and environmental risk assessment. Aquat Toxicol 275:. https://doi.org/10.1016/j.aquatox.2024.107068

Egghe L (2006) Theory and practise of the g-index. Scientometrics 69:131–152. https://doi.org/10.1007/s11192-006-0144-7

Ehrhart AL, Granek EF, Nielsen-Pincus M, Horn DA (2020) Leftover drug disposal: Customer behavior, pharmacist recommendations, and obstacles to drug take-back box implementation. Waste Manag 118:416–425. https://doi.org/10.1016/j.wasman.2020.08.038

Estrada-Almeida AG, Castrejón-Godínez ML, Mussali-Galante P, et al (2024)

Pharmaceutical pollutants: ecotoxicological impacts and the use of agro-industrial waste for their removal from aquatic environments. J Xenobiotics 14:1465–1518. https://doi.org/10.3390/jox14040082

Flórez-Restrepo MA, López-Legarda X, Segura-Sánchez F (2025) Bioremediation of emerging pharmaceutical pollutants acetaminophen and ibuprofen by white-rot fungi – A review. Sci Total Environ 977:. https://doi.org/10.1016/j.scitotenv.2025.179379

Freitas L de AA, Radis-Baptista G (2021) Pharmaceutical pollution and disposal of expired, unused, and unwanted medicines in the Brazilian context. J Xenobiotics 11:61–76. https://doi.org/10.3390/jox11020005

Gao N, Liu CX, Xu QM, et al (2018) Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of Phanerochaete chrysosporium and Pycnoporus sanguineus. Chemosphere 195:146–155. https://doi.org/10.1016/j.chemosphere.2017.12.062

García-Delgado C, Eymar E, Camacho-Arévalo R, et al (2018) Degradation of tetracyclines and sulfonamides by stevensite- and biochar-immobilized laccase systems and impact on residual antibiotic activity. J Chem Technol Biotechnol 93:3394–3409. https://doi.org/10.1002/jctb.5697

Garduño-Jiménez AL, Gomes RL, López-Maldonado Y, Carter LJ (2025) Addressing the global data imbalance of contaminants of emerging concern in the context of the United Nations sustainable development goals. RSC Sustain 3384–3391. https://doi.org/10.1039/d5su00144g

Garfield E (2006) The history and meaning of the journal impact factor. JAMA 295:90–93. https://doi.org/10.1001/jama.295.1.90

Gonçalves MCP, Kieckbusch TG, Perna RF, et al (2019) Trends on enzyme immobilization researches based on bibliometric analysis. Process Biochem 76:95–110. https://doi.org/10.1016/j.procbio.2018.09.016

Gracia-Lor E, Sancho J V., Serrano R, Hernández F (2012) Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere 87:453–462. https://doi.org/10.1016/j.chemosphere.2011.12.025

Guerra P, Kim M, Shah A, et al (2014) Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci Total Environ 473–474:235–243.

https://doi.org/10.1016/j.scitotenv.2013.12.008

Guo Y, Qi PS, Liu YZ (2017) A review on advanced treatment of pharmaceutical wastewater. IOP Conf Ser Earth Environ Sci 63:12025. https://doi.org/10.1088/1755-1315/63/1/012025

Heberer T (2002) Tracking persistent pharmaceutical residues from municipal sewage to drinking water. J Hydrol 266:175–189. https://doi.org/10.1016/S0022-1694(02)00165-8

Hirsch JE, Buela-Casal G (2014) The meaning of the h-index. Int J Clin Heal Psychol 14:161–164. https://doi.org/10.1016/S1697-2600(14)70050-X

Isfandyari-Moghaddam A, Saberi MK, Tahmasebi-Limoni S, et al (2023) Global scientific collaboration: A social network analysis and data mining of the co-authorship networks. J Inf Sci 49:1126–1141. https://doi.org/10.1177/01655515211040655

Iyer P V., Ananthanarayan L (2008) Enzyme stability and stabilization-aqueous and non-aqueous environment. Process Biochem 43:1019–1032. https://doi.org/10.1016/j.procbio.2008.06.004

Jesionowski T, Zdarta J, Krajewska B (2014) Enzyme immobilization by adsorption: A review. Adsorption 20:801–821. https://doi.org/10.1007/s10450-014-9623-y

Khan MF (2025) Fungi for sustainable pharmaceutical remediation: enzymatic innovations, challenges, and applications - A review. Processes 13:1–37. https://doi.org/10.3390/pr13041034

Kijeńska-Gawrońska E, Zdarta J, Thabit HSAM, et al (2024) From PET bottle waste to enzyme support for removal of estrogens from wastewaters. Environ Technol Innov 34:. https://doi.org/10.1016/j.eti.2024.103555

Kyomuhimbo HD, Brink HG (2023) Applications and immobilization strategies of the copper-centred laccase enzyme; a review. Heliyon 9:e13156. https://doi.org/10.1016/j.heliyon.2023.e13156

Liu X, Yuan J, Feng Y, et al (2024) Knowledge graph and development hotspots of biochar as an emerging aquatic antibiotic remediator: A scientometric exploration based on VOSviewer and CiteSpace. J Environ Manage 360:121165. https://doi.org/10.1016/j.jenvman.2024.121165

Lotka AJ (1926) The frequency distribution of scientific productivity. J Washingt Acad Sci 16:317–323

Maryskova M, Vrsanska M, Sevcu A, et al (2022) Laminated PAA nanofibers as a

practical support for crude laccase : A new perspective for biocatalytic treatment of micropollutants in wastewaters. Environ Technol Innov 26:102316. https://doi.org/10.1016/j.eti.2022.102316

Mutegoa E (2024) Efficient techniques and practices for wastewater treatment: an update. Discov Water 4:. https://doi.org/10.1007/s43832-024-00131-8

Naghdi M, Taheran M, Brar SK, et al (2018) Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes. Environ Pollut 234:190–213. https://doi.org/10.1016/j.envpol.2017.11.060

Nguyen LN, Hai FI, Yang S, et al (2014) Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: Role of biosorption and biodegradation. Int Biodeterior Biodegrad 88:169–175. https://doi.org/10.1016/j.ibiod.2013.12.017

ONU (2025) The United Nations Sustainable Development Goals. In: United Nations. https://sdgs.un.org/goals

Ortúzar M, Esterhuizen M, Olicón-Hernández DR, et al (2022) Pharmaceutical pollution in aquatic environments: a concise review of environmental impacts and bioremediation systems. Front Microbiol 13:. https://doi.org/10.3389/fmicb.2022.869332

Page MJ, McKenzie JE, Bossuyt PM, et al (2021) The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372:. https://doi.org/10.1136/bmj.n71

Patel M, Kumar R, Kishor K, et al (2019) Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem Rev 119:3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Petrie B, Barden R, Kasprzyk-Hordern B (2015) A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Res 72:3–27. https://doi.org/10.1016/j.watres.2014.08.053

Prieto A, Moeder M, Rodil R, et al (2011) Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. Bioresour Technol 102:10987–10995. https://doi.org/10.1016/j.biortech.2011.08.055

Puspita K, Chiari W, Abdulmadjid SN, et al (2023) Four decades of laccase research for wastewater treatment: insights from bibliometric analysis. Int J Environ Res

Public Health 20:. https://doi.org/10.3390/ijerph20010308

R Core DT (2023) R: A language and environment for statistical computing.

Rodríguez-delgado M, Orona-navar C, García-morales R, et al (2016) Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from Pycnoporus sanguineus CS43 fungi. Int Biodeterior Biodegradation 108:34–41. https://doi.org/10.1016/j.ibiod.2015.12.003

Sharma K, Thakur IS, Kaushik G (2021) Occurrence and distribution of pharmaceutical compounds and their environmental impacts: A review. Bioresour Technol Reports 16:100841. https://doi.org/10.1016/j.biteb.2021.100841

Sheldon RA, van Pelt S (2013) Enzyme immobilisation in biocatalysis: Why, what and how. Chem Soc Rev 42:6223–6235. https://doi.org/10.1039/c3cs60075k

Silori R, Shrivastava V, Singh A, et al (2022) Global groundwater vulnerability for Pharmaceutical and Personal care products (PPCPs): The scenario of second decade of 21st century. J. Environ. Manage. 320

Singh AK, Abellanas-Perez P, de Andrades D, et al (2025) Laccase-based biocatalytic systems application in sustainable degradation of pharmaceutically active contaminants. J. Hazard. Mater. 485:136803

Singh NS, Sharma R, Kumari L (2024) Tracing the evolution of enzymatic bioremediation using scientometric analysis. J Scientometr Res 13:517–534. https://doi.org/10.5530/jscires.13.2.41

Sodhi AS, Bhatia S, Batra N (2024) Laccase: Sustainable production strategies, heterologous expression and potential biotechnological applications. Int J Biol Macromol 280:135745. https://doi.org/10.1016/j.ijbiomac.2024.135745

Sybuia PA, Contato AG, de Araújo CAV, et al (2024) Application of the white-rot fungus Trametes sp. (C3) laccase in the removal of acetaminophen from aqueous solutions. J Water Process Eng 57:104677. https://doi.org/10.1016/j.jwpe.2023.104677

Tarcomnicu I, Van Nuijs ALN, Simons W, et al (2011) Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. Talanta 83:795–803. https://doi.org/10.1016/j.talanta.2010.10.045

Thathola P, Melchor-Martinez EM, Adhikari P, et al (2024) Laccase-mediated degradation of emerging contaminants: unveiling a sustainable solution. Environ

Sci. https://doi.org/10.1039/d4va00173g

Torres-Farradá G, Thijs S, Rineau F, et al (2024) White rot fungi as tools for the bioremediation of xenobiotics: a review. J Fungi 10:. https://doi.org/10.3390/jof10030167

Tran NH, Reinhard M, Gin KYH (2018) Occurrence and fate of emerging contaminants in municipal treatment wastewater plants from different geographical regions-a review. Water Res 133:182–207. https://doi.org/10.1016/j.watres.2017.12.029

Upadhyay P, Shrivastava R, Agrawal PK (2016) Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 1–12. https://doi.org/10.1007/s13205-015-0316-3

Vaudin P, Augé C, Just N, et al (2022) When pharmaceutical drugs become environmental pollutants: Potential neural effects and underlying mechanisms. Environ Res 205:. https://doi.org/10.1016/j.envres.2021.112495

Viswanath B, Rajesh B, Janardhan A, et al (2014) Fungal laccases and their applications in bioremediation. Enzyme Res 2014:. https://doi.org/10.1155/2014/163242

Wang F, Xiang L, Sze-Yin Leung K, et al (2024) Emerging contaminants: A One Health perspective. Innovation 5:100612. https://doi.org/10.1016/j.xinn.2024.100612

Wang J, Chu L, Wojnárovits L, Takács E (2020) Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview. Sci Total Environ 744:140997. https://doi.org/10.1016/j.scitotenv.2020.140997

Wysokowski M, Chmielewska Z, Sandomierski M, et al (2024) Chitin membrane for efficient laccase immobilization and synergistic removal of 17α-ethynylestradiol from water-based solutions. Int J Biol Macromol 282:136599. https://doi.org/10.1016/j.ijbiomac.2024.136599

Yang J, Li W, Bun Ng T, et al (2017) Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation. Front Microbiol 8:. https://doi.org/10.3389/fmicb.2017.00832

Supplementary Information

Supplementary Table 1 Consolidated list of synonyms adopted for co-occurrence network construction

Label	Synonymous compiled
laccase	laccases; enzyme; enzymatic; lacasse; laccase enzyme; crude enzyme laccase; enzymes; fungal laccase; fungal laccases; laccase a; laccase b; ligninolytic enzymes; crude laccase
wastewater	wastewaters; wastewater treatment; pharmaceutical wastewater; water pollution; water treatment; municipal wastewater; biocatalytic wastewater treatment
immobilization	enzyme immobilization
micropollutant	micropollutants; micropollutant degradation; pharmaceutical micropollutants; micro-pollutant; micro-pollutants; micropollutants removal; pharmaceutic micropollutant; pharmaceutical pollutants; trace pollutant; pollutants; pharmaceutical micropollutants
enzymatic membrane bioreactor	enzymatic membrane; enzymatic membrane reactor; enzymatic membrane bioreactor (embr); enzymatic membrane bioreactor laccase-catalyzed; enzymatic membrane reactors
redox mediator	redox-mediator
tetracycline	tetracyclines;tetracycline antibiotics; tetracycline degradation; tetracycline/antibiotic degradation; chlortetracycline
antibiotics	antibiotic; antibiotic pollutants; antimicrobial
carbamazepine	carbamazepine (cbz)
ciprofloxacin	ciprofloxacin hydrochloride
diclofenac	diclofenac sodium (diclofenac; or diclofenac salt
enzymatic elimination	enzymatic decontamination;
estrogens	estrogen; estrogen bioremediation; estrogen removal; -ethynylestradiol; 17 \& alpha; 17 alpha-ethinylestradiol; 17 beta-estradiol; 17 beta-estradiol (e2); 17?-ethynylestradiol; estrogen bioremediation; estrogen removal
fluroquinolone	fluoroquinolones; fluoroquinolone antibiotics
endocrine disrupting chemical	endocrine-disrupting chemicals; endocrine disrupting compound (edc); endocrine disruptors
mediator	redox mediator; redox mediators; redox-mediators; mediator; redox mediator
immobilization	immobilized laccase; laccase immobilization; laccase immobilisation
metabolite	metabolites; metabolic
transformation pathway	transformation pathways

Supplementary Table 2 List of documents excluded due to their publication type

Type	Title	Authors	DOI
RV	Application of hairy roots for phytoremediation: what makes them an interesting tool for this purpose?	Agostini E;Talano M;González P;Oller A;Medina M	10.1007/S00253-012-4658-Z
RV	Regio- and stereoselective intermolecular phenol coupling enzymes in secondary metabolite biosynthesis.	Hüttel W;Müller M	10.1039/D0NP00010H
RV	Enzyme-based formulations for decontamination: current state and perspectives.	Grover N;Dinu C;Kane R;Dordick J	10.1007/S00253-013-4797-X
RV	Bioremediation of organic pollutants by laccase-metal-organic framework composites: a review of current knowledge and future perspective.	Aghaee M;Salehipour M;Rezaei S;Mogharabi-Manzari M	S0960-8524(24)00776-4
RV	Catalytic roles, immobilization and management of recalcitrant environmental pollutants by laccases: significance in sustainable green chemistry.	Zofair S;Ahmad S;Hashmi M;Khan S;Khan M;Younus H	S0301-4797(22)00249-3
RV	Enzymatic modification of polysaccharides: mechanisms, properties, and potential applications: a review.	Karaki N;Aljawish A;Humeau C;Muniglia L;Jasniewski J	S0141-0229(16)30057-6
RV	Exploring current tendencies in techniques and materials for immobilization of laccases - a review.	Alvarado-Ramírez L;Rostro-Alanis M;Rodríguez-Rodríguez J;Castillo-Zacarías C;Sosa-Hernández J;Barceló D;Iqbal H;Parra-Saldívar R	S0141-8130(21)00721-2
RV	Feasibility and potential of laccase-based enzyme in wastewater treatment through sustainable approach: a review.	Sutaoney P;Pandya S;Gajarlwar D;Joshi V;Ghosh P	10.1007/S11356-022-21565-4
RV	Genome-based engineering of ligninolytic enzymes in fungi.	Asemoloye M;Marchisio M;Gupta V;Pecoraro L	10.1186/S12934-021-01510-9
RV	Impact of antibiotics as waste, physical, chemical, and enzymatical degradation: use of laccases.	Mora-Gamboa M;Rincón-Gamboa S;Ardila-Leal L;Poutou-Piñales R;Pedroza-Rodríguez A;Quevedo-	10.3390/MOLECULES27144436

		Hidalgo B	
RV	It is the mix that matters: substrate-specific enzyme production from filamentous fungi and bacteria through solid-state fermentation.	Steudler S;Werner A;Walther T	10.1007/10_2019_85
RV	Laccase catalysis for the synthesis of bioactive compounds.	Kudanga T;Nemadziva B;Le R M	10.1007/S00253-016-7987-5
RV	Laccase: various types and applications.	Khatami S;Vakili O;Movahedpour A;Ghesmati Z;Ghasemi H;Taheri-Anganeh M	10.1002/BAB.2313
RV	Medicinal properties of the genus clitocybe and of lectins from the clouded funnel cap mushroom, c. Nebularis (agaricomycetes): a review.	Pohleven J;Kos J;Sabotic J	10.1615/INTJMEDMUSHROOMS.V18.I11.20
RV	Nano-reduction of gold and silver ions: a perspective on the fate of microbial laccases as potential biocatalysts in the synthesis of metals (gold and silver) nano-particles.	Chaurasia P;Bharati S;Yadava S	10.1016/J.CRMICR.2021.100098
RV	Recent developments in the immobilization of laccase on carbonaceous supports for environmental applications - a critical review.	Adamian Y;Lonappan L;Alokpa K;Agathos S;Cabana H	10.3389/FBIOE.2021.778239
RV	Research progress of bioactive proteins from the edible and medicinal mushrooms.	Zhou R;Liu Z;Zhang Y;Wong J;Ng T;Liu F	10.2174/1389203719666180613090710
RV	Thermophiles and the applications of their enzymes as new biocatalysts.	Atalah J;Cáceres-Moreno P;Espina G;Blamey J	S0960-8524(19)30187-7
RV	Biological significance of edible mushrooms in mycoremediation.	Muszyńska B;Lazur J;Dobosz K	
RV	A comprehensive review on incredible renewable carriers as promising platforms for enzyme immobilization & thereof strategies	Aggarwal S;Chakravarty A;Ikram S	10.1016/j.ijbiomac.2020.11.052
RV	A crucial review on polycyclic aromatic hydrocarbons - environmental occurrence and strategies for microbial degradation	Premnath N;Mohanrasu K;Rao R;Dinesh G;Prakash G;Ananthi V;Ponnuchamy K;Muthusamy ;Govarthanan G;Arun A	10.1016/j.chemosphere.2021.130608

RV	A review of polymeric refabrication techniques to modify polymer properties for biomedical and drug delivery applications	Pillay V;Seedat A;Choonara Y;Du T L;Kumar P;Ndesendo V	10.1208/s12249-013-9955-z
RV	A review on biodegradation of bisphenol-a (bpa) with bacteria and fungi under laboratory conditions	Razia S;Hadibarata T;Lau S	10.1016/j.ibiod.2024.105893
RV	Advanced applications in enzyme-induced electrospun nanofibers	Fan L;Mei X;Huang Y;Zheng W;Wei P;Jiang M;Dong W	10.1039/d4nr03404j
RV	Advances on (+)-nootkatone microbial biosynthesis and its related enzymes	Li X;Ren J;Fan G;Zhang L;Pan S	10.1093/jimb/kuab046
RV	Aerobic biodegradation of phenols: a comprehensive review	Al-Khalid T;El-Naas M	10.1080/10643389.2011.569872
RV	Applications and immobilization strategies of the copper-centred laccase enzyme; a review	Kyomuhimbo H;Brink H	10.1016/j.heliyon.2023.e13156
RV	Applications and mechanisms of free and immobilized laccase in detoxification of phenolic compounds - a review	Rostami A;Abdelrasoul A;Shokri Z;Shirvandi ;Zeinab Z	10.1007/s11814-021-0984-0
RV	Biocatalysis for the synthesis of active pharmaceutical ingredients in deep eutectic solvents: state-of-the-art and prospects	Zhang N;Dominguez D M P;Kara S	10.3390/catal14010084
RV	Biocatalytic degradation/redefining ``removal'' fate of pharmaceutically active compounds and antibiotics in the aquatic environment	Bilal M;Ashraf S;Barcelo D;Iqbal ;Hafiz M N H	10.1016/j.scitotenv.2019.07.224
RV	Biocatalytic membranes for combating the challenges of membrane fouling and micropollutants in water purification: a review	Barbhuiya N;Misra U;Singh S	10.1016/j.chemosphere.2021.131757
RV	Biodegradation and biotransformation of polycyclic non-steroidal anti-inflammatory drugs	Domaradzka D;Guzik U;Wojcieszynska D	10.1007/s11157-015-9364-8
RV	Biodegradation of endocrine-disrupting compounds by ligninolytic fungi: mechanisms involved in the degradation	Cajthaml T	10.1111/1462-2920.12460
RV	Biodegradation strategies of veterinary medicines in the environment: enzymatic degradation	Xu X;Lin X;Ma W;Huo M;Tian ;Xiaoyuan X;Wang H;Huang L	10.1016/j.scitotenv.2023.169598

RV	Biological oxidation methods for the removal of organic and inorganic contaminants from wastewater: a comprehensive review	Mohammadi S;Naja H;Zolgharnian S;Shari S;Asasian-Kolur N	10.1016/j.scitotenv.2022.157026
RV	Biomedical and pharmaceutical-related applications of laccases	Mohit E;Tabarzad M;Faramarzi M	10.2174/1389203720666191011105624
RV	Bioremediation and microbial metabolism of benzo(a)pyrene	Loss E;Yu J	10.1111/mmi.14062
RV	Critical review of catalysis-assisted nanofiltration for micropollutants removal: catalytic coupled nanofiltration system *vs* catalytic nanofiltration membrane	Zhu T;Li X;Zhu X;Liu B;Zhu J;Luo J	10.1080/10643389.2022.2113319
RV	Current challenges for biological treatment of pharmaceutical-based contaminants with oxidoreductase enzymes: immobilization processes, real aqueous matrices and hybrid techniques	Sa H;Michelin M;Tavares T;Silva B	10.3390/biom12101489
RV	Degradation of antibiotics in wastewater: new advances in cavitational treatments	Calcio G E;Canova E;Liu P;Wu ;Zhilin Z;Cravotto G	10.3390/molecules26030617
RV	Degradation of pharmaceuticals and personal care products by white-rot fungi-a critical review	Asif M;Hai F;Singh L;Price ;William E W;Nghiem L	10.1007/s40726-017-0049-5
RV	Developments in enzyme and microalgae based biotechniques to remediate micropollutants from aqueous systems—a review	Usmani Z;Sharma M;Lukk T;Karpichev Y;Thakur V;Kumar V;Allaoui A;Awasthi A;Gupta V	10.1080/10643389.2020.1862551
RV	Diclofenac biodegradation by microorganisms and with immobilised systems-a review	Wojcieszynska D;Lagoda K;Guzik U	10.3390/catal13020412
RV	Emerging contaminants of high concern and their enzyme-assisted biodegradation – a review	Bilal M;Adeel M;Rasheed T;Zhao Y;Iqbal H	10.1016/j.envint.2019.01.011
RV	Emerging field of nanotechnology in environment	Laxmi V;Singhvi N;Ahmad N;Sinha S;Negi T;Gupta V;Mubashshir M;Ahmad ;Adnan A;Sharma S	10.1007/s12088-023-01092-7
RV	Eminent industrial and biotechnological applications of laccases from bacterial source: a current overview	Akram F;Ashraf S;Ul H I;Shah F;Iftikhar I;Aqeel A	10.1007/s12010-021-03781-9

RV	Endocrine disrupting compounds (nonylphenol and bisphenol a)-sources, harmfulness and laccase-assisted degradation in the aquatic environment	Galazka A;Jankiewicz U	10.3390/microorganisms10112236
RV	Engineering biocatalytic and biosorptive materials for environmental applications	Zhu B;Chen Y;Wei N	10.1016/j.tibtech.2018.11.005
RV	Enhanced oxidation of organic contaminants by mn(vii) in water	Guan C;Guan C;Guo Q;Huang R;Duan J;Wang Z;Wei X;Jiang J	10.1016/j.watres.2022.119265
RV	Environmental concerns and bioaccumulation of psychiatric drugs in water bodies-conventional versus biocatalytic systems of mitigation	Martinez S;Melchor-Martinez E;Gonzalez-Gonzalez R;Sosa-Hernandez J;Araujo R;Rodriguez-Hernandez J;Barcelo ;Damia D;Parra-Saldivar R;Iqbal H	10.1016/j.envres.2023.115892
RV	Enzymatic modification of polysaccharides: mechanisins, properties, and potential applications: a review	Karaki N;Aljawish A;Humeau C;Muniglia L;Jasniewski J	10.1016/j.enzmictec.2016.04.004
RV	Enzymatic reactors for the removal of recalcitrant compounds in wastewater	Arca-Ramos A;Eibes G;Feijoo G;Lema ;Juan M J;Teresa M M	10.1080/10242422.2017.1315411
RV	Enzymatic synthesis of bioactive compounds with high potential for cosmeceutical application	Antonopoulou I;Varriale S;Topakas E;Rova ;Ulrika U;Christakopoulos P;Faraco V	10.1007/s00253-016-7647-9
RV	Enzymatic synthesis of chitosan derivatives and their potential applications	Aljawish A;Chevalot I;Jasniewski J;Scher J;Muniglia L	10.1016/j.molcatb.2014.10.014
RV	Enzyme immobilization as a sustainable approach toward ecological remediation of organic-contaminated soils: advances, issues, and future perspectives	Wang L;Du X;Li Y;Bai Y;Tang T;Wu J;Liang H;Gao D	10.1080/10643389.2023.2180285
RV	Enzyme immobilization by amperometric biosensors with tio^2 nanoparticles used to detect phenol compounds	Romero-Arcos M;Garnica-Romo M;Martinez-Flores H;Vazquez-Marrufo G;Ramirez-Bon R;Gonzalez-Hernandez J;Barbosa-Canovas G	10.1007/s12393-015-9129-8
RV	Enzyme-catalyzed atom transfer radical polymerization	Wang H;Hu X;Hu Y;Zhu N;Guo K	10.7536/PC211009
RV	Enzyme-immobilized porous crystals for environmental	Wang H;Kou X;Gao R;Huang S;Chen	10.1021/acs.est.4c01273

	applications	;Guosheng G;Ouyang G	
RV	Enzymes in ``green'' synthetic chemistry: laccase and lipase	Scheibel D;Gitsov I;Gitsov I	10.3390/molecules29050989
RV	Enzymes in removal of pharmaceuticals from wastewater: a critical review of challenges, applications and screening methods for their selection	Stadlmair L;Letzel T;Drewes J;Grassmann J	10.1016/j.chemosphere.2018.04.142
RV	Exolaccase-boosted humification for agricultural applications	Chu H;Li S;Sun K;Si Y;Gao ;Yanzheng Y	10.1016/j.isci.2022.104885
RV	Fate of bisphenol a in terrestrial and aquatic environments	Im J;Loffler F	10.1021/acs.est.6b00877
RV	Fungal enzymes for environmental management	Kuees U	10.1016/j.copbio.2015.03.006
RV	Fungi in mangrove ecosystems and their potential applications	Jia S;Chi Z;Liu G;Hu Z;Chi Z	10.1080/07388551.2020.1789063
RV	Harnessing bio and (photo)catalysts for microplastics degradation and remediation in soil environment	Adamu H;Bello U;Tafida U;Garba ;Zaharaddeen N Z;Galadima A;Lawan M;Abba ;Sani I S;Qamar M	10.1016/j.jenvman.2024.122543
RV	Harnessing fungal bio-electricity: a promising path to a cleaner environment	Umar A;Mubeen M;Ali I;Iftikhar Y;Sohail M;Sajid A;Kumar A;Solanki M;Kumar D P;Zhou L	10.3389/fmicb.2023.1291904
RV	Harnessing the biocatalytic attributes and applied perspectives of nanoengineered laccases-a review	Bilal M;Ashraf S;Cui J;Lou ;Wen-Yong W;Franco M;Mulla S;Iqbal H;N. N	10.1016/j.ijbiomac.2020.10.195
RV	Hazardous contaminants in the environment and their laccase-assisted degradation – a review	Bilal M;Rasheed T;Nabeel F;Iqbal H;Zhao Y	10.1016/j.jenvman.2019.01.001
RV	How to enjoy laccases	Pezzella C;Guarino L;Piscitelli A	10.1007/s00018-014-1823-9
RV	Immobilizing white-rot fungi laccase: toward bio-derived supports as a circular economy approach in organochlorine removal	Serbent M;Magario I;Saux C	10.1002/bit.28591
RV	Impact of wastewater derived dissolved interfering compounds on growth, enzymatic activity and trace organic contaminant removal of white rot fungi – a critical review	Asif M;Hai F;Hou J;Price W;Nghiem L	10.1016/j.jenvman.2017.06.014
RV	Insects to the rescue? Insights into applications,	Gwenzi W;Gufe C;Alufasi	10.1016/j.scitotenv.2024.1711

	mechanisms, and prospects of insect-driven remediation of organic contaminants	R;Makuvara Z;Marumure J;Shanmugam S;Selvasembian R;Halabowski D	16
RV	Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants	Tran N;Urase T;Ngo H;Hu J;Ong S	10.1016/j.biortech.2013.07.083
RV	Insight into pharmaceutical and personal care products removal using constructed wetlands: a comprehensive review	Salah M;Zheng Y;Wang Q;Li C;Li Y;Li F	10.1016/j.scitotenv.2023.163721
RV	Insights into metabolic engineering of bioactive molecules in tetrastigma hemsleyanum diels & gilg: a traditional medicinal herb	Krishna T;Maharajan T;Krishna T;Ceasar S	10.2174/0113892029251472230921053135
RV	Insights into the applications of extracellular laccase-aided humification in livestock manure composting	Li S;Sun K;Latif A;Si Y;Gao ;Yanzheng Y;Huang Q	10.1021/acs.est.1c08042
RV	Laccase immobilization and insolubilization: from fundamentals to applications for the elimination of emerging contaminants in wastewater treatment	Ba S;Arsenault A;Hassani T;Jones J;Peter P;Cabana H	10.3109/07388551.2012.725390
RV	Laccase immobilization and its degradation of emerging pollutants: a comprehensive review	Wang H;Tang L;Ye Y;Ma J;Li X;Si J;Cui B	10.1016/j.jenvman.2024.120984
RV	Laccase immobilized on nanocomposites for wastewater pollutants degradation: current status and future prospects	Zhang W;Zhang Z;Ji L;Lu Z;Liu R;Nian B;Hu Y	10.1007/s00449-023-02907-z
RV	Laccase-assisted degradation of emerging recalcitrant compounds - a review	Bhardwaj P;Kaur N;Selvaraj M;Ghramh H;Al-Shehri B;Singh G;Arya ;Shailendra K S;Bhatt K;Ghotekar S;Mani R;Chang S;Ravindran B;Awasthi M;Kumar K	10.1016/j.biortech.2022.128031
RV	Laccase-evoked removal of antibiotics: reaction kinetics, conversion mechanisms, and ecotoxicity assessment	Sun K;Chen M;Qi X;Hong D;Dai ;Ling-Zhi L;Li S;Lu Y;Yu H	10.1080/10643389.2023.2224612
RV	Laccase-mediated synthesis of bioactive natural products and their analogues	Cardullo N;Muccilli V;Tringali C	10.1039/d1cb00259g

RV	Laccases and peroxidases: the smart, greener and futuristic biocatalytic tools to mitigate recalcitrant emerging pollutants	Morsi R;Bilal M;Iqbal H;Ashraf S	10.1016/j.scitotenv.2020.136572
RV	Laccases and their applications: a patent review	Kunamneni A;Plou F;Ballesteros A;Alcalde M	10.2174/18722080878333096 5
RV	Laccases and tyrosinases in organic synthesis	Martinkova L;Kristkova B;Kren V	10.3390/ijms23073462
RV	Laccases as effective tools in the removal of pharmaceutical products from aquatic systems	Chmelova D;Ondrejovic M;Miertus S	10.3390/life14020230
RV	Laccases to take on the challenge of emerging organic contaminants in wastewater	Gasser C;Ammann E;Shahgaldian P;Corvini P	10.1007/s00253-014-6177-6
RV	Laccases: production, expression regulation, and applications in pharmaceutical biodegradation	Yang J;Li W;Ng T;Deng X;Lin ;Juan J;Ye X	10.3389/fmicb.2017.00832
RV	Laccases: structure, function, and potential application in water bioremediation	Arregui L;Ayala M;Gomez-Gil X;Gutierrez-Soto G;Eduardo H C;Herrera H;De L S M;Levin L;Rojo-Dominguez A;Romero-Martinez D;Saparrat M;Trujillo-Roldan ;Mauricio A M;Valdez-Cruz N	10.1186/s12934-019-1248-0
RV	Landfill leachate treatment using fungi and fungal enzymes: a review	Nalladiyil A;Sughosh P;Babu G;Ramaswami S	10.1007/s10532-023-10052-3
RV	Lignin-modifying enzymes: a green and environmental responsive technology for organic compound degradation	Da S V D;Bilal M;Bharagava R;Kumar A;Kumar N A;Salazar-Banda G;Eguiluz K;Romanholo F L	10.1002/jctb.6751
RV	Ligninolytic enzymes: versatile biocatalysts for the elimination of endocrine-disrupting chemicals in wastewater	Falade A;Mabinya L;Okoh A;Nwodo U	10.1002/mbo3.722
RV	Mechano-chemical and biological energetics of immobilized enzymes onto functionalized polymers and their applications	Sharma T;Xia C;Sharma A;Raizada P;Singh P;Sharma S;Sharma P;Kumar S;Lam S;Nadda A	10.1080/21655979.2022.2062526
RV	Medicines as an emergent contaminant: the review of microbial biodegration potential	Grignet R;Barros M;Panatta A;S. S;Bernal S;Ottoni J;Passarini M;Z.	10.1007/s12223-021-00941-6

		Z;Goncalves C	
RV	Metabolism of non-steroidal anti-inflammatory drugs by non-target wild-living organisms	Mulkiewicz E;Wolecki D;Świacka K;Kumirska J;Stepnowski P;Caban M	10.1016/j.scitotenv.2021.148251
RV	Mycoremediation: expunging environmental pollutants	Akhtar N;Mannan M	10.1016/j.btre.2020.e00452
RV	Overview on the biochemical potential of filamentous fungi to degrade pharmaceutical compounds	Olicón-Hernández D;González-López J;Aranda E	10.3389/fmicb.2017.01792
RV	Pharmaceutical pollution in aquatic environments: a concise review of environmental impacts and bioremediation systems	Ortúzar M;Esterhuizen M;Olicón-Hernández D;González-López J;Aranda E	10.3389/fmicb.2022.869332
RV	Pharmaceutically active micropollutants: origin, hazards and removal	Gupta A;Kumar S;Bajpai Y;Chaturvedi K;Johri P;Tiwari R;Vivekanand V;Trivedi M	10.3389/fmicb.2024.1339469
RV	Phytoremediation of small organic contaminants using transgenic plants	James C;Strand S	10.1016/j.copbio.2009.02.014
RV	Plastic biodegradation: frontline microbes and their enzymes	Amobonye A;Bhagwat P;Singh S;Pillai S	10.1016/j.scitotenv.2020.143536
RV	Polymerization of micropollutants in natural aquatic environments: a review	Zhong C;Zhao H;Cao H;Huang Q	10.1016/j.scitotenv.2019.133751
RV	Potential applications of laccase-mediated coupling and grafting reactions: a review	Kudanga T;Nyanhongo G;Guebitz G;Burton S	10.1016/j.enzmictec.2010.11.007
RV	Potential of enzymatic process as an innovative technology to remove anticancer drugs in wastewater	Pereira C;Kelbert M;Daronch N;Michels C;De O D;Soares H	10.1007/s00253-019-10229-y
RV	Potential of laccase as a tool for biodegradation of wastewater micropollutants	Janusz G;Skwarek E;Pawlik A	10.3390/w15213770
RV	Production and purification strategies for laccase	Dhull N;Michael M;Simran P;Gokak V;Venkatanagaraju E	10.13040/IJPSR.0975-8232.11(6).2617-25
RV	Progress and trend on the regulation methods for nanozyme activity and its application	Hou L;Jiang G;Sun Y;Zhang X;Huang ;Juanjuan J;Liu S;Lin T;Ye F;Zhao ;Shulin S	10.3390/catal9121057
RV	Progressive biocatalysts for the treatment of aqueous	Efremenko E;Stepanov N;Senko	10.3390/life13030841

	systems containing pharmaceutical pollutants	O;Maslova O;Lyagin I;Aslanli A	
RV	Recent development in the application of immobilized oxidative enzymes for bioremediation of hazardous micropollutants – a review	Shakerian F;Zhao J;Li S	10.1016/j.chemosphere.2019.124716
RV	Recent developments in the use of tyrosinase and laccase in environmental applications	Ba S;Kumar V	10.1080/07388551.2016.1261081
RV	Recent progress and prospects in catalytic water treatment	Parvulescu V;Epron F;Garcia H;Granger P	10.1021/acs.chemrev.1c00527
RV	Recent strategies and applications for l-asparaginase confinement	Nunes J;Cristovao R;Freire M;Santos-Ebinuma V;Faria J;Silva C;Tavares A	10.3390/molecules25245827
RV	Removal of emerging contaminants by degradation during filtration: a review of experimental procedures and modeling	Undabeytia T;Jimenez-Barrera J;Nir S	10.3390/w16010110
RV	Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes	Naghdi M;Taheran M;Brar S;Kermanshahi-Pour A;Verma M;Surampalli R	10.1016/j.envpol.2017.11.060
RV	Role of fungal enzymes in the synthesis of pharmaceutically important scaffolds: a green approach	Kumar D;Narula A;Deswal D	10.1039/d3gc02384b
RV	Role of various enzymes for deinking paper: a review	Saxena A;Chauhan P	10.1080/07388551.2016.1207594
RV	Synthesis and biological activities of dehydrodiisoeugenol: a review	Godinez-Chaparro B;Perez-Gutierrez S;Perez-Ramos ;Julia J;Heyerdahl-Viau I;Hernandez-Vazquez L	10.3390/ph15111351
RV	The use of algae and fungi for removal of pharmaceuticals by bioremediation and biosorption processes: a review	Silva A;Delerue-Matos C;Figueiredo S;Freitas O	10.3390/w11081555
RV	Thermophilic and alkaliphilic actinobacteria: biology and potential applications	Shivlata L;Satyanarayana T	10.3389/fmicb.2015.01014
RV	Thermozymes: adaptive strategies and tools for their biotechnological applications	Kumar S;Dangi A;Shukla P;Baishya D;Khare S	10.1016/j.biortech.2019.01.088
RV	Toward the development of a molecular toolkit for the	Hu M;Scott C	10.1128/aem.00157-24

	microbial remediation of per-and polyfluoroalkyl substances		
RV	Towards oxidoreductase-based processes for the removal of antibiotics from wastewater	De B S;Schaeffer A;Moreira M	10.1007/s11157-023-09676-x
RV	Trade-off effect of dissolved organic matter on degradation and transformation of micropollutants: a review in water decontamination	Chen X;Wang J;Wu H;Zhu Z;Zhou ;Jianfei J;Guo H	10.1016/j.jhazmat.2023.130996
RV	Trends in predictive biodegradation for sustainable mitigation of environmental pollutants: recent progress and future outlook	Singh A;Bilal M;Iqbal H;Raj ;Abhay A	10.1016/j.scitotenv.2020.144561
RV	Unlocking the potential of soil microbial communities for bioremediation of emerging organic contaminants: omics-based approaches	Alidoosti F;Giyahchi M;Moien S;Moghimi ;Hamid H	10.1186/s12934-024-02485-z
RV	Valorization of agro-industrial wastes and residues through the production of bioactive compounds by macrofungi in liquid state cultures: growing circular economy	Pilafidis S;Diamantopoulou P;Gkatzionis ;Konstantinos K;Sarris D	10.3390/app122211426
RV	White rot fungi can be a promising tool for removal of bisphenol a, bisphenol s, and nonylphenol from wastewater	Grelska A;Noszczynska M	10.1007/s11356-020-10382-2
RV	Whole-cell biocatalytic, enzymatic and green chemistry methods for the production of resveratrol and its derivatives	Jeandet P;Sobarzo-Sanchez E;Silva A;Clement C;Nabavi S;Battino M;Rasekhian M;Belwal T;Habtemariam S;Koffas M;Nabavi S	10.1016/j.biotechadv.2019.107461
RV	Laccase-mediated degradation of emerging contaminants: unveiling a sustainable solution	Thathola P;Melchor-Martinez E;Adhikari P;Martinez S;Pandey A;Parra-Saldivar ;Roberto R	10.1039/d4va00173g
RV	An overview of different strategies involved in an efficient control of emerging contaminants: promising enzymes and the related reaction process	Ouyang B;Xu W;Zhang W;Guang C;Mu ;Wanmeng W	10.1016/j.jece.2022.108211
RV	Biocatalytic remediation of pharmaceutically active micropollutants for environmental sustainability*	Bilal M;Lam S;Iqbal H	10.1016/j.envpol.2021.118582
RV	Biotechnological potential of ligninolytic enzymes for	Mendez-Hernandez J;Loera O	10.24275/uam/izt/dcbi/revme

	pollutant biodegradation in water: from test-tubes to full-scale enzymatic reactors		xingguim/2019v18n2/Mendez
RV	Emerging pollutant treatments in wastewater: cases of antibiotics and hormones	Méndez E;González-Fuentes M;Rebollar-Perez G;Méndez-Albores A;Torres E	10.1080/10934529.2016.1253391
RV	Endocrine disrupting chemicals (EDCS) in environmental matrices: occurrence, fate, health impact, physio-chemical and bioremediation technology	Ismanto A;Hadibarata T;Kristanti R;Maslukah L;Safinatunnajah N;Kusumastuti W	10.1016/j.envpol.2022.119061
RV	Enzyme conjugation - a promising tool for bio-catalytic and biotransformation applications - a review	Muneer M;Fatima S;Hussain N;Mashifana T;Sayed A;Boczkaj G;Rajoka ;Muhammad S R M	10.1007/s11244-024-01986-w
RV	Enzyme immobilization technologies and industrial applications	Maghraby Y;El-Shabasy R;Ibrahim A;Azzazy H	10.1021/acsomega.2c07560
RV	Four decades of laccase research for wastewater treatment: insights from bibliometric analysis	Puspita K;Chiari W;Abdulmadjid S;Idroes R;Iqhrammullah M	10.3390/ijerph20010308
RV	Lance: laccase-nanoparticle conjugates for the elimination of micropollutants (endocrine disrupting chemicals) from wastewater in bioreactors	Corvini P;Shahgaldian P	10.1007/s11157-009-9182-y
RV	Spent waste from edible mushrooms offers innovative strategies for the remediation of persistent organic micropollutants: a review	Ghose A;Mitra S	10.1016/j.envpol.2022.119285
RV	Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review	Yang S;Hai F;Nghiem L;Price W;E. E;Roddick F;Moreira M;Magram S	10.1016/j.biortech.2013.01.173
BC	Alternative and new sources of feedstuffs	Romano N	10.1016/B978-0-12-805419-2.00019-8
BC	Application of white-rot fungi in transformation, detoxification, or revalorization of agriculture wastes: role of laccase in the processes	Jurado M;Martinèz T;Martinez M;Saparrat M	10.1016/B978-0-444-64046-8.00378-5
BC	Application, current and future trends of bacterial and	Sharma P;Tamang S;Thakur N;Das S	10.1515/9783111033525-008

	fungal polyphenol oxidases (laccases)		
BC	Applications of microbial laccases in bioremediation of environmental pollutants: potential issues, challenges, and prospects	Unuofin J;Falade A;Aladekoyi O	10.1016/B978-0-12-820524-2.00021-3
BC	Bacterial polyphenol oxidases and their applications in bioremediation	Kumari B;Golla N;Kumar K;Geetha K;Reddy B	10.1515/9783111033525-002
BC	Current trends and perspectives in microbial bioconversions of steroids	Donova M	10.1007/978-1-0716-3385-4_1
BC	Emerging contaminants and their possible bioremediation through bacterial laccases	Romero-Martínez D;Parra-Saldivar R;Trujillo-Roldán M;Valdez-Cruz N	10.1016/B978-0-323-91889-3.00008-X
BC	Enzymatic approach for phytoremediation	Pathak A;Gupta M;Rabani M;Tripathi S;Pandey S;Gupta C;Shrivastav M	10.1002/9781119989318.ch8
BC	Fungal polyphenol oxidases and their applications in bioremediation	Kumari B;Golla N;Kumar K;Geetha K;Reddy B	10.1515/9783111033525-003
BC	General perspectives of enzymes, environment preservation, and scarce natural resources-conclusions	Nunes C	10.1016/B978-0-12-805419-2.00027-7
BC	Laccase-mediated treatment of pharmaceutical wastes	Forootanfar H;Arjmand S;Behzadi M;Faramarzi M	10.4018/978-1-5225-5237-6.ch010
BC	Leveraging the pharmaceutical area through multidisciplinary synergy: from prescription to disintegration	Razak N;Diba R;Xin F;Salem A;Jayadeep R;Malik I;Manap A;Rhofita E;Qi N;Xin L;Emran A;Zain D;Jayaram N;Kandandapani S;Zia U;Hang N;Thoe N	10.4018/979-8-3693-3699-1.ch008
BC	Nanotubes tethered laccase biosensor for sensing of chlorophenol substances	Kaya S;Corman M;Cetinkaya A;Karasu T;Uzun L;Ozkan S	10.1016/B978-0-323-90553-4.00008-1
BC	Nonaqueous catalysis: a way forward for the intermediation of phenolic environmental pollutant bisphenol a	Trivedi J;Chhaya U;Patel Y;Rudakiya D	10.1007/978-981-15-7455-9_12
BC	Organopollutant degradation by wood decay basidiomycetes	Hadar Y;Cullen D	10.1007/978-3-642-36821-9_5
BC	Perspectives on the feasibility of using enzymes for	Bilal M;Iqbal H;Barceló D	10.1007/698_2020_661

	pharmaceutical removal in wastewater		
BC	Prospecting bio-enzymes for a greener environment	Shahbaz A;Hussain N;Saba S;Gul I;Khurshid M;Derakhshan Z;Hadibarata T;Bilal M	10.1016/B978-0-323-99476-7.00009-0
BC	Treatment of landfill leachate using fungi: an efficient and cost-effective strategy	Ghosh P;Thakur I	10.1007/978-981-10-4768-8_18
BC	Trends in downstream processing approaches, laccase mediator systems and biotechnological applications of laccases	Akinyemi O;Ahuekwe E;Oziegbe O;Nwinyi O	10.1007/978-3-030-96721-5_15
BC	Vermicompost derived from spent coffee grounds: assessing the potential for enzymatic bioremediation	Sanchez-Hernandez J;Domínguez J	10.1016/B978-0-12-811290-8.00012-8
CP	Hydrophilic metal-chelated membrane for biocatalytic membrane reactor application	Ummi A M R N;Marpani F;Hashimah A N;Hidayati O N;Shafiq M S M	10.1016/j.matpr.2023.02.381
CP	Investigating the presence of trace organic contaminants in hospital wastewater and their treatment by laccase	Alokpa K;Saibi S;Haroune L;Cabana H	10.11159/iceptp23.176
CP	Sequestration, phytoreduction, and phytooxidation of halogenated organic chemicals by aquatic and terrestrial plants	Nzengung V;Jeffers P	10.1080/15226510108500048
SS	Nootkatone-a biotechnological challenge	Fraatz M;Berger R;Zorn H	10.1007/s00253-009-1968-x
SS	Stereoselective pharmacokinetics and chiral inversions of some chiral hydroxy group drugs	Chen F;Bai Q;Wang Q;Chen S;Ma X;Cai C;Wang D;Waqas A;Gong P	10.2174/13892010216662007 27144053
RP	Molecular docking exploration of the degradation activity of some synthetic hydrocarbons polymers by the laccase enzyme of *Streptomyces*	Mansouri N;Benslama O	10.1016/j.matpr.2021.12.169

RV: Review - BC: Book Chapter - CP: Conference Paper - SS: Short Survey - RP: Retracted Publication

Supplementary Table 3 Articles included in the scientometric analysis

N	Title	Authors	DOI
1	3d chitin scaffolds from the marine demosponge aplysina archeri as a support for laccase immobilization and its use in the removal of pharmaceuticals	Zdarta J;Machalowski T;Degorska O;Bachosz ;Karolina K;Fursov A;Ehrlich H;Ivanenko ;Viatcheslav N V;Jesionowski T	10.3390/biom10040646
2	3d printed polylactide scaffolding for laccase immobilization to improve enzyme stability and estrogen removal from wastewater	Rybarczyk A;Smu W;Grzywaczyk A;Kaczorek E;Jesionowski T;Nghiem L;Zdarta ;Jakub J	10.1016/j.biortech.2023.129144
3	A customizable 3d printed device for enzymatic removal of drugs in water	Xu X;Pose-Boirazian T;Eibes G;Mccoubrey ;Laura E L;Martinez-Costas J;Gaisford S;Goyanes ;Alvaro A;Basit A	10.1016/j.watres.2021.117861
4	A hybrid bioreactor based on insolubilized tyrosinase and laccase catalysis and microfiltration membrane remove pharmaceuticals from wastewater	Ba S;Haroune L;Soumano L;Bellenger ;Jean-Phillipe J;Jones J;Cabana H	10.1016/j.chemosphere.2018.03.022
5	A new laccase-mediator system facing the biodegradation challenge: insight into the nsaids removal	Apriceno A;Astolfi M;Girelli A;Scuto F	10.1016/j.chemosphere.2018.10.086
6	A novel and sustainable composite of l@psac for superior removal of pharmaceuticals from different water matrices: production, characterization, and application	Al-Sareji O;Al-Samarrai S;Grmasha R;Meiczinger M;Al-Juboori R;Jakab M;Somogyi V;Miskolczi N;Hashim K	10.1016/j.envres.2024.118565

7	A novel approach in crude enzyme laccase production and application in emerging contaminant bioremediation	Nguyen L;Vu M;Johir M;Pathak N;Zdarta J;Jesionowski T;Semblante G;Hai F;Nguyen H;Nghiem L	10.3390/PR8060648
8	A novel process for the covalent immobilization of laccases on silica gel and its application for the elimination of pharmaceutical micropollutants	Guardado A;Druon-Bocquet S;Belleville ;Marie-Pierre M;Sanchez-Marcano J	10.1007/s11356-021-12394-y
9	A sustainable nano-hybrid system of laccase@m-mwcnts for multifunctional pahs and phacs removal from water, wastewater, and lake water	Grmasha R;Al-Sareji O;Meiczinger M;Stenger-Kov C;Al-Juboori R;Jakab M;Lengyel E;Somogyi V;Khan M;Hashim ;Khalid S K	10.1016/j.envres.2024.118097
10	Activated carbon-gravity driven biomimetic membrane (ac-gdbm) for organic micro-polluted water treatment	Chen W;Luo J;Du X;Ding L;Zhang W	10.1016/j.jclepro.2021.128224
11	Adsorptive immobilization of agro-industrially produced crude laccase on various micro-biochars and degradation of diclofenac	Lonappan L;Liu Y;Rouissi T;Brar S;Kaur K;Verma M;Surampalli R	10.1016/j.scitotenv.2018.06.005
12	Agro-industrial-produced laccase for degradation of diclofenac and identification of transformation products	Lonappan L;Rouissi T;Laadila M;Brar ;Satinder K S;Hernandez G L;Verma M;Suranripalli R	10.1021/acssuschemeng.7b00390
13	Application of laccase and hydrolases for trace organic contaminants removal from contaminated water	Alokpa K;Lafortune F;Cabana H	10.1016/j.envadv.2022.100243
14	Application of pleurotus ostreatus to efficient removal of selected antidepressants and immunosuppressant	Kózka B;Nałęcz-Jawecki G;Turło J;Giebułtowicz J	S0301-4797(20)31058-6
15	Application of response surface methodology to study the removal of estrogens in a laccase-mediated continuous membrane reactor	Lloret L;Eibes G;Feijoo G;Teresa T;Moreira M;Lema J	10.3109/10242422.2013.815745
16	Application of the white-rot fungus trametes sp. (c3) laccase in the removal of acetaminophen from aqueous solutions	Sybuia P;Contato A;De A C;Aparecida V A;Zanzarin D;Maciel G;Pilau E;Peralta R;De S C;Giatti M G	10.1016/j.jwpe.2023.104677
17	Assessing the environmental risk potential of transformation byproducts formed during fungal enzymatic treatment of a	Oh S;Nguyen H;Shola O K;Ilahi S S	10.1016/j.jiec.2024.08.015

	pharmaceutical mixture		
18	Assessing the fungal simultaneous removal efficiency of carbamazepine, diclofenac and ibuprofen in aquatic environment	Kasonga T;Coetzee M;Kamika I;Momba M	10.3389/fmicb.2021.755972
19	Assessing the use of nanoimmobilized laccases to remove micropollutants from wastewater	Arca-Ramos A;Ammann E;Gasser C;Nastold P;Eibes G;Feijoo G;Lema J;Moreira M;Corvini ;P. F - P	10.1007/s11356-015-5564-6
20	Assessment of the biodegradation of doxycycline by biostimulation with addition of glucose, phenol or/and copper	Djelal H;Martinez P;Haddouche D;Chabani M	10.19040/ecocycles.v6i2.175
21	Bacterial versus fungal laccase: potential for micropollutant degradation	Margot J;Bennati-Granier C;Maillard J;Blánquez P;Barry D;Holliger C	10.1186/2191-0855-3-63
22	Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor	Ji C;Hou J;Wang K;Zhang Y;Chen V	10.1016/j.memsci.2015.12.043
23	Biocatalytic degradation of pharmaceuticals, personal care products, industrial chemicals, steroid hormones and pesticides in a membrane distillation-enzymatic bioreactor	Asif M;Hai F;Kang J;Van D M J;Leusch F;Price W;Nghiem L	10.1016/j.biortech.2017.09.129
24	Biocatalytic elimination of pharmaceutics found in water with hierarchical silica monoliths in continuous flow	Sebai W;Ahmad S;Belleville M;Boccheciampe A;Chaurand P;Levard C;Brun ;Nicolas N;Galarneau A;Sanchez-Marcano J	10.3389/fceng.2022.823877
25	Biocatalytic properties of cell surface display laccase for degradation of emerging contaminant acetaminophen in water reclamation	Wu Y;Chen Y;Wei N	10.1002/bit.27214
26	Biochemical and physicochemical processes contributing to the removal of endocrine-disrupting chemicals and pharmaceuticals by the aquatic ascomycete phoma sp uhh 5-1-03	Hofmann U;Schlosser D	10.1007/s00253-015-7113-0
27	Biodegradation of chlortetracycline by trametes versicolor - produced laccase: by-product identification	Taheran M;Naghdi M;Brar S;Knystautas E;Verma M;Tyagi	10.1061/(ASCE)EE.1943-7870.0001362

		R;Surampalli R	
28	Biodegradation of nsaids and their effect on the activity of ligninolytic enzymes from pleurotus djamor	Cruz-Ornelas R;Sanchez-Vazquez J;Amaya-Delgado ;Lorena L;Guillen-Navarro K;Calixto-Romo A	10.1007/s13205-019-1904-4
29	Biodegradation of the analgesic naproxen by trametes versicolor and identification of intermediates using hplc-dad-ms and nmr	Marco-Urrea E;Perez-Trujillo M;Blanquez P;Vicent T;Caminal G	10.1016/j.biortech.2009.11.019
30	Biological removal of pharmaceutical compounds using white-rot fungi with concomitant fame production of the residual biomass	Vasiliadou I;Sanchez-Vazquez R;Molina R;Martinez ;F. F;Melero J;Bautista L;Iglesias J;Morales ;G. G	10.1016/j.jenvman.2016.05.035
31	Bioremoval and biodetoxification of ciprofloxacin from hospital wastewater effluent by the efficient and recyclable laccase@hercynite magnetic nanoparticles	Mojtabavi S;Rezayaraghi F;Shahverdi M;Golroudbari H;Moshiri F;Faramarzi M;Ali A	10.1016/j.jece.2023.111599
32	Bioremoval of estrogens by laccase immobilized onto polyacrylonitrile/polyethersulfone material: effect of inhibitors and mediators, process characterization and catalytic pathways determination	Zdarta J;Jankowska K;Strybel U;Marczak ;Lukasz L;Nguyen L;Oleskowicz-Popiel P;Jesionowski T	10.1016/j.jhazmat.2022.128688
33	Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from pycnoporus sanguineus cs43 fungi	Rodriguez-Delgado M;Orona-Navar C;Garcia-Morales ;Raul R;Hernandez-Luna C;Parra R;Mahlknecht ;Juergen J;Ornelas-Soto N	10.1016/j.ibiod.2015.12.003
34	Biotransformation of 4, 4'-dihydroxybiphenyl and dienestrol by laccase from trametes versicolor	Lzaod S;Dutta T	10.1016/j.hazadv.2022.100169
35	Biotransformation of emerging pollutants in groundwater by laccase from p. Sanguineus cs43 immobilized onto titania nanoparticles	Garcia-Morales R;Garcia-Garcia A;Orona-Navar ;Carolina C;Osma J;Nigam K;Ornelas-Soto N	10.1016/j.jece.2017.12.006
36	Biotransformation of endocrine-disrupting compounds in groundwater: bisphenol a, nonylphenol, ethynylestradiol and triclosan by a laccase cocktail from pycnoporus sanguineus cs43	Garcia-Morales R;Rodriguez-Delgado M;Gomez-Mariscal K;Orona-Navar C;Hernandez-Luna C;Torres E;Parra R;Cardenas-Chavez D;Mahlknecht	10.1007/s11270-015-2514-3

		J;Ornelas-Soto N	
37	Biotransformnation of carbamazepine by laccase-mediator system: kinetics, by-products and toxicity assessment	Naghdi M;Taheran M;Brar S;Kermanshahi-Pour A;Verma M;Surampalli R	10.1016/j.procbio.2018.02.009
38	Cell surface display fungal laccase as a renewable biocatalyst for degradation of persistent micropollutants bisphenol a and sulfamethoxazole	Chen Y;Stemple B;Kumar M;Wei N	10.1021/acs.est.6b01641
39	Characterisation of electron beam irradiation-immobilised laccase for application in wastewater treatment	Jahangiri E;Thomas I;Schulze A;Seiwert ;Bettina B;Cabana H;Schlosser D	10.1016/j.scitotenv.2017.12.127
40	Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals	Touahar I;Haroune I;Ba S;Bellenger ;Jean-Phillipe J;Cabana H	10.1016/j.scitotenv.2014.01.132
41	Characterization of laccase-grafted ceramic membranes for pharmaceuticals degradation	De C M;Belleville M;Mougel M;Kellner H;Sanchez-Marcano J	10.1016/j.memsci.2014.11.044
42	Characterization of laccases from trametes hirsuta in the context of bioremediation of wastewater treatment plant effluent	El Y Y;Lemieux B;Segura P;Cabana ;Hubert H	10.1016/j.enzmictec.2023.110308
43	Chitin membrane for efficient laccase immobilization and synergistic removal of 17α-ethynylestradiol from water-based solutions	Wysokowski M;Chmielewska Z;Sandomierski M;Zdarta J;Jesionowski T	10.1016/j.ijbiomac.2024.136599
44	Combining enzymatic membrane bioreactor and ultraviolet photolysis for enhanced removal of trace organic contaminants: degradation efficiency and by-products formation	Tufail A;Alharbi S;Alrifai J;Ansari A;Price W;Hai F	10.1016/j.psep.2020.08.001
45	Comparison of acetaminophen degradation by laccases immobilized by two different methods via a continuous flow microreactor process scheme	Sotelo L;Sotelo D;Ornelas-Soto N;Cruz ;Juan C J;Osma J	10.3390/membranes12030298
46	Comprehensive ms-based screening and identification of	Stadlmair L;Grosse S;Letzel T;Drewes	10.1007/s00216-018-1442-7

	pharmaceutical transformation products formed during enzymatic conversion	;Joerg E J;Grassmann J	
47	Concentration of diclofenac sodium using the nanofiltration combined with laccase degradation from trametes versicolor	Haouche R;Innocent C;Akretche D	10.1007/s13369-018-3371-3
48	Construction of a continuous packed bed laccase reactor for the elimination of tetracyclines in seawater	Wang X;Meng F	10.1016/j.jece.2024.111939
49	Continuous adsorption and biotransformation of micropollutants by granular activated carbon-bound laccase in a packed-bed enzyme reactor	Nguyen L;Hai F;Dosseto A;Richardson C;Price W;Nghiem L	10.1016/j.biortech.2016.01.014
50	Continuous biotransformation of bisphenol a and diclofenac bylaccase in an enzymatic membrane reactor	Nguyen L;Hai F;Price W;Leusch F;Roddick F;Mcadam E;Magram S;Nghiem L	10.1016/j.ibiod.2014.05.017
51	Covalent immobilization of laccase onto nanofibrous membrane for degradation of pharmaceutical residues in water	Taheran M;Naghdi M;Brar S;Knystautas E;Verma M;Surampalli R	10.1021/acssuschemeng.7b02465
52	Covalent organic framework in-situ immobilized laccase for the covalent polymerization removal of sulfamethoxazole in the presence of natural phenols: prominent enzyme stability and activity	Xu J;Zhang X;Zhou Z;Ye G;Wu ;Deli D	10.1016/j.jhazmat.2023.132714
53	Cross-linked carbon nanotubes-based biocatalytic membranes for micro-pollutants degradation: performance, stability, and regeneration	Ji C;Hou J;Chen V	10.1016/j.memsci.2016.08.056
54	Cupuacu theobroma grandiflorum residue and its potential application in the bioremediation of 17--ethinylestradiol as a pycnoporus sanguineus laccase inducer	Golveia J;Santiago M;Sales P;F. F;Sartoratto A;Ponezi A;Thomaz D;V. V;Gil E;Bara M	10.1080/10826068.2018.1466161
55	Degradation of a broad spectrum of trace organic contaminants by an enzymatic membrane reactor: complementary role of membrane retention and enzymatic degradation	Nguyen L;Hai F;Price W;Kang ;Jinguo J;Leusch F;Roddick F;Van D;Merwe J;Magram S;Nghiem L	10.1016/j.ibiod.2014.12.004
56	Degradation of chlortetracycline using immobilized laccase	Taheran M;Naghdi M;Brar	10.1016/j.scitotenv.2017.06.1

	on polyacrylonitrile-biochar composite nanofibrous membrane	S;Knystautas E;Verma M;Surampalli R	85
57	Degradation of diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole by laccase from trametes versicolor: transformation products and toxicity of treated effluent	Alharbi S;Nghiem L;Van D M J;Leusch F;Asif M;Hai F;Price W	10.1080/10242422.2019.1580268
58	Degradation of endocrine-disrupting chemicals in wastewater by new thermophilic fungal isolates and their laccases	Daassi D;Alharbi S	10.1007/s13205-022-03439-1
59	Degradation of selected pharmaceuticals detected in wastewater systems using an enzyme-mediator system and identification of resulting transformation products	Caraene I;Gruchlik Y;Busetti F;Linge K;Joll C	10.1080/10242422.2021.2003344
60	Degradation of sodium metamizole by enzymatic extracts from some white rot fungi	Mayorga R;Sanchez J;Gutierrez R;Calixto-Romo M	10.12905/0380.sydowia69-2017-0205
61	Degradation of some benzodiazepines by a laccase-mediated system in aqueous solution	Ostadhadi-Dehkordi S;Tabatabaei-Sameni M;Forootanfar H;Kolahdouz S;Ghazi-Khansari M;Faramarzi M	10.1016/j.biortech.2012.09.039
62	Degradation of tetracyclines and sulfonamides by stevensite- and biochar-immobilized laccase systems and impact on residual antibiotic activity	Garcia-Delgado C;Eymar E;Camacho-Arevalo R;Petruccioli M;Crognale S;D'annibale ;Alessandro A	10.1002/jctb.5697
63	Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products	Prieto A;Moeder M;Rodil R;Adrian L;Marco-Urrea E	10.1016/j.biortech.2011.08.055
64	Degradation of trace organic contaminants by a membrane distillation-enzymatic bioreactor	Asif M;Hai F;Kang J;Van D M;Jason P J;Leusch F;Yamamoto K;Price ;William E W;Nghiem L	10.3390/app7090879
65	Design and optimization of an enzymatic membrane reactor for tetracycline degradation	De C M;Belleville M;Petit E;Llorca M;Rodriguez-Mozaz S;De G J;Barcelo D;Sanchez-Marcano J	10.1016/j.cattod.2014.02.051
66	Design and optimization of laccase immobilization in cellulose acetate microfiltration membrane for	Varga B;Meiczinger M;Jakab M;Somogyi V	10.3390/catal13020222

	micropollutant remediation		
67	Design, economic evaluation and optimization of enzymatic membrane reactors for antibiotics degradation in wastewaters	Abejon R;Belleville M;Sanchez-Marcano J	10.1016/j.seppur.2015.09.072
68	Development of an advanced multifunctional portable water purifier	Taheran M;Kumar P;Naghdi M;Brar S;Knystautas E;Verma M;Surampalli R	10.1007/s41204-019-0054-6
69	Direct immobilization of laccase on titania nanoparticles from crude enzyme extracts of p. ostreatus culture for micro-pollutant degradation	Ji C;Nguyen L;Hou J;Hai F;Chen V	10.1016/j.seppur.2017.01.043
70	Ecofriendly laccases treatment to challenge micropollutants issue in municipal wastewaters	Spina F;Gea M;Bicchi C;Cordero C;Schilirò T;Varese G	10.1016/j.envpol.2019.113579
71	Effect of redox mediators in pharmaceuticals degradation by laccase: a comparative study	Guardado A;Belleville M;Rostro R;Alanis M;Parra S R;Sanchez-Marcano J	10.1016/j.procbio.2018.12.032
72	Efficient ibuprofen removal using enzymatic activated zif-8-pvdf membranes	Hassan A;Hasan S;Van D B B;Al-Zuhair S	10.1016/j.clet.2024.100824
73	Efficient remediation of 17α-ethinylestradiol by lentinula edodes (shiitake) laccase	Eldridge H;Milliken A;Farmer C;Hampton A;Wendland N;Coward L;Gregory D;Johnson C	10.1016/j.bcab.2017.02.004
74	Efficient removal of pharmaceutical contaminants from water and wastewater using immobilized laccase on activated carbon derived from pomegranate peels	Al-Sareji O;Meiczinger M;Al-Juboori R;Grmasha R;Andredaki M;Somogyi V;Idowu I;Stenger-Kovacs C;Jakab M;Lengyel E;Hashim K	10.1038/s41598-023-38821-3
75	Elimination of tetracyclines in seawater by laccase-mediator system	Wang X;Meng F;Zhang B;Xia Y	10.1016/j.chemosphere.2023.138916
76	Elucidating the performance of an integrated laccase- and persulfate-assisted process for degradation of trace organic contaminants (trocs)	Asif M;Van D M J;Leusch F;Pramanik B;Price W;Hai F	10.1039/c9ew01022j
77	Endocrine disrupting chemicals (edcs) in municipal wastewaters: effective degradation and detoxification by	Spina F;Cordero C;Sgorbini B;Schiliro ;Tiziana T;Gilli G;Bicchi C;Varese G	10.3303/CET1332066

	fungal laccases		
78	Enhanced laccase-mediated transformation of diclofenac and flufenamic acid in the presence of bisphenol a and testing of an enzymatic membrane reactor	Hahn V;Meister M;Hussy S;Cordes A;Enderle G;Saningong A;Schauer F	10.1186/s13568-018-0546-y
79	Enhanced removal of aqueous acetaminophen by a laccase-catalyzed oxidative coupling reaction under a dual-ph optimization strategy	Wang K;Huang K;Jiang G	10.1016/j.scitotenv.2017.10.191
80	Enhanced removal of ibuprofen in water using dynamic dialysis of laccase catalysis	Zhang J;Cai Q;Chen J;Lu Y;Ren X;Liu Q;Wen L;Mateen M	10.1016/j.jwpe.2022.102791
81	Enhancement of trace organic contaminant degradation by crude enzyme extract from trametes versicolor culture: effect of mediator type and concentration	Nguyen L;Hai F;Kang J;Leusch ;Frederic D L F;Roddick F;Magram S;Price ;William E W;Nghiem L	10.1016/j.jtice.2014.03.021
82	Enzymatic degradation of chloramphenicol by laccase from trametes hirsuta and comparison among mediators	Navada K;Kulal A	10.1016/j.ibiod.2018.12.012
83	Enzymatic degradation of micropollutants in water: the case of tetracycline degradation by enzymes immobilized on monoliths	Ahmad S;Sebai W;Belleville M;Brun N;Galarneau A;Sanchez-Marcano J	10.3303/CET2079068
84	Enzymatic monolithic reactors for micropollutants degradation	Ahmad S;Sebai W;Belleville M;Brun N;Galarneau ;A. A;Sanchez-Marcano J	10.1016/j.cattod.2020.04.048
85	Enzymatic transformation and bonding of sulfonamide antibiotics to model humic substances	Schwarz J;Knicker H;Schaumann G;Thiele-Bruhn S	10.1155/2015/829708
86	Enzyme polymer engineered structure strategy to enhance cross-linked enzyme aggregate stability: a step forward in laccase exploitation for cannabidiol removal from wastewater	Ariste A;Haroune L;Saibi S;Cabana H	10.1007/s11356-021-13746-4
87	Evaluation of bezafibrate, gemfibrozil, indomethacin, sulfamethoxazole, and diclofenac removal by ligninolytic enzymes	Camarillo R D;Loera C O;Gonzalez-Martinez ;Ignacio I;Chan C W;Rodriguez N C	10.1080/10826068.2020.1721532
88	Experimental and modeling of tetracycline degradation in water in a flow-through enzymatic monolithic reactor	Ahmad S;Sebai W;Belleville M;Brun ;Nicolas N;Galarneau A;Sanchez-	10.1007/s11356-022-21204-y

		Marcano J	
89	Facile fabrication of flexible ceramic nanofibrous membranes for enzyme immobilization and transformation of emerging pollutants	Zhao D;Leth M;Abou H M;Aziz I;Jancic N;Luxbacher T;Helix-Nielsen C;Zhang W	10.1016/j.cej.2022.138902
90	Formulation and characterization of an immobilized laccase biocatalyst and its application to eliminate organic micropollutants in wastewater	Nair R;Demarche P;Agathos S	10.1016/j.nbt.2012.12.004
91	From pet bottle waste to enzyme support for removal of estrogens from wastewaters	Kijenska-Gawronska E;Zdarta J;Thabit H;Jesionowski T;Swieszkowski W	10.1016/j.eti.2024.103555
92	Fungal laccase-triggered 17β-estradiol humification kinetics and mechanisms in the presence of humic precursors	Liu Q;Liu J;Hong D;Sun K;Li S;Latif A;Si X;Si Y	10.1016/j.jhazmat.2021.125197
93	Genipin and glutaraldehyde based laccase two-layers immobilization with improved properties: new biocatalysts with high potential for enzymatic removal of trace organic contaminants	Pinheiro B;Saibi S;Haroune L;Rios ;Nathalia S N;Goncalves L;Cabana H	10.1016/j.enzmictec.2023.110261
94	Graphene facilitated removal of labetalol in laccase-abts system: reaction efficiency, pathways and mechanism	Dong S;Xiao H;Huang Q;Zhang J;Mao L;Gao S	10.1038/srep21396
95	Green remediation potential of immobilized oxidoreductases to treat halo-organic pollutants persist in wastewater and soil matrices-a way forward	Sharma A;Bravo V L;Mora H;Erick O E;Moreno B M;Oza G;Ahmed S;Ramalingam S;Iqbal H;N. N	10.1016/j.chemosphere.2021.133305
96	Hierarchically-structured laccase@ni3(po4)2 hybrid nanoflowers for antibiotic degradation: application in real wastewater effluent and toxicity evaluation	Jafari-Nodoushan H;Fazeli M;Faramarzi ;Mohammad A M;Samadi N	10.1016/j.ijbiomac.2023.123574
97	High efficiency of osmotically stable laccase for biotransformation and micro-detoxification of levofloxacin in the urea-containing solution: catalytic performance and mechanism	Najafabadipour N;Mojtabavi S;Jafari-Nodoushan ;Hossein H;Samadi N;Faramarzi M	10.1016/j.colsurfb.2021.112022
98	Highly permeable biocatalytic membrane prepared by 3d modification: metal-organic frameworks ameliorate its	Ren Z;Luo J;Wan Y	10.1016/j.cej.2018.04.203

	stability for micropollutants removal		
99	Hybrid bioreactor (hbr) of hollow fiber microfilter membrane and cross-linked laccase aggregates eliminate aromatic pharmaceuticals in wastewaters	Ba S;Jones J;Cabana H	10.1016/j.jhazmat.2014.08.062
100	Hybridization of laccase with dendrimer-grafted silica-coated hercynite-copper phosphate magnetic hybrid nanoflowers and its application in bioremoval of gemifloxacin	Rezayaraghi F;Jafari-Nodoushan H;Mojtabavi ;Somayeh S;Golshani S;Jahandar H;Faramarzi M;Ali A	10.1007/s11356-022-21959-4
101	Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow liquid-chromatography coupled to a high resolution mass spectrometer ltq-orbitrap	Llorca M;Rodriguez-Mozaz S;Couillerot O;Panigoni K;De G J;Bayer S;Czaja R;Barcelo D	10.1016/j.chemosphere.2014.05.072
102	Immobilized laccase in the form of (magnetic) cross-linked enzyme aggregates for sustainable diclofenac (bio) degradation	Primozic M;Kravanja G;Knez Z;Crnjac A;Leitgeb M	10.1016/j.jclepro.2020.124121
103	Immobilized laccase on oxygen functionalized nanobiochars through mineral acids treatment for removal of carbamazepine	Naghdi M;Taheran M;Brar S;Kermanshahi-Pour A;Verma M;Surampalli R	10.1016/j.scitotenv.2017.01.021
104	Immobilized laccase on polyimide aerogels for removal of carbamazepine	Simon-Herrero C;Naghdi M;Taheran M;Brar ;Satinder K S;Romero A;Valverde J;Ramirez ;Antonio A A;Sanchez-Silva L	10.1016/j.jhazmat.2019.05.032
105	Impact of inorganic salts on degradation of bisphenol a and diclofenac by crude extracellular enzyme from pleurotus ostreatus	Chapple A;Nguyen L;Hai F;Dosseto ;Anthony A;Rashid M;Oh S;Price W;Nghiem L	10.1080/10242422.2017.1415332
106	Impact of simultaneous retention of micropollutants and laccase on micropollutant degradation in enzymatic membrane bioreactor	Asif M;Hai F;Dhar B;Ngo H;Guo W;Jegatheesan V;Price W;Nghiem L;Yamamoto K	10.1016/j.biortech.2018.07.066
107	Impacts of redox-mediator type on trace organic	Ashe B;Nguyen L;Hai F;Lee D;Van D	10.1016/j.ibiod.2016.04.027

	contaminants degradation by laccase: degradation efficiency, laccase stability and effluent toxicity	M J;Leusch F;Price W;E. E;Nghiem L	
108	Improvement of carbamazepine removal through biodegradation coupled with peroxymonosulfate-based fenton oxidation	Hien T N H;Adil S;Cho K;Jeong S;Kim E	10.1016/j.jece.2022.108150
109	Influence of treatment conditions on the oxidation of micropollutants by trametes versicolor laccase	Margot J;Maillard J;Rossi L;Barry D;Holliger C	10.1016/j.nbt.2013.06.004
110	Inorganic oxide systems as platforms for synergistic adsorption and enzymatic conversion of estrogens from aqueous solutions: mechanism, stability and toxicity studies	Zdarta J;Ciesielczyk F;Bilal M;Jankowska ;Katarzyna K;Bachosz K;Degorska O;Rybarczyk ;Agnieszka A;Jesionowski T;Meyer A	10.1016/j.jece.2023.109443
111	Insolubilization of tramates versicolor laccase as cross-linked enzyme aggregates for the remediation of trace organic contaminants from municipal wastewater	George J;Rajendran D;Venkataraman S;Rathankumar A;Saikia K;Muthusamy S;Singh I;Singh I;Sinha S;Ramkumar S;Cabana H;Vaidyanathan V	10.1016/j.envres.2022.112882
112	Integration of an enzymatic bioreactor with membrane distillation for enhanced biodegradation of trace organic contaminants	Asif M;Nguyen L;Hai F;Price ;William E W;Nghiem L	10.1016/j.ibiod.2017.06.012
113	Ketoprofen and aspirin removal by laccase immobilized on date stones	Al-Sareji O;Meiczinger M;Salman J;Al-Juboori R;Hashim K;Somogyi V;Jakab M	10.1016/j.chemosphere.2022.137133
114	Laccase and horseradish peroxidase for green treatment of phenolic micropollutants in real drinking water and wastewater	Maryskova M;Linhartova L;Novotny V;Rysova ;Miroslava M;Cajthaml T;Sevcu A	10.1007/s11356-021-12910-0
115	Laccase catalyzed elimination of morphine from aqueous systems	Huber D;Bleymaier K;Pellis A;Vielnascher R;Daxbacher A;Greimel K;Guebitz G	10.1016/j.nbt.2018.01.003
116	Laccase immobilization in polyelectrolyte multilayer membranes for 17α-ethynylestradiol removal: biocatalytic	Zdarta J;Sigurdardottir S;Jankowska K;Pinelo M	10.1016/j.chemosphere.2022.135374

	approach for pharmaceuticals degradation		
117	Laccase mediated diclofenac transformation and cytotoxicity assessment on mouse fibroblast 3t3-l1 preadipocytes	Sathishkumar P;Mythili A;Hadibarata T;Jayakumar R;Kanthimathi M;Palvannan ;Thayumanavan T;Ponraj M;Salim M;Yusoff ;Abdull R M A	10.1039/c3ra46014b
118	Laccase mediated transformation of fluoroquinolone antibiotics: analyzing degradation pathways and assessing algal toxicity	Mathur P;Kochar M;Conlan X;Pfeffer F;Dubey M;Callahan D	10.1016/j.envpol.2024.124700
119	Laccase removal of 2-chlorophenol and sulfamethoxazole in municipal wastewater	Haugland J;Kinney K;Johnson W;Atilano C M;Whitman C;Lawler D	10.1002/wer.1006
120	Laccase-assisted degradation of anticancer drug etoposide: by-products and cytotoxicity	Pereira C;Kelbert M;Daronch N;Cordeiro A;Cesca K;Michels C;De O D;Soares H	10.1007/s12155-023-10604-8
121	Laccase-catalyzed degradation of anti-inflammatories and estrogens	Lloret L;Eibes G;Lu-Chau T;Moreira M;Feijoo G;Lema J	10.1016/j.bej.2010.06.005
122	Laccase-catalyzed reactions of 17β-estradiol in the presence of humic acid: resolved by high-resolution mass spectrometry in combination with ¹³c labeling	Sun K;Luo Q;Gao Y;Huang Q	10.1016/j.chemosphere.2015.11.117
123	Laccase-catalyzed treatment of ketoconazole, identification of biotransformed metabolites, determination of kinetic parameters, and evaluation of micro-toxicity	Yousefi-Ahmadipour A;Bozorgi-Koshalshahi M;Mogharabi M;Amini M;Ghazi-Khansari M;Faramarzi M	10.1016/j.molcatb.2016.07.015
124	Laccase-induced decontamination and humification mechanisms of estrogen in water-crop matrices	Sun K;Dai L;Chen M;Si Y;Fang ;Guo-Dong G;Li S;Yu H	10.1093/pnasnexus/pgae118
125	Laccase-loaded magnetic dialdehyde inulin nanoparticles as an efficient heterogeneous natural polymer-based biocatalyst for removal and detoxification of ofloxacin	Shokri M;Mojtabavi S;Jafari-Nodoushan H;Vojdanitalab K;Golshani S;Jahandar H;Faramarzi M	10.1007/s10532-022-09994-x
126	Laccase-syringaldehyde-mediated degradation of trace organic contaminants in an enzymatic membrane reactor: removal efficiency and effluent toxicity	Nguyen L;Van D M J;Hai F;Leusch F;Kang J;Price W;Roddick F;Magram S;Nghiem L	10.1016/j.biortech.2015.10.054
127	Laminated paa nanofibers as a practical support for crude	Maryskova M;Vrsanska M;Sevcu	10.1016/j.eti.2022.102316

	laccase: a new perspective for biocatalytic treatment of micropollutants in wastewaters	A;Novotny ;Vit V;Blahutova A;Voberkova S	
128	Large-scale enzymatic membrane reactors for tetracycline degradation in wwtp effluents	Abejon R;De C M;Belleville M;Sanchez-Marcano ;J. J	10.1016/j.watres.2015.01.012
129	Mass spectrometry based in vitro assay investigations on the transformation of pharmaceutical compounds by oxidative enzymes	Stadlmair L;Letzel T;Drewes J;Grassmann J	10.1016/j.chemosphere.2017.01.140
130	Micropollutants (ciprofloxacin and norfloxacin) remediation from wastewater through laccase derived from spent mushroom waste: fate, toxicity, and degradation	Ghose A;Nuzelu V;Gupta D;Kimoto H;Takashima S;Harlin E;Sonu S;Ueda ;Hiroshi H;Koketsu M;Rangan L;Mitra S	10.1016/j.jenvman.2024.121857
131	Monitoring enzymatic degradation of emerging contaminants using a chip-based robotic nano-esi-ms tool	Stadlmair L;Letzel T;Grassmann J	10.1007/s00216-017-0729-4
132	Natural silicates encapsulated enzymes as green biocatalysts for degradation of pharmaceuticals	Vardanyan A;Agback T;Golovko O;Dietre ;Quentin Q;Seisenbaeva G	10.1021/acsestwater.3c00811
133	Nmr investigation of enzymatic coupling of sulfonamide antimicrobials with humic substances	Bialk H;Pedersen J	10.1021/es070779d
134	Non-covalent assembled laccase-graphene composite: property, stability and performance in beta-blocker removal	Dong S;Jing X;Cao Y;Xia E;Gao ;Shixiang S;Mao L	10.1016/j.envpol.2019.05.053
135	Novel laccase from xylaria polymorpha and its efficiency in the biotransformation of pharmaceuticals: optimization of operational conditions, comparative effect of redox-mediators and toxicity studies	Bankole P;Omoni V;Tennison-Omovoh ;Chidinma A C;Adebajo S;Mulla S;Adekunle A;Semple K	10.1016/j.colsurfb.2022.112675
136	On the use of a high-redox potential laccase as an alternative for the transformation of non-steroidal anti-inflammatory drugs (nsaids)	Lloret L;Eibes G;Moreira M;Feijoo G;Lema J;M. M	10.1016/j.molcatb.2013.08.021
137	Optimization of growth conditions for enhancing the production of microbial laccase and its application in treating antibiotic contamination in wastewater	Mathur P;Sanyal D;Dey P	10.1007/s13205-020-02627-1
138	Oxytetracycline removal by biological/chemical activated	Blair-Gonzalez J;Contreras-Villacura	10.1016/j.micromeso.2021.11

	mesoporous carbon	E;Guevara ;Andrea C A;Toloza C	1384
139	Performance evaluation of biocatalytic and biostimulation approaches for the remediation of trace organic contaminants in municipal biosolids	Vaithyanathan V;Savary O;Cabana H	10.1016/j.wasman.2020.11.046
140	Pharmaceuticals removal by immobilized laccase on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes	Masjoudi M;Golgoli M;Nejad Z;Sadeghzadeh S;Borghei S	10.1016/j.chemosphere.2020.128043
141	Pleurotus ostreatus and lentinus sajor-caju laccases for sulfamethoxazole biotransformation: enzymatic degradation, toxicity and cost analysis	Sa H;Michelin M;Silverio S;Polizeli ;Maria D L T M M;Silva A;Pereira L;Tavares T;Silva B	10.1016/j.jwpe.2024.104943
142	Polymerization and formation of insoluble byproducts of diclofenac using trametes versicolor laccases - experimental study and modelling	Pype R;Septavaux J;Amar B;Debaste F	10.1016/j.jwpe.2019.100948
143	Potential of agro-industrial produced laccase to remove ciprofloxacin	Cuprys A;Thomson P;Suresh G;Roussi ;Tarek T;Brar S;Drogui P	10.1007/s11356-021-13578-2
144	Preliminary study on enzymatic degradation of model of pharmaceutical waste by laccase from trametes sp	Yusuf Y;Fitriani F;Yuniarti F	10.31838/jcr.07.01.37
145	Recyclable cross-linked laccase aggregates coupled to magnetic silica microbeads for elimination of pharmaceuticals from municipal wastewater	Arca-Ramos A;Kumar V;Eibes G;Moreira M;Cabana H	10.1007/s11356-016-6139-x
146	Removal and degradation of sodium diclofenac via radical-based mechanisms using s. Sclerotiorum laccase	Coman C;Hadade N;Pesek S;Silaghi-Dumitrescu R;Mot A	10.1016/j.jinorgbio.2023.112400
147	Removal of 17β-estradiol in laccase catalyzed treatment processes	Xia Q;Kong D;Liu G;Huang Q;Alalewi A;Lu J	10.1007/s11783-013-0567-3
148	Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from trametes hirsuta	Hachi M;Chergui A;Yeddou A;Selatnia A;Cabana H	10.1080/10242422.2017.1280032
149	Removal of acetaminophen using enzyme-mediated oxidative coupling processes: i. Reaction rates and pathways	Lu J;Huang Q;Mao L	10.1021/es9002422
150	Removal of acetaminophen using enzyme-mediated	Lu J;Huang Q	10.1021/es9001295

	oxidative coupling processes: ii. Cross-coupling with natural organic matter		
151	Removal of antibiotics in wastewater by enzymatic treatment with fungal laccase - degradation of compounds does not always eliminate toxicity	Becker D;Varela D G S;Rodriguez-Mozaz ;Sara S;Schoevaart R;Barcelo D;De C M;Belleville M;Sanchez-Marcano J;De G J;Couillerot O;Voelker J;Oehlmann J;Wagner M	10.1016/j.biortech.2016.08.004
152	Removal of carbamazepine and diclofenac by laccase-based membrane bioreactor	Sadeghzadeh S;Golgoli M;Masjoudi M;Nejad Z;Zargar M;Borghei S	10.1007/s13762-023-05453-z
153	Removal of diclofenac using microbiochar fixed-bed column bioreactor	Lonappan L;Rouissi T;Liu Y;Brar S;Kaur K;Surampalli R	10.1016/j.jece.2019.102894
154	Removal of emerging pollutants from water using enzyme-immobilized activated carbon from coconut shell	Al-Sareji O;Meiczinger M;Somogyi V;Al-Juboori R;Grmasha R;Stenger-Kovács C;Jakab M;Hashim K	10.1016/j.jece.2023.109803
155	Removal of meloxicam, piroxicam and cd⁺² by fe₃o₄/sio₂/glycidyl methacrylate-s-sh nanocomposite loaded with laccase	Siddeeg S;Amari A;Tahoon M;Alsaiari N;Ben R F	10.1016/j.aej.2020.03.018
156	Removal of micropollutants by fungal laccases in model solution and municipal wastewater: evaluation of estrogenic activity and ecotoxicity	Spina F;Cordero C;Schilirò T;Sgorbini B;Pignata C;Gilli G;Bicchi C;Varese G	10.1016/j.jclepro.2015.03.047
157	Removal of pharmaceutical' estrogenic activity of sequencing batch reactor effluents assessed in the t47d-kbluc reporter gene assay	Kasonga T;Coetzee M;Van Z C;Momba M	10.1016/j.jenvman.2019.03.113
158	Removal of pharmaceuticals and personal care products using native fungal enzymes extracted during the ligninolytic process	Kang B;Kim S;Kang M;Lee T	10.1016/j.envres.2021.110878
159	Removal of pharmaceuticals from water using laccase immobilized on orange peels waste-derived activated carbon	Al-Sareji O;Abdulzahra M;Hussein T;Shafi S;Shlakaa A;Karhib M;Meiczinger ;Monika M;Grmasha	10.3390/w15193437

		R;Al-Juboori R;Somogyi ;Viola V;Domokos E;Idowu I;Andredaki M;Hashim K	
160	Removal of sulfamethoxazole and methylparaben using hydrocolloid and fiber industry wastes: comparison with biochar and laccase-biocomposite	Fernandez-Sanroman A;Acevedo-Garcia V;Pazos M;Angeles S M;Rosales E	10.1016/j.jclepro.2020.122436
161	Removal of trace organic contaminants by enzymatic membrane bioreactors: role of membrane retention and biodegradation	Asif M;Hou J;Price W;Chen ;Vicki V;Hai F	10.1016/j.memsci.2020.118345
162	Removal of trace organic contaminants by nitrifying activated sludge and whole-cell and crude enzyme extract of trametes versicolor	Yang S;Hai F;Nghiem L;Roddick ;Felicity F;Price W	10.2166/wst.2013.684
163	Response surface methodology mediated optimization of diclofenac and norfloxacin biodegradation using laccase immobilized on metal–organic frameworks	Lungelo D M;Lesaoana M;Kotzé I;Richards H	10.1016/j.seppur.2023.124709
164	Robust biodegradation of naproxen and diclofenac by laccase immobilized using electrospun nanofibers with enhanced stability and reusability	Zdarta J;Jankowska K;Wyszowska M;Kijenska-Gawronska E;Zgala-Grzeskowiak A;Pinelo ;Manuel M;Meyer A;Moszynski D;Jesionowski T	10.1016/j.msec.2019.109789
165	Simultaneous removal and degradation characteristics of sulfonamide, tetracycline, and quinolone antibiotics by laccase-mediated oxidation coupled with soil adsorption	Ding H;Wu Y;Zou B;Lou Q;Zhang ;Weihao W;Zhong J;Lu L;Dai G	10.1016/j.jhazmat.2015.12.062
166	Stability improvement of laccase for micropollutant removal of pharmaceutical origins from municipal wastewater	Meiczinger M;Varga B;Wolmarans L;Hajba L;Somogyi V	10.1007/s10098-022-02336-8
167	Study of membrane-immobilized oxidoreductases in wastewater treatment for micropollutants removal	Zdarta A;Zdarta J	10.3390/ijms232214086
168	Sulfamethoxazole and isoproturon degradation and detoxification by a laccase-mediator system: influence of treatment conditions and mechanistic aspects	Margot J;Copin P;Von G U;Barry D;A. A;Holliger C	10.1016/j.bej.2015.06.008

169	Synthesis and characterization of combined cross-linked laccase and tyrosinase aggregates transforming acetaminophen as a model phenolic compound in wastewaters	Ba S;Haroune L;Cruz-Morato C;Jacquet C;Touahar I;Bellenger J;Legault C;Jones J;Cabana H	10.1016/j.scitotenv.2013.10.004
170	The effects of mediator and granular activated carbon addition on degradation of trace organic contaminants by an enzymatic membrane reactor	Nguyen L;Hai F;Price W;Leusch ;Frederic D L F;Roddick F;Ngo H;Guo W;Magram S;Nghiem L	10.1016/j.biortech.2014.05.125
171	The optimization of enzymatic oxidation of levofloxacin, a fluoroquinolone antibiotic for wastetwater treatment	Mathur P;Sanyal D;Dey P	10.1007/s10532-021-09946-x
172	The utilization of laccase-functionalized graphene oxide as an effective biodegradation of pharmaceutical industry waste: diclofenac and ibuprofen	Yusuf Y	10.5530/srp.2020.1.67
173	Transformation and products of captopril with humic constituents during laccase-catalyzed oxidation: role of reactive intermediates	Du P;Zhao H;Liu C;Huang Q;Cao ;Hongbin H	10.1016/j.watres.2016.10.036
174	Transformation and toxicity evaluation of tetracycline in humic acid solution by laccase coupled with 1-hydroxybenzotriazole	Sun K;Huang Q;Li S	10.1016/j.jhazmat.2017.02.058
175	Transformation of 17α-ethinylestradiol by simultaneous photo-enzymatic process in humic water	Yang Y;Li J;Lu K;Shi H;Gao ;Shixiang S	10.1016/j.chemosphere.2017.03.077
176	Transformation of atenolol by a laccase-mediator system: efficiencies, effect of water constituents, and transformation pathways	Feng Y;Shen M;Wang Z;Liu G	10.1016/j.ecoenv.2019.109555
177	Ultrafast synthesis of laccase-copper phosphate hybrid nanoflowers for efficient degradation of tetracycline antibiotics	Han Z;Wang H;Zheng J;Wang S;Yu ;Shuyu S;Lu L	10.1016/j.envres.2022.114690
178	Ultrasound assisted enzyme catalyzed degradation of cetirizine dihydrochloride	Sutar R;Rathod V	10.1016/j.ultsonch.2014.10.016
179	Ultrasound assisted laccase catalyzed degradation of ciprofloxacin hydrochloride	Sutar R;Rathod V	10.1016/j.jiec.2015.06.037

180	Use of sawdust for production of ligninolytic enzymes by white-rot fungi and pharmaceutical removal	Hultberg M;Golovko O	10.1007/s00449-024-02976-8
181	Utilization of trametes versicolor for the production of laccase and its application in oxytetracycline degradation from wastewater	Suboh S;Setapar S;Alshammari ;Mohammed B M;Ahmad A	10.5004/dwt.2022.28654
182	Zeolitic imidazolate frameworks as effective crystalline supports for aspergillus-based laccase immobilization for the biocatalytic degradation of carbamazepine	Dlamini M;Lesaoana M;Kotze I;Richards H	10.1016/j.chemosphere.2022.137142

Supplementary Table 4 Lotka's law distribution of author productivity generated using Bibliometrix

Documents written	N. of Authors	Proportion of Authors
1	572	0.784
2	87	0.119
3	29	0.04
4	12	0.016
5	6	0.008
6	5	0.007
7	6	0.008
8	4	0.005
9	2	0.003
10	2	0.003
12	2	0.003
14	1	0.001
16	1	0.001
19	1	0.001

Supplementary Table 5 Metrics of the top 10 most productive authors in pharmaceutical bioremediation research involving fungal laccases

Rank	Authors	NP*	TC#	h_index	g_index	m_index
1	Hai F	19	1018	17	19	1.417
2	Nghiem L	16	854	15	16	1.25
3	Price W	14	799	13	14	1.083
4	Nguyen L	12	739	12	12	1.091
5	Cabana H	12	444	8	12	0.727
6	Belleville M	10	482	8	10	0.727
7	Sanchez-Marcano J	10	444	8	10	0.727
8	Brar S	9	470	8	9	1
9	Zdarta J	9	156	5	9	0.833
10	Leusch F	8	501	8	8	0.727

* NP: number of publications # TC: total citations

Supplementary Table 6 List of journal productivity identified by Bradford's law using Bibliometrix

Source	Rank	FQ	CFQ	Zone
Chemosphere	1	12	12	Zone 1
Bioresource Technology	2	10	22	Zone 1
Environmental Science And Pollution Research	3	8	30	Zone 1
Journal Of Environmental Chemical Engineering	4	7	37	Zone 1
Journal Of Hazardous Materials	5	7	44	Zone 1
Science Of The Total Environment	6	7	51	Zone 1

Biocatalysis And Biotransformation	7	5	56	Zone 1
Environmental Research	8	5	61	Zone 1
International Biodeterioration & Biodegradation	9	5	66	Zone 2
Environmental Science & Technology	10	4	70	Zone 2
Journal Of Cleaner Production	11	4	74	Zone 2
Journal Of Environmental Management	12	4	78	Zone 2
Journal Of Membrane Science	13	4	82	Zone 2
Journal Of Water Process Engineering	14	4	86	Zone 2
3 Biotech	15	3	89	Zone 2
Environmental Pollution	16	3	92	Zone 2
New Biotechnology	17	3	95	Zone 2
Separation And Purification Technology	18	3	98	Zone 2
Water Research	19	3	101	Zone 2
Acs Sustainable Chemistry & Engineering	20	2	103	Zone 2
Amb Express	21	2	105	Zone 2
Analytical And Bioanalytical Chemistry	22	2	107	Zone 2
Biochemical Engineering Journal	23	2	109	Zone 2
Biodegradation	24	2	111	Zone 2
Catalysis Today	25	2	113	Zone 2
Chemical Engineering Journal	26	2	115	Zone 2
Chemical Engineering Transactions	27	2	117	Zone 2
Colloids And Surfaces B-Biointerfaces	28	2	119	Zone 2
Environmental Technology & Innovation	29	2	121	Zone 2
Enzyme And Microbial Technology	30	2	123	Zone 2
International Journal Of Biological Macromolecules	31	2	125	Zone 3
Journal Of Industrial And Engineering Chemistry	32	2	127	Zone 3
Journal Of Molecular Catalysis B-Enzymatic	33	2	129	Zone 3
Preparative Biochemistry & Biotechnology	34	2	131	Zone 3
Process Biochemistry	35	2	133	Zone 3
Scientific Reports	36	2	135	Zone 3
Acs Es&T Water	37	1	136	Zone 3
Alexandria Engineering Journal	38	1	137	Zone 3
Applied Microbiology And Biotechnology	39	1	138	Zone 3
Applied Sciences-Basel	40	1	139	Zone 3
Arabian Journal For Science And Engineering	41	1	140	Zone 3
Biocatalysis And Agricultural Biotechnology	42	1	141	Zone 3
Bioenergy Research	43	1	142	Zone 3
Biomolecules	44	1	143	Zone 3
Bioprocess And Biosystems Engineering	45	1	144	Zone 3
Biotechnology And Bioengineering	46	1	145	Zone 3
Catalysts	47	1	146	Zone 3
Clean Technologies And Environmental Policy	48	1	147	Zone 3
Cleaner Engineering And Technology	49	1	148	Zone 3
Desalination And Water Treatment	50	1	149	Zone 3
Ecocycles	51	1	150	Zone 3
Ecotoxicology And Environmental Safety	52	1	151	Zone 3
Environmental Advances	53	1	152	Zone 3
Environmental Science-Water Research & Technology	54	1	153	Zone 3
Frontiers In Chemical Engineering	55	1	154	Zone 3

Frontiers In Microbiology	56	1	155	Zone 3
Frontiers Of Environmental Science & Engineering	57	1	156	Zone 3
International Biodeterioration And Biodegradation	58	1	157	Zone 3
International Journal Of Environmental Science And Technology	59	1	158	Zone 3
International Journal Of Molecular Sciences	60	1	159	Zone 3
Journal Of Chemical Technology And Biotechnology	61	1	160	Zone 3
Journal Of Chemistry	62	1	161	Zone 3
Journal Of Critical Reviews	63	1	162	Zone 3
Journal Of Environmental Engineering (United States)	64	1	163	Zone 3
Journal Of Hazardous Materials Advances	65	1	164	Zone 3
Journal Of Inorganic Biochemistry	66	1	165	Zone 3
Journal Of The Taiwan Institute Of Chemical Engineers	67	1	166	Zone 3
Materials Science & Engineering C-Materials For Biological Applications	68	1	167	Zone 3
Membranes	69	1	168	Zone 3
Microporous And Mesoporous Materials	70	1	169	Zone 3
Nanotechnology For Environmental Engineering	71	1	170	Zone 3
Pnas Nexus	72	1	171	Zone 3
Process Safety And Environmental Protection	73	1	172	Zone 3
Processes	74	1	173	Zone 3
Rsc Advances	75	1	174	Zone 3
Sydowia	76	1	175	Zone 3
Systematic Reviews In Pharmacy	77	1	176	Zone 3
Ultrasonics Sonochemistry	78	1	177	Zone 3
Waste Management	79	1	178	Zone 3
Water	80	1	179	Zone 3
Water Air And Soil Pollution	81	1	180	Zone 3
Water Environment Research	82	1	181	Zone 3
Water Sci Technol	83	1	182	Zone 3

FQ: Frequency – CFQ: Cumulative Frequency

4.2 Capítulo 2: Fungal laccase crude extracts: production and pharmaceutical bioremediation

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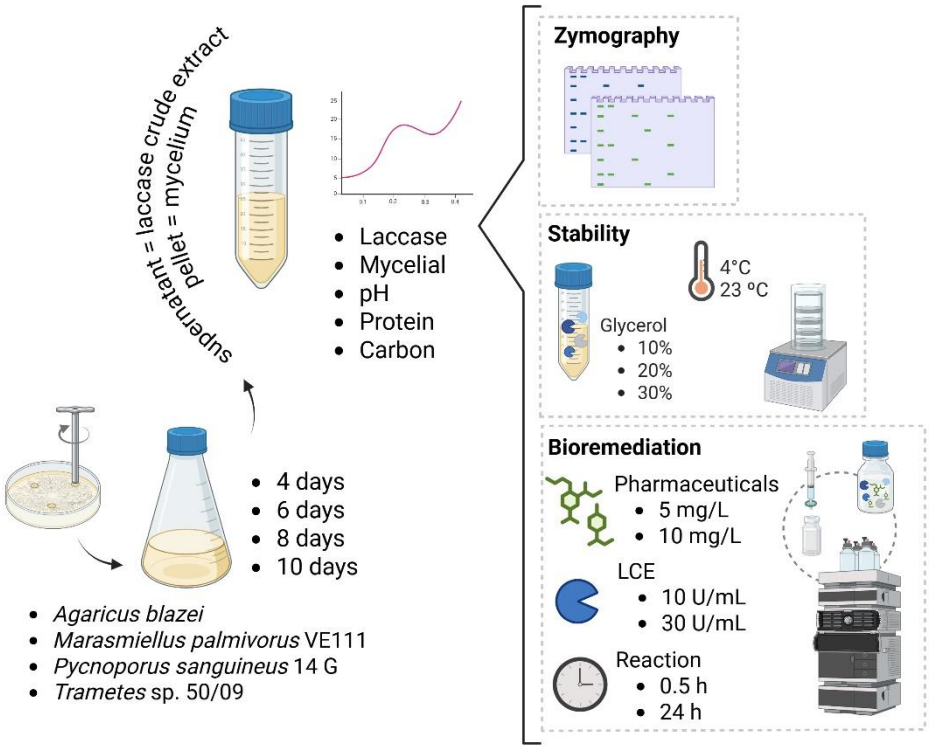
Graphical Abstract



Abstract

Fungal laccases are eco-friendly biocatalysts capable of oxidizing a broad spectrum of compounds. In this study, four fungal species were cultivated to evaluate laccase production and their application in the bioremediation of acetaminophen and diclofenac. Among these, *Pycnoporus sanguineus* exhibited the highest laccase activity (1116.94 U/mL). SDS-PAGE profiling of laccase crude extracts (LCEs) revealed multiple protein bands, while zymograms confirmed the presence of distinct isoforms. Stability assays demonstrated that glycerol protected laccase activity in a concentration- and source-dependent manner, with laccase from *Trametes* sp. 50/08 showing the most

gradual decline over 60 days. When examining the efficacy of bioremediation, the LCE from *Marasmiellus palmivorus* at 10 U/mL achieved 96% acetaminophen removal within 24 hours, whereas the LCE from *Agaricus blazei* at 30 U/mL was the most effective for diclofenac, achieving 82% removal. However, increasing laccase concentration did not enhance pharmaceutical degradation, suggesting possible substrate saturation or inhibition. These findings highlight the efficiency of crude enzyme extracts in removing acetaminophen and diclofenac, reinforcing their potential as sustainable tools for treating pharmaceutical pollutants in aquatic environments.

keywords: enzyme, bioremediation, stability, pharmaceutical, diclofenac, acetaminophen, water

1. Introduction

Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are multicopper oxidases that catalyze the oxidation of phenolic and aromatic amine compounds, using molecular oxygen as the terminal electron acceptor and producing water as the only by-product (Brugnari et al., 2021; Sodhi et al., 2024). These enzymes are widely distributed among fungi, bacteria, and plants (Singh et al., 2024). Fungal laccases are the most extensively studied due to their high redox potential and low substrate specificity (Sharma and Gupta, 2024).

Fungal laccases are involved in various physiological and ecological functions, including lignin degradation, pigment biosynthesis, sporulation, stress defense, and pathogenesis (Singh et al., 2024). The capacity to oxidize a broad array of compounds and the environmentally eco-friendly catalytic mechanism make these enzymes highly attractive for biotechnological applications (Sodhi et al., 2024).

In recent years, fungal laccases have gained attention as promising biocatalysts for the degradation of micropollutants, including synthetic dyes, pesticides, and pharmaceutically active contaminants, from water and soil systems (Cantele et al., 2017; Chmelová et al., 2024; Singh et al., 2025; Thathola et al., 2024). Pharmaceuticals, in particular, have emerged as a global concern due to the physicochemical characteristics (e.g., molecular weight, pKa, logP) that contribute to recalcitrant behavior (Patel et al., 2019). These compounds are continuously released into the environment through domestic sewage, hospital effluents, and drug manufacturing waste (Estrada-Almeida et al., 2024).

Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, and analgesics like acetaminophen are among the most frequently detected pharmaceuticals in water matrices (Guerra et al., 2014; Placova et al., 2023). These compounds are widely consumed and exhibit moderate to high environmental persistence (Lonappan et al., 2016; Phong Vo et al., 2019). Once in the environment, these compounds may induce toxic and genotoxic effects in soil microbial communities and aquatic organisms, causing oxidative stress, endocrine disruption, and developmental abnormalities (Kummerová et al., 2016). Their ecological relevance and ubiquity have made acetaminophen and diclofenac model pollutants in bioremediation studies (Lonappan et al., 2016; Phong Vo et al., 2019).

Conventional wastewater treatment plants are not specifically designed to eliminate pharmaceuticals, leading to their incomplete removal and subsequent detection in surface waters, sediments, and even drinking water (Estrada-Almeida et al., 2024; Patel et al., 2019). Advanced treatment technologies such as ozonation, membrane filtration, and advanced oxidation processes have been proposed for wastewater treatment (Bouabadi et al., 2024; Mutegoa, 2024). However, their high operational costs, energy demands, and generation of toxic by-products limit their scalability and sustainability (Guo et al., 2017; Phong Vo et al., 2019). In contrast, enzymatic bioremediation offers a greener and more cost-effective alternative, operating under mild conditions with minimal secondary pollution (Brugnari et al., 2021; Singh et al., 2024).

Laccases, in particular, have been studied for the bioremediation of pharmaceuticals in model systems and real wastewater matrices (Chmelová et al., 2024; Singh et al., 2025). However, their large-scale industrial application is limited by enzymatic stability (Brugnari et al., 2021). While strategies such as enzyme purification or immobilization have been explored, they often involve complex and expensive processes (Brugnari et al., 2021; Singh et al., 2025). An alternative is the use of laccase crude extracts (LCEs) derived directly from fungal cultures, which provide a practical and low-cost approach for large-scale bioremediation. Furthermore, exploring fungal strains beyond the extensively studied white-rot fungi may reveal novel enzymatic profiles with broader degradation capabilities.

Although studies have reported the use of crude fungal laccases for the oxidation of pharmaceuticals, few have systematically compared enzymatic extracts from different basidiomycete species under standardized conditions. In this study, *Agaricus blazei*, *Marasmiellus palmivorus* VE111 (MIUCS 2025), *Pycnoporus sanguineus* 14G, and *Trametes* sp. 50/09 were evaluated to assess their potential for enzymatic bioremediation

of acetaminophen and diclofenac. Moreover, the study examined the main factors influencing enzyme activity and stability, as well as the structural features of these pharmaceuticals that determine their susceptibility to enzymatic degradation.

2. Materials and methods

2.1 Laccase crude extracts production

The fungal strains *Agaricus blazei*, *Marasmiellus palmivorus* VE111 (MIUCS 2025), *Pycnoporus sanguineus* 14G, and *Trametes* sp. 50/09 were obtained from the Microorganism Collection of the Laboratory of Enzymes and Biomass at the University of Caxias do Sul. The strains were maintained on potato dextrose agar (PDA; Kasvi®) in Petri dishes and incubated in a germination chamber with a photoperiod (Oxylab) at 28°C for seven days.

For inoculum preparation, three mycelial disks (Ø = 1.5 cm) were transferred from actively growing PDA cultures into 500-mL Erlenmeyer flasks containing 100 mL of culture medium. The composition of the medium was based on the protocol described by (Schneider et al., 2018). It contained 2 g glucose (Quimidrol®), 0.15 g pure casein (Synth®), 5 mL of a 20× concentrated mineral solution, and potato broth prepared by boiling 200 g of chopped potatoes in 1 L of water. Cultivation was conducted under shaking conditions at 180 rpm and 28°C in a reciprocal agitation system.

Culture samples were collected at 4, 6, 8, and 10 days of cultivation and centrifuged at 9600×g for 20 min at 4 °C. To explore the stability, zymography, and the role of extracellular enzymes in pharmaceutical bioremediation, supernatants from 8-day-old cultures of *M. palmivorus* VE111 and 10-day-old cultures of *A. blazei*, *P. sanguineus* 14G, and *Trametes* sp. 50/09 were collected and used as lacase crude extracts (LCEs).

2.2. Mycelial biomass, protein, pH, and consumption of carbon sources

Mycelial biomass was used to assess fungal growth, following the method described by Rapp et al. (1981). For this, the entire contents of the Erlenmeyer flasks were processed. After removal of the supernatant, the biomass was washed with distilled water and dried at 40°C until a constant weight was reached.

Supernatants were evaluated for protein concentration, pH, laccase activity, and carbon source consumption. Protein concentration was determined using the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as the standard. The pH was measured using a glass electrode pH meter.

For carbon source analysis, supernatants were filtered through 0.20 µm membranes and analyzed by high-performance liquid chromatography (HPLC; Shimadzu), according to the protocol of the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008).

2.3 Enzymatic laccases assay

Laccase activity was determined by kinetically measuring the oxidation of 5 mmol/L 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) to its radical cation ($ABTS^+$) at 420 nm, in 0.2 mmol/L sodium acetate buffer (pH 5.0) at 25 °C. Absorbance was recorded over 90 s using a spectrophotometer (Spectra MAX 190 - Molecular Devices®). One unit (U) of laccase activity was defined as the amount of enzyme required to produce 1 µmol of $ABTS^+$ per minute under the assay conditions (Wolfenden and Willson, 1982).

2.4 Zymogram of laccase crude extract

The LCEs were subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE), according to the method described by Laemmli (1970). After electrophoresis, the gels were stained with Coomassie Brilliant Blue R in an ethanol:acetic acid:water solution (5:1:4, v/v/v) for 2 min and then destained using the same solution without dye.

For laccase zymography, the gels were sequentially washed at room temperature: first in 2.5% Triton X-100 (solution A), followed by 0.1 mmol/L sodium acetate buffer (pH 5.0; solution B), each for 10 min. To visualize laccase activity, the gels were placed on an ABTS–agar layer prepared with 0.02 g ABTS, 0.4 g agar, and 40 mL distilled heated water. Green bands, indicative of ABTS oxidation, became visible within the first 5 min of incubation at 25 °C (Camassola et al., 2013). Laccase bands were visualized, and molecular mass was estimated by comparison with standard protein markers (Bio-Rad®, USA). Images were documented by Molecular Imager ® (Gel Doc XR + ™ Imaging System - Bio-Rad®, USA).

2.5 Storage stability assessment

The LCEs were subjected to storage stability assessment under different conditions: refrigeration (4 °C), room temperature (23 ± 2 °C), with and without a stabilizer, and freeze-drying. Glycerol was used as the stabilizing agent and tested at

concentrations of 10%, 20%, and 30%. Residual laccase activity was measured every 10 days up to 60 days; all assays were performed in triplicate.

2.6 Enzymatic pharmaceutical bioremediation

To assess the degradation efficiency of each LCE, reaction mixtures were prepared using the LCE adjusted to a laccase activity of 10 U/mL and acetaminophen (Anqiu Lu'An, China) or diclofenac (Elam Pharma, India) at final concentrations of 5 or 10 mg/L. An additional condition using 30 U/mL of LCE was evaluated with pharmaceutical concentrations of 10 mg/L. All assays were performed in triplicate in 50 mL Duran® bottles, with a final reaction volume of 20 mL made up of ultrapure water. The mixtures were incubated at room temperature (23 ± 2 °C) under constant agitation (160 rpm). Each pharmaceutical compound was tested individually. Samples were collected at 0.5 and 24 h and immediately placed on ice to stop enzymatic activity. Subsequently, they were filtered through 0.2 µm PVDF syringe filters. Control reactions without enzyme addition were included to assess the intrinsic stability of the pharmaceutical compounds.

The analytical method for quantifying acetaminophen and diclofenac was adapted from García-Morales et al. (2018). Analyses were carried out using high-performance liquid chromatography (HPLC-UV, Shimadzu, Kyoto, Japan), equipped with a reverse-phase C18 column (Discovery®, 5 µm, 150 × 4.6 mm), using a 20 µL injection volume and a flow rate of 0.5 mL/min. For acetaminophen, the mobile phase consisted of acetonitrile and phosphoric acid (pH 3) in a 40:60 (v/v) ratio, with detection at 243 nm. Diclofenac was analyzed using a mobile phase composed of formic acid and acetonitrile (40:60, v/v), with detection at 250 nm. In both cases, compound concentrations were quantified using external calibration curves.

2.7 Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical differences were evaluated using one-way analysis of variance (ANOVA) at a 95% confidence level. Results with $p < 0.05$ were considered statistically significant. Statistical analyses were performed using JASP software (version 0.16.1), and graphical representations were generated using GraphPad Prism (version 8.0.1).

3. Results and discussion

The profiles of laccase activity, pH, protein concentration, mycelial biomass, and glucose content in the culture medium are shown in Figure 1. After ten days of cultivation, the maximum laccase activities observed were 221.06 U/mL for *A. blazei*, 239.28 U/mL for *Trametes* sp. 50/09, and 1116.94 U/mL for *P. sanguineus* 14G. In contrast, for *M. palmivorus* VE111, the highest laccase activity (994.36 U/mL) was reached on the eighth day. Notably, *M. palmivorus* VE111 exhibited elevated laccase activity as early as the fourth day, maintaining high levels until day eight, after which a sharp decline occurred. Similar temporal patterns have been reported in previous studies and may be attributed to a cascade of metabolic events (Cantele et al., 2017; Schneider et al., 2018).

In *M. palmivorus* VE111 cultures, the carbon source was almost completely depleted by the tenth day (glucose: 0.066 g/L), coinciding with the peak of mycelial biomass. However, organic acid production by fungal metabolism led to a pH drop from 6.74 on day eight to 5.65 on day ten. Although a strong inverse correlation was observed between pH and mycelial growth ($r = –0.891$; $p < 0.001$), this acidification likely contributed to the sharp decline in enzymatic activity during the later stages of cultivation. Overall, the data indicate that pH is a critical factor: while slightly acidic conditions favored biomass accumulation, a near-neutral pH appeared more suitable for maximizing laccase production (Cantele et al., 2017; Schneider et al., 2018).

A near-neutral pH also favored laccase biosynthesis in *A. blazei* cultures, although this condition does not necessarily correspond to the commonly observed optimal pH for catalytic activity. These production profiles primarily reflect the physiological and regulatory mechanisms that promote enzyme secretion rather than the intrinsic catalytic properties of the enzymes. Similar patterns have been reported in previous studies, where neutral or slightly alkaline pH conditions favored laccase production, whereas the enzymes exhibited their highest catalytic efficiency under acidic conditions(Schneider et al., 2019; Ullrich et al., 2005; Valle et al., 2014). This distinction between production and activity optima underscores the importance of decoupling physiological and catalytic parameters when designing bioprocesses for fungal laccase production and application.

Soluble carbon compounds, such as glucose, can be readily absorbed by fungal mycelium and used both for growth and as an energy source (Kim et al., 2004). However, for *A. blazei*, a culture medium containing 2% glucose was found to be unsuitable for both mycelial biomass production and enzymatic activity. Only 4% of the initial glucose was consumed after ten days of cultivation, indicating low carbon uptake compared to the other fungal species tested (Figure 1). This result, when considered together with the other

evaluated parameters, suggests that *A. blazei* was in the stationary growth phase. Glucose was still available in the medium, no reduction in mycelial biomass or change in pH was observed, and laccase production, typically associated with secondary metabolism, was increasing. Similar behavior has been previously reported for this species, which consumed only about 50% of the available glucose after 26 days of cultivation (Argyropoulos et al., 2022). Although prolonged incubation would likely result in further glucose depletion and higher laccase activity, the cultivation period was standardized to 10 days for all fungal species to ensure experimental consistency.

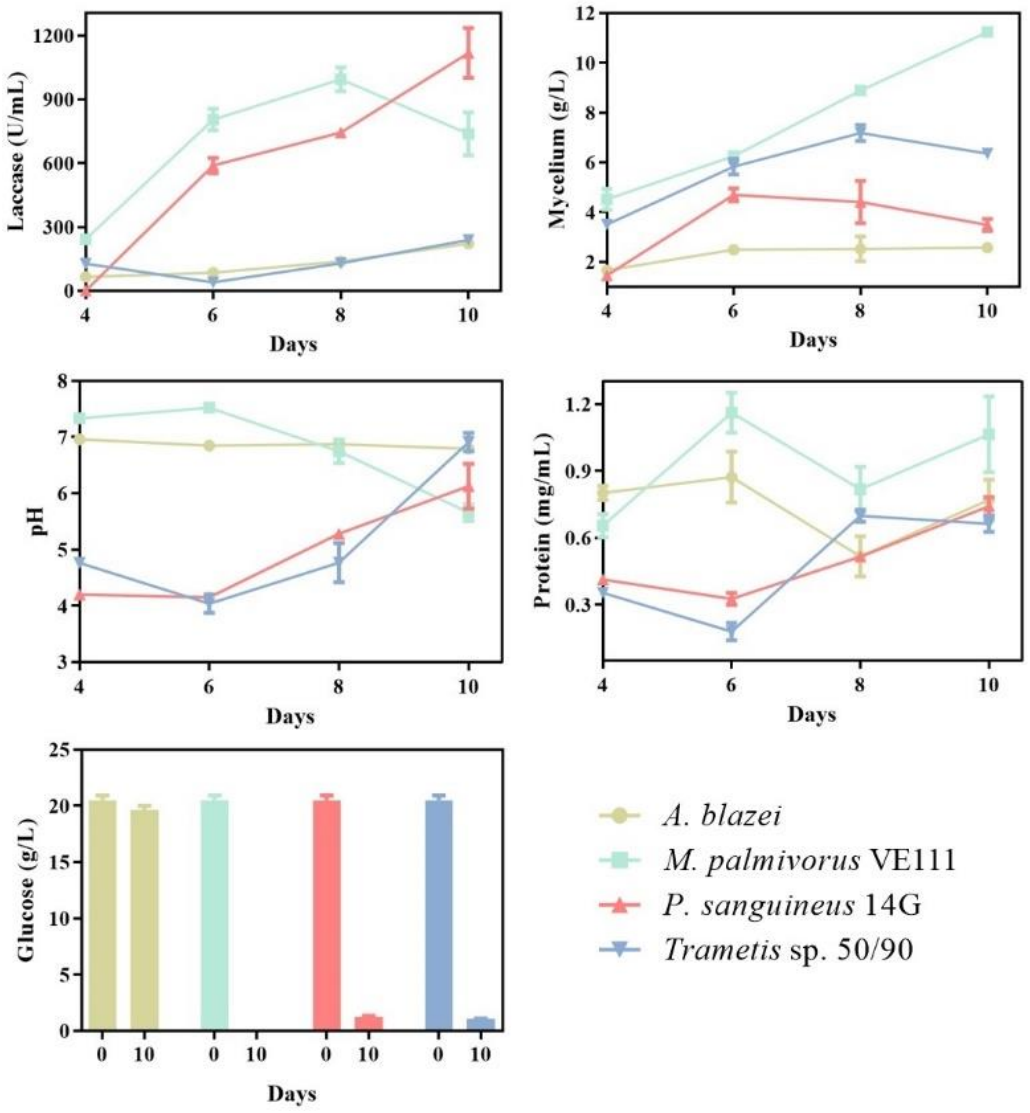


Figure 1. Kinetics of laccase production and physiological cultivation parameters of four fungal species in shaking flasks. Parameters monitored over 10 days include. Values are expressed as mean ± standard deviation (n = 3).

Mycelial growth peaked at 2.48 g/L on day six and remained nearly unchanged until day ten (2.57 g/L). Previous studies have reported higher mycelial biomass values for *A. blazei*, likely due to differences in culture media composition and inoculum concentration. Kim et al. (2004) obtained 8.10 g/L of mycelium after five days of cultivation with 5 g/L glucose, while Liu and Wang (2007) reported 13.91 ± 0.71 g/L using 20 g/L glucose over nine days. Additionally, Hamedi et al. (2007) tested various carbon sources and found that starch (20 g/L) was more effective than glucose.

Regarding laccase production, *A. blazei* exhibited the lowest activity among the tested species in this study (221.05 U/mL), yet this value is significantly higher than those reported in the literature for this fungus. Ullrich et al., (2005) obtained 5 U/mL after seven days of cultivation. Valle et al., (2014) reported 9.7 U/mL using sugarcane molasses as the main carbon source over ten days and reached 43.8 U/mL after 21 days by supplementing the culture with aromatic compounds known to induce laccase expression.

Among the evaluated species, *P. sanguineus* 14G stood out by producing the LCE with the highest enzymatic activity. Laccase activity was first detected after six days of cultivation (588.62 ± 63.13 U/mL) and continued to increase thereafter. This pattern contrasts with the mycelial biomass profile, which had already reached a plateau by this time, and aligns with the observed depletion of glucose in the culture medium. Previous studies have demonstrated that laccase production in bioreactors can continue for an extended period even after glucose depletion and the cessation of biomass growth (Saat et al., 2013). Therefore, future experiments should evaluate this hypothesis for the *P. sanguineus*.

The high laccase production observed in *P. sanguineus* 14G is consistent with previous studies reporting that *Pycnoporus* strains can exhibit substantial enzyme yields depending on the composition of the culture medium and the presence of specific inducers (Gioia et al., 2014; Ramírez-Cavazos et al., 2014a; Vikineswary et al., 2006). To the best of our knowledge, this is the first study to cultivate *P. sanguineus* using casein as a nitrogen source, which likely contributed to the exceptionally high enzymatic activity observed compared with values previously reported in the literature. Casein may have promoted fungal growth and enzyme secretion by providing readily assimilable amino acids and peptides, thereby stimulating secondary metabolism and laccase synthesis. Regarding nitrogen sources employed in previous studies with basidiomycetes, organic nitrogen sources have generally been reported to be more favourable than inorganic ones (Schneider et al., 2018). Mikiashvili et al. (2006) found that peptone, followed by casein,

was the most effective nitrogen source for laccase production by *Pleurotus ostreatus* (Mikiashvili et al., 2006). Similarly, Schneider (2018) reported an approximately tenfold increase in laccase activity when the culture medium of *M. palmivorus* VE111 was supplemented with casein. Consistent with these findings, the present results support the hypothesis that protein-based nitrogen sources may play a key regulatory role in the expression of ligninolytic enzymes.

Trametes sp. 50/09 exhibited good mycelial development, second only to *M. palmivorus* VE111. However, its laccase activity was comparatively low among the studied species. Several strategies may be employed to enhance enzyme production (Cantele et al., 2017). For instance, the addition of metal ions or lignin-related compounds, such as 2,5-xylidine, to the culture medium has shown promising results in inducing laccase synthesis by this species (Jang et al., 2006; Yang et al., 2013). Additionally, another proposed strategy involves the immobilization of the mycelium, which may improve enzyme yield and operational stability during bioprocesses (Wang et al., 2013).

The Pearson correlation coefficients between laccase activity and mycelial biomass, protein concentration, and pH are presented in Table 1. No significant correlation was found between mycelial biomass and laccase activity. Although *M. palmivorus* VE111 exhibited notable performance in terms of both biomass accumulation and enzyme production, the correlation analysis indicates that mycelial growth alone is not a reliable predictor of laccase activity. This observation aligns with previous findings for *P. sanguineus*, where laccase production has been associated with the stationary growth phase, a period typically characterized by limited or no mycelial expansion (Saat et al., 2013). Likewise, no significant correlation was found between enzyme activity and protein concentration in the cultures of *A. blazei* and *M. palmivorus* VE111. In contrast, moderate positive correlations were observed for *P. sanguineus* 14G ($r = 0.652$) and *Trametes* sp. 50/09 ($r = 0.732$). These findings indicate that total protein concentration is not a consistent estimate of laccase content in LCEs. This interpretation is further corroborated by the data presented in Figure 2A, which reports the protein secretome profiles of the LCEs obtained from each fungal species. The extracts contain proteins distributed across a wide range of molecular weights, with the most prominent bands observed between 50 and 100 kDa.

Table 1.

Pearson correlation coefficient between laccase activity and mycelium, protein or pH.

Laccase	Mycelium	Protein	pH
A. blazei	0.520	0.200	0.568
M. palmivorus VE111	0.612*	0.310	0.299
P. sanguineus 14 G	0.545	0.652*	0.778**
Trametes sp. 50/09	0.161	0.732***	0.920***

*p<0.05 **p<0.01 ***p<0.001

In the zymogram (Figure 2B), bands corresponding to laccases were detected for all LCEs. For *A. blazei* LCE, a single band with an approximate molecular weight of ~50 kDa was observed. Ullrich et al. (2005) previously reported a laccase from this species with a molecular weight of ~66 kDa, when cultivated in a tomato-based medium. Such variability likely reflects the occurrence of distinct laccase isoforms, whose expression is modulated by environmental and nutritional factors. Fungal laccases are encoded by multigene families, and the transcription of each gene can be differentially regulated depending on medium composition, pH, temperature, and the presence of metal ions or aromatic inducers (Yang et al., 2017). Accordingly, different isoenzyme patterns have been described for *Pleurotus ostreatus* and *M. palmivorus* VE111 under variable nutrient or copper concentrations (Grace et al., 2012; Schneider et al., 2018). Therefore, the isoform profile observed for *A. blazei* may result from the differential expression and post-translational modification of laccase genes favored by the specific conditions of the potato-based medium used in this study.

For *M. palmivorus* VE111, two distinct bands were identified, with estimated molecular weights of ~55 and ~75 kDa. In contrast, Schneider et al. (2018) reported a single laccase isoform of ~50 kDa under slightly acidic to neutral pH and low glucose concentration (0.18%), conditions similar to those used in this study, except for the higher glucose content.

Trametes sp. is one of the most extensively studied fungal genera for laccase production, primarily due to its strong secretion capacity under optimized cultivation conditions (Alharbi et al., 2019; Brugnari et al., 2021). In the present zymogram analysis, the LCE from *Trametes* sp. 50/09 exhibited a single band with an apparent molecular weight of 50 kDa. Similar results have been reported in the literature: Ling et al. (2015) purified a laccase of ~59 kDa from *Trametes* sp. LAC-01; Chaurasia et al., (2014) observed a 55 kDa band from *T. hirsuta* MTCC-1171; and Riedi et al. (2022) reported a

45 kDa laccase from *T. villosa*. These variations in molecular mass may reflect species-specific differences and potential isoform diversity among *Trametes* sp.

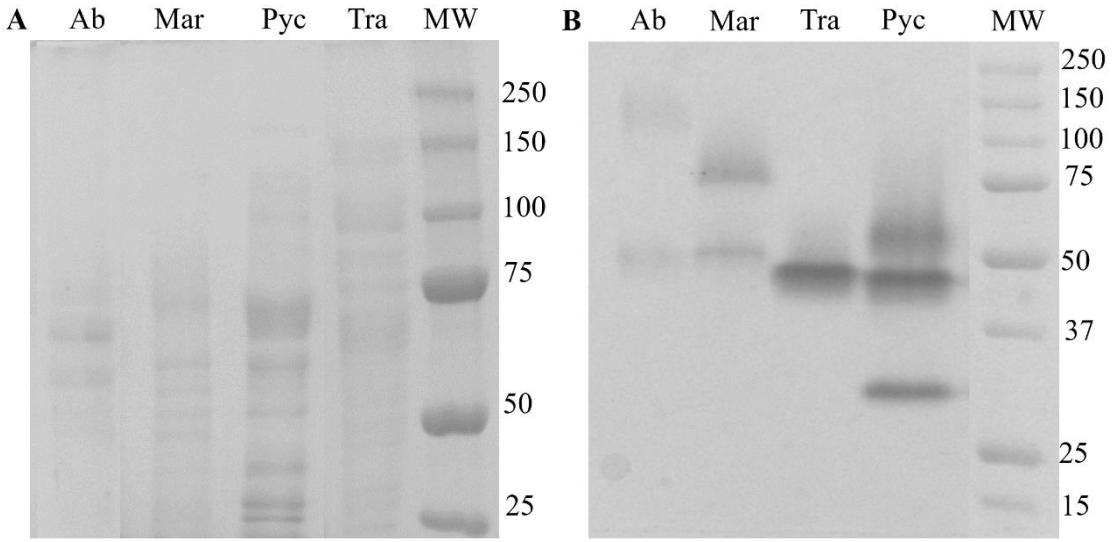


Figure 2. Characterization of laccase crude extracts (LCEs) produced by *Agaricus blazei* (Ab), *Marasmiellus palmivorus* VE111 (Mar), *Pycnoporus sanguineus* 14G (Pyc), and *Trametes* sp. 50/09 (Tra). (A) SDS-PAGE analysis showing total protein profiles stained with Coomassie Brilliant Blue R-250. (B) Zymogram analysis revealing laccase isoforms based on oxidation of ABTS. MW: molecular weight marker (kDa).

For *P. sanguineus* 14G, three bands were observed at approximately 60, 40, and 30 kDa, suggesting the presence of multiple isoforms. Previous reports described purified laccases from *P. sanguineus* with molecular weights of 68 and 66 kDa (Ramírez-Cavazos et al., 2014b), and another at 61.4 kDa with dye decolorization potential (Lu et al., 2007). The multiplicity of bands detected in our study likely reflects the unpurified nature of the LCEs and the coexistence of different laccase isoforms. Nevertheless, although most laccases from these species exhibit molecular weights between 55 and 70 kDa, similar low-molecular-weight isoforms have been reported in other basidiomycetes. For instance, Camassola et al. (2013) described laccases of approximately 30 kDa from *Pleurotus salmoneo-stramineus* and *Pleurotus citrinopileatus*, while Isanapong et al. (2024) purified and characterized a laccase of around 34 kDa from *Pleurotus ostreatus* HK35 (Camassola et al., 2013; Isanapong et al., 2024). Such bands may also correspond to partially degraded or deglycosylated forms that preserve the trinuclear copper site, as

previously observed for *P. sanguineus* by Vite-Vallejo et al. (2014) (Vite-vallejo et al., 2009).

The LCEs from each fungal species were stored under different conditions to evaluate their ability to retain laccase activity: refrigeration (4 °C), room temperature (23 ± 2 °C), supplementation with glycerol (10%, 20%, or 30%), or freeze-drying. The results are presented in Figure 3.

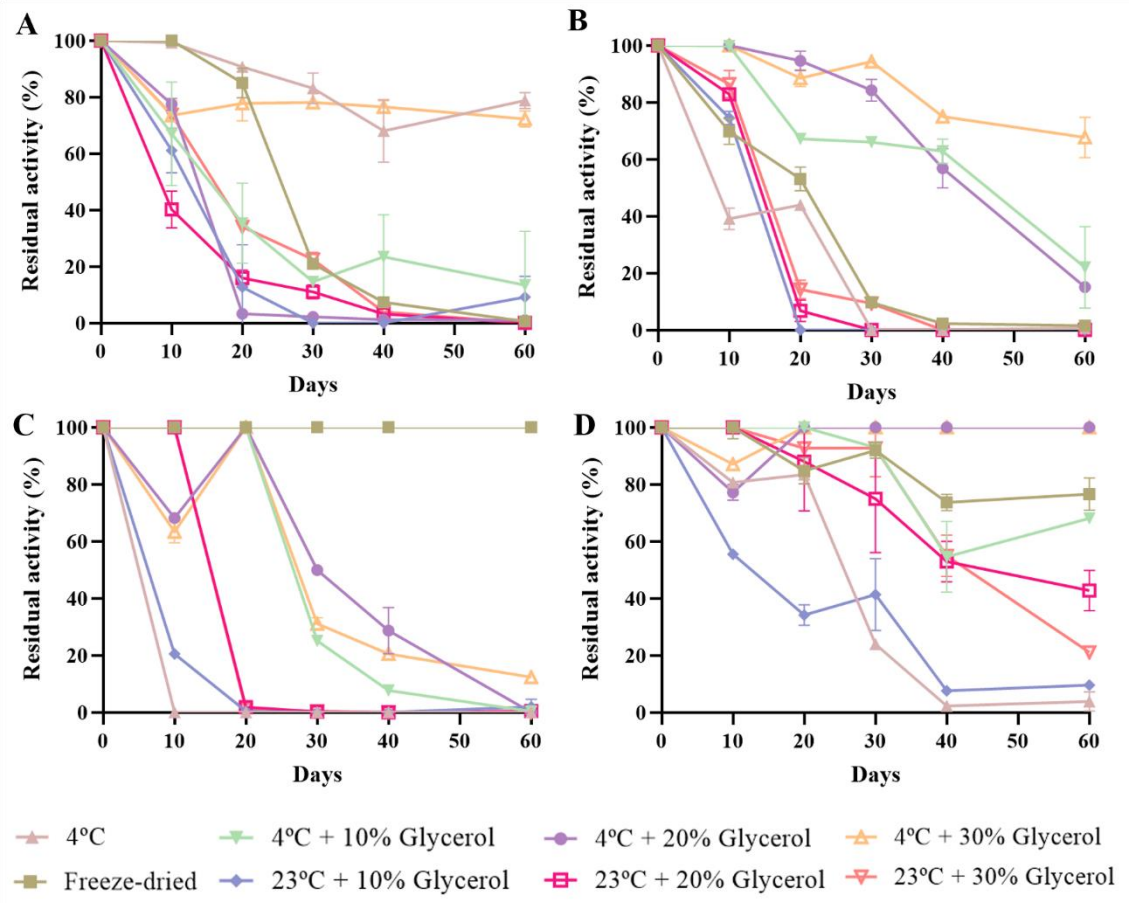


Figure 3. Storage stability of laccase crude extracts (LCEs) from (A) *Agaricus blazei*, (B) *Marasmiellus palmivorus* VE111, (C) *Pycnoporus sanguineus* 14G, and (D) *Trametes* sp. 50/09 (Tra) under different storage conditions. LCEs were stored at 4 °C, 23 °C (±2 °C), or freeze-dried, with or without glycerol at 10%, 20%, and 30% (v/v). Residual laccase activity was measured over 60 days and expressed as a percentage of initial activity. Error bars represent standard deviation (n = 3).

The storage method was critical for preserving enzymatic activity during the first 10 days (Figure 3). The subsequent decline in laccase activity is mainly associated with

protein aggregation and residual protease activity, key processes underlying enzyme instability during storage (Gianfreda et al., 1985; Iyer and Ananthanarayan, 2008). Understanding these mechanisms is essential for optimizing storage conditions and extending the shelf life of LCEs, particularly for commercial applications.

For *A. blazei* LCE (Figure 3A), both refrigeration and freeze-drying effectively preserved laccase activity in the early stages. However, over 60 days, refrigeration combined with 30% glycerol proved most effective, maintaining approximately 75% of the initial activity. Similarly, for *M. palmivorus* VE111 (Figure 3B), enzymatic activity declined rapidly under most conditions; nonetheless, refrigeration with 30% glycerol maintained up to 67% of the initial activity.

Freeze-drying proved to be the most effective long-term storage method for the LCE from *P. sanguineus* 14G (Figure 3C) preserving high laccase activity throughout the 60-day evaluation period. This highlights lyophilization as a promising stabilization strategy, capable of preventing enzymatic degradation, autolysis, and microbial contamination. Although previous studies have reported the preservation of *P. sanguineus* laccase through immobilization techniques (Cheute et al., 2025; Chmelová et al., 2024; García-Morales et al., 2018), our findings demonstrate that freeze-drying offers a simpler and comparably effective alternative.

For *Trametes* sp. 50/09 LCE (Figure 3D), laccase activity declined more gradually over the 60-day period. Refrigeration combined with 20% or 30% glycerol preserved up to 70% of the initial activity at the end of the storage period. This aligns with previous reports showing that 10% glycerol effectively preserves laccase activity in *T. versicolor* during cryopreservation (Eichlerová et al., 2015). These results illustrate that low-cost glycerol supplementation can stabilize *Trametes* sp. laccases comparably to more complex immobilization approaches.

The observed differences in stability likely reflect species-specific biochemical properties of laccase isoforms, such as hydrophobicity, glycosylation, and structural resilience. Glycerol stabilizes these enzymes by reinforcing hydrogen bonds and reducing unfolding, particularly in more hydrophobic or less glycosylated isoforms (Bîtcan et al., 2023; Braham et al., 2021). However, higher glycerol concentrations may increase viscosity or hinder substrate diffusion, potentially impacting catalytic performance in practical applications.

The ability of LCEs to retain 65-75% of their enzymatic activity after 60 days under refrigerated conditions underscores their potential as robust biocatalysts for

decentralized or cost-sensitive bioremediation systems, particularly in settings with basic cold chain logistics and limited access to enzyme purification or immobilization technologies. This stability profile also supports the use of LCEs in modular, field-deployable bioreactors for on-site pharmaceutical degradation in wastewater treatment systems. The results of this study represent the stability of the extracts under ideal storage conditions, established to minimize thermal degradation reactions previously reported in the literature and to ensure that the product retains its properties over the evaluated period. Although environmental conditions may vary across different regions of the world, seasonal temperature variations could be addressed in future experimental designs, particularly for extracts that exhibited promising performance. Future work should include kinetic modeling of inactivation rates, assessments of enzyme reuse and rehydration performance of freeze-dried extracts, and the development of tailored stabilization protocols for each fungal species. To the best of our knowledge, no prior study has reported on the stability of LCEs from *A. blazei* and *M. palmivorus* VE111 under glycerol-enriched storage conditions, highlighting the uniqueness and significance of our findings.

Laccases offer clear advantages for bioremediation, operating with low energy input and minimal use of auxiliary reagents (Thathola et al., 2024). Fungal laccases are particularly relevant due to their broad substrate specificity and strong oxidative potential (Chmelová et al., 2024). These properties enhance their versatility as biocatalysts and support their integration with conventional remediation systems to improve pollutant degradation (Chmelová et al., 2024; Singh et al., 2025). In this context, experiments were conducted to assess the pharmaceutical biotransformation potential of LCEs derived from the four cultivated fungal species.

Acetaminophen is among the most frequently detected pharmaceuticals in surface waters and wastewater effluents worldwide, reflecting its extensive use and incomplete removal by conventional treatment technologies (Guerra et al., 2014; Phong Vo et al., 2019). Even at trace levels, it has been shown to exert toxic effects on diverse organisms, including bacteria, algae, macrophytes, protozoa, and fish (Phong Vo et al., 2019; Placova et al., 2023). To address these concerns, experiments were conducted using LCEs to evaluate their potential for paracetamol bioremediation. At an initial acetaminophen concentration of 5 mg/L, all LCEs tested at 10 U/mL demonstrated the ability to biotransform within 24 hours (Figure 4A). The LCE from *M. palmivorus* VE111 was the most effective, achieving 96% biotransformation, followed by *P. sanguineus* 14G (91%),

Trametes sp. 50/09 (86%), and *A. blazei* (66%). However, increasing the acetaminophen concentration to 10 mg/L (Figure 4B) resulted in a time-dependent decline in degradation efficiency across all species. For *M. palmivorus*, VE111 degradation dropped from 89% at 0.5 h to 39% at 24 h. *P. sanguineus* 14G showed a decline from 93% to 82%, and *Trametes* sp. 50/09 exhibited only a modest reduction (88% to 81%). *A. blazei,* however, exhibited the most pronounced decline, with biotransformation dropping from 41% at 0.5 h to undetectable levels at 24 h, matching the control condition. Increasing LCEs concentration threefold did not enhance acetaminophen degradation (Figure 4C). This can be explained by substrate saturation or inhibitory effects.

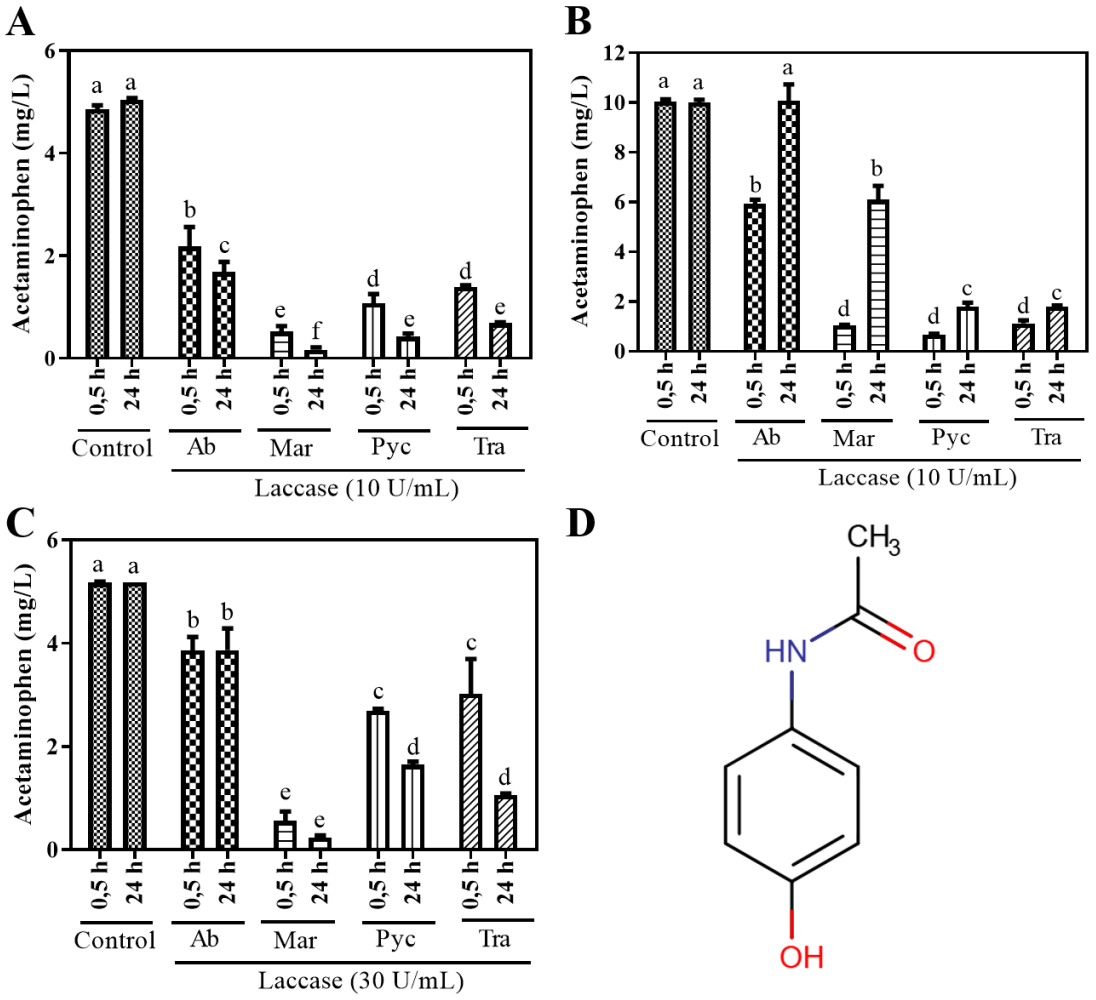


Figure 4. Bioremediation of acetaminophen by fungal laccase crude extracts (LCEs). Residual concentrations of the pharmaceutical were determined after 0.5 and 24 h of incubation with LCEs under the following conditions: (A) 5 mg/L acetaminophen and 10 U/mL laccase, (B) 10 mg/L acetaminophen and 10 U/mL laccase, and (C) 10 mg/L

acetaminophen and 30 U/mL laccase. Fungal species: *Agaricus blazei* (Ab), *Marasmiellus palmivorus* VE111 (Mar), *Pycnoporus sanguineus* 14G (Pyc), and *Trametes* sp. 50/09 (Tra). Control samples contained no enzyme. (D) Chemical structure of acetaminophen. Different letters denote statistically significant differences between treatments (Tukey's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

Structurally, acetaminophen contains a phenolic group (Figure 4D), rendering it a suitable laccase substrate (Hachi et al., 2017; Phong Vo et al., 2019). Laccases catalyze single-electron oxidation of phenolic and aromatic amine compounds, forming reactive quinone-imine intermediates that are more hydrophilic and prone to polymerization or further oxidation, thus reducing the parent compound's persistence and toxicity. These intermediates can undergo coupling and ring-opening reactions, leading ultimately to smaller, more polar degradation products such as hydroxyquinones and short-chain organic acids. Peroxidases and cytochrome P450 monooxygenases, commonly secreted by fungi, may also contribute to acetaminophen transformation through hydroxylation and subsequent oxidative cleavage (Phong Vo et al., 2019; Pylypchuk et al., 2018). However, while this mechanism is generally efficient at moderate substrate concentrations, our results indicate that laccase activity may decline under higher substrate loads. This time-dependent loss of activity, particularly observed in *M. palmivorus* VE111 and *A. blazei*, is likely due to a combination of factors, including enzyme inactivation and covalent modification by reactive oxidative byproducts such as quinones. These byproducts can interact with amino acid residues or alter enzyme conformation, leading to irreversible catalytic loss (Shu et al., 2019). Additionally, although not fully characterized, interactions with other fungal redox-active enzymes could potentially contribute to the observed decrease in activity, representing a hypothesis that warrants further investigation.

Interestingly, despite the observed decrease in laccase activity, prolonged incubation in some LCE treatments led to the partial reappearance of acetaminophen (Figure 4B), suggesting that reductive pathways may contribute to the regeneration of the parent compound. This phenomenon could involve redox cycling of quinone or iminoquinone intermediates, which might be reduced back to acetaminophen by fungal reductases. Fungi are known to secrete NAD(P)H-dependent quinone reductases, azo reductases, and nitroreductases capable of catalyzing such reactions (Gómez-Toribio et al., 2009). In species such as *Pleurotus eryngii*, extracellular reductases have been

reported to operate in tandem with oxidative enzymes, forming coupled redox cycles that facilitate detoxification (Gómez-Toribio et al., 2009). Although direct evidence for acetaminophen regeneration remains limited, these observations highlight the complexity of enzymatic biotransformation pathways in fungal enzyme crude extracts and underscore the need for further mechanistic studies to elucidate interactions between laccases, redox-active metabolites, and potential reductases.

All LCEs were further evaluated for their ability to degrade diclofenac, with noteworthy results presented in Figure 5. When diclofenac was applied at an initial concentration of 5 mg/L (Figure 5A), the LCE from *P. sanguineus* 14G achieved a degradation rate of approximately 30% at both 0.5 and 24 hours. In contrast, the LCEs from *M. palmivorus* VE111 and *Trametes* sp. 50/09 exhibited comparable degradation rates only after 24 h. Upon doubling the initial diclofenac concentration, the LCE from *M. palmivorus* VE111 demonstrated significantly enhanced performance, achieving a degradation rate of approximately 75% (Figure 5B). Under both experimental conditions, the LCE derived from *A. blazei* did not produce statistically significant degradation relative to the control. However, when the pharmaceutical was applied at 5 mg/L and the LCE dosage increased to 30 U/mL, an approximate degradation rate of 82% was observed (Figure 5C). The findings presented here are particularly significant, as no previous research has assessed the ability of *M. palmivorus* VE111 and *A. blazei* laccases to degrade diclofenac.

The susceptibility of diclofenac to laccase-mediated degradation can be attributed to its molecular structure (Figure 5D), which exhibits pronounced chemical reactivity and a high affinity for interactions with biomolecules (Devi et al., 2019). Diclofenac features a biphenyl backbone, incorporating a substituted aniline ring with two ortho-chlorine atoms and a carboxylic acid moiety. The presence of the aromatic amine, a well-known electron-donating group, facilitates single-electron oxidation by laccases, leading to the formation of reactive iminoquinone or aryl cation intermediates. These unstable intermediates may subsequently undergo hydrolysis, ring-opening, or coupling reactions, ultimately reducing the toxicity and environmental persistence of the parent molecule (Chmelová et al., 2024; Devi et al., 2019). Moreover, the ortho-chlorine substituents, although potentially deactivating for electrophilic aromatic substitution, may contribute to the stabilization of radical intermediates through inductive effects, thereby enabling regioselective enzymatic oxidation (Devi et al., 2019; Lonappan et al., 2016).

Furthermore, laccase activity is not limited to phenolic substrates; it can oxidize a broader range of aromatic amines and electron-rich structures, especially in the presence of redox mediators or reactive oxidation products formed in situ (Lonappan et al., 2017). This mechanistic versatility underscores the enzyme's potential to transform structurally diverse pharmaceuticals such as diclofenac and highlights the importance of exploring enzyme–substrate structural compatibility in bioremediation research.

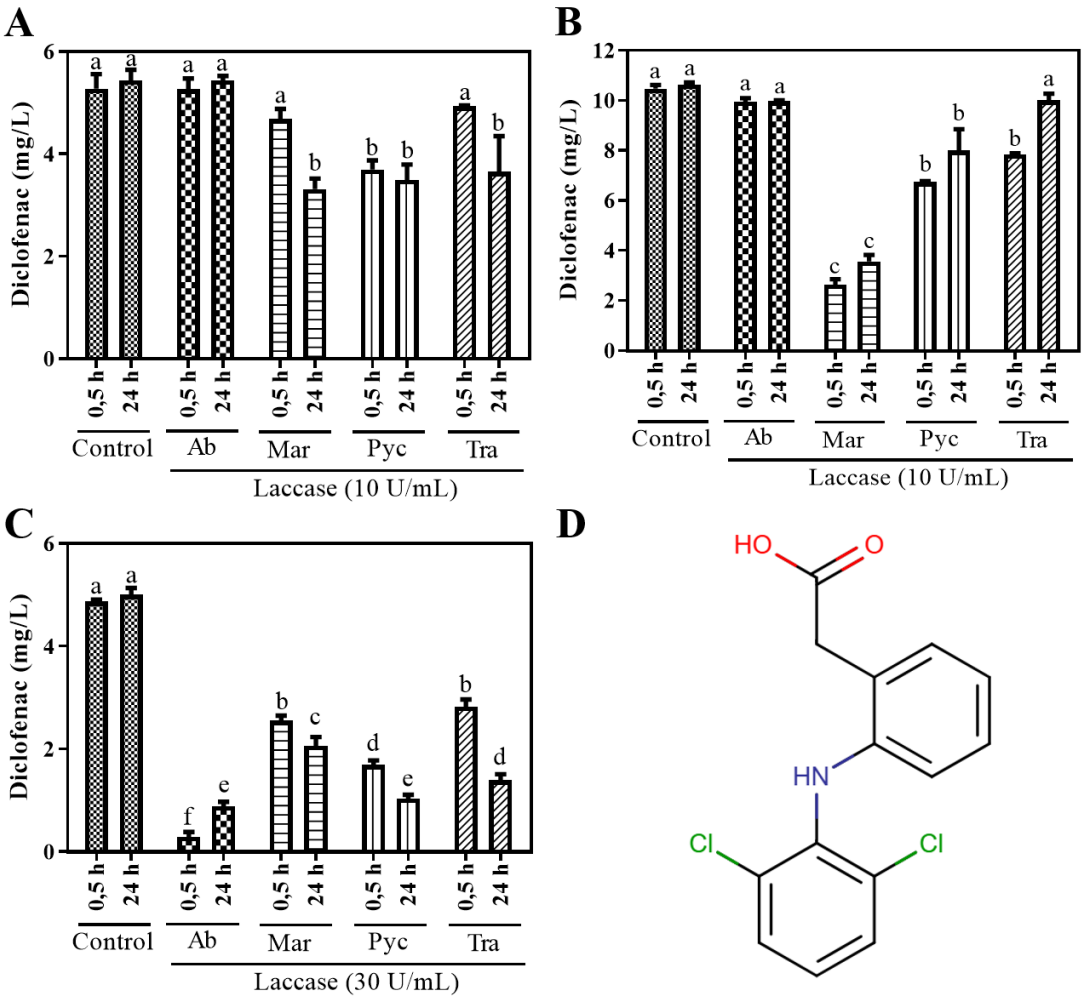


Figure 5. Bioremediation of diclofenac by fungal laccase crude extracts (LCEs). Residual concentrations of the pharmaceutical were determined after 0.5 and 24 h of incubation with LCEs under the following conditions: (A) 5 mg/L diclofenac and 10 U/mL laccase, (B) 10 mg/L diclofenac and 10 U/mL laccase, and (C) 10 mg/L diclofenac and 30 U/mL laccase. Fungal species: *Agaricus blazei* (Ab), *Marasmiellus palmivorus* VE111 (Mar), *Pycnoporus sanguineus* 14G (Pyc), and *Trametes* sp. 50/09 (Tra). Control samples contained no enzyme. (D) Chemical structure of diclofenac. Different letters denote

statistically significant differences between treatments (Tukey's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

Future investigations should focus on identifying transformation intermediates using LC-MS/MS, which will be crucial for elucidating the degradation pathways of the pharmaceuticals evaluated in this study. Such analyses will also improve the predictability of the toxicity of the resulting products, which can subsequently be assessed in dedicated toxicological assays. Additionally, integrating comprehensive kinetic studies into the degradation experiments could substantially enhance the scientific relevance of laccase-mediated processes. Together, these approaches are essential to verify that enzymatic degradation does not lead to the formation of harmful by-products.

To better contextualize the degradation performance observed in this study, Table 2 summarizes bioremediation data from previously reported laccase crude extracts derived from the same fungal species used here for the degradation of acetaminophen and diclofenac, together with the best results obtained in this work. To the best of our knowledge, no previous studies have reported the use of crude extracts or purified laccases from *A. blazei* or *M. palmivorus* for the degradation of these pharmaceuticals, underscoring the novelty of the present findings.

The acetaminophen and diclofenac concentrations used in this study (5 and 10 mg/L) were selected based on previous bioremediation assays employing fungal laccases under controlled conditions (Alharbi et al., 2019; García-Morales et al., 2018; Lonappan et al., 2017; Masjoudi et al., 2021). These concentrations were intentionally chosen to simulate a model scenario of high pollutant load, as typically observed in pharmaceutical manufacturing effluents, hospital wastewater, or bench-scale enzymatic screening assays. Such levels ensure detectable analytical signals and facilitate the assessment of enzymatic degradation patterns and stability over time. However, they are higher than those typically found in surface waters or wastewater treatment plant effluents, where concentrations usually range from ng L^{-1} to low µg L^{-1} levels (Chmelová et al., 2024; Patel et al., 2019). Given that laccases can effectively oxidize substrates even at trace concentrations, future studies should investigate more environmentally relevant levels across diverse matrices. Moreover, future experiments should evaluate simultaneous exposure to acetaminophen and diclofenac, since these compounds

frequently co-occur in aquatic environments. Such combined treatments may produce synergistic or antagonistic effects that influence pollutant removal efficiency.

Table 2.
Bioremediation of acetaminophen and diclofenac by laccase crude extracts.

Fungi	Laccase Extract	Enzyme Activity	PCL	Concentration	Effectiveness of treatment	Reference
Agaricus blazei	Crude extract	10 U/mL	ACE	5 mg/L	67% (24h)	PS
Agaricus blazei	Crude extract	30 U/mL	DCF	5 mg/L	94% (0,5h)	PS
Marasmiellus palmivorus VE111	Crude extract	10 U/mL	ACE	5 mg/L	97% (24h)	PS
Marasmiellus palmivorus VE111	Crude extract	10 U/mL	DCF	10 mg/L	75% (0,5h)	PS
Pycnoporus sanguineus CS43	Crude extract	100 U/L	DCF	10 m/L	50% (8h)	(Rodríguez-Delgado et al., 2016)
Pycnoporus sanguineus CS43	Crude extract	100 U/L	ACE	10 mg/L	90% (2h)	(García-Morales et al., 2018)
Pycnoporus sanguineus CS43	Crude extract	100 U/L	DCF	10 mg/L	80% (8h)	(García-Morales et al., 2018)
Pycnoporus sanguineus 14G	Crude extract	10 U/mL	ACE	5 mg/L	92% (24h)	PS
Pycnoporus sanguineus 14G	Crude extract	30 U/mL	DCF	5 mg/L	79% (0,5h)	PS
Trametes sp. (C3)	Crude extract/ Concentrated	40 U/L	ACE	80 mg/L	76% (1,5h)	(Sybuia et al., 2024)
Trametes hirsuta	Crude extract/Immobilized	1,5 U/L	ACE	1 mg/L	30% (6h)	(Hachi et al., 2017)
Trametes hirsuta	Crude extract/ Cross-linked enzyme aggregates	200 U/L	ACE	10 µg/L	97% (24h)	(Alokpa et al., 2022)
Trametes pubescens	Crude extract	100 U/L	DCF	0.84 µg/L	39% (24h)	(Spina et al., 2020)
Trametes versicolor	Crude extract	210 U/mL	DCF	20 mg/L	100% (144h)	(Margot et al., 2013)
Trametes versicolor	Crude extract	35 µM/min	DCF	50 µg/L	60% (24h)	(Nguyen et al., 2014)
Trametes sp. 50/90	Crude extract	10 U/mL	ACE	5 mg/L	86% (24h)	PS
Trametes sp. 50/90	Crude extract	30 U/mL	DCF	5 mg/L	72% (24h)	PS

PCL: pharmaceutical ACE: acetaminophen DCF: diclofenac PS: present study

Understanding and quantifying enzymatic activity is essential for effective bioremediation. Therefore, this study aimed to assess whether the enzymes retained their catalytic performance following exposure to pharmaceutical compounds. To achieve this, an aliquot of the reaction mixture, containing 10 U/mL of enzyme and 5 mg/L of either acetaminophen or diclofenac, was analyzed to evaluate the enzymatic activity, using ABTS as a substrate for the reaction. In Figure 6, we can see that in both the biotransformation reaction of acetaminophen (Figure 6A) and diclofenac (Figure 6B), the enzyme maintains its activity after 0.5 h and 24 h of reaction. Researchers demonstrates that enzymatic activity and stability are preserved when enzymes are immobilized on a range of supports (Cheute et al., 2025; Chmelová et al., 2024; Zdarta et al., 2019). Our findings demonstrate that the enzymes retain their catalytic activity after exposure to these pharmaceutical compounds, even when present in a free state within the reaction medium. This highlights their potential for continued use in biotransformation processes. However, further research is needed to explore the turnover mechanisms involved in the biotransformation of these pharmaceuticals upon their reintroduction into the reaction medium, as well as their potential to degrade other pharmaceutical contaminants. Moreover, pharmaceuticals that are not typical laccase substrates may still be biodegraded via oxidative byproducts generated from low-molecular-weight compounds, such as acetaminophen. These intermediates can act as redox mediators, thereby enhancing the enzymatic transformation of more recalcitrant pollutants, for example carbamazepine (Hachi et al., 2017).

It is well-established that variations in pH can alter the molecular conformation of enzymes, influencing their catalytic efficiency, substrate specificity, and affinity (Iyer and Ananthanarayan, 2008). Consequently, pH was systematically evaluated in the reaction environment involving the pharmaceuticals acetaminophen (Figure 6C) and diclofenac (Figure 6D). The results indicated that the pH remained stable after both 0.5 and 24 hours of reaction time, suggesting that pH did not significantly impact the biotransformation of the pharmaceuticals under the conditions tested. Further investigation is warranted to examine other factors, such as temperature and enzyme concentration, which may offer additional insights into their effects on the efficiency of biotransformation processes for these specific pharmaceuticals.

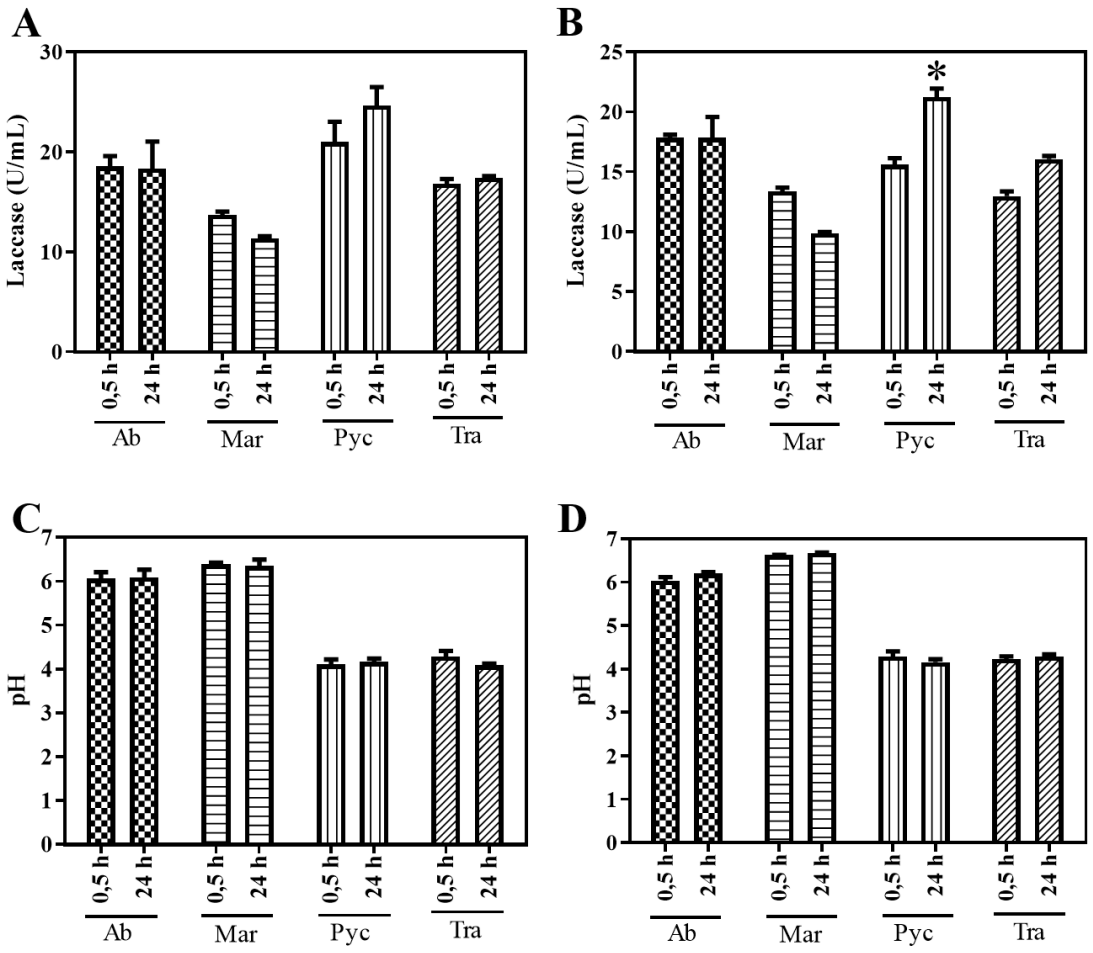


Figure 6. Laccase activity (U/mL) and pH variations during pharmaceutical biotransformation reactions. (A) and (C): acetaminophen; (B) and (D): diclofenac. Enzyme activity and pH were evaluated at 0.5 and 24 h of reaction using LCEs from *Agaricus blazei* (Ab), *Marasmiellus palmivorus* VE111 (Mar), *Pycnoporus sanguineus* 14G (Pyc), and *Trametes* sp. 50/09 (Tra). * Statistically significant differences between time points (Tukey's test, $p < 0.05$). Error bars represent standard deviation ($n = 3$).

4. Conclusion

This study demonstrates the potential of crude laccase extracts for pharmaceutical bioremediation, particularly acetaminophen and diclofenac. *P. sanguineus* 14G produced the highest laccase levels, while *Trametes* sp. 50/09 exhibited a gradual decline in activity during storage. In bioremediation, *M. palmivorus* VE111 efficiently transformed acetaminophen, and *A. blazei* showed notable activity against diclofenac, both reported here for the first time. Laccases retained performance at elevated pharmaceutical

concentrations, highlighting their robustness and applicability in wastewater treatment. Preliminary evidence suggests redox cycling, possibly mediated by fungal reductases, contributing to acetaminophen reappearance, a mechanism deserving further investigation.

5. References

Alharbi, S.K., Nghiem, L.D., van de Merwe, J.P., Leusch, F.D.L., Asif, M.B., Hai, F.I., Price, W.E., 2019. Degradation of diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole by laccase from Trametes versicolor: Transformation products and toxicity of treated effluent. Biocatal. Biotransformation 37, 399–408. https://doi.org/10.1080/10242422.2019.1580268

Alokpa, K., Lafortune, F., Cabana, H., 2022. Application of laccase and hydrolases for trace organic contaminants removal from contaminated water. Environ. Adv. 8. https://doi.org/https://doi.org/10.1016/j.envadv.2022.100243

Argyropoulos, D., Psallida, C., Sitareniou, P., Flemetakis, E., Diamantopoulou, P., 2022. Biochemical Evaluation of Agaricus and Pleurotus Strains in Batch Cultures for Production Optimization of Valuable Metabolites.

Bîtcan, I., Petrovici, A., Pellis, A., Klébert, S., Károly, Z., Bereczki, L., Péter, F., Todea, A., 2023. Enzymatic route for selective glycerol oxidation using covalently immobilized laccases. Enzyme Microb. Technol. 163. https://doi.org/10.1016/j.enzmictec.2022.110168

Bouabadi, I., Miyah, Y., Benjelloun, M., El-habacha, M., El Addouli, J., 2024. Advanced strategies for the innovative treatment of hospital liquid effluents: A comprehensive review. Bioresour. Technol. Reports 28, 101990. https://doi.org/10.1016/j.biteb.2024.101990

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999

Braham, S.A., Siar, E.H., Arana-Peña, S., Bavandi, H., Carballares, D., Morellon-Sterling, R., de Andrades, D., Kornecki, J.F., Fernandez-Lafuente, R., 2021. Positive effect of glycerol on the stability of immobilized enzymes: Is it a universal fact?

Process Biochem. 102, 108–121. https://doi.org/10.1016/j.procbio.2020.12.015

Brugnari, T., Braga, D.M., dos Santos, C.S.A., Torres, B.H.C., Modkovski, T.A., Haminiuk, C.W.I., Maciel, G.M., 2021. Laccases as green and versatile biocatalysts: from lab to enzyme market—an overview. Bioresour. Bioprocess. 8, 1–29. https://doi.org/10.1186/s40643-021-00484-1

Camassola, M., Rosa, L.O., Calloni, R., Gaio, T.A., Dillon, A.J.P., 2013. Secretion of laccase and manganese peroxidase by Pleurotus strains cultivate in solid-state using Pinus spp . sawdust. Brazilian J. Microbiol. 213, 207–213.

Cantele, C., Fontana, R.C., Mezzomo, A.G., da Rosa, L.O., Poleto, L., Camassola, M., Dillon, A.J.P., 2017. Production, characterization and dye decolorization ability of a high level laccase from Marasmiellus palmivorus. Biocatal. Agric. Biotechnol. 12, 15–22. https://doi.org/10.1016/j.bcab.2017.08.012

Chaurasia, P.K., Yadav, R.S.S., Yadava, S., 2014. Purification and characterization of yellow laccase from Trametes hirsuta MTCC-1171 and its application in synthesis of aromatic aldehydes. Process Biochem. 49, 1647–1655. https://doi.org/10.1016/j.procbio.2014.06.016

Cheute, V.M.S., Backes, E., Pateis, V. de O., de Oliveira Junior, V.A., Uber, T.M., dos Santos Filho, J.R., dos Santos, L.F.O., Castoldi, R., de Souza, C.G.M., Polonio, J.C., Contato, A.G., Bracht, A., Peralta, R.M., 2025. Optimization of immobilization, characterization, and environmental applications of Llccases from Pycnoporus sanguineus UEM-20. Processes 13, 1–24. https://doi.org/10.3390/pr13061800

Chmelová, D., Ondrejovič, M., Miertuš, S., 2024. Laccases as effective tools in the removal of pharmaceutical products from aquatic systems. Life 14. https://doi.org/10.3390/life14020230

Devi, R.N., Stephen, A.D., Justin, P., Saravanan, K., Macchi, P., Jelsch, C., 2019. Topological and electrostatic properties of diclofenac molecule as a non-steroidal anti-inflammatory drug: An experimental and theoretical study. J. Mol. Struct. 1196, 42–53. https://doi.org/10.1016/j.molstruc.2019.06.027

Eichlerová, I., Homolka, L., Tomšovský, M., Lisá, L., 2015. Long term storage of Pleurotus ostreatus and Trametes versicolor isolates using different cryopreservation techniques and its impact on laccase activity. Fungal Biol. 119, 1345–1353.

https://doi.org/10.1016/j.funbio.2015.10.004

Estrada-Almeida, A.G., Castrejón-Godínez, M.L., Mussali-Galante, P., Tovar-Sánchez, E., Rodríguez, A., 2024. Pharmaceutical pollutants: ecotoxicological impacts and the use of agro-industrial waste for their removal from aquatic environments. J. Xenobiotics 14, 1465–1518. https://doi.org/10.3390/jox14040082

García-Morales, R., García-García, A., Orona-Navar, C., Osma, J.F., Nigam, K.D.P., Ornelas-Soto, N., 2018. Biotransformation of emerging pollutants in groundwater by laccase from P. sanguineus CS43 immobilized onto titania nanoparticles. J. Environ. Chem. Eng. 6, 710–717. https://doi.org/10.1016/j.jece.2017.12.006

Gianfreda, L., Modafferi, M., Greco, G., 1985. Enzyme stabilization towards thermal, chemical and proteolytic deactivation. Enzyme Microb. Technol. 7, 78–82. https://doi.org/10.1016/0141-0229(85)90017-1

Gioia, L., Manta, C., Ovsejevi, K., Burgueño, J., Menéndeza, P., Rodriguez-Couto, S., 2014. Enhancing laccase production by a newly-isolated strain of Pycnoporus sanguineus with high potential for dye decolouration. RSC Adv. 4, 34096–34103. https://doi.org/10.1039/C4RA06039C

Gómez-Toribio, V., García-Martín, A.B., Martínez, M.J., Martínez, Á.T., Guillén, F., 2009. Enhancing the production of hydroxyl radicals by Pleurotus eryngii via quinone redox cycling for pollutant removal. Appl. Environ. Microbiol. 75, 3954–3962. https://doi.org/10.1128/AEM.02138-08

Grace, S., Faraco, V., Amore, A., Birolo, L., Giangrande, C., Thomaz, V., Pandey, A., Ricardo, C., 2012. Characterization of laccase isoforms produced by Pleurotus ostreatus in solid state fermentation of sugarcane bagasse. Bioresour. Technol. 114, 735–739. https://doi.org/10.1016/j.biortech.2012.03.058

Guerra, P., Kim, M., Shah, A., Alaee, M., Smyth, S.A., 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci. Total Environ. 473–474, 235–243. https://doi.org/10.1016/j.scitotenv.2013.12.008

Guo, Y., Qi, P.S., Liu, Y.Z., 2017. A review on advanced treatment of pharmaceutical wastewater. IOP Conf. Ser. Earth Environ. Sci. 63, 12025. https://doi.org/10.1088/1755-1315/63/1/012025

Hachi, M., Chergui, A., Yeddou, A.R., Selatnia, A., Cabana, H., 2017. Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from Trametes hirsuta. Biocatal. Biotransformation 35, 51–62. https://doi.org/10.1080/10242422.2017.1280032

Hamedi, A., Vahid, H., Ghanati, F., 2007. Optimization of the medium composition for production of mycelial biomass and exo-polysaccharide by Agaricus blazei murill DPPh 131 using response surface methodology. Biotechnology 6, 456–464. https://doi.org/10.3923/biotech.2007.456.464

Isanapong, J., Suwannoi, K., Lertlattanapong, S., Panchal, S., 2024. Purification , characterization of laccase from Pleurotus ostreatus HK35 , and optimization for congo red biodecolorization using Box – Behnken design. 3 Biotech. https://doi.org/10.1007/s13205-024-03926-7

Iyer, P. V., Ananthanarayan, L., 2008. Enzyme stability and stabilization-aqueous and non-aqueous environment. Process Biochem. 43, 1019–1032. https://doi.org/10.1016/j.procbio.2008.06.004

Jang, M.Y., Ryu, W.Y., Cho, M.H., Zhang, J., Li, Z., Riemensberger, J., Huang, G., Kippenberg, T.J., 2006. Enhanced production of laccase from Trametes sp. by combination of various inducers. Biotechnol. Bioprocess Eng. 11, 96–99. https://doi.org/10.1007/BF02931890

Kim, H.H., Na, J.G., Chang, Y.K., Chun, G.T., Lee, S.J., Jeong, Y.H., 2004. Optimization of submerged culture conditions for mycelial growth and exopolysaccharides production by Agaricus blazei. J. Microbiol. Biotechnol. 14, 944–951.

Kummerová, M., Zezulka, Š., Babula, P., Tříska, J., 2016. Possible ecological risk of two pharmaceuticals diclofenac and paracetamol demonstrated on a model plant Lemna minor. J. Hazard. Mater. 302, 351–361. https://doi.org/10.1016/j.jhazmat.2015.09.057

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685. https://doi.org/10.1038/227680a0

Ling, Z.R., Wang, S.S., Zhu, M.J., Ning, Y.J., Wang, S.N., Li, B., Yang, A.Z., Zhang, G.Q., Zhao, X.M., 2015. An extracellular laccase with potent dye decolorizing ability from white rot fungus Trametes sp. LAC-01. Int. J. Biol. Macromol. 81, 785–

793. https://doi.org/10.1016/j.ijbiomac.2015.09.011

Liu, G.Q., Wang, X.L., 2007. Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide production by Agaricus blazei. Appl. Microbiol. Biotechnol. 74, 78–83. https://doi.org/10.1007/s00253-006-0661-6

Lonappan, L., Brar, S.K., Das, R.K., Verma, M., Surampalli, R.Y., 2016. Diclofenac and its transformation products: Environmental occurrence and toxicity - A review. Environ. Int. 96, 127–138. https://doi.org/10.1016/j.envint.2016.09.014

Lonappan, Linson, Rouissi, Tarek, Laadila, Mohamed Amine, Brar, Satinder Kaur, Galan, L.H., Verma, Mausam, Surampalli, R.Y., Lonappan, L, Rouissi, T, Laadila, M A, Brar, S K, Hernandez Galan, L., Verma, M, Surampalli, R.Y., 2017. Agro-industrial-produced laccase for degradation of diclofenac and identification of transformation products. ACS Sustain. Chem. Eng. 5, 5772–5781. https://doi.org/10.1021/acssuschemeng.7b00390

Lu, L., Zhao, M., Zhang, B.B., Yu, S.Y., Bian, X.J., Wang, W., Wang, Y., 2007. Purification and characterization of laccase from Pycnoporus sanguineus and decolorization of an anthraquinone dye by the enzyme. Appl. Microbiol. Biotechnol. 74, 1232–1239. https://doi.org/10.1007/s00253-006-0767-x

Margot, J., Bennati-Granier, C., Maillard, J., Blánquez, P., Barry, D.A., Holliger, C., 2013. Bacterial versus fungal laccase: potential for micropollutant degradation. AMB Express 3, 63. https://doi.org/10.1186/2191-0855-3-63

Masjoudi, M., Golgoli, M., Ghobadi Nejad, Z., Sadeghzadeh, S., Borghei, S.M., 2021. Pharmaceuticals removal by immobilized laccase on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes. Chemosphere 263, 128043. https://doi.org/10.1016/j.chemosphere.2020.128043

Mikiashvili, N., Wasser, S.P., Nevo, E., Elisashvili, V., 2006. Effects of carbon and nitrogen sources on Pleurotus ostreatus ligninolytic enzyme activity. World J. Microbiol. Biotechnol. 22, 999–1002. https://doi.org/10.1007/s11274-006-9132-6

Mutegoa, E., 2024. Efficient techniques and practices for wastewater treatment: an update. Discov. Water 4. https://doi.org/10.1007/s43832-024-00131-8

Nguyen, L.N., Hai, F.I., Yang, S., Kang, J., Leusch, F.D.L., Roddick, F., Price, W.E., Nghiem, L.D., 2014. Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: Role of biosorption and biodegradation. Int. Biodeterior. Biodegrad. 88, 169–175. https://doi.org/10.1016/j.ibiod.2013.12.017

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Phong Vo, H.N., Le, G.K., Nguyen, T.M.H., Bui, X.T., Nguyen, K.H., Rene, E.R., Vo, T.D.H., Cao, N.D.T., Mohan, R., 2019. Acetaminophen micropollutant: Historical and current occurrences, toxicity, removal strategies and transformation pathways in different environments. Chemosphere 236, 15. https://doi.org/10.1016/j.chemosphere.2019.124391

Placova, K., Halfar, J., Brozova, K., Heviankova, S., 2023. Issues of non-steroidal anti-inflammatory drugs in aquatic environments: a review study. Eng. Proc. 57. https://doi.org/10.3390/engproc2023057013

Pylypchuk, I. V., Kessler, V.G., Seisenbaeva, G.A., 2018. Simultaneous Removal of Acetaminophen, Diclofenac, and Cd(II) by Trametes versicolor Laccase Immobilized on Fe3O4/SiO2-DTPA Hybrid Nanocomposites. ACS Sustain. Chem. Eng. 6, 9979–9989. https://doi.org/10.1021/acssuschemeng.8b01207

Ramírez-Cavazos, L.I., Junghanns, C., Nair, R., Cárdenas-Chávez, D.L., Hernández-Luna, C., Agathos, S.N., Parra, R., 2014a. Enhanced production of thermostable laccases from a native strain of Pycnoporus sanguineus using central composite design. J. Zhejiang Univ. Sci. B 15, 343–352. https://doi.org/10.1631/jzus.B1300246

Ramírez-Cavazos, L.I., Junghanns, C., Ornelas-Soto, N., Cárdenas-Chávez, D.L., Hernández-Luna, C., Demarche, P., Enaud, E., García-Morales, R., Agathos, S.N., Parra, R., 2014b. Purification and characterization of two thermostable laccases from Pycnoporus sanguineus and potential role in degradation of endocrine disrupting chemicals. J. Mol. Catal. B Enzym. 108, 32–42. https://doi.org/10.1016/j.molcatb.2014.06.006

Rapp, P., Grote, E., Wagner, F., 1981. Formation and location of 1,4-β-glucanases and 1,4-β-glucosidases from Penicillium janthinellum. Appl. Environ. Microbiol. 41, 857–866. https://doi.org/10.1128/aem.41.4.857-866.1981

Riedi, H. de P., de Liz, M.V., Braga, D.M., Ianoski, A.B., de Freitas Pereira, T., Brugnari, T., Haminiuk, C.W.I., Maciel, G.M., 2022. Synthesis and characterization of laccase enzyme aggregates from Trametes villosa for simultaneous elimination of rifampicin and isoniazid. Int. J. Environ. Res. 16, 1–12. https://doi.org/10.1007/s41742-022-00403-y

Rodríguez-Delgado, M., Orona-Navar, C., García-Morales, R., Hernandez-Luna, C., Parra, R., Mahlknecht, J., Ornelas-Soto, N., 2016. Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from Pycnoporus sanguineus CS43 fungi. Int. Biodeterior. Biodegrad. 108, 34–41. https://doi.org/10.1016/j.ibiod.2015.12.003

Saat, M.N., Annuar, M.S.M., Alias, Z., Chuan, L.T., Chisti, Y., 2013. Modeling of growth and laccase production by Pycnoporus sanguineus. Bioprocess Biosyst. Eng. 1–11. https://doi.org/10.1007/s00449-013-1046-8

Schneider, W.D.H., Cavion Costa, P., Fontana, R.C., de Siqueira, F.G., Pinheiro Dillon, A.J., Camassola, M., 2019. Upscale and characterization of lignin-modifying enzymes from Marasmiellus palmivorus VE111 in a bioreactor under parameter optimization and the effect of inducers. J. Biotechnol. 295, 1–8. https://doi.org/10.1016/j.jbiotec.2019.03.002

Schneider, W.D.H., Fontana, R.C., Mendonça, S., de Siqueira, F.G., Dillon, A.J.P., Camassola, M., 2018. High level production of laccases and peroxidases from the newly isolated white-rot basidiomycete Marasmiellus palmivorus VE111 in a stirred-tank bioreactor in response to different carbon and nitrogen sources. Process Biochem. 69, 1–11. https://doi.org/10.1016/j.procbio.2018.03.005

Sharma, D., Gupta, R., 2024. Laccase: exploring structural insights and functional versatility for applications. Biologia (Bratisl). 79, 3381–3393. https://doi.org/10.1007/s11756-024-01787-2

Shu, N., Lorentzen, L.G., Davies, M.J., 2019. Reaction of quinones with proteins: Kinetics of adduct formation, effects on enzymatic activity and protein structure, and

potential reversibility of modifications. Free Radic. Biol. Med. 137, 169–180. https://doi.org/10.1016/j.freeradbiomed.2019.04.026

Singh, A.K., Abellanas-Perez, P., de Andrades, D., Cornet, I., Fernandez-Lafuente, R., Bilal, M., 2025. Laccase-based biocatalytic systems application in sustainable degradation of pharmaceutically active contaminants. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2024.136803

Singh, N.S., Sharma, R., Kumari, L., 2024. Tracing the evolution of enzymatic bioremediation using scientometric analysis. J. Scientometr. Res. 13, 517–534. https://doi.org/10.5530/jscires.13.2.41

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Tech. Rep. NREL/TP-510-42623.

Sodhi, A.S., Bhatia, S., Batra, N., 2024. Laccase: Sustainable production strategies, heterologous expression and potential biotechnological applications. Int. J. Biol. Macromol. 280, 135745. https://doi.org/10.1016/j.ijbiomac.2024.135745

Spina, F., Gea, M., Bicchi, C., Cordero, C., Schilir, T., Cristina, G., 2020. Ecofriendly laccases treatment to challenge micropollutants issue in. Environ. Pollut. 257. https://doi.org/10.1016/j.envpol.2019.113579

Sybuia, P.A., Contato, A.G., de Araújo, C.A.V., Zanzarin, D.M., Maciel, G.M., Pilau, E.J., Peralta, R.M., de Souza, C.G.M., 2024. Application of the white-rot fungus Trametes sp. (C3) laccase in the removal of acetaminophen from aqueous solutions. J. Water Process Eng. 57, 104677. https://doi.org/10.1016/j.jwpe.2023.104677

Thathola, P., Melchor-Martinez, E.M., Adhikari, P., Martinez, S.A.H., Pandey, A., Parra-Saldivar, R., 2024. Laccase-mediated degradation of emerging contaminants: unveiling a sustainable solution. Environ. Sci. https://doi.org/10.1039/d4va00173g

Ullrich, R., Le, M.H., Nguyen, L.D., Hofrichter, M., 2005. Laccase from the medicinal mushroom Agaricus blazei: Production, purification and characterization. Appl. Microbiol. Biotechnol. 67, 357–363. https://doi.org/10.1007/s00253-004-1861-6

Valle, J.S. do, Vandenberghe, L.P. de S., Santana, T.T., Linde, G.A., Colauto, N.B., Soccol, C.R., 2014. Optimization of Agaricus blazei laccase production by

submerged cultivation with sugarcane molasses. African J. Microbiol. Res. 8, 939–946. https://doi.org/10.5897/ajmr2013.6508

Vikineswary, S., Abdullah, N., Renuvathani, M., Sekaran, M., Pandey, a., Jones, E.B.G., 2006. Productivity of laccase in solid substrate fermentation of selected agro-residues by Pycnoporus sanguineus. Bioresour. Technol. 97, 171–177. https://doi.org/10.1016/j.biortech.2005.02.015

Vite-vallejo, O., Palomares, L.A., Dantán-gonzález, E., Ayala-castro, H.G., Martínez-anaya, C., Valderrama, B., Folch-mallol, J., 2009. The role ofN-glycosylation on the enzymatic activity of a Pycnoporus sanguineus laccase. Enzym. Microb. Technol. J. 45, 233–239. https://doi.org/10.1016/j.enzmictec.2009.05.007

Wang, F., Guo, C., Liu, C.Z., 2013. Immobilization of Trametes versicolor cultures for improving laccase production in bubble column reactor intensified by sonication. J. Ind. Microbiol. Biotechnol. 40, 141–150. https://doi.org/10.1007/s10295-012-1214-x

Wolfenden, B.S., Willson, R.L., 1982. Radical-cations as reference chromogens in kinetic studies of ono-electron transfer reactions: pulse radiolysis studies of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate). J. Chem. Soc. Perkin Trans. 2 805–812. https://doi.org/10.1039/P29820000805

Yang, J., Li, W., Bun Ng, T., Deng, X., Lin, J., Ye, X., 2017. Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00832

Yang, Y., Wei, F., Zhuo, R., Fan, F., Liu, H., Zhang, C., Ma, L., Jiang, M., Zhang, X., 2013. Enhancing the laccase production and laccase gene expression in the white-rot fungus Trametes velutina 5930 with great potential for biotechnological applications by different metal ions and aromatic compounds. PLoS One 8. https://doi.org/10.1371/journal.pone.0079307

Zdarta, J., Jankowska, K., Wyszowska, M., Kijeńska-Gawrońska, E., Zgoła-Grześkowiak, A., Pinelo, M., Meyer, A.S., Moszyński, D., Jesionowski, T., 2019. Robust biodegradation of naproxen and diclofenac by laccase immobilized using electrospun nanofibers with enhanced stability and reusability. Mater. Sci. Eng. C 103. https://doi.org/10.1016/j.msec.2019.109789

5. DISCUSSÃO GERAL

As questões ambientais têm se tornado centrais nas agendas governamentais e científicas em nível global, especialmente diante do aumento da poluição de matrizes naturais por contaminantes emergentes, como fármacos, pesticidas e produtos químicos industriais (Wang et al., 2024). Esses compostos apresentam alta persistência, baixa biodegradabilidade e efeitos adversos sobre ecossistemas aquáticos e terrestres, configurando desafios significativos para a manutenção da saúde ambiental e humana (Estrada-Almeida et al., 2024; Placova et al., 2023).

Nesse contexto, o desenvolvimento de tecnologias sustentáveis para mitigação de poluentes tornou-se uma prioridade. Entre essas, destacam-se as enzimas, que catalisam reações bioquímicas específicas sob condições brandas, com elevada eficiência e baixo impacto ambiental (Singh et al., 2024). Diferentemente dos processos químicos convencionais, as abordagens biocatalíticas reduzem a geração de subprodutos tóxicos e o consumo energético (Steinbüchel, 2020).

A aplicação de lacases fúngicas evidencia o potencial da biotecnologia na transformação de poluentes em produtos menos nocivos, alinhando-se às metas dos Objetivos de Desenvolvimento Sustentável (ODS), em especial o ODS 6 (água potável e saneamento) e o ODS 15 (vida terrestre). Essa estratégia contribui para a descontaminação de corpos hídricos e a recuperação de ambientes impactados.

Além disso, a abordagem de Saúde Única, que integra as dimensões humana, animal e ambiental, reforça a importância de intervenções que considerem os impactos transversais entre diferentes sistemas biológicos. A presença de fármacos no meio ambiente afeta não apenas organismos aquáticos ou terrestres, mas também a saúde humana, por meio da contaminação da água potável ou da bioacumulação na cadeia alimentar (Wang et al., 2024). Nesse sentido, a biorremediação enzimática oferece soluções compatíveis com a complexidade desses sistemas, fortalecendo o papel das biotecnologias sustentáveis.

O estudo do potencial das lacases fúngicas na biorremediação de fármacos, portanto, insere-se em um cenário de relevância global, combinando inovação tecnológica, responsabilidade ambiental e compromisso com a sustentabilidade e a saúde integrada.

Até o presente, não havia um levantamento sistemático sobre as tendências de pesquisa envolvendo as lacases fúngicas aplicadas à biorremediação de fármacos. Essa lacuna dificultava a visualização de padrões de produção científica, redes de colaboração e principais focos temáticos, limitando o direcionamento estratégico de futuras pesquisas e investimentos. O estudo cienciométrico desenvolvido nesta tese preencheu essa lacuna, oferecendo uma análise abrangente do panorama científico e identificando oportunidades e desafios para o avanço da área.

Os resultados indicaram que o campo encontra-se em expansão, refletindo o crescente interesse acadêmico e o potencial estratégico das lacases no desenvolvimento de tecnologias sustentáveis. As publicações concentram-se nas áreas de Ciências Ambientais, Engenharia Ambiental e Microbiologia Aplicada à Biotecnologia, confirmando a natureza interdisciplinar da temática.

A análise das redes de colaboração revelou comunidades científicas coesas, mas com conectividade limitada entre os *clusters*, o que indica que grande parte das colaborações ainda ocorre de forma regionalizada ou restrita a fronteiras disciplinares específicas. Isso reforça a necessidade de fomentar programas de intercâmbio internacional e redes interinstitucionais para fortalecer a integração e a inovação colaborativa.

Do ponto de vista dos periódicos, *Chemosphere*, *Bioresource Technology* e *Environmental Science and Pollution Research* se destacaram tanto pelo número de publicações quanto pelo número de citações. As métricas desses periódicos evidenciam indexações expressivas, elevados fatores de impacto e CiteScore, consolidando-os como veículos centrais para a disseminação do conhecimento na área.

O mapeamento de palavras-chave revelou termos recorrentes como “imobilização”, “águas residuais” e “degradação”, evidenciando a forte relação entre lacases e o tratamento de contaminantes emergentes. Entre os fármacos mais estudados estão diclofenaco, estrogênios, tetraciclina e carbamazepina, compostos que podem servir de referência para delineamentos experimentais futuros. Os dados também indicam a necessidade de ampliar o espectro de fármacos investigados e explorar aplicações em matrizes sólidas, como solos e sedimentos.

A diversidade de espécies fúngicas estudadas para a produção de lacases voltadas à biorremediação de fármacos é limitada. Entre 2012 e 2020, observou-se um aumento constante na utilização de enzimas de *Trametes versicolor*, consolidando seu papel como organismo de referência para a produção de lacase. Espécies como *Agaricus blazei*,

Marasmiellus palmivorus, *Pleurotus sanguineus*, *Ganoderma lucidum*, *Lentinula edodes*, *Phanerochaete chrysosporium* e *Pycnoporus sanguineus*, reconhecidas como secretoras enzimáticas, ainda carecem de investigação sistemática, especialmente no que se refere à biorremediação.

Com base nesses achados, o estudo experimental desta tese foi planejado para incluir espécies pouco exploradas (*A. blazei* e *M. palmivorus* VE111), visando preencher lacunas da literatura, e espécies já consolidadas (*P. sanguineus* 14G e *Trametes* sp. 50/90), para fins de comparação e validação de protocolos. Essa estratégia permitiu combinar inovação e consolidação, ampliando o escopo de investigação.

A seleção dos fármacos considerou critérios de relevância ambiental e informações disponíveis na literatura. Paracetamol e diclofenaco foram escolhidos por sua ampla utilização clínica e baixa biodegradabilidade, sendo reconhecidos como contaminantes emergentes de preocupação global (Kummerová et al., 2016; Sathishkumar et al., 2020). Além disso, possuem estruturas químicas distintas, o que possibilita avaliar a versatilidade catalítica das lacases.

Os extratos brutos de lacases produzidos pelas quatro espécies foram avaliados quanto a parâmetros bioquímicos e enzimáticos durante o cultivo, incluindo atividade enzimática, pH, biomassa micelial, concentração proteica e consumo de glicose. Essa abordagem permitiu caracterizar o comportamento de cada espécie e avaliar a aplicabilidade direta dos extratos em biorremediação.

A opção por utilizar extratos brutos, em vez de enzimas purificadas, fundamenta-se em sua viabilidade prática e econômica, especialmente em processos de tratamento de águas residuais, nos quais a utilização de enzimas purificadas eleva os custos e a complexidade operacional sem ganhos significativos em eficiência sob condições reais (Di Cosimo et al., 2013).

Os extratos obtidos apresentaram características distintas para cada espécie, refletindo nas diferenças nos padrões de produção enzimática, no crescimento micelial e no metabolismo secundário. Durante o cultivo, observou-se que *M. palmivorus* VE111 apresentou alta atividade enzimática, enquanto *A. blazei* se destacou pelo crescimento micelial e conteúdo proteico. *P. sanguineus* 14G e *Trametes* sp. 50/90 exibiram padrões intermediários, consistentes com os relatados para organismos modelo. A caracterização detalhada fornece informações essenciais para a aplicação prática dos extratos, permitindo selecionar espécies ou até mesmo combinações mais adequadas para diferentes fármacos ou matrizes ambientais.

O monitoramento do pH e do consumo de glicose confirmou a influência do metabolismo fúngico na estabilidade do cultivo e na produção enzimática, fornecendo subsídios para a padronização de protocolos e a escalabilidade do processo.

Considerando a suscetibilidade das enzimas à desnaturação, foram avaliadas as seguintes estratégias de estabilização para os extratos obtidos: liofilização, armazenamento sob refrigeração, estabilização com glicerol em temperatura ambiente e sob refrigeração. A combinação de glicerol e refrigeração manteve a atividade enzimática ao longo de 60 dias, evidenciando o efeito protetor do poliol na estrutura tridimensional das lacases. A liofilização também se mostrou eficaz, especialmente para o extrato de *P. sanguineus* 14G, permitindo conservação prolongada sem a necessidade de aditivos. Do ponto de vista tecnológico, a estabilidade enzimática observada para os extratos mantidos sob refrigeração ou liofilizados amplia as possibilidades de armazenamento e transporte, favorecendo o desenvolvimento de biocatalisadores comerciais e formulações de enzimas secas aplicáveis em larga escala.

A capacidade de biorremediação dos extratos foi testada frente ao paracetamol e ao diclofenaco. *M. palmivorus* VE111 apresentou melhor desempenho na degradação do paracetamol, enquanto *A. blazei* foi mais eficiente frente ao diclofenaco. *P. sanguineus* 14G e *Trametes* sp. 50/90 também exibiram desempenho satisfatório, confirmando o potencial de organismos modelo. Essas variações refletem diferenças metabólicas e reforçam a importância da escolha adequada da espécie conforme o fármaco alvo.

Os resultados obtidos fornecem base sólida para o desenvolvimento de estratégias aplicadas de biorremediação envolvendo diferentes espécies, fármacos e matrizes ambientais. Pesquisas futuras poderão avaliar o uso de mediadores, a imobilização enzimática e o controle de variáveis físico-químicas (pH, temperatura e substratos) visando otimizar os processos de degradação e caracterizar os produtos formados quanto à toxicidade e impacto ambiental.

Apesar dos resultados promissores, este estudo apresenta algumas limitações que devem ser consideradas na interpretação dos dados e no planejamento de trabalhos futuros. Primeiramente, as reações de biorremediação foram conduzidas em condições controladas de laboratório, o que não reflete integralmente a complexidade das matrizes ambientais reais, que podem conter compostos interferentes e variações de pH, temperatura e carga orgânica. Além disso, não foi realizada a identificação detalhada dos produtos de transformação gerados nas reações enzimáticas, o que limita a compreensão dos mecanismos de degradação e da possível regeneração dos compostos-alvo. Ensaios

ecotoxicológicos complementares e estudos de toxicidade dos produtos formados bem como do próprio extrato enzimático seriam necessários para confirmar a efetividade ambiental dos processos.

Em síntese, os resultados confirmam que os extratos brutos de lacases fúngicas representam uma abordagem promissora para amenizar o impacto dos contaminantes emergentes, com relevância prática e potencial de aplicação na biorremediação de fármacos. A simplicidade operacional e o baixo custo de produção desses extratos reforçam sua viabilidade para uso em estações de tratamento de águas residuais e em tecnologias descentralizadas de saneamento. A integração dos achados cienciométricos e experimentais evidencia que escolhas estratégicas de espécies, fármacos e métodos de conservação influenciam diretamente a eficiência e a aplicabilidade das enzimas em diferentes contextos ambientais, apontando caminhos concretos para o desenvolvimento de soluções biotecnológicas sustentáveis.

6. CONCLUSÕES

O presente trabalho integrou abordagens cienciométricas e experimentais para ampliar o entendimento e a aplicação de lacases fúngicas na biorremediação de fármacos. As principais conclusões e contribuições podem ser sintetizadas da seguinte forma:

- O estudo cienciométrico mapeou de forma abrangente as tendências globais de pesquisa envolvendo o uso de lacases fúngicas na degradação de fármacos. A análise evidenciou o crescimento contínuo do interesse científico na área, identificando os países, instituições e autores de maior relevância, bem como as palavras-chave mais recorrentes. Esses resultados consolidam o estado atual do conhecimento e fornecem subsídios estratégicos para orientar novas investigações e promover colaborações internacionais voltadas à aplicação biotecnológica de lacases na biorremediação.

- O cultivo submerso das espécies *Agaricus blazei, Marasmiellus palmivorus* VE111, *Pycnoporus sanguineus* 14G e *Trametes* sp. 50/09 possibilitou a caracterização detalhada de seu desempenho biotecnológico. Foram determinados os parâmetros de produção de lacases, variação de pH, desenvolvimento micelial, consumo de carboidratos e concentração proteica, permitindo compreender diferenças interespécies relevantes para a seleção de linhagens mais promissoras.

- Os extratos enzimáticos obtidos apresentaram atividade lacásica detectável e perfis de estabilidade distintos. Observou-se que a adição de glicerol influenciou positivamente a preservação da atividade em determinadas condições de armazenamento, ampliando as perspectivas de uso prático desses extratos em processos biotecnológicos.

- Na avaliação da capacidade de biorremediação, os extratos enzimáticos demonstraram potencial para a transformação dos fármacos diclofenaco e paracetamol, ainda que em níveis distintos entre espécies e condições de reação. Esses resultados confirmam a viabilidade do uso de lacases fúngicas em processos de biorremediação e apontam a necessidade de explorar mecanismos complementares, como reações redutivas e ciclos redox acoplados, que podem influenciar a eficiência global do processo.

7. PERSPECTIVAS

Com base nos resultados obtidos, evidenciam-se diversas oportunidades de continuidade e aprofundamento científico, voltadas ao avanço do conhecimento e à aplicação tecnológica das lacases fúngicas na biorremediação de fármacos:

- Otimização de cultivo e produção enzimática: explorar diferentes condições de cultivo submerso, substratos e indutores, bem como parâmetros de aeração e pH, para maximizar a expressão e o rendimento de lacases fúngicas.
- Caracterização enzimática avançada: purificar e caracterizar as isoformas de lacases obtidas, determinando parâmetros cinéticos, estabilidade térmica, comportamento frente a mediadores redox e potenciais inibidores.
- Elucidação de mecanismos de transformação: investigar detalhadamente os mecanismos de degradação e possível regeneração de fármacos, considerando a atuação de redutases fúngicas e ciclos redox acoplados que possam modular a eficiência do processo.
- Avaliação em condições complexas: aplicar os extratos enzimáticos em efluentes reais de estações de tratamento de águas residuais ou sistemas laboratoriais multicomponentes, avaliando a eficiência de remoção, os produtos formados e sua toxicidade residual.
- Estabilização e conservação enzimática: ampliar as estratégias de preservação da atividade enzimática, incluindo o uso de diferentes aditivos estabilizantes e métodos físicos, como a secagem por aspersão, visando maior durabilidade e redução de custos operacionais.
- Imobilização e reuso enzimático: desenvolver técnicas de imobilização de lacases em diferentes suportes, com foco em estabilidade operacional, reuso sucessivo e viabilidade econômica em processos contínuos de biorremediação.
- Escalonamento e validação tecnológica: conduzir estudos em escala piloto e avaliar a integração dos processos enzimáticos em sistemas reais de tratamento, aproximando os resultados laboratoriais das condições industriais e ambientais.
- Integração com tecnologias complementares: investigar a sinergia das lacases com outros processos de tratamento, como fotocatálise, ozonização, eletroquímica e biodegradação microbiana, visando aumentar a eficiência global e reduzir impactos ambientais.

8. REFERÊNCIAS BIBLIOGRÁFICAS

Abejón, R., De Cazes, M., Belleville, M.P., Sanchez-Marcano, J., 2015. Large-scale enzymatic membrane reactors for tetracycline degradation in WWTP effluents. Water Res. 73, 118–131. https://doi.org/10.1016/j.watres.2015.01.012

Abla, K.K., Mehanna, M.M., 2022. Freeze-drying: A flourishing strategy to fabricate stable pharmaceutical and biological products. Int. J. Pharm. 628, 122233. https://doi.org/10.1016/j.ijpharm.2022.122233

Aghaee, M., Salehipour, M., Rezaei, S., Mogharabi-Manzari, M., 2024. Bioremediation of organic pollutants by laccase-metal-organic framework composites: A review of current knowledge and future perspective. Bioresour. Technol. 406, 131072. https://doi.org/10.1016/j.biortech.2024.131072

Al-Sareji, O.J., Meiczinger, M., Al-Juboori, R.A., Grmasha, R.A., Andredaki, M., Somogyi, V., Idowu, I.A., Stenger-Kovács, C., Jakab, M., Lengyel, E., Hashim, K.S., 2023. Efficient removal of pharmaceutical contaminants from water and wastewater using immobilized laccase on activated carbon derived from pomegranate peels. Sci. Rep. 13, 11933. https://doi.org/10.1038/s41598-023-38821-3

Alharbi, Sultan K., Nghiem, L.D., van de Merwe, J.P., Leusch, F.D.L., Asif, M.B., Hai, F.I., Price, W.E., 2019. Degradation of diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole by laccase from Trametes versicolor: Transformation products and toxicity of treated effluent. Biocatal. Biotransformation 37, 399–408. https://doi.org/10.1080/10242422.2019.1580268

Alharbi, Sultan K, Nghiem, L.D., van de Merwe, J.P., Leusch, F.D.L., Asif, M.B., Hai I, F., Price, W.E., 2019. Degradation of diclofenac, trimethoprim, carbamazepine, andsulfamethoxazole by laccase from *Trametes versicolor*:Transformation products and toxicity of treated effluent. Biocatal. Biotransformation 37, 399–408. https://doi.org/10.1080/10242422.2019.1580268

Almeida, P.H., De Oliveira, A.C.C., De Souza, G.P.N., Friedrich, J.C., Linde, G.A., Colauto, N.B., Do Valle, J.S., 2018. Decolorization of remazol brilliant blue R with laccase from *Lentinus crinitus* grown in agro-industrial by-products. An. Acad. Bras. Cienc. 90, 3463–3473. https://doi.org/10.1590/0001-3765201820170458 WE - Science Citation Index Expanded (SCI-EXPANDED)

ANVISA, A.N. de V.S., 2019. Farmacopeia Brasileira. 6a Edição - Volume 1. Anvisa 1, 874.

Aquino, S.F. de, Brandt, E.M.F., Chernicharo, C.A. de L., 2013. Remoção de fármacos e desreguladores endócrinos em estações de tratamento de esgoto: Revisão da literatura. Eng. Sanit. e Ambient. 18, 187–204. https://doi.org/10.1590/S1413-41522013000300002

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174, 437–446. https://doi.org/10.1016/j.chemosphere.2017.01.101

Aria, M., Cuccurullo, C., 2017. Bibliometrix: An R-tool for comprehensive science mapping analysis. J. Informetr. 11, 959–975. https://doi.org/10.1016/j.joi.2017.08.007

Arregui, L., Ayala, M., Gómez-Gil, X., Gutiérrez-Soto, G., Hernández-Luna, C.E., Herrera De Los Santos, M., Levin, L., Rojo-Domínguez, A., Romero-Martínez, D., Saparrat, M.C.N., Trujillo-Roldán, M.A., Valdez-Cruz, N.A., 2019. Laccases: structure, function, and potential application in water bioremediation. Microb. Cell Fact. 18, 1–33. https://doi.org/10.1186/s12934-019-1248-0

Arsand, J.B., Hoff, R.B., Jank, L., Bussamara, R., Dallegrave, A., Bento, F.M., Kmetzsch, L., Falção, D.A., do Carmo Ruaro Peralba, M., de Araujo Gomes, A., Pizzolato, T.M., 2020. Presence of antibiotic resistance genes and its association with antibiotic occurrence in Dilúvio River in southern Brazil. Sci. Total Environ. 738, 139781. https://doi.org/10.1016/j.scitotenv.2020.139781

Assress, H.A., Nyoni, H., Mamba, B.B., Msagati, T.A.M., 2020. Occurrence and risk assessment of azole antifungal drugs in water and wastewater. Ecotoxicol. Environ. Saf. 187, 109868. https://doi.org/10.1016/j.ecoenv.2019.109868

Aulton, M.E., Taylor, K.M.G., 2016. Aulton delineamento de formas farmacêuticas, 4th ed. Rio de Janeiro.

aus der Beek, T., Weber, F.A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster, A., 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823–835. https://doi.org/10.1002/etc.3339

Aza, P., Camarero, S., 2023. Fungal laccases: fundamentals, engineering and classification update. Biomolecules 13. https://doi.org/10.3390/biom13121716

Ba, S., Arsenault, A., Hassani, T., Jones, J.P., Cabana, H., 2013. Laccase immobilization and insolubilization: From fundamentals to applications for the elimination of

emerging contaminants in wastewater treatment. Crit. Rev. Biotechnol. 33, 404–418. https://doi.org/10.3109/07388551.2012.725390

Back, J.F., Oakenfull, D., Smith, M.B., 1979. Incresed thermal stability of proteins in the presence of sugar and polyols. Biochemistry 18. https://doi.org/10.1021/bi00590a025

Baldrian, P., 2006. Fungal laccases-occurrence and properties. FEMS Microbiol. Rev. 30, 215–242. https://doi.org/10.1111/j.1574-4976.2005.00010.x

Barreiro, E.J., Fraga, C.A.M., 2015. Química medicinal: as bases moleculares da ação dos fármacos, 3rd ed, Artmed. Artmed, Porto Alegre. https://doi.org/10.15517/rc_ucr-hsjd.v3i11.13145

Beltrán-Flores, E., Sarrà, M., Blánquez, P., 2021. Pesticide bioremediation by Trametes versicolor: Application in a fixed-bed reactor, sorption contribution and bioregeneration. Sci. Total Environ. 794, 148386. https://doi.org/10.1016/J.SCITOTENV.2021.148386

Beretta, M., Britto, V., Tavares, T.M., da Silva, S.M.T., Pletsch, A.L., 2014. Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J. Soils Sediments 14, 1278–1286. https://doi.org/10.1007/s11368-014-0884-6

Bhatt, P., Bhatt, K., Chen, W.J., Huang, Y., Xiao, Y., Wu, S., Lei, Q., Zhong, J., Zhu, X., Chen, S., 2023. Bioremediation potential of laccase for catalysis of glyphosate, isoproturon, lignin, and parathion: Molecular docking, dynamics, and simulation. J. Hazard. Mater. 443, 130319. https://doi.org/10.1016/j.jhazmat.2022.130319

Białk, A., Łukasz, B., Ewa, G., Alan, M., Stefan, P., 2022. Mixture toxicity of six pharmaceuticals towards Aliivibrio fischeri , Daphnia magna , and Lemna minor. Environ. Sci. Pollut. Res. 26977–26991. https://doi.org/10.1007/s11356-021-17928-y

Bialk, H.M., Pedersen, J.A., 2008. NMR investigation of enzymatic coupling of sulfonamide antimicrobials with humic substances. Environ. Sci. Technol. 42, 106–112. https://doi.org/10.1021/es070779d

Bilal, M., Adeel, M., Rasheed, T., Zhao, Y., Iqbal, H.M.N.N., 2019a. Emerging contaminants of high concern and their enzyme-assisted biodegradation – A review. Environ. Int. 124, 336–353. https://doi.org/10.1016/j.envint.2019.01.011

Bilal, M., Rasheed, T., Nabeel, F., Iqbal, H.M.N., Zhao, Y., 2019b. Hazardous contaminants in the environment and their laccase-assisted degradation - A review.

J. Environ. Manage. 234, 253–264. https://doi.org/10.1016/j.jenvman.2019.01.001

Bîtcan, I., Petrovici, A., Pellis, A., Klébert, S., Károly, Z., Bereczki, L., Péter, F., Todea, A., 2023. Enzymatic route for selective glycerol oxidation using covalently immobilized laccases. Enzyme Microb. Technol. 163. https://doi.org/10.1016/j.enzmictec.2022.110168

Bjelošević, M., Seljak, K.B., Trstenjak, U., Logar, M., Brus, B., Ahlin Grabnar, P., 2018. Aggressive conditions during primary drying as a contemporary approach to optimise freeze-drying cycles of biopharmaceuticals. Eur. J. Pharm. Sci. 122, 292–302. https://doi.org/10.1016/j.ejps.2018.07.016

Borger, J.G., 2024. The power of networking in science and academia. Immunol. Cell Biol. 102, 871–877. https://doi.org/10.1111/imcb.12832

Bornmann, L., Daniel, H.H.D., Bornmann, L., Daniel, H.H.D., 2008. What do citation counts measure? A review of studies on citing behavior. J. Doc. 64, 45–80. https://doi.org/10.1108/00220410810844150

Bornmann, L., Mutz, R., 2015. Growth rates of modern science: A bibliometric analysis based on the number of publications and cited references. J. Assoc. Inf. Sci. Technol. 66, 2215–2222. https://doi.org/10.1002/asi.23329

Bou-Mitri, C., Kermasha, S., 2018. Lyoprotection and stabilization of laccase extract from Coriolus hirsutus, using selected additives. AMB Express 8. https://doi.org/10.1186/s13568-018-0683-3

Bouabadi, I., Miyah, Y., Benjelloun, M., El-habacha, M., El Addouli, J., 2024. Advanced strategies for the innovative treatment of hospital liquid effluents: A comprehensive review. Bioresour. Technol. Reports 28, 101990. https://doi.org/10.1016/j.biteb.2024.101990

Bourbonnais, R., Leech, D., Paice, M.G., 1998. Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. Biochim. Biophys. Acta - Gen. Subj. 1379, 381–390. https://doi.org/10.1016/S0304-4165(97)00117-7

Bourbonnais, Robert, Paice, M.G., 1990. Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. FEBS Lett. 267, 99–102. https://doi.org/10.1016/0014-5793(90)80298-W

Bourbonnais, R., Paice, M.G., 1990. Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. FEBS Lett. 267, 99–102. https://doi.org/10.1016/0014-5793(90)80298-W

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram

quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999

Bradford, S.C., 1934. Sources of information on specific subjects. J. Inf. Sci. 137, 85–86. https://doi.org/doi.org/10.1177/016555158501000407

Braham, S.A., Siar, E.H., Arana-Peña, S., Bavandi, H., Carballares, D., Morellon-Sterling, R., de Andrades, D., Kornecki, J.F., Fernandez-Lafuente, R., 2021. Positive effect of glycerol on the stability of immobilized enzymes: Is it a universal fact? Process Biochem. 102, 108–121. https://doi.org/10.1016/j.procbio.2020.12.015

Brijwani, K., Rigdon, A., Vadlani, P. V., 2010. Fungal laccases: Production, function, and applications in food processing. Enzyme Res. 2010. https://doi.org/10.4061/2010/149748

Brugnari, T., Braga, D.M., dos Santos, C.S.A., Torres, B.H.C., Modkovski, T.A., Haminiuk, C.W.I., Maciel, G.M., 2021. Laccases as green and versatile biocatalysts: from lab to enzyme market—an overview. Bioresour. Bioprocess. 8, 1–29. https://doi.org/10.1186/s40643-021-00484-1

Bruton, L.L. (org), 2013. As bases farmacológicas da terapêutica de Goodman & Gilman, 12ª. ed. McGraw-Hill, Porto Alegre.

Çaloglu, B., Binay, B., 2023. Utilization potential of agro-industrial by-products and waste sources: Laccase production in bioreactor with *Pichia pastoris*. Biochem. Eng. J. 193. https://doi.org/10.1016/j.bej.2023.108854

Camarero, S., Ibarra, D., Martínez, Á.T., Romero, J., Gutiérrez, A., del Río, J.C., 2007. Paper pulp delignification using laccase and natural mediators. Enzyme Microb. Technol. 40, 1264–1271. https://doi.org/10.1016/j.enzmictec.2006.09.016

Camassola, M., Rosa, L.O., Calloni, R., Gaio, T.A., Dillon, A.J.P., 2013. Secretion of laccase and manganese peroxidase by Pleurotus strains cultivate in solid-state using Pinus spp . sawdust. Brazilian J. Microbiol. 213, 207–213.

Cañas, A.I., Camarero, S., 2010. Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol. Adv. 28, 694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002

Cantele, C., Fontana, R.C., Mezzomo, A.G., da Rosa, L.O., Poleto, L., Camassola, M., Dillon, A.J.P., 2017. Production, characterization and dye decolorization ability of a high level laccase from Marasmiellus palmivorus. Biocatal. Agric. Biotechnol. 12, 15–22. https://doi.org/10.1016/j.bcab.2017.08.012

Carpenter, J.F., Arakawa, T., Crowe, J.H., 1992. Interactions of stabilizing additives with

proteins during freeze-thawing and freeze-drying. Dev. Biol. Stand.

Chang, S., Miles, P.G., 2004. Mushrooms - cultivation, nutritional value, medicinal effect, and environmental impact, 2 nd. ed. Washington.

Chaurasia, P.K., Yadav, R.S.S., Yadava, S., 2014. Purification and characterization of yellow laccase from Trametes hirsuta MTCC-1171 and its application in synthesis of aromatic aldehydes. Process Biochem. 49, 1647–1655. https://doi.org/10.1016/j.procbio.2014.06.016

Cheetham, P.S.J., 1984. Stabilization of immobilized enzymes with glycerol.

Chen, X., Bhandari, B., Zhou, P., 2019. Insight into the effect of glycerol on stability of globular proteins in high protein model system. Food Chem. 278, 780–785. https://doi.org/10.1016/j.foodchem.2018.11.117

Cheute, V.M., Uber, T.M., dos Santos, L.F., Backes, E., Dantas, M.P., Contato, A.G., Castoldi, R., de Souza, C.G., Corrêa, R.C., Bracht, A., Peralta, R.M., 2024. Biotransformation of pollutants by Pycnoporus spp. in submerged and solid-state fermentation: mechanisms, achievements, and perspectives. Biomass. https://doi.org/10.3390/biomass4020015

Cheute, V.M.S., Backes, E., Pateis, V. de O., de Oliveira Junior, V.A., Uber, T.M., dos Santos Filho, J.R., dos Santos, L.F.O., Castoldi, R., de Souza, C.G.M., Polonio, J.C., Contato, A.G., Bracht, A., Peralta, R.M., 2025. Optimization of immobilization, characterization, and environmental applications of Llccases from Pycnoporus sanguineus UEM-20. Processes 13, 1–24. https://doi.org/10.3390/pr13061800

Chiarello, M., Minetto, L., Giustina, S.V.D., Beal, L.L., Moura, S., 2016. Popular pharmaceutical residues in hospital wastewater: quantification and qualification of degradation products by mass spectroscopy after treatment with membrane bioreactor. Environ. Sci. Pollut. Res. 23, 16079–16089. https://doi.org/10.1007/s11356-016-6766-2

Chmelová, D., Ondrejovič, M., Miertuš, S., 2024. Laccases as effective tools in the removal of pharmaceutical products from aquatic systems. Life 14. https://doi.org/10.3390/life14020230

Christopher, L.P., Yao, B., Ji, Y., 2014. Lignin biodegradation with laccase-mediator systems. Front. Energy Res. 2. https://doi.org/10.3389/fenrg.2014.00012

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H., 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res. 39,

4797–4807. https://doi.org/10.1016/j.watres.2005.09.015

Copeland, R.A., 2023. Enzymes: A Practical introduction to structure, mechanism, and data analysis, 3ª. ed. Wiley.

Cordi, L., Minussi, R.C., Freire, R.S., Durán, N., 2007. Fungal laccase: Copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. African J. Biotechnol. 6, 1255–1259.

Costa, S.A., Tzanov, T., Filipa Carneiro, A., Paar, A., Gübitz, G.M., Cavaco-Paulo, A., 2002. Studies of stabilization of native catalase using additives. Enzyme Microb. Technol. 30, 387–391. https://doi.org/10.1016/S0141-0229(01)00505-1

Couto, S.R., Herrera, J.L.T., 2006. Industrial and biotechnological applications of laccases: A review. Biotechnol. Adv. 24, 500–513. https://doi.org/10.1016/j.biotechadv.2006.04.003

Couto, S.R., Toca-Herrera, J.L., 2007. Laccase production at reactor scale by filamentous fungi. Biotechnol. Adv. 25, 558–569. https://doi.org/10.1016/j.biotechadv.2007.07.002

Daronch, N.A., Kelbert, M., Pereira, C.S., de Araújo, P.H.H., de Oliveira, D., 2020. Elucidating the choice for a precise matrix for laccase immobilization: A review. Chem. Eng. J. 397, 125506. https://doi.org/10.1016/j.cej.2020.125506

Dashnau, J.L., Vanderkooi, J.M., 2007. Computational approaches to investigate how biological macromolecules can be protected in extreme conditions. J. Food Sci. 72, R001-R010. https://doi.org/10.1111/j.1750-3841.2006.00242.x

Datta, S., Christena, L.R., Rajaram, Y.R.S., 2013. Enzyme immobilization: an overview on techniques and support materials. 3 Biotech 3, 1–9. https://doi.org/10.1007/s13205-012-0071-7

Datta, S., Veena, R., Samuel, M.S., Selvarajan, E., 2021. Immobilization of laccases and applications for the detection and remediation of pollutants: a review. Environ. Chem. Lett. 19, 521–538. https://doi.org/10.1007/s10311-020-01081-y

Davarazar, M., Mostafaie, A., Jahanianfard, D., Davarazar, P., Ghiasi, S.A.B., Gorchich, M., Nemati, B., Kamali, M., Aminabhavi, T.M., 2020. Treatment technologies for pharmaceutical effluents-A scientometric study. J. Environ. Manage. 254, 109800. https://doi.org/10.1016/j.jenvman.2019.109800

de Wilde, C., Uzan, E., Zhou, Z., Kruus, K., Andberg, M., Buchert, J., Record, E., Asther, M., Lomascolo, A., 2008. Transgenic rice as a novel production system for Melanocarpus and Pycnoporus laccases. Transgenic Res. 17, 515–527.

https://doi.org/10.1007/s11248-007-9124-9

Debnath, R., Saha, T., 2020. An insight into the production strategies and applications of the ligninolytic enzyme laccase from bacteria and fungi. Biocatal. Agric. Biotechnol. 26, 101645. https://doi.org/10.1016/j.bcab.2020.101645

Delorme, A.E., Andanson, J.M., Verney, V., 2020. Improving laccase thermostability with aqueous natural deep eutectic solvents. Int. J. Biol. Macromol. 163, 919–926. https://doi.org/10.1016/j.ijbiomac.2020.07.022

Devi, R.N., Stephen, A.D., Justin, P., Saravanan, K., Macchi, P., Jelsch, C., 2019. Topological and electrostatic properties of diclofenac molecule as a non-steroidal anti-inflammatory drug: An experimental and theoretical study. J. Mol. Struct. 1196, 42–53. https://doi.org/10.1016/j.molstruc.2019.06.027

Di Cosimo, R., Mc Auliffe, J., Poulose, A.J., Bohlmann, G., 2013. Industrial use of immobilized enzymes. Chem. Soc. Rev. 42, 6437–6474. https://doi.org/10.1039/c3cs35506c

Dias, R.V.A., 2014. Avaliação da ocorrência de microcontaminantes emergentes em sistemas de abastecimento de água e da atividade estrogênica do estinilestradiol. Univ. Fed. Minas Gerais, Programa Pós-Graduação em Saneam. Meio Ambient. e Recur. Hídricos.

Dinakarkumar, Y., Ramakrishnan, G., Gujjula, K.R., Vasu, V., Balamurugan, P., Murali, G., 2024. Fungal bioremediation: An overview of the mechanisms, applications and future perspectives. Environ. Chem. Ecotoxicol. 6, 293–302. https://doi.org/10.1016/j.enceco.2024.07.002

Dong, S., Jing, X., Cao, Y., Xia, E., Gao, S., Mao, L., 2019. Non-covalent assembled laccase-graphene composite: Property, stability and performance in beta-blocker removal. Environ. Pollut. 252, 907–916. https://doi.org/10.1016/j.envpol.2019.05.053

dos Santos, C.R., Rosa e Silva, G.O., Valias, C. de F., Santos, L.V. de S., Amaral, M.C.S., 2024. Ecotoxicological study of seven pharmaceutically active compounds: Mixture effects and environmental risk assessment. Aquat. Toxicol. 275. https://doi.org/10.1016/j.aquatox.2024.107068

Duarte, C., Di Lorenzo, T., Reboleira, A.S.P.S., 2024. Environmental risk of diclofenac in European groundwaters and implications for environmental quality standards. Sci. Rep. 14. https://doi.org/10.1038/s41598-024-71747-y

Dwivedi, U.N., Singh, P., Pandey, V.P., Kumar, A., 2011. Structure-function relationship

among bacterial, fungal and plant laccases. J. Mol. Catal. B Enzym. 68, 117–128. https://doi.org/10.1016/j.molcatb.2010.11.002

Ebele, A.J., Abou-Elwafa Abdallah, M., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg. Contam. 3, 1–16. https://doi.org/10.1016/j.emcon.2016.12.004

Edens, W.A., Goins, T.Q., Dooley, D., Henson, J.M., 1999. Purification and characterization of a secreted laccase of Gaeumannomyces graminis var. tritici. Appl. Environ. Microbiol. 65, 3071–3074. https://doi.org/10.1128/aem.65.7.3071-3074.1999

EEA, 2010. Pharmaceuticals in the environment - Results of an EEA workshop. Eur. Environ. Agency 1–34.

Eggert, C., 1997. Laccase-catalyzed formation of cinnabarinic acid is responsible for antibacterial activity of Pycnoporus cinnabarinus. Microbiol. Res. 152, 315–318. https://doi.org/10.1016/S0944-5013(97)80046-8

Eggert, C., Temp, U., Eriksson, K.E.L., 1996. The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: Purification and characterization of the laccase. Appl. Environ. Microbiol. 62, 1151–1158. https://doi.org/10.1128/aem.62.4.1151-1158.1996

Egghe, L., 2006. Theory and practise of the g-index. Scientometrics 69, 131–152. https://doi.org/10.1007/s11192-006-0144-7

Ehrhart, A.L., Granek, E.F., Nielsen-Pincus, M., Horn, D.A., 2020. Leftover drug disposal: Customer behavior, pharmacist recommendations, and obstacles to drug take-back box implementation. Waste Manag. 118, 416–425. https://doi.org/10.1016/j.wasman.2020.08.038

Eichlerová, I., Homolka, L., Tomšovský, M., Lisá, L., 2015. Long term storage of Pleurotus ostreatus and Trametes versicolor isolates using different cryopreservation techniques and its impact on laccase activity. Fungal Biol. 119, 1345–1353. https://doi.org/10.1016/j.funbio.2015.10.004

El Yagoubi, Y., Lemieux, B., Segura, P.A., Cabana, H., 2023. Characterization of laccases from Trametes hirsuta in the context of bioremediation of wastewater treatment plant effluent. Enzyme Microb. Technol. 171. https://doi.org/10.1016/j.enzmictec.2023.110308

Estrada-Almeida, A.G., Castrejón-Godínez, M.L., Mussali-Galante, P., Tovar-Sánchez, E., Rodríguez, A., 2024. Pharmaceutical pollutants: ecotoxicological impacts and the

use of agro-industrial waste for their removal from aquatic environments. J. Xenobiotics 14, 1465–1518. https://doi.org/10.3390/jox14040082

Farhan, M., Hasani, I.W., Khafaga, D.S.R., Ragab, W.M., Nazir, R., Kazi, A., Aatif, M., Muteeb, G., Fahim, Y.A., 2025. Enzymes as Catalysts in Industrial Biocatalysis : Advances in Engineering , Applications , and Sustainable Integration 1–31.

Feng, Y., Shen, M., Wang, Z., Liu, G., 2019. Transformation of atenolol by a laccase-mediator system: Efficiencies, effect of water constituents, and transformation pathways. Ecotoxicol. Environ. Saf. 183. https://doi.org/10.1016/j.ecoenv.2019.109555

Fenice, M., Giovannozzi Sermanni, G., Federici, F., D'Annibale, A., 2003. Submerged and solid-state production of laccase and Mn-peroxidase by Panus tigrinus on olive mill wastewater-based media. J. Biotechnol. 100, 77–85. https://doi.org/10.1016/S0168-1656(02)00241-9

Flórez-Restrepo, M.A., López-Legarda, X., Segura-Sánchez, F., 2025. Bioremediation of emerging pharmaceutical pollutants acetaminophen and ibuprofen by white-rot fungi – A review. Sci. Total Environ. 977. https://doi.org/10.1016/j.scitotenv.2025.179379

Fram, M.S., Belitz, K., 2011. Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. Sci. Total Environ. 409, 3409–3417. https://doi.org/10.1016/j.scitotenv.2011.05.053

Freitas, L. de A.A., Radis-Baptista, G., 2021. Pharmaceutical pollution and disposal of expired, unused, and unwanted medicines in the Brazilian context. J. Xenobiotics 11, 61–76. https://doi.org/10.3390/jox11020005

Fu, S.Y., Yu, H.S., Buswell, J.A., 1997. Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by Pleurotus sajor-caju. FEMS Microbiol. Lett. 147, 133–137. https://doi.org/10.1016/S0378-1097(96)00516-2

Gao, N., Liu, C.-X.X., Xu, Q.-M.M., Cheng, J.-S.S., Yuan, Y.-J.J., 2018a. Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of Phanerochaete chrysosporium and Pycnoporus sanguineus. Chemosphere 195, 146–155. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.12.062

Gao, N., Liu, C.X., Xu, Q.M., Cheng, J.S., Yuan, Y.J., 2018b. Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of Phanerochaete chrysosporium and Pycnoporus sanguineus. Chemosphere

195, 146–155. https://doi.org/10.1016/j.chemosphere.2017.12.062

García-Morales, R., García-García, A., Orona-Navar, C., Osma, J.F., Nigam, K.D.P., Ornelas-Soto, N., 2018. Biotransformation of emerging pollutants in groundwater by laccase from P. sanguineus CS43 immobilized onto titania nanoparticles. J. Environ. Chem. Eng. 6, 710–717. https://doi.org/10.1016/j.jece.2017.12.006

Garduño-Jiménez, A.L., Gomes, R.L., López-Maldonado, Y., Carter, L.J., 2025. Addressing the global data imbalance of contaminants of emerging concern in the context of the United Nations sustainable development goals. RSC Sustain. 3384–3391. https://doi.org/10.1039/d5su00144g

Garfield, E., 2006. The history and meaning of the journal impact factor. JAMA 295, 90–93. https://doi.org/10.1001/jama.295.1.90

Gianfreda, L., Modafferi, M., Greco, G., 1985. Enzyme stabilization towards thermal, chemical and proteolytic deactivation. Enzyme Microb. Technol. 7, 78–82. https://doi.org/10.1016/0141-0229(85)90017-1

Gianfreda, L., Scarfi, M.R., 1991. Enzyme stabilization: state of the art. Mol. Cell. Biochem. 100, 97–128. https://doi.org/10.1007/BF00234161

Gianfreda, L., Xu, F., Bollag, J.M., 1999. Laccases: A useful group of oxidoreductive enzymes. Bioremediat. J. 3, 1–26. https://doi.org/10.1080/10889869991219163

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111, 418–426. https://doi.org/10.1016/j.chemosphere.2014.03.132

Gómez-Toribio, V., García-Martín, A.B., Martínez, M.J., Martínez, Á.T., Guillén, F., 2009. Enhancing the production of hydroxyl radicals by Pleurotus eryngii via quinone redox cycling for pollutant removal. Appl. Environ. Microbiol. 75, 3954–3962. https://doi.org/10.1128/AEM.02138-08

Gonçalves, M.C.P., Kieckbusch, T.G., Perna, R.F., Fujimoto, J.T., Morales, S.A.V., Romanelli, J.P., 2019. Trends on enzyme immobilization researches based on bibliometric analysis. Process Biochem. 76, 95–110. https://doi.org/10.1016/j.procbio.2018.09.016

Gonçalves, V.D., Meirelles-Pereira, F., Cataldo, M., Fonseca, B. de O., Nogueira, B.A., Olivella, J.G.B., Esteves, F. de A., Mattos-Guaraldi, A.L., de Andrade, A.F.B., Bello, A.R., Pereira, J.A.A., 2019. Detection of multidrug-resistant Enterobacteria isolated from river waters flowing to Guanabara Bay (Rio de Janeiro, Brazil) and from

clinical samples of hospital origin. Biomedica 39, 135–149. https://doi.org/10.7705/biomedica.v39i0.4391

Gracia-Lor, E., Sancho, J. V., Serrano, R., Hernández, F., 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere 87, 453–462. https://doi.org/10.1016/j.chemosphere.2011.12.025

Griffin, E.G., Nelson, J.M., 1916. Adsorption of invertase. J. Am. Chem. Soc. 38, 1109–1115. https://doi.org/10.1021/ja02262a018

Guerra, P., Kim, M., Shah, A., Alaee, M., Smyth, S.A., 2014. Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci. Total Environ. 473–474, 235–243. https://doi.org/10.1016/j.scitotenv.2013.12.008

Guo, Y., Qi, P.S., Liu, Y.Z., 2017. A review on advanced treatment of pharmaceutical wastewater. IOP Conf. Ser. Earth Environ. Sci. 63, 12025. https://doi.org/10.1088/1755-1315/63/1/012025

Gutfreud, H., 1976. Wilhelm Friedrich Kühne: an appreciation. FEBS Lett. 62.

Hachi, M., Chergui, A., Yeddou, A.R., Selatnia, A., Cabana, H., 2017. Removal of acetaminophen and carbamazepine in single and binary systems with immobilized laccase from Trametes hirsuta. Biocatal. Biotransformation 35, 51–62. https://doi.org/10.1080/10242422.2017.1280032

Hahn, V., 2023. Potential of the enzyme laccase for the synthesis and derivatization of antimicrobial compounds. World J. Microbiol. Biotechnol. 39, 1–27. https://doi.org/10.1007/s11274-023-03539-x

Hakulinen, N., Rouvinen, J., 2015. Three-dimensional structures of laccases. Cell. Mol. Life Sci. 72, 857–868. https://doi.org/10.1007/s00018-014-1827-5

Hamedi, A., Vahid, H., Ghanati, F., 2007. Optimization of the medium composition for production of mycelial biomass and exo-polysaccharide by Agaricus blazei murill DPPh 131 using response surface methodology. Biotechnology 6, 456–464. https://doi.org/10.3923/biotech.2007.456.464

Haque, I., Singh, R., Ahmad, F., Moosavi-Movahedi, A.A., 2005. Testing polyols' compatibility with Gibbs energy of stabilization of proteins under conditions in which they behave as compatible osmolytes. FEBS Lett. 579, 3891–3898. https://doi.org/10.1016/j.febslet.2005.06.005

Harkin, J.M., Larsen, M.J., Obst, J.R., 1974. Use of syringaldazine for detection of

laccase in sporophores of wood rotting fungi. Mycologia 66, 469–476.

He, H., Li, Y., Fang, M., Li, T., Liang, Y., Mei, Y., 2021. Carbon source affects synthesis , structures , and sctivities of mycelial polysaccharides from medicinal fungus Inonotus obliquus. J. Microbiol. Biotechnol. 31, 855–866.

Heberer, T., 2002. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. J. Hydrol. 266, 175–189. https://doi.org/10.1016/S0022-1694(02)00165-8

Hirsch, J.E., Buela-Casal, G., 2014. The meaning of the h-index. Int. J. Clin. Heal. Psychol. 14, 161–164. https://doi.org/10.1016/S1697-2600(14)70050-X

Inamuddin, 2023. Bioremediation for Environmental Pollutants. Bentham Books imprint, Aligarh, India.

IQVIA, 2024. The global use of medicines 2024: outlook to 2028, Iqvia.

Isfandyari-Moghaddam, A., Saberi, M.K., Tahmasebi-Limoni, S., Mohammadian, S., Naderbeigi, F., 2023. Global scientific collaboration: A social network analysis and data mining of the co-authorship networks. J. Inf. Sci. 49, 1126–1141. https://doi.org/10.1177/01655515211040655

Iyer, P. V., Ananthanarayan, L., 2008. Enzyme stability and stabilization-aqueous and non-aqueous environment. Process Biochem. 43, 1019–1032. https://doi.org/10.1016/j.procbio.2008.06.004

Janeček, Š., 1993. Strategies for obtaining stable enzymes. Process Biochem. 28, 435–445. https://doi.org/10.1016/0032-9592(93)85026-C

Jang, M.Y., Ryu, W.Y., Cho, M.H., Zhang, J., Li, Z., Riemensberger, J., Huang, G., Kippenberg, T.J., 2006. Enhanced production of laccase from Trametes sp. by combination of various inducers. Biotechnol. Bioprocess Eng. 11, 96–99. https://doi.org/10.1007/BF02931890

Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., Paszczyński, A., 2020. Laccase properties, physiological functions, and evolution. Int. J. Mol. Sci. 21. https://doi.org/10.3390/ijms21030966

Jesionowski, T., Zdarta, J., Krajewska, B., 2014. Enzyme immobilization by adsorption: A review. Adsorption 20, 801–821. https://doi.org/10.1007/s10450-014-9623-y

Johannes, C., Majcherczyk, A., 2000. Laccase activity tests and laccase inhibitors. J. Biotechnol. 78, 193–199. https://doi.org/10.1016/S0168-1656(00)00208-X

Jones, S.M., Solomon, E.I., 2015. Electron transfer and reaction mechanism of laccases. Cell. Mol. Life Sci. 72, 869–883. https://doi.org/10.1007/s00018-014-1826-6

Junqueira, L.C., Carneiro, J., 2023. Biologia Celular e Molecular, 10ª. ed. Guanabara Koogan.

Kalichak, F., Idalencio, R., Da Rosa, J.G.S., De Alcântara Barcellos, H.H., Fagundes, M., Piato, A., Barcellos, L.J.G., 2017. Psychotropic in the environment: Risperidone residues affect the behavior of fish larvae. Sci. Rep. 7, 1–7. https://doi.org/10.1038/s41598-017-14575-7

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Khan, M.F., 2025. Fungi for sustainable pharmaceutical remediation: enzymatic innovations, challenges, and applications - A review. Processes 13, 1–37. https://doi.org/10.3390/pr13041034

Kim, H.H., Na, J.G., Chang, Y.K., Chun, G.T., Lee, S.J., Jeong, Y.H., 2004. Optimization of submerged culture conditions for mycelial growth and exopolysaccharides production by Agaricus blazei. J. Microbiol. Biotechnol. 14, 944–951.

Klibanov, A.M., 2001. Improving enzymes by using them in organic solvents. Nature 409.

Koroleva, O. V., Stepanova, E. V., Binukov, V.I., Timofeev, V.P., Pfeil, W., 2001. Temperature-induced changes in copper centers and protein conformation of two fungal laccases from Coriolus hirsutus and Coriolus zonatus. Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1547, 397–407. https://doi.org/10.1016/S0167-4838(01)00209-6

Kózka, B., Nałęcz-Jawecki, G., Turło, J., Giebułtowicz, J., 2020. Application of Pleurotus ostreatus to efficient removal of selected antidepressants and immunosuppressant. J. Environ. Manage. 273. https://doi.org/10.1016/j.jenvman.2020.111131

Kummerová, M., Zezulka, Š., Babula, P., Tříska, J., 2016. Possible ecological risk of two pharmaceuticals diclofenac and paracetamol demonstrated on a model plant Lemna minor. J. Hazard. Mater. 302, 351–361. https://doi.org/10.1016/j.jhazmat.2015.09.057

Kyomuhimbo, H.D., Brink, H.G., 2023. Applications and immobilization strategies of the copper-centred laccase enzyme; a review. Heliyon. 9, e13156. https://doi.org/10.1016/j.heliyon.2023.e13156

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685. https://doi.org/10.1038/227680a0

Lakhtar, H., Ismaili-Alaoui, M., Philippoussis, A., Perraud-Gaime, I., Roussos, S., 2010. Screening of strains of Lentinula edodes grown on model olive mill wastewater in solid and liquid state culture for polyphenol biodegradation. Int. Biodeterior. Biodegrad. 64, 167–172. https://doi.org/10.1016/j.ibiod.2009.10.006

Langfelder, K., Streibel, M., Jahn, B., Haase, G., Brakhage, A.A., 2003. Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet. ans Biol. 38, 143–158. https://doi.org/10.1016/S1087-1845(02)00526-1

Leontievsky, A.A., Vares, T., Lankinen, P., Shergill, J.K., Pozdnyakova, N.N., Myasoedova, N.M., Kalkkinen, N., Golovleva, L.A., Cammack, R., Thurston, C.F., Hatakka, A., 1997. Blue and yellow laccases of ligninolytic fungi. FEMS Microbiol. Lett. 156, 9–14. https://doi.org/10.1016/S0378-1097(97)00393-5

Lettera, V., Pezzella, C., Cicatiello, P., Piscitelli, A., Giacobelli, V.G., Galano, E., Amoresano, A., Sannia, G., 2016. Efficient immobilization of a fungal laccase and its exploitation in fruit juice clarification. Food Chem. 196, 1272–1278. https://doi.org/10.1016/j.foodchem.2015.10.074

Lima, D.R.S., Tonucci, M.C., Libânio, M., de Aquino, S.F., 2017. Fármacos e desreguladores endócrinos em águas Brasileiras: Ocorrência e técnicas de remoção. Eng. Sanit. e Ambient. 22, 1043–1054. https://doi.org/10.1590/s1413-41522017165207

Linde, G.A., Luciani, A., Lopes, A.D., Valle, J.S. do, Colauto, N.B., 2018. Long-term cryopreservation of basidiomycetes. Brazilian J. Microbiol. 49, 220–231. https://doi.org/10.1016/j.bjm.2017.08.004

Ling, Z.R., Wang, S.S., Zhu, M.J., Ning, Y.J., Wang, S.N., Li, B., Yang, A.Z., Zhang, G.Q., Zhao, X.M., 2015. An extracellular laccase with potent dye decolorizing ability from white rot fungus Trametes sp. LAC-01. Int. J. Biol. Macromol. 81, 785–793. https://doi.org/10.1016/j.ijbiomac.2015.09.011

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2012. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 64, 4–17. https://doi.org/10.1016/j.addr.2012.09.019

Liu, G.Q., Wang, X.L., 2007. Optimization of critical medium components using response surface methodology for biomass and extracellular polysaccharide production by Agaricus blazei. Appl. Microbiol. Biotechnol. 74, 78–83. https://doi.org/10.1007/s00253-006-0661-6

Liu, Jiayang, Yu, Z., Liao, X., Liu, Junhe, Mao, F., Huang, Q., 2016. Scalable production, fast purification, and spray drying of native Pycnoporus laccase and circular dichroism characterization. J. Clean. Prod. 127, 600–609. https://doi.org/10.1016/j.jclepro.2016.03.154

Liu, X., Yuan, J., Feng, Y., Zhang, Z., Tang, L., Chen, H., 2024. Knowledge graph and development hotspots of biochar as an emerging aquatic antibiotic remediator: A scientometric exploration based on VOSviewer and CiteSpace. J. Environ. Manage. 360, 121165. https://doi.org/10.1016/j.jenvman.2024.121165

Lonappan, L., Brar, S.K., Das, R.K., Verma, M., Surampalli, R.Y., 2016. Diclofenac and its transformation products: Environmental occurrence and toxicity - A review. Environ. Int. 96, 127–138. https://doi.org/10.1016/j.envint.2016.09.014

Lonappan, L., Liu, Y., Rouissi, T., Brar, S.K., Verma, M., Surampalli, R.Y., 2018a. Adsorptive immobilization of agro-industrially produced crude laccase on various micro-biochars and degradation of diclofenac. Sci. Total Environ. 640–641, 1251–1258. https://doi.org/10.1016/j.scitotenv.2018.06.005

Lonappan, L., Liu, Y., Rouissi, T., Pourcel, F., Brar, S.K., Verma, M., Surampalli, R.Y., 2018b. Covalent immobilization of laccase on citric acid functionalized micro-biochars derived from different feedstock and removal of diclofenac. Chem. Eng. J. 351, 985–994. https://doi.org/10.1016/j.cej.2018.06.157

Lonappan, Linson, Rouissi, Tarek, Laadila, Mohamed Amine, Brar, Satinder Kaur, Galan, L.H., Verma, Mausam, Surampalli, R.Y., Lonappan, L, Rouissi, T, Laadila, M A, Brar, S K, Hernandez Galan, L., Verma, M, Surampalli, R.Y., 2017. Agro-industrial-produced laccase for degradation of diclofenac and identification of transformation products. ACS Sustain. Chem. Eng. 5, 5772–5781. https://doi.org/10.1021/acssuschemeng.7b00390

Lotas, K.M., Fonseca, R.S.K., da Costa, J.C.M., Cortez, A.C.A., Souza, F.D.D., Barreto, M.R., Carneiro, L.M., Silva, J.P.A., Canto, E.S.M., Fernandes, F.D., de Souza, J.V.B., de Souza, E.S., 2024. Optimization of laccase production by *Pleurotus ostreatus (Jacq.)* P. Kumm. using agro-industrial residues: a comparative study on peels of tucumã (*Astrocaryum* aculeatum G. Mey.) and pupunha (*Bactris* gasipaes Kunth) fru. AIMS Bioeng. 11, 561–573. https://doi.org/10.3934/bioeng.2024025 WE - Emerging Sources Citation Index (ESCI)

Lotka, A.J., 1926. The frequency distribution of scientific productivity. J. Washingt. Acad. Sci. 16, 317–323.

Lu, L., Zhao, M., Zhang, B.B., Yu, S.Y., Bian, X.J., Wang, W., Wang, Y., 2007. Purification and characterization of laccase from Pycnoporus sanguineus and decolorization of an anthraquinone dye by the enzyme. Appl. Microbiol. Biotechnol. 74, 1232–1239. https://doi.org/10.1007/s00253-006-0767-x

Lubick, N., 2010. Do pharmaceutical take-back programs make a difference? Environ. Health Perspect. 118, 707–720. https://doi.org/10.1289/ehp.118-a210

Lundell, T.K., Mäkelä, M.R., Hildén, K., 2010. Lignin-modifying enzymes in filamentous basidiomycetes - Ecological, functional and phylogenetic review. J. Basic Microbiol. 50, 5–20. https://doi.org/10.1002/jobm.200900338

Maroneze, M.M., Zepka, L.Q., Vieira, J.G., Queiroz, M.I., Jacob-Lopes, E., 2012. Evaluation of the use of Pycnoporus sanguineus fungus for phenolics and genotoxicity decay of a pharmaceutical effluent treatment. Rev. Ambient. Água - An Interdiscip. J. Appl. Sci. 7, 445–458. https://doi.org/10.4136/1980-993X

Masjoudi, M., Golgoli, M., Ghobadi Nejad, Z., Sadeghzadeh, S., Borghei, S.M., 2021. Pharmaceuticals removal by immobilized laccase on polyvinylidene fluoride nanocomposite with multi-walled carbon nanotubes. Chemosphere 263, 128043. https://doi.org/10.1016/j.chemosphere.2020.128043

Mate, D.M., Alcalde, M., 2017. Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microb. Biotechnol. 10, 1457–1467. https://doi.org/10.1111/1751-7915.12422

Mate, D.M., Alcalde, M., 2015. Laccase engineering: From rational design to directed evolution. Biotechnol. Adv. 33, 25–40. https://doi.org/10.1016/j.biotechadv.2014.12.007

Matsumoto, M., Kida, K., Kondo, K., 1997. Effects of polyols and organic solvents on thermostability of lipase. J. Chem. Technol. Biotechnol. 70, 188–192. https://doi.org/10.1002/(SICI)1097-4660(199710)70:2<188::AID-JCTB745>3.0.CO;2-X

Mehra, R., Muschiol, J., Meyer, A.S., Kepp, K.P., 2018. A structural-chemical explanation of fungal laccase activity. Sci. Rep. 8, 1–16. https://doi.org/10.1038/s41598-018-35633-8

Mikiashvili, N., Wasser, S.P., Nevo, E., Elisashvili, V., 2006. Effects of carbon and nitrogen sources on Pleurotus ostreatus ligninolytic enzyme activity. World J. Microbiol. Biotechnol. 22, 999–1002. https://doi.org/10.1007/s11274-006-9132-6

Mikolasch, A., Schauer, F., 2009. Fungal laccases as tools for the synthesis of new hybrid

molecules and biomaterials. Appl. Microbiol. Biotechnol. 82, 605–624. https://doi.org/10.1007/s00253-009-1869-z

Milosavić, N.B., Prodanović, R.M., Velićković, D., Dimitrijević, A., 2017. Enzyme Stabilization and immobilization: methods and protocols, Methods in Molecular Biology. https://doi.org/10.1007/978-1-4939-6499-4_11

Mogharabi, M., Faramarzi, M.A., 2014. Laccase and laccase-mediated systems in the synthesis of organic compounds. Adv. Synth. Catal. 356, 897–927. https://doi.org/10.1002/adsc.201300960

Mutegoa, E., 2024. Efficient techniques and practices for wastewater treatment: an update. Discov. Water 4. https://doi.org/10.1007/s43832-024-00131-8

Naghdi, M., Taheran, M., Brar, S.K., Kermanshahi-pour, A., Verma, M., Surampalli, R.Y., 2018. Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes. Environ. Pollut. 234, 190–213. https://doi.org/10.1016/j.envpol.2017.11.060

Nakagawa, Y., Sakamoto, Y., Kikuchi, S., Sato, T., Yano, A., 2010. A chimeric laccase with hybrid properties of the parental Lentinula edodes laccases. Microbiol. Res. 165, 392–401. https://doi.org/https://doi.org/10.1016/j.micres.2009.08.006

Nakamura, T., 1960. On the mechanism of the reaction of the reduced laccase with oxygen. BBA - Biochim. Biophys. Acta 42, 499–505. https://doi.org/10.1016/0006-3002(60)90829-5

Navada, K.K., Kulal, A., 2019. Enzymatic degradation of chloramphenicol by laccase from Trametes hirsuta and comparison among mediators. Int. Biodeterior. Biodegrad. 138, 63–69. https://doi.org/10.1016/j.ibiod.2018.12.012

Nelson, D.L., Cox, M.M., 2011. Princípios de bioquímica de Lehninger, 5th ed. Artmed, Porto Alegre.

Nguyen, L.N., Hai, F.I., Dosseto, A., Richardson, C., Price, W.E., Nghiem, L.D., 2016. Continuous adsorption and biotransformation of micropollutants by granular activated carbon-bound laccase in a packed-bed enzyme reactor. Bioresour. Technol. 210, 108–116. https://doi.org/10.1016/j.biortech.2016.01.014

Nguyen, L.N., Hai, F.I., Yang, S., Kang, J., Leusch, F.D.L., Roddick, F., Price, W.E., Nghiem, L.D., 2014. Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: Role of biosorption and biodegradation. Int. Biodeterior. Biodegrad. 88, 169–175. https://doi.org/10.1016/j.ibiod.2013.12.017

Nguyen, L.N., Hai, F.I., Yang, S., Kang Jinguoand Leusch, F.D.L., Roddick, F., Price William E.and Nghiem, L.D., 2013. Removal of trace organic contaminants by an MBR comprising a mixedculture of bacteria and white-rot fungi. Bioresour. Technol. 148, 234–241. https://doi.org/10.1016/j.biortech.2013.08.142

Nguyen, L.N., Vu, M.T., Johir, M.A.H., Pathak, N., Zdarta, J., Jesionowski, T., Semblante, G.U., Hai I, F., Nguyen, H.K.D., Nghiem, L.D., Bioremediation, C., Nguyen, L.N., Vu, M.T., Johir, A.H., Pathak, N., 2020. A novel approach in crude enzyme laccase production and application in emerging contaminat bioremediation. Processes 8. https://doi.org/10.3390/pr8060648

Nirmal, J., Jain, G.K., 2018. Pharmaceutical formulation: The science and technology of dosage forms, Pharmacology of Ocular Therapeutics.

Ó'Fágáin, C., Colliton, K., 2023. Storage and lyophilization of pure proteins, in: Loughran, S.T., Milne, J.J. (Eds.), Protein Chromatography: Methods and Protocols. Springer US, New York, NY, pp. 421–475. https://doi.org/10.1007/978-1-0716-3362-5_19

Okazaki, S.Y., Goto, M., Wariishi, H., Tanaka, H., Furusaki, S., 2000. Characterization and catalytic property of surfactant-laccase complex in organic media. Biotechnol. Prog. 16, 583–588. https://doi.org/10.1021/bp000042r

Omura, T., 1961. Studies on laccases of lacquer trees: I. comparison of laccases obtained from rhus vernicifera and rhus succedanea. J. Biochem. 50, 264–272. https://doi.org/10.1093/oxfordjournals.jbchem.a127442

ONU, 2025. The United Nations Sustainable Development Goals [WWW Document]. United Nations. URL https://sdgs.un.org/goals

Ortúzar, M., Esterhuizen, M., Olicón-Hernández, D.R., González-López, J., Aranda, E., 2022. Pharmaceutical pollution in aquatic environments: a concise review of environmental impacts and bioremediation systems. Front. Microbiol. 13. https://doi.org/10.3389/fmicb.2022.869332

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372. https://doi.org/10.1136/bmj.n71

Papinutti, L., Dimitriu, P., Forchiassin, F., 2008. Stabilization studies of Fomes scleroderme laccases. Bioresour. Technol. 99, 419–424. https://doi.org/10.1016/j.biortech.2006.11.061

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem. Rev. 119, 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Patel, N., Shahane, S., Shivam, Majumdar, R., Mishra, U., 2018. Mode of action, properties, production, and application of laccase: a review. Recent Pat. Biotechnol. 13, 19–32. https://doi.org/10.2174/1872208312666180821161015

Peng, Y., Gautam, L., Hall, S.W., 2019. The detection of drugs of abuse and pharmaceuticals in drinking water using solid-phase extraction and liquid chromatography-mass spectrometry. Chemosphere 223, 438–447. https://doi.org/10.1016/j.chemosphere.2019.02.040

Peralta, R.M., 2022. Biodegradação e Biorremediação, Biodegradação e Biorremediação. Mérida, Canoas. https://doi.org/10.4322/mp.978-65-84548-03-9

Peralta, R.M., da Silva, B.P., Gomes Côrrea, R.C., Kato, C.G., Vicente Seixas, F.A., Bracht, A., 2017. Enzymes from basidiomycetes-peculiar and efficient tools for biotechnology, in: Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications. Elsevier Inc., pp. 119–149. https://doi.org/10.1016/B978-0-12-803725-6.00005-4

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27. https://doi.org/10.1016/j.watres.2014.08.053

Phong Vo, H.N., Le, G.K., Nguyen, T.M.H., Bui, X.T., Nguyen, K.H., Rene, E.R., Vo, T.D.H., Cao, N.D.T., Mohan, R., 2019. Acetaminophen micropollutant: Historical and current occurrences, toxicity, removal strategies and transformation pathways in different environments. Chemosphere 236, 15. https://doi.org/10.1016/j.chemosphere.2019.124391

Placova, K., Halfar, J., Brozova, K., Heviankova, S., 2023. Issues of non-steroidal anti-inflammatory drugs in aquatic environments: a review study. Eng. Proc. 57. https://doi.org/10.3390/engproc2023057013

Polaina, J., MacCabe, A.P., 2007. Industrial enzymes: structure, function and

applications, Library. Springer.

Pomati, F., 2007. Pharmaceuticals in drinking water: is the cure worse than the disease? Environ. Sci. Technol. 41, 8204. https://doi.org/10.1021/es072659b

Porter, L.K., 2016. Enzymes, Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. https://doi.org/10.2134/agronmonogr9.2.c61

Prieto, A., Moeder, M., Rodil, R., Adrian Lorenzand Marco-Urrea, E., Möder, M., Rodil, R., Adrian, L., Marco-Urrea, E., 2011. Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. Bioresour. Technol. 102, 10987–10995. https://doi.org/10.1016/j.biortech.2011.08.055

Priev, A., Almagor, A., Yedgar, S., Gavish, B., 1996. Glycerol decreases the volume and compressibility of protein interior. Biochemistry 35, 2061–2066. https://doi.org/10.1021/bi951842r

Puspita, K., Chiari, W., Abdulmadjid, S.N., Idroes, R., Iqhrammullah, M., 2023. Four decades of laccase research for wastewater treatment: insights from bibliometric analysis. Int. J. Environ. Res. Public Health 20. https://doi.org/10.3390/ijerph20010308

Quadra, G.R., Oliveira de Souza, H., Costa, R. dos S., Fernandez, M.A. dos S., 2017. Do pharmaceuticals reach and affect the aquatic ecosystems in Brazil? A critical review of current studies in a developing country. Environ. Sci. Pollut. Res. 24, 1200–1218. https://doi.org/10.1007/s11356-016-7789-4

R Core, D.T., 2023. R: A language and environment for statistical computing.

Ramírez-Cavazos, L.I., Junghanns, C., Ornelas-Soto, N., Cárdenas-Chávez, D.L., Hernández-Luna, C., Demarche, P., Enaud, E., García-Morales, R., Agathos, S.N., Parra, R., 2014. Purification and characterization of two thermostable laccases from Pycnoporus sanguineus and potential role in degradation of endocrine disrupting chemicals. J. Mol. Catal. B Enzym. 108, 32–42. https://doi.org/10.1016/j.molcatb.2014.06.006

Rapp, P., Grote, E., Wagner, F., 1981. Formation and location of 1,4-β-glucanases and 1,4-β-glucosidases from Penicillium janthinellum. Appl. Environ. Microbiol. 41, 857–866. https://doi.org/10.1128/aem.41.4.857-866.1981

Ren, D., Wang, Z., Jiang, S., Yu, H., Zhang, S., Zhang, X., 2020. Recent environmental applications of and development prospects for immobilized laccase: a review. Biotechnol. Genet. Eng. Rev. 36, 81–131.

https://doi.org/10.1080/02648725.2020.1864187

Rey, L., May, J.C. (Ed.)., 2023. Freeze drying/lyophilization of pharmaceutical and biological products, 3rd ed. CRC Press. https://doi.org/https://doi.org/10.3109/9781439825761

Riedi, H. de P., de Liz, M.V., Braga, D.M., Ianoski, A.B., de Freitas Pereira, T., Brugnari, T., Haminiuk, C.W.I., Maciel, G.M., 2022. Synthesis and characterization of laccase enzyme aggregates from Trametes villosa for simultaneous elimination of rifampicin and isoniazid. Int. J. Environ. Res. 16, 1–12. https://doi.org/10.1007/s41742-022-00403-y

Robinson, P.K., 2015. Enzymes: principles and biotechnological applications. Essays Biochem. 59, 1–41. https://doi.org/10.1042/BSE0590001

Rodriguez-Rodriguez, C.E., Jelić, A., Llorca, M., Farré, M., Caminal, G., Petrović, M., Barceló, D., Vicent, T., 2011. Solid-phase treatment with the fungus Trametes versicolor substantially reduces pharmaceutical concentrations and toxicity from sewage sludge. Bioresour. Technol. 102, 5602–5608. https://doi.org/10.1016/j.biortech.2011.02.029

Saat, M.N., Annuar, M.S.M., Alias, Z., Chuan, L.T., Chisti, Y., 2013. Modeling of growth and laccase production by Pycnoporus sanguineus. Bioprocess Biosyst. Eng. 1–11. https://doi.org/10.1007/s00449-013-1046-8

Sakamoto, Y., Nakade, K., Yano, A., Nakagawa, Y., Hirano, T., Irie, T., Watanabe, H., Nagai, M., Sato, T., 2008. Heterologous expression of lcc1 from Lentinula edodes in tobacco BY-2 cells results in the production an active, secreted form of fungal laccase. Appl. Microbiol. Biotechnol. 79, 971–980. https://doi.org/10.1007/s00253-008-1507-1

Saloheimo, M., Niku-Paavola, M.-L., 1991. Heterologous production of a ligninolytic enzyme: Expression of the Phlebia radiata laccase gene in Trichoderma reesei. Nat. Biotechnol. 9, 1067–1071.

Sathishkumar, P., Meena, R.A.A., Palanisami, T., Ashokkumar, V., Palvannan, T., Gu, F.L., 2020. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota - a review. Sci. Total Environ. 698, 134057. https://doi.org/10.1016/j.scitotenv.2019.134057

Schneider, W.D.H., Cavion Costa, P., Fontana, R.C., de Siqueira, F.G., Pinheiro Dillon, A.J., Camassola, M., 2019. Upscale and characterization of lignin-modifying enzymes from Marasmiellus palmivorus VE111 in a bioreactor under parameter

optimization and the effect of inducers. J. Biotechnol. 295, 1–8. https://doi.org/10.1016/j.jbiotec.2019.03.002

Schneider, W.D.H., Fontana, R.C., Mendonça, S., de Siqueira, F.G., Dillon, A.J.P., Camassola, M., 2018. High level production of laccases and peroxidases from the newly isolated white-rot basidiomycete Marasmiellus palmivorus VE111 in a stirred-tank bioreactor in response to different carbon and nitrogen sources. Process Biochem. 69, 1–11. https://doi.org/10.1016/j.procbio.2018.03.005

Servili, M., Stefano, G., Piacquadio, P., Sciancalepore, V., 2000. A novel method for removing phenols from grape must. Am. J. Enol. Vitic. 51, 357–361. https://doi.org/10.5344/ajev.2000.51.4.357

Sharma, D., Gupta, R., 2024. Laccase: exploring structural insights and functional versatility for applications. Biologia (Bratisl). 79, 3381–3393. https://doi.org/10.1007/s11756-024-01787-2

Sharma, K., Thakur, I.S., Kaushik, G., 2021. Occurrence and distribution of pharmaceutical compounds and their environmental impacts: A review. Bioresour. Technol. Reports 16, 100841. https://doi.org/10.1016/j.biteb.2021.100841

Sheldon, R.A., van Pelt, S., 2013. Enzyme immobilisation in biocatalysis: Why, what and how. Chem. Soc. Rev. 42, 6223–6235. https://doi.org/10.1039/c3cs60075k

Shleev, S., Klis, M., Wang, Y., Rogalski, J., Bilewicz, R., Gorton, L., 2007. Comparative spectroelectrochemical studies of lyophilized and nonlyophilized laccases from Cerrena unicolor basidiomycete. Electroanalysis 19, 1039–1047. https://doi.org/10.1002/elan.200603841

Shu, N., Lorentzen, L.G., Davies, M.J., 2019. Reaction of quinones with proteins: Kinetics of adduct formation, effects on enzymatic activity and protein structure, and potential reversibility of modifications. Free Radic. Biol. Med. 137, 169–180. https://doi.org/10.1016/j.freeradbiomed.2019.04.026

Silori, R., Shrivastava, V., Singh, A., Sharma, P., Aouad, M., Mahlknecht, J., Kumar, M., 2022. Global groundwater vulnerability for Pharmaceutical and Personal care products (PPCPs): The scenario of second decade of 21st century. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2022.115703

Silva, K.E.R., Alves, L.D.S., Soares, M.F.R., Passos, R.C.S., Faria, A.R., Rolim Neto, P.J., 2009. Modelos de avaliação da estabilidade de fármacos e medicamentos para a indústria farmacêutica. Rev. Ciencias Farm. Basica e Apl. 30, 129–135.

Silveira, T.R., Ebling, C.D., Magro, L.D., Rodrigues, R.C., Hahn Schneider, W.D.,

Camassola, M., Weber de Menezes, E., Meneguzzi, Á., Klein, M.P., 2020. An efficient decolorization of methyl orange dye by laccase from Marasmiellus palmivorus immobilized on chitosan-coated magnetic particles. Biocatal. Agric. Biotechnol. 30, 1–10. https://doi.org/10.1016/j.bcab.2020.101859

Simón-Herrero, C., Naghdi, M., Taheran, M., Kaur Brar, S., Romero, A., Valverde, J.L., Avalos Ramirez, A., Sánchez-Silva, L., 2019. Immobilized laccase on polyimide aerogels for removal of carbamazepine. J. Hazard. Mater. 376, 83–90. https://doi.org/10.1016/j.jhazmat.2019.05.032

Singh, A.K., Abellanas-Perez, P., de Andrades, D., Cornet, I., Fernandez-Lafuente, R., Bilal, M., 2025. Laccase-based biocatalytic systems application in sustainable degradation of pharmaceutically active contaminants. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2024.136803

Singh, N.S., Sharma, R., Kumari, L., 2024. Tracing the evolution of enzymatic bioremediation using scientometric analysis. J. Scientometr. Res. 13, 517–534. https://doi.org/10.5530/jscires.13.2.41

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. Tech. Rep. NREL/TP-510-42623.

Sodhi, A.S., Bhatia, S., Batra, N., 2024. Laccase: Sustainable production strategies, heterologous expression and potential biotechnological applications. Int. J. Biol. Macromol. 280, 135745. https://doi.org/10.1016/j.ijbiomac.2024.135745

Sonoki, T., Kajita, S., Ikeda, S., Uesugi, M., Tatsumi, K., Katayama, Y., Iimura, Y., 2005. Transgenic tobacco expressing fungal laccase promotes the detoxification of environmental pollutants. Appl. Microbiol. Biotechnol. 67, 138–142. https://doi.org/10.1007/s00253-004-1770-8

Souza, F.S., Da Silva, V. V., Rosin, C.K., Hainzenreder, L., Arenzon, A., Pizzolato, T., Jank, L., Féris, L.A., 2017. Determination of pharmaceutical compounds in hospital wastewater and their elimination by advanced oxidation processes. J. Environ. Sci. Heal. 53, 213–221. https://doi.org/10.1080/10934529.2017.1387013

Steinbüchel, A., 2020. Laccases in Bioremediation and Waste Valorisation, Springer. Springer.

Stepanova, E. V., Koroleva, O. V., Gavrilova, V.P., Landesman, E.O., Makower, A., Papkovskii, D.B., 2003. Comparative stability assessment of laccases from the basidiomycetes Coriolus hirsutus and Coriolus zonatus in the presence of effectors.

Prikl. Biokhim. Mikrobiol. 39, 549–554.

Suleiman, M., Le Lay, N., Demaria, F., Kolvenbach, B.A., Cretoiu, M.S., Petchey, O.L., Jousset, A., Corvini, P.F.X., 2024. Pollutant profile complexity governs wastewater removal of recalcitrant pharmaceuticals. ISME J. 18. https://doi.org/10.1093/ismejo/wrae033

Tarcomnicu, I., Van Nuijs, A.L.N., Simons, W., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011. Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. Talanta 83, 795–803. https://doi.org/10.1016/j.talanta.2010.10.045

Tausz, J., Donath, P., 1930. Über die Oxydation des Wasserstoffs und der Kohlenwasserstoffe mittels Bakterien. Biological Chemistry 190, 141–168. https://doi.org/doi:10.1515/bchm2.1930.190.3-6.141

Thathola, P., Melchor-Martinez, E.M., Adhikari, P., Martinez, S.A.H., Pandey, A., Parra-Saldivar, R., 2024. Laccase-mediated degradation of emerging contaminants: unveiling a sustainable solution. Environ. Sci. https://doi.org/10.1039/d4va00173g

Torres-Farradá, G., Thijs, S., Rineau, F., Guerra, G., Vangronsveld, J., 2024. White rot fungi as tools for the bioremediation of xenobiotics: a review. J. Fungi 10. https://doi.org/10.3390/jof10030167

Tran, N.H., Reinhard, M., Gin, K.Y.H., 2018. Occurrence and fate of emerging contaminants in municipal treatment wastewater plants from different geographical regions-a review. Water Res. 133, 182–207. https://doi.org/10.1016/j.watres.2017.12.029

Ullrich, R., Le, M.H., Nguyen, L.D., Hofrichter, M., 2005. Laccase from the medicinal mushroom Agaricus blazei: Production, purification and characterization. Appl. Microbiol. Biotechnol. 67, 357–363. https://doi.org/10.1007/s00253-004-1861-6

Upadhyay, P., Shrivastava, R., Agrawal, P.K., 2016. Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 1–12. https://doi.org/10.1007/s13205-015-0316-3

Vagenende, V., Yap, M.G.S., Trout, B.L., 2009. Mechanisms of protein stabilization and prevention of protein aggregation by glycerol. Biochemistry 48, 11084–11096. https://doi.org/10.1021/bi900649t

Vahidi, H., Mojab, F., Taghavi, N., 2006. Effects of Carbon Sources on Growth and Production of Antifungal Agents by Gymnopilus Spectabilis 219–222.

Valle, J.S. do, Vandenberghe, L.P. de S., Santana, T.T., Linde, G.A., Colauto, N.B., Soccol, C.R., 2014. Optimization of Agaricus blazei laccase production by submerged cultivation with sugarcane molasses. African J. Microbiol. Res. 8, 939–946. https://doi.org/10.5897/ajmr2013.6508

Valle, J.S., Vandenberghe, L.P.S., Oliveira, A.C.C., Tavares, M.F., Linde, G.A., Colauto, N.B., Soccol, C.R., 2015. Effect of different compounds on the induction of laccase production by Agaricus blazei. Genet. Mol. Res. 14, 15882–15891. https://doi.org/10.4238/2015.December.1.40

Varriale, S., Delorme, A.E., Andanson, J.M., Devemy, J., Malfreyt, P., Verney, V., Pezzella, C., 2022. Enhancing the Thermostability of Engineered Laccases in Aqueous Betaine-Based Natural Deep Eutectic Solvents. ACS Sustain. Chem. Eng. 10, 572–581. https://doi.org/10.1021/acssuschemeng.1c07104

Vaudin, P., Augé, C., Just, N., Mhaouty-Kodja, S., Mortaud, S., Pillon, D., 2022. When pharmaceutical drugs become environmental pollutants: Potential neural effects and underlying mechanisms. Environ. Res. 205. https://doi.org/10.1016/j.envres.2021.112495

Vieira, Y., Spode, J.E., Dotto, G.L., Georgin, J., Franco, D.S.P., dos Reis, G.S., Lima, E.C., 2024. Paracetamol environmental remediation and ecotoxicology: a review. Environ. Chem. Lett. 22, 2343–2373. https://doi.org/10.1007/s10311-024-01751-1

Viniegra-González, G., Favela-Torres, E., Aguilar, C.N., Rómero-Gomez, S. de J., Díaz-Godínez, G., Augur, C., 2003. Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem. Eng. J. 13, 157–167. https://doi.org/10.1016/S1369-703X(02)00128-6

Viswanath, B., Rajesh, B., Janardhan, A., Kumar, A.P., Narasimha, G., 2014. Fungal laccases and their applications in bioremediation. Enzyme Res. 2014. https://doi.org/10.1155/2014/163242

Vitolo, M., Jr., A.P., Souza, G.M. de, Carvalho, J.C.M. de, Stephano, M.A., Sato, S., 2015. Biotecnologia farmacêutica: Aspectos sobre aplicação industrial, 1ª. ed. São Paulo.

Wang, F., Guo, C., Liu, C.Z., 2013. Immobilization of Trametes versicolor cultures for improving laccase production in bubble column reactor intensified by sonication. J. Ind. Microbiol. Biotechnol. 40, 141–150. https://doi.org/10.1007/s10295-012-1214-x

Wang, F., Xiang, L., Sze-Yin Leung, K., Elsner, M., Zhang, Y., Guo, Y., Pan, B., Sun, H., An, T., Ying, G., Brooks, B.W., Hou, D., Helbling, D.E., Sun, J., Qiu, H., Vogel,

T.M., Zhang, Wei, Gao, Yanzheng, Simpson, M.J., Luo, Yi, Chang, S.X., Su, G., Wong, B.M., Fu, T.M., Zhu, D., Jobst, K.J., Ge, C., Coulon, F., Harindintwali, J.D., Zeng, X., Wang, H., Fu, Y., Wei, Z., Lohmann, R., Chen, C., Song, Y., Sanchez-Cid, C., Wang, Y., El-Naggar, A., Yao, Y., Huang, Y., Cheuk-Fung Law, J., Gu, Chenggang, Shen, H., Gao, Yanpeng, Qin, C., Li, Hao, Zhang, Tong, Corcoll, N., Liu, M., Alessi, D.S., Li, Hui, Brandt, K.K., Pico, Y., Gu, Cheng, Guo, J., Su, J., Corvini, P., Ye, M., Rocha-Santos, T., He, H., Yang, Y., Tong, M., Zhang, Weina, Suanon, F., Brahushi, F., Wang, Z., Hashsham, S.A., Virta, M., Yuan, Q., Jiang, G., Tremblay, L.A., Bu, Q., Wu, J., Peijnenburg, W., Topp, E., Cao, X., Jiang, X., Zheng, M., Zhang, Taolin, Luo, Yongming, Zhu, L., Li, X., Barceló, D., Chen, J., Xing, B., Amelung, W., Cai, Z., Naidu, R., Shen, Q., Pawliszyn, J., Zhu, Y. guan, Schaeffer, A., Rillig, M.C., Wu, F., Yu, G., Tiedje, J.M., 2024. Emerging contaminants: A One Health perspective. Innovation 5, 100612. https://doi.org/10.1016/j.xinn.2024.100612

Wang, G.-D., Li, Q.-J., Luo, B., Chen, X.-Y., 2004. Ex planta phytoremediation of trichlorophenol and phenolic allelochemicals via an engineered secretory laccase. Nat. Biotechnol. 22, 893–897. https://doi.org/10.1038/nbt982

Wang, J., Chu, L., Wojnárovits, L., Takács, E., 2020. Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview. Sci. Total Environ. 744, 140997. https://doi.org/10.1016/j.scitotenv.2020.140997

Wang, W., 2000. Lyophilization and development of solid protein pharmaceuticals, International Journal of Pharmaceutics. https://doi.org/10.1016/S0378-5173(00)00423-3

White, N.A., Boddy, L., 1992. Differential extracellular enzyme production in colonies of Coriolus versicolor, Phlebia radiata and Phlebia rufa: Effect of gaseous regime. J. Gen. Microbiol. 138, 2589–2598. https://doi.org/10.1099/00221287-138-12-2589

Wisniak, J., 2014. Gabriel Bertrand. Rev. CENIC. Ciencias Biológicas 45, 230–245. https://doi.org/10.1051/978-2-7598-0315-6.c013

Wolfenden, B.S., Willson, R.L., 1982. Radical-cations as reference chromogens in kinetic studies of ono-electron transfer reactions: pulse radiolysis studies of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate). J. Chem. Soc. Perkin Trans. 2 805–812. https://doi.org/10.1039/P29820000805

Wong, A.W., Datla, A., 2005. Assay and stability testing. Sep. Sci. Technol. 6, 335–358.

https://doi.org/10.1016/S0149-6395(05)80057-1

Yang, J., Li, W., Bun Ng, T., Deng, X., Lin, J., Ye, X., 2017. Laccases: Production, expression regulation, and applications in pharmaceutical biodegradation. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00832

Yang, Y., Wei, F., Zhuo, R., Fan, F., Liu, H., Zhang, C., Ma, L., Jiang, M., Zhang, X., 2013. Enhancing the laccase production and laccase gene expression in the white-rot fungus Trametes velutina 5930 with great potential for biotechnological applications by different metal ions and aromatic compounds. PLoS One 8. https://doi.org/10.1371/journal.pone.0079307

Yoshida, H., 1883. Chemistry of Lacquer (Urushi). J. Chem. Soc. Trans. 43, 472–486.

Zaks, A., Klibanov, A.M., 1988. The effect of water on enzyme action in organic media. J. Biol. Chem. 263, 8017–8021. https://doi.org/10.1016/s0021-9258(18)68435-2

Zdarta, J., Jankowska, K., Wyszowska, M., Kijeńska-Gawrońska, E., Zgoła-Grześkowiak, A., Pinelo, M., Meyer, A.S., Moszyński, D., Jesionowski, T., 2019. Robust biodegradation of naproxen and diclofenac by laccase immobilized using electrospun nanofibers with enhanced stability and reusability. Mater. Sci. Eng. C 103. https://doi.org/10.1016/j.msec.2019.109789

Zhang, W., Zhang, Z., Ji, L., Lu, Z., Liu, R., Nian, B., Hu, Y., 2023. Laccase immobilized on nanocomposites for wastewater pollutants degradation: current status and future prospects. Bioprocess Biosyst. Eng. 46, 1513–1531. https://doi.org/10.1007/s00449-023-02907-z

Zhou, W., Zhang, W., Cai, Y., 2021. Laccase immobilization for water purification: A comprehensive review. Chem. Eng. J. 403, 126272. https://doi.org/10.1016/j.cej.2020.126272