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AVALIAÇÃO DO POTENCIAL DE MICROALGAS NA OBTENÇÃO DE BIOPRODUTOS: ESTUDO DE PROPRIEDADES METABÓLICAS E ENZIMÁTICAS

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia Ambiental – PPGCTA da Universidade Federal da Fronteira Sul – UFFS Campus Erechim, como requisito para obtenção do título de Mestre em Ciência e Tecnologia Ambiental.

Orientadora: Profª. Drª. Helen Treichel

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Dedico este trabalho a todos aqueles que acreditam na ciência e que por meio de suas pesquisas buscam alternativas para um mundo melhor.

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Seja corajoso, seja curioso, seja determinado, supere as probabilidades. É possível!

Stephen Hawking

RESUMO

As mudanças climáticas sentidas atualmente pelo mundo todo é uma consequência do uso desenfreado dos recursos naturais, o que fez necessário a instituição de metas globais para preservação desses recursos que incluem o gerenciamento de resíduos e métodos de produção mais sustentáveis para que os impactos causados ao meio ambiente sejam mitigados. Os setores da agricultura e farmacêutico estão entre os principais geradores de resíduos contaminantes como compostos xenobióticos que são responsáveis pela contaminação de ecossistemas aquáticos, e portanto, sendo necessários processos terciários de descontaminação de águas residuárias provenientes desses setores. As microalgas são bastante utilizadas em processos terciários de tratamento de efluentes por conseguirem remover esses contaminantes e ainda utilizar os compostos presentes nas águas residuárias para seu desenvolvimento. Além disso, a biomassa proveniente do processo de biorremediação pode ser utilizada como matéria prima na obtenção de produtos de valor agregado devido a sua composição rica em carboidratos, proteínas e bioativos, promovendo a economia circular. Nesse contexto o objetivo deste estudo foi avaliar o potencial das microalgas no setor ambiental por meio do seu uso na biorremediação de contaminantes em água e o uso da biomassa microalgal para a obtenção de biocompostos de valor agregado. Para a avaliação do potencial das microalgas para a biorremediação de poluentes, inicialmente foi realizada a escrita de um capítulo de livro que demonstrou os mecanismos de biorremediação de poluentes e possíveis produtos que podem ser obtidos da biomassa advindas desse processo. Após, foi realizado a caracterização do biocompósito obtido por fermentação em estado submerso utilizando biomassa microalgal proveniente de águas residuárias e o microrganismo *Trichoderma koningiopsis*. Como resultados, a caracterização demonstrou que o biocompósito obtido possui um *pool* enzimático com proteínas de elevada atividade enzimática como catalase (1274,79 μmol/min.mL), peroxidase (52,08 U/mL) e protease (97,50 U/mL) que são estáveis em diferentes temperaturas (20°C, 04°C e -80°C) e tempo de armazenamento (30,60 e 90 dias). Além disso, por meio análise UHPLC-ESI-qTOF-MS/MS foi constatada a presença de metabólitos como leucina, tirosina, vitamina B3 e ácido d-glucônico que possibilitam o uso do composto fúngico obtido neste estudo para a produção de bioinsumos. Ainda foi constatada a presença de compostos de valor agregado tanto na agricultura como na área da saúde no biocompósito, como as coninginas a, b e d e tricodermina que são antifúngicos e agentes antitumorais. Portanto, por meio deste estudo foi possível obter um composto fúngico com variada aplicação, principalmente na área ambiental e na agricultura auxiliando no comprimento de metas globais de gerenciamento de resíduos e produção sustentável.

Palavras-chave: Biorremediação; Enzimas; *Trichoderma koninigiopsis*; Biocomposito; pH.

ABSTRACT

The climate change currently felt around the world is a consequence of the unbridled use of natural resources, which has made it necessary to establish global goals for the preservation of these resources, which include waste management and more sustainable production methods so that the impacts caused to the environment can be mitigated. The agriculture and pharmaceutical sectors are among the main generators of contaminant waste, which include organic and inorganic compounds and medicines of different classes responsible for the contamination of aquatic ecosystems; therefore, tertiary processes of decontaminating wastewater from these sectors are necessary. Microalgae are widely used in tertiary effluent treatment processes because they can remove these contaminants and use the compounds in sewage for their development. In addition, the biomass from the bioremediation process can be used as a raw material to obtain value-added products due to its composition rich in carbohydrates, proteins, and bioactives, promoting the circular economy. In this context, the objective of this study was to evaluate the potential of microalgae in the environmental sector through their use in the bioremediation of contaminants in water and the use of microalgal biomass to obtain value-added biocompounds. To evaluate the potential of microalgae for the bioremediation of pollutants, a book chapter was written that demonstrated the mechanisms of bioremediation of pollutants and possible products that can be obtained from biomass from this process. After that, the biocomposite obtained by fermentation in a submerged state using microalgal biomass from wastewater and the microorganism *Trichoderma koningiopsis*, was characterized . As a result, the characterization showed that the biocomposite obtained has an enzymatic pool with proteins of high enzymatic activity such as catalase (1274.79 µmol/min.mL), peroxidase (52.08 U/mL), and protease (97.50 U/mL) that are stable at different temperatures (20°C, 04°C and -80°C) and storage time (30, 60 and 90 days). In addition, through UHPLC-ESI-qTOF-MS/MS analysis, the presence of metabolites such as leucine, tyrosine, vitamin B3, and d-gluconic acid was observed, which allow the use of fungal composite obtained in this study for the production of bioinputs. The presence of value-added compounds in agriculture and health was also observed in the biocomposite, such as koninginin A, B and D and trichodermin, antifungal and antitumor agents. Therefore, through this study, it was possible to obtain a fungal composite with varied applications, mainly in the environmental area and in agriculture, helping to achieve global goals for waste management and sustainable production.

Keywords: Bioremediation; Enzymes; *Trichoderma koninigiopsis*; Biocomposite; pH.

SUMÁRIO

1 INTRODUÇÃO GERAL

Os avanços econômicos e tecnológicos nos setores agrícola e farmacêutico têm suas vantagens em termos de produção de alimentos e bem-estar à saúde humana, no entanto, seu processo produtivo, se realizado de forma inadequada, pode causar efeitos adversos ao meio ambiente e à saúde humana, principalmente se os resíduos e águas residuárias gerados durante a produção serem descartados sem o tratamento adequado. As consequências da expansão produtiva de ambos os setores e de outras indústrias já podem ser observadas nas mudanças climáticas que estão ocorrendo em nosso planeta, como os altos índices de UV e precipitações elevadas que ocasionaram em desastres climáticos no ano de 2024 . Para mitigar esses efeitos, foi necessário o estabelecimento de metas globais, que incluem o gerenciamento adequado dos resíduos para reduzir a contaminação de seu descarte e métodos de produção mais sustentáveis, considerando o uso dos recursos naturais de forma equilibrada.

Diante disso, é necessário ressaltar que o setor agrícola e farmacêutico é responsável pela geração de resíduos sólidos e águas residuárias com elevado teor de matéria orgânica e inorgânica, substâncias tóxicas, sólidos dissolvidos, antibióticos, analgésicos, anti-inflamatórios, antidepressivos entre outros compostos que acarretam na eutrofização de rios e lagos (Alavianghavanini *et al*., 2024; Matsumura e Mierzwa, 2008; Nguyen *et al*., 2024). Os compostos xenobióticos advindos desses dois setores veem sendo monitorados por meio de estudos e no Brasil alguns dos contaminantes encontrados em águas residuárias são compostos farmacêuticos, compostos advindos de produtos de higiene pessoal e compostos como 17β-estradiol, bisfenol A, estrona e pesticidas químicos (Oliveira *et al*., 2020; Reichert *et al*., 2019; Caldas *et al*., 2016) sendo portanto necessários processos terciários de tratamento, além dos primários e secundários para a remoção desses poluentes de águas residuárias.

Uma alternativa bastante empregada no tratamento terciário de águas residuárias é o uso de microalgas devido os seus mecanismos de biorremediação e por serem mais acessíveis economicamente em comparação com o uso de tratamentos convencionais como os físico-químicos que podem incluir cloração, UV, filtração por membrana e processos oxidativos avançados (Zagklis e Bampos, 2022). As microalgas são organismos fotossintéticos unicelulares que podem ser encontrados naturalmente em rios, lagos, oceanos e solos úmidos, e por serem organismos fotossintéticos realizam a bioconversão de CO_2 em biomassa rapidamente (Anyaoha *et al*., 2024; Brasil, Silva e Siqueira, 2017). O Brasil tem grande potencial para a obtenção desses organismos, pois possui uma área costeira tropical de cerca de 10.959 km^2 e a maior porcentagem de água doce do mundo (Brasil, Silva e Siqueira, 2017).

Atualmente, cerca de 3.400 espécies de algas estão catalogadas no país, sendo mantidas em instituições e laboratórios para fins de pesquisa, principalmente na área de recursos energéticos, como a produção de biocombustíveis e obtenção de bioprodutos (Brasil, Silva e Siqueira, 2017; Forzza *et al*., 2012).

O cultivo de microalgas em efluentes para a biorremediação de poluentes e obtenção de biomassa teve sua primeira aplicação há mais de 50 anos, tornando-se uma prática consolidada (Oswald e Golueke, 1966; Alavianghavanini *et al*., 2024). A remoção de poluentes presentes nas águas residuárias por esses organismos ocorre por meio de mecanismos de biossorção, bioadsorção e bioacumulação, nos quais durante esses processos as microalgas utilizam contaminantes como nitrogênio, fósforo, agroquímicos, compostos orgânicos e inorgânicos como fonte de nutrientes para seu desenvolvimento ao mesmo tempo em que os retira do efluente, possibilitando o descarte dessas águas de forma adequada (Fayaz *et al*., 2024; Goh *et al*., 2023).

Além de atuar na biorremediação de contaminantes, ao final do processo, a biomassa recuperada pode se tornar matéria-prima para diferentes finalidades como obtenção de bioprodutos e produção de energia, promovendo a economia circular. A biomassa de microalgas é uma matéria-prima alternativa devido à sua rica composição de lipídios, proteínas, carboidratos e outros metabólitos do processo fotossintético (Ansari *et al*., 2017; Hosseini, Shang e Scott, 2018). Ainda, com base em seu modo de produção, que inclui a incidência de luz, CO_2, sais e nitrogênio, entre outros compostos, as microalgas podem sintetizar diversas enzimas, porém, por serem intracelulares, ao visarem apenas a produção da enzima a partir de seu cultivo, é necessário um maior tempo de cultivo e métodos de ruptura celular que facilitem o acesso à enzima (Brasil *et al*., 2017).

A biomassa microalgal proveniente de processos de biorremediação também pode servir de matéria prima em processos fermentativos que visam a produção de bioprodutos, podendo ser fonte de carbono e nitrogênio para microrganismos fermentadores, além de ser utilizada para a produção de biocombustíveis, bioinsumos e biopolímeros (Bhatt *et al*., 2022; Camargo *et al.*, 2024; Morais Junior *et al.*, 2020). No entanto, é necessário ressaltar que para se obter um bioproduto com uma variedade de aplicabilidades com base em sua composição, alguns parâmetros devem ser considerados, como a escolha do microrganismo ideal quando se trata de processos fermentativos com biomassa de microalgas como substrato.

Em termos de produção de enzimas industriais com aplicabilidade em diversos setores econômicos, o uso de fungos endofíticos tem sido amplamente explorado por serem reconhecidos como depósitos enzimáticos. Presentes na estrutura intercelular das plantas, nas

folhas, caules e sistemas radiculares, os fungos endofíticos são responsáveis pela produção de metabólitos, prevenção de doenças e aumento da resistência das plantas, esses microrganismos possuem em sua estrutura molecular diversas enzimas relevantes para sua ação na planta e que podem ser utilizadas em setores industriais (Raghav *et al*., 2022).

Há uma diversidade de microrganismos oriundos de plantas com potencial para produção enzimática, dentre eles os fungos do gênero *Trichoderma* reconhecidos por seu uso na agricultura como agentes de controle biológico em lavouras e amplamente utilizados para a produção de enzimas (Harman, 2006; Schuster e Schmoll, 2010). Os microrganismos do gênero *Trichoderma* se adaptam rapidamente ao ambiente em que estão inseridos, utilizando de forma eficiente o substrato disponível e consequentemente secretando metabólitos e enzimas, e por isso, são considerados promissores para seu uso em processos biotecnológicos para obtenção de produtos de valor agregado (Schuster e Schmoll, 2010).

A utilização de microrganismos do gênero *Trichoderma* em conjunto com a biomassa de microalgas em processos fermentativos possibilita a obtenção de biocompostos constituídos por pools enzimáticos devido à capacidade das microalgas de sintetizar enzimas como lacases, celulases, amilases, proteases e enzimas antioxidantes, e o fungo *Trichoderma* spp. produzir também enzimas como amilases, celulases, oxirredutases e proteases, além de metabólitos como fitohormônios, ácidos orgânicos e álcoois (Brasil *et al*., 2017; Camargo *et al*., 2023; Camargo *et al*., 2024; Spier *et al*., 2020; Sutaoney *et al*., 2024). Portanto, um biocomposto obtido a partir de processos fermentativos que utilizam esse tipo de biomassa em conjunto com *Trichoderma* spp., como microrganismo fermentador tem potencial para ser utilizado para diversos fins nas áreas agrícola e ambiental, contribuindo para o cumprimento das metas globais dadas pela Agenda 2030, por ser um método sustentável de obtenção e sem o uso de produtos nocivos ao meio ambiente.

Neste contexto, este estudo pretende contribuir para a ciência através da avaliação do papel das microalgas no setor ambiental, desde a biorremediação de contaminantes até à utilização da sua biomassa como substrato em processos fermentativos com *Trichoderma koningiopsis*, promovendo a economia circular.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Destacar o uso das microalgas no setor ambiental e avaliar as propriedades enzimáticas e metabólicas do biocomposto produzido utilizando biomassa microalgal obtida a partir de águas residuárias como substrato em processos fermentativos em estado submerso em biorreator *Airlif* com *Trichoderma koningiopsis*.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar, por meio de um capítulo de revisão, o potencial das microalgas na biorremediação de poluentes xenobióticos e no uso de biomassa residual para a obtenção de produtos de valor agregado;
- Avaliar a presença e propriedades enzimáticas, como efeitos de pH e temperatura na atividade enzimática por meio de delineamento composto central rotacional (DCCR) 2^2, no biocomposto obtido por fermentação submersa usando biomassa de microalgas e *Trichoderma koningiopsis*;
- Avaliar a estabilidade da atividade enzimática em diferentes temperaturas e tempos de armazenamento;
- Determinar os compostos orgânicos e metabólitos presentes no biocomposto pré e pós-fermentação.

3 ESTRUTURA DA DISSERTAÇÃO

A dissertação está estruturada em dois capítulos, o primeiro é uma revisão abrangente dos usos de microalgas na biorremediação de poluentes xenobióticos e o potencial da biomassa residual como matéria-prima para a obtenção de produtos de valor agregado. Este capítulo é parte de um livro da editora CRC Press e contempla o primeiro objetivo específico deste estudo.

O segundo capítulo é um artigo científico que contempla os outros objetivos desta dissertação e foi aceito para publicação na revista internacional Biocatalysis and Agricultural Biotechnology.

CAPÍTULO 01: Linking microalgae-based processes and conversion of water pollutants for valuable compounds obtaining

ABSTRACT

The use of microalgae in the bioremediation of contaminants has been widely discussed as a viable alternative for reducing environmental impacts due to their metabolic versatility and ability to grow in different environments. Therefore, when applied in effluent treatment and using the nutrients in the environment for cell growth, microalgal biomass acts in biosorption and bioadsorption processes on pollutants, such as xenobiotics. The presence of these compounds in the aquatic environment causes accumulation in invertebrate organisms, passing them to other levels of the food chain. In this sense, this chapter will address the state of the art regarding water remediation processes of xenobiotic pollutants based on the use of microalgae, such as pesticides and pharmaceutical products, and also explore the value-added products that can be obtained through this biomass.

Keywords: wastewater; bioremediation; nutrients; biomass; organisms

1 INTRODUCTION

The bioremediation processes are an environmentally viable alternative for removing contaminants in water. The use of microalgae as a bioremediator of polluting compounds has been discussed to reduce environmental impacts due to their metabolic versatility and adaptability to different environments. Emerging contaminants in water bodies have increasingly been reported due to anthropogenic actions involving improper disposal of effluents, inappropriate application of chemical inputs, lack of monitoring, and poor sanitation conditions. Among the primary sources of pollution are the agricultural sector and wastewater treatment plants. The inappropriate application of chemical inputs causes the leaching of these compounds and reaches groundwater. At the same time, wastewater treatment plants discharge water into aquatic environments with high levels of chemical and biochemical demand for oxygen, suspended solids, oils and greases, and xenobiotic substances (Bhatt et al. 2022).

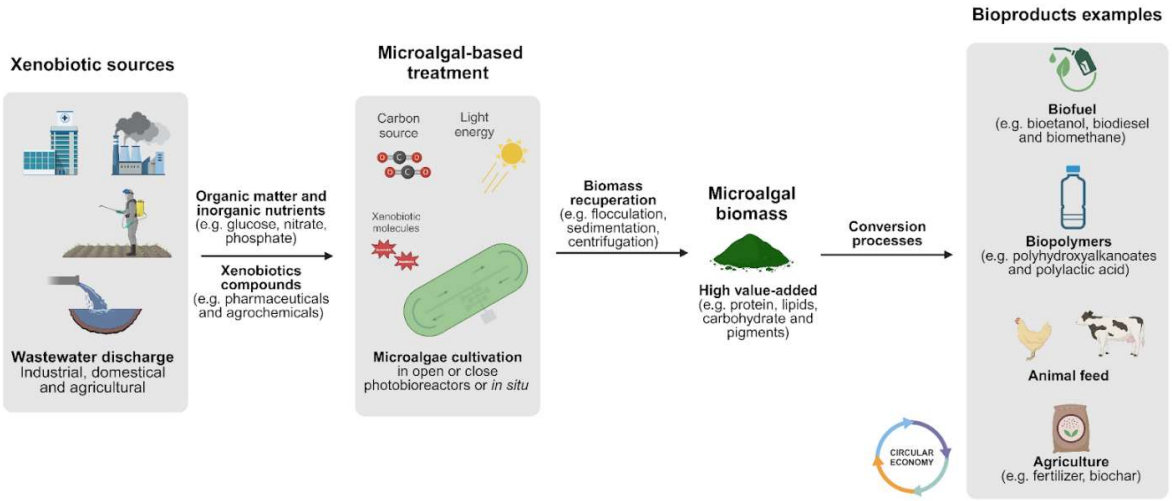
Xenobiotic compounds are chemical substances found in the environment or organisms from industrial processes and the use of chemical compounds that can cause interference in ecosystems, especially pharmaceutical-based compounds, agricultural pesticides, and insecticides, already considered toxic to animals and human health (Goswami, Agrawal, and Verma 2022a; Štefanac, Grgas, and Dragičević 2021). Pesticides and insecticides such as diazinon and trichlorfon applied directly in the soil and which end up contaminating rivers and surface waters due to the transport of rain, are considered Class II chemicals according to the

World Health Organization, and therefore toxic to organisms, had their metabolites detected in wastewater and urban water. The pharmaceuticals are introduced into water bodies through the disposal of domestic effluents because they are not able to metabolize entirely and, like chemical inputs, are converted into several metabolites that are more toxic than their initial formulation (Štefanac, Grgas, and Dragičević 2021). The presence of these contaminants in water bodies raises nitrogen and phosphorus levels in water, causing eutrophication, bioaccumulation, and biomagnification in invertebrate organisms (Bhatt et al. 2022; Ray and Shaju 2023)

Microalgae are chosen as an environmentally friendly alternative for removing these contaminants from effluents due to their characteristics. Known as cell factories, microalgae use photosynthetic processes for their growth and biomass production, fixing the CO_2 in the environment and generating lipids, carbohydrates, and proteins in their composition (Bhatt et al. 2022). When exposed to wastewater containing pollutants, they use carbon, nitrogen, and phosphorus available in high quantities, such as nutrients for growth and based on processes of accumulation, precipitation, adsorption, complexation, and bioaccumulation, microalgae remove contaminants (Bhatt et al. 2022; Goswami, Agrawal, and Verma 2022a).

In addition, microalgae continue to be a preference in wastewater treatment due to the possibility of using biomass produced post-treatment. In a circular economy context that aims at reduction, reuse, and recycling, the use of microalgal biomass from xenobiotic bioremediation meets the objectives by making the obtaining of valuable compounds through bioconversion such as fuels, fertilizers, biopolymers, and animal feed supplements more sustainable (Nagarajan et al. 2020). In this sense, this chapter aims to address the state-of-the-art remediation processes based on the use of microalgae of xenobiotics pollutants such as pharmaceutical-based and agricultural-based present in wastewater and to explore the compounds and value-added products that can be obtained from the bioconversion of this biomass, addressing the aspects described in Figure 1 below.

Figure 1: Representation of a system for removing xenobiotic pollutants based on usingmicroalgae and bioconversion into valuable compounds. Create with BioRender.com



2 MAIN SOURCES OF XENOBIOTIC POLLUTION IN THE AQUATIC ENVIRONMENT

The occurrence of xenobiotics in environmental matrices, such as surface waters, groundwater, and sediments, can be attributed to society's increasing chemical production and consumption. Wastewater is the primary pollution source for other aqueous matrices, specifically when generated by industries, households, hospitals, and agriculture (Burri et al. 2019; Islam et al. 2022). This is due to the higher concentrations of chemicals that these activities release compared to decentralized sources such as municipal wastewater. Because of the low efficiency of conventional treatment technologies to degrade and remove these xenobiotics from wastewater, these chemicals reach natural aquatic environments (Islam et al. 2022; Goswami, Agrawal, and Verma 2022a).

With this emerges the necessity of constantly and systematically monitoring these compounds, aiming to understand the extension of such environmental pollution. For this, financial support and creating research networks can be a way to exchange information and collect data. The information has been used to create lists of prioritization compounds, which are valuable as a guideline for future research, monitoring campaigns, and mitigation actions (Islam et al. 2022; Burri et al. 2019; Wilkinson et al. 2022).

An example is the NORMAN network, formed by reference laboratories, research centers, and related organizations in Europe. This network has been monitoring emerging environmental substances since 2005 with the financial support of the European Commission.

In addition, they maintain an open-access database with geo-referenced monitoring data on emerging substances on samples from several countries and ecosystems. The network has monitored more than four thousand compounds, mainly in aquatic environments (98% of the samples) (https://www.norman-network.net/).

When searching at NORMAN Chemical Occurrence Database (https://www.norman-network.com/nds/empodat/chemicalSearch.php?s=new) for wastewater samples from 2019 to 2024, it was found 580 xenobiotics (8,396 samples). The country that had more number (n) of samples at concentrations higher than the limit of quantification (LoQ) was Switzerland (n = 1,296), followed by Germany (n = 820), Austria (n = 631), Slovenia (n = 552), and Romania (n = 550).

From these 580 xenobiotics, 23 compounds had $n \geq 50$, totalizing 1,283 samples. When analyzing these compounds, it was found that 4 of them (benzothiazole-2-sulfonic acid, ensulizole, N-Acetylaminoantipyrine, and telmisartan) are not part of the list of prioritization, determined by the NORMAN Prioritisation framework for emerging substances. Thus, the remaining 19 compounds (n = 1,069) were detailed in Table 01, which compiles general information, physicochemical properties, and concentration data of these xenobiotics found in wastewater samples from 14 European countries.

In a first look at Table 01, it is possible to observe the prevalence of compounds used as pharmaceuticals (10 substances) and agrochemicals (5 substances). These two categories of substances can reach the aquatic environment through different routes. Still, the ones with significant concern are those related to anthropogenic activities, such as industries, urbanization, and agriculture. Once in contact with water, these chemicals can migrate or accumulate because of natural water cycles processes, such as infiltration, surface and subsurface runoff, percolation, and leaching (Burri et al. 2019; Islam et al. 2022).

Pharmaceuticals have been considered xenobiotics only in recent decades, and with the advances in the medical field, the abundance of new compounds to treat human and animal diseases has been growing. They are mainly found in anthropogenically impacted water bodies, and the serious concern regarding these chemicals is that they are designed to be bioavailable to living organisms. Thus, reaching aquatic environments can potentially negatively affect the life cycle of untargeted organisms (Burri et al. 2019).

Agrochemicals, on the other hand, were recognized as environmental pollutants in the early 1960s. These substances have a crucial role in providing food security for society, which is one reason for a continued increase in their production and consumption. Meanwhile, the increasing awareness of their environmental impacts induced the creation of regulations

regarding their use over the past two decades. The restrictions are advancing, particularly in Europe and North America, where several agrochemicals have been banned; however, they continue to be measured in aquatic environments, including groundwater.

Table 01: General information and concentration data of xenobiotics most found in urban wastewater samples collected during 2019 in Europe.

Compound name (acronym)	**CAS number**	**Chemical formula**	**Use category[a]**	**Physicochemical properties[b]**					**Concentration (µg L^{-1})[a]**		
				Mass (g mol^{-1})	pK_a	log K_{ow}	K_{oc} (L kg^{-1})	Biodeg. half-time (days)	Ave ± SD (*n*)	Min	Max
1,2,3-Benzotriazole (BTA)	95-14-7	$C_6H_5N_3$	Industrial chemical	119.13	8.38	1.37	55.7	3.98	3.03 ± 4.91 (63)	0.02	25.92
5-Methyl-1H-benzotriazole (MBT)	136-85-6	$C_7H_7N_3$	Industrial chemical	133.15	8.90	1.56	63.1	2.51	1.79 ± 4.06 (53)	0.02	22.21
Carbamazepine (CBZ)	298-46-4	$C_{15}H_{12}N_2O$	Pharmaceutical	236.27	5.07	2.41	550.0	6.61	0.29 ± 0.33 (67)	5.0×10^{-4}	1.27
Carbendazim (CAR)	10605-21-7	$C_9H_9N_3O_2$	PPP* / Biocide	191.19	7.12	1.55	172.0	4.47	1.21 ± 4.45 (52)	0.01	22.02
Diclofenac (DIC)	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	Pharmaceutical	296.15	0.91	4.27	5750.0	4.68	1.21 ± 1.14 (67)	0.01	6.46
Hydrochlorothiazide	58-93-5	$C_7H_8ClN_3O_4S_2$	Pharmaceutical	297.73	9.25	-0.06	52.5	7.24	1.06 ± 1.20 (54)	4.2×10^{-3}	4.91

(HCTZ)											
Imidacloprid (IMI)	138261-41-3	$C_9H_{10}ClN_5O_2$	PPP / Biocide	255.66	1.10	0.43	74.2	3.55	0.07 ± 0.15 (52)	2.4×10^{-3}	0.97
Lamotrigine (LTG)	84057-84-1	$C_9H_7Cl_2N_5$	Pharmaceutical	256.09	5.22	1.26	178.0	6.17	0.39 ± 0.42 (55)	0.02	2.60
Metformin (MET)	657-24-9	$C_4H_{11}N_5$	Pharmaceutical	129.17	13.3	-1.42	9.5	4.79	15.83 ± 33.93 (56)	4.1×10^{-3}	139.96
N,N-Diethyl-meta-toluamide (DEET)	134-62-3	$C_{12}H_{17}NO$	PCP** / Biocide	191.27	2.14	2.16	191.0	3.39	0.71 ± 1.69 (50)	1.7×10^{-3}	10.17
Sucralose (SCL)	56038-13-2	$C_{12}H_{19}Cl_3O_8$	Food additive	397.63	7.31	-0.29	28.2	4.37	9.75 ± 11.22 (56)	0.02	47.08
Sulfamethoxazole (SMX)	723-46-6	$C_{10}H_{11}N_3O_3S$	Pharmaceutical	253.28	6.18	0.73	91.2	3.31	0.25 ± 0.35 (67)	9.7×10^{-4}	1.64
Sulfapyridine (SPD)	144-83-2	$C_{11}H_{11}N_3O_2S$	Pharmaceutical	249.29	5.37	0.35	148.0	3.39	0.16 ± 0.32 (53)	1.0×10^{-3}	2.07
Tebuconazole (TEB)	107534-96-3	$C_{16}H_{22}ClN_3O$	PPP / Biocide	307.82	7.03	3.73	715.0	3.16	2.78 ± 9.71 (53)	8.0×10^{-4}	45.89

Terbutryn (TER)	886-50-0	$C_{10}H_{19}N_5S$	PPP / Biocide	241.36	4.83	3.53	657.0	3.39	1.40 ± 5.94 (62)	4.1×10^{-4}	37.22
Tramadol (TRA)	27203-92-5	$C_{16}H_{25}NO_2$	Pharmaceutical	263.38	8.69	2.52	562.0	3.39	0.46 ± 0.56 (50)	0.01	2.59
Triethyl phosphate (TEP)	78-40-0	$C_6H_{15}O_4P$	Flame retardant	182.16	–	0.94	60.3	4.90	0.53 ± 0.88 (52)	1.2×10^{-3}	4.74
Trimethoprim (TMP)	738-70-5	$C_{14}H_{18}N_4O_3$	Pharmaceutical	290.32	7.11	0.89	115.0	4.27	0.12 ± 0.12 (51)	0.01	0.41
Valsartan (VAL)	137862-53-4	$C_{24}H_{29}N_5O_3$	Pharmaceutical	435.53	1.71	3.18	589.0	7.41	2.13 ± 7.24 (56)	3.0×10^{-3}	49.40

Average (Ave), standard deviation (SD), maximum (Max), and minimum (Min) concentrations. The number of analyzed samples (*n*) is given in parentheses. pK_a: Dissociation constant. Log K_{ow}: Octanol-water partition coefficient. K_{oc}: Soil adsorption coefficient.

[a] NORMAN EMPODAT Database (https://www.norman-network.com/nds/empodat/chemicalSearch.php?s=new), restricting the search for wastewater samples, period 2019 to 2024, concentrations >LOQ, and samples per compound ≥ 50.

[b] CompTox predicted average (https://comptox.epa.gov/dashboard/).

*PPP: Plant protection product.

**PCP: personal care product.

Although the scientific community does not fully understand why, their physical and chemical properties and environmental parameters seem to drive the persistence of these substances. In addition, they have the potential to be transported over long distances, being found far away from the pollution source (Burri et al. 2019; Islam et al. 2022; Ray and Shaju 2023). On the other hand, several pharmaceuticals, including CBZ and DIC reported in Table 01, do not have regulations and restrictions in leading chemical-producing countries (Europe, USA, China, Canada, and Switzerland) (Burri et al. 2019).

The compounds reported in Table 01 present a wide variation of chemical composition and physicochemical properties. As a consequence, these chemicals will behave differently when reaching aquatic environments. Indeed, some are more likely to achieve and persist in these environments because of their characteristics. For example, the pK_a values vary from 9.25 (HCTZ) to 0.91 (DIC). Both compounds belong to the pharmaceutical class, but the first one is more likely to dissociate completely in aqueous solution. The pK_a is related to the pH of the environment, and thus, when a compound has a high pK_a value, it will adsorb better when in acidic conditions (National Research Council 2014; Jjemba, 2018).

Log Kow's values are related to a compound's mobility in aquatic environments. Negative values, such as for HCTZ (-0.06), MET (-1.42), and SCL (-0.29), indicate that these substances are more likely to remain dissolved in water rather than adsorbing onto soil, sediment, and living organisms. Compared to these compounds, the xenobiotics DIC, TEB, and TER have higher Log K_{ow} values (see Table 01), thus presenting hydrophobic behavior and lower bioaccumulation and toxicity potential (National Research Council 2014).

The K_{oc} is also related to mobility, but it has a particular relationship with soil adsorption in this case. Higher K_{oc} values, such as 5,750 for DIC, 715 for TEB, and 657 for TER, indicate a high affinity for soil particles, which is a good characteristic when considering water contamination. When the compound has a low K_{oc}, as is the case of MET, SCL, and HCTZ (see Table 01), it increases its mobility in soil and raises concerns about surface and subsurface water contamination.

Note that the physicochemical properties above are related and corroborate synergistically for a compound to migrate to the aqueous phase rather than adsorb on surfaces and pores. Besides, the environmental conditions (e.g., temperature, pH, microbial community composition, and nutrient availability) are essential for understanding the behavior of a compound once it reaches aquatic environments (National Research Council 2014).

The implications of this behavior in terms of human and environmental health will be discussed in detail in the next section of this chapter. For instance, we can highlight (i) the

biodegradation half-time and (ii) the concentration data on environmental samples. These two factors are dependent, i.e., the rate at which the concentration of a compound decreases by half due to biological processes depends on the initial concentration of the compound; in the same way, the initial concentration will drive the decay rate and can induce or inhibit biological activity (Mattoli et al. 2022; Hammershøj et al. 2020).

As presented in Table 01, biodegradation half-time values of the 19 compounds can vary from 2.51 (MBT) to 7.41 (VAL) days, indicating that natural biological processes are inefficient in degrading these xenobiotics. It is important to note that these chemicals are often released into aquatic environments in a complex chemical mixture, i.e., the active ingredient of a pharmaceutical or biocide product is part of a formulation combining several chemicals. This fact is often ignored when estimating the biodegradation half-time, which results in the wrong output, as the global result is not the sum of the parts (Mattoli et al. 2022).

Regarding the concentration data on wastewater samples, the data presented in Table 01 reinforces that conventional wastewater treatment technologies have not been efficient in degrading these persistent xenobiotics. The average concentration ranged from 0.07 $\mu g\ L^{-1}$ for the biocide imidacloprid (IMI, n= 52 samples) to 15.83 $\mu g\ L^{-1}$ for the pharmaceutical metformin (MET, n= 56 samples). In the case of MET, there is a high standard deviation between the minimum ($4.1\times10^{-3}\ \mu g\ L^{-1}$) and maximum (139.96 $\mu g\ L^{-1}$) concentrations.

By searching for the location of the wastewater sample that presented the higher concentration of MET, using the geo-referenced data, it was found that it is from a WWTP that collects and treats municipal wastewater from Hodonín District, Czech Republic (https://vak-hod.cz/). The plant comprises a pretreatment step, a primary sedimentation tank, and a sequencing batch reactor (SBR) with activated sludge and operates at a daily flow of 6549 m^3/day. When analyzing the complete dataset of this treatment plant (n= 8,396), it was found that the sample that contained a high concentration of MET (collected in August 2019) also had 167 other compounds, which resulted in a summed concentration of 1,015.62 $\mu g\ L^{-1}$ of mixed xenobiotics.

A similar case was reported for a municipal WWTP located in Giurgiu District (Romania), which comprises the exact treatment steps previously mentioned and operates at a daily flow of 9600 m^3/day. The sample collected in August 2019 contained low concentrations of the compounds listed in Table 01, but it had a total of 152 compounds, resulting in a summed concentration of 3,441.13 $\mu g\ L^{-1}$. The case of these two WWTPs mentioned above highlights the importance of considering that the environmental pollutants are found as a complex mixture

that sometimes reaches bulk concentrations. This fact creates a cumulative effect on the environment (Burri et al. 2019)

From a socioeconomic perspective, it is essential to highlight the differences between low-, middle-, and high-income countries. While monitoring campaigns, regulations, and awareness are moving in the right direction in regions such as North America and Europe, in countries from sub-Saharan Africa, south Asia, and South America, the scenarios of wastewater management and availability of safe drinking water are alarming. The study by Wilkinson et al. (2022) of pharmaceutical occurrence along 258 rivers in 104 countries of all continents (1,052 sampling sites) found that the most contaminated samples were from low- and middle-income countries (e.g., Ethiopia, Tunisia, Pakistan, and India). Besides the reality of poor sanitation conditions, these regions had received limited or no previous monitoring campaigns of the aquatic environment up to this study (conducted between 2018 and 2020).

3 THE CONSEQUENCES FOR AQUATIC ECOSYSTEMS AND HUMAN HEALTH DUE TO EXPOSURE TO XENOBIOTICS

Aquatic ecosystems can be divided into two large groups: freshwater and marine environments. Both are home to many plant and animal species and are also an essential part of the life cycle of some species. On the other hand, aquatic environments suffer a series of effects due to exposure to different substances from different sources of pollution (AbuQamar et al. 2024).

More specifically, when it comes to runoff pollution, pesticides used in agricultural management increase the concentration of nitrogen and phosphorus, which in aquatic environments, in addition to creating dead zones, affect the biochemical processes of organisms that inhabit these environments. More specifically, these chemical compounds can break the cell membranes of aquatic organisms, hindering their reproduction and survival, and may even contribute to the extinction of species. As for human health, studies provide information on the relationship between contact with pesticides and the occurrence of cancer, reproductive problems, and cardiovascular and neurological diseases (long-term consequences) (Cavalier, Trasande, and Porta 2023).

The stability and solubility of these contaminants will guide their behavior and bioavailability in the aquatic environment. As a rule, when the marine environment is contaminated with pesticides, these compounds enter the human body through skin contact or ingesting contaminated food (via irrigation with contaminated water or direct application).

After contact, poisoning may present acute symptoms, such as burning eyes, skin rashes, nausea, and dizziness. On the other hand, in long-term manifestations, the list of associations with chronic diseases is extensive, some of which are leukemia, autism, childhood cancer, Alzheimer's disease, Parkinson's disease, endocrine system disruption, neurological and developmental toxicity, among others (Xu et al. 2023). Still focusing on the effects of pesticides, studies have demonstrated their impact on the male reproductive system, including damage to sperm DNA, directly affecting the number, mobility, density, and viability of sperm (Islam et al. 2022).

As for aquatic life, the improper disposal of pesticides mainly affects mammals, corals, and fish. The main effects include DNA damage, rupture of cellular components, thinning of eggshells, reproductive failure, suppression of the immune system, excessive production of slime on scales and gills, and development of tumors. In addition to the organisms themselves, pesticides strongly impact the entire cycle of the aquatic ecosystem, limiting the flow of the food chain, causing ecological balance, and, unfortunately, extinction (AbuQamar et al. 2024).

When the aquatic environment is exposed to these compounds, the main mechanisms involved are bioaccumulation and biomagnification. Initially, contaminants can adhere to suspended particles (adsorption) and form accumulation layers, directly affecting, for example, benthic invertebrates that usually inhabit these places. The adsorption process can occur directly through contact with water, when the contaminants dissolve in the water and are absorbed by the gills and skin of fish, amphibians, and primary producers such as algae and aquatic plants. In a more complex way, these contaminants can be transmitted via the food chain, with some organisms able to metabolize and eliminate the pollutants, while others, more limited, favor bioaccumulation over time. As contaminants move up the food chain, their concentrations also rise, accumulating pollutants in the bodies of organisms. This phenomenon is called biomagnification, which is highly harmful to the health and biodiversity of the aquatic ecosystem and can have implications for humans through the consumption of fish and other contaminated organisms (Ray and Shaju 2023).

4 MICROALGAE AS BIOREMEDIATORS OF XENOBIOTIC POLLUTANTS

Microalgae are considered unique bioremediators because they present metabolic diversity and their ability to change in response to the environment (Kurade et al. 2016). Based on these characteristics and the need to establish more sustainable treatments, systems using microalgae have been implemented to remove a variety of pollutants, such as pesticides,

pharmaceuticals, personal care products, heavy metals, and oils, in different environments (Goswami, Agrawal, and Verma 2022a)

Some reports showing the efficiency of using microalgae to remove these pollutants in different effluents have already been published. Removal efficiency changes according to the concentration of compounds present in the effluent, the wastewater's origin and components, and the microalgae species used (Goswami, Agrawal, and Verma 2022a).

Pollutant remediation by microalgae occurs through different pathways, mainly bioadsorption, bioaccumulation, and biodegradation. Nitrogen, phosphorus, and carbon, both from organic and inorganic molecules, are assimilated and serve as nutrients for microalgae, being used by cells in the construction of carbohydrates, proteins, and lipids that contribute to the growth of these microalgae (Sutherland and Ralph 2019; Goswami, Agrawal, and Verma 2022). Extracellular degradation of pollutants through photodegradation, hydroxyl ion reaction, and enzymatic reactions can also occur (Goswami, Agrawal, and Verma 2022a).

Bioadsorption occurs through a non-metabolic association between the surface of microalgae and pollutants. The cell wall of microalgae has negatively charged functional groups, such as carboxyl, phosphoryl, and amine, while pollutants have cationic groups, thus being attracted to the cell wall of microalgae. Among the chemical processes observed in this pathway are adsorption, complexation, ion exchange, precipitation, and chelation (Bhatt et al. 2022). Bioadsorption is carried out even by dead microalgae cells, with reports of adsorption of up to 16.7% for some drugs, such as ibuprofen and paracetamol (Goswami, Agrawal, and Verma 2022a).

In bioaccumulation, pollutants are transported from the cell surface into the cell. The bio-concentration factor (BCF) indicates the accumulation of these compounds, which varies according to factors such as the ionization state, bioavailability of pollutants, metabolic rate of microalgae, and environmental parameters. This pathway is only observed in living microalgae cells and can occur through passive, passive-facilitated, or active transport. In passive transport, pollutants cross the cell wall from a region with high concentration to an area with low concentration of these compounds. In contrast, these pollutants diffuse through the cell membrane using transport proteins in passive-facilitated transport. Active transport is done with the application of energy (Bhatt et al. 2022). Reactive oxygen species, important in signaling different cellular activities of microalgae, can be generated in this process. When in very high concentrations, these free radicals can damage proteins, lipids, and even DNA, increasing the rate of mutagenesis (Goswami, Agrawal, and Verma 2022a).

Unlike bioadsorption and bioaccumulation, which filter and remove pollutants from the effluent, biodegradation transforms these pollutants into simpler, non-toxic molecules (Wu et al. 2021). In this type of remediation, the pollutant is degraded by the microalgae's metabolism. It serves as a source of nutrients and electrons, producing algal biomass free of contaminants compared to those generated in the abovementioned mechanisms. Pollutant degradation can occur outside the cell, inside the cell, or both through catalytic reactions divided into two phases. In the first phase, pollutants are degraded into more polar compounds through hydroxylation, carboxylation, decarboxylation, methylation, demethylation, hydrogenation, ring cleavage, and oxidation-reduction. This hydrophilic nature of the metabolites formed facilitates their removal from the cells. In the second phase, the compounds formed in the first phase are transformed into larger polar molecules, increasing their solubilization in water (Bhatt et al. 2022).

Pollutants can also undergo photodegradation and photo-oxidation, both assisted by microalgae. In these cases, microalgae secrete dissolved organic matter, contributing to increased degradation of xenobiotics. In photodegradation, the compounds absorb light and undergo chemical changes, resulting in their decomposition. Already in photo-oxidation, the pollutants react with hydroxyl radicals (Bhatt et al. 2022; Goswami, Agrawal, and Verma 2022a).

Therefore, some examples of microalgae considered for the removal of contaminants are *Desmodesmus serratus* and *Parachlorella kessleri* which are capable of degrading pharmaceutical metabolites present in ibuprofen, paracetamol, and diclofenac (Jiménez-Bambague et al. 2020), as a well such as *Scenedesmus* sp. wich as effective in removing xenobiotics contaminants including carbamazepine and 4-octylphenol (Gojkovic et al. 2019), and *Chlamydomonas reinhardtii* which as capable biodegradable of trichlorfon (Wan et al. 2020).

Still, other microalgae that deserve to be highlighted when removing contaminants are *Chlorella* sp*., Neochloris* sp., and *Chlorococcum* sp. as they have high efficiency in BOD removal. The biomass of *Neochloris sp.* stands out in the production of the highest amount of lipids, as well as in the removal of BOD (<90%) (Singh and Ummalyma 2020). *Chlorococcum* sp. is also known for its versatility and resilience, standing out in the field of biodegradation mitigating damage from organic compounds. In addition, it stands out for its ability to form biofilms that can aid in treatment efficiency, increasing the surface area available for pollutant removal (Singh and Ummalyma 2020).

The use of microalgae in wastewater remediation is a sustainable and efficient strategy. In addition to purifying water, they can produce biomass and biofuels, among other bioproducts with high value-added. This strategy helps to reduce pollution and promotes the circular economy and environmental sustainability (Singh and Ummalyma 2020).

5 BIOCONVERSION OF POLLUTANT-REMEDIATING MICROALGAE INTO VALUE-ADDED PRODUCTS

In addition to acting in the bioremediation of xenobiotic pollutants, microalgae from these processes can be exploited to obtain value-added products. In a circular bioeconomy, the use of microalgal biomass from the treatment of contaminated water can be explored to remove pollutants and recover bioproducts, such as carbohydrates, proteins, lipids, and pigments (Nagarajan et al. 2020).

It is worth mentioning that, for biomass from the bioremediation of xenobiotic pollutants to be used for bioconversion into value-added products, biomass recovery methods such as flocculation, sedimentation, centrifugation, filtration, and flotation are necessary. The flocculation process consists of the accumulation of microalgae utilizing charge neutralization, bridging, and net capture, followed by solid-liquid separation of the biomass from the medium. This method involves flocculants that can be chemicals, physical, or biological and can be used as a single recovery method or as a pre-step for physicochemical recovery processes such as sedimentation (Morais Junior et al. 2020; Liu and Hong 2021). Gravitational sedimentation is among the most used methods of microalgal biomass recovery due to its low operating cost; however, to be efficient in the process, its use is only recommended for microalgae with particles larger than 70 μm and high cell density (Liu and Hong 2021).

Following sedimentation, centrifugation is another widely used recovery method due to its speed and recovery yields of up to 98%. However, even if there is a significant recovery yield, some peculiarities arise from the process that must be considered, such as the operating cost, which ends up becoming high compared to sedimentation and cell damage caused by shear forces (Morais Junior et al. 2020). Filtration, another method of biomass recovery, consists of separating solid biomass from the liquid phase employing filters or membranes, which can be performed in various ways such as vacuum filtration, pressure filtration, microfiltration, ultrafiltration, tangential flow filtration, and dead-end filtration. However, even though it is an effective process, using filter membranes increases its cost since it is often necessary to replace them due to their clogging (Morais Junior et al. 2020). On the other hand, to the recovery

methods already mentioned, flotation is a simple process in which microbubbles of air are dispersed in the aqueous medium, causing the adsorbed cells to move to the surface for recovery (Morais Junior et al. 2020; Liu and Hong 2021).

Once the biomass recovery is carried out through one of the methods mentioned above and based on its composition, these bioproducts can be converted into biofuels, biofertilizers, and biopolymers, which will be addressed below (Bhatt et al. 2022; Shahid et al. 2020).

5.1 BIOFUELS

Obtaining biofuels from microalgal biomass is a widely explored field, especially for converting carbohydrates into bioethanol and lipids into biodiesel. Biomass for biofuels is an attractive alternative since it is cultivated and obtained from alternative media, fixing CO_2, and can be converted into bioenergy, such as biodiesel, bioethanol, and biomethane (Bhatt et al. 2022).

Conversion to biodiesel is of interest since most microalgae have a high content of lipids such as triglycerides, glycolipids, and phospholipids in their composition, which, after being extracted, are subjected to the transesterification process, in which they are converted into methyl esters and fatty acids for the production of biodiesel (Bhatt et al. 2022). Biomass from cultivation in contaminated water media tends to have a higher lipid content due to the presence of chemical compounds that cause oxidative stress, increasing the accumulation of lipids for cellular protection and, as a consequence, increasing the yield in biodiesel production (Okeke et al. 2022; Goswami, Agrawal, and Verma 2022a).

This behavior was observed in the removal of phenol by *Tetradesmus obliquus,* with a 1.6-fold increase in lipid content, which, when converted to biodiesel, showed characteristics by international standards (Gomaa et al. 2022). Even so, it should be noted that oxidative stress needs to be controlled to be beneficial in the accumulation of lipids and prevent cell death. Strategies for the cell to be protected from oxidative damage with high removal of xenobiotics are reported, such as the use of exogenous phytohormones, such as gibberellin, which promoted the protection of cells from *Chlorella vulgaris* that removed 91.8% of sulfamethoxazole (Yang et al. 2023).

The carbohydrate fraction of microalgae can be converted to ethanol by fermentation processes, being considered one of the most sustainable ways to obtain the chemical and produce energy. The carbohydrates in the microalgae's cell wall are broken down by hydrolysis

processes to monosaccharides, followed by conversion by fermentation, usually by yeasts (Serrà et al. 2020).

Microalgal biomass can also produce biogas through anaerobic digestion that converts the carbon in the biomass into methane and CO_2 by the activity of bacteria and archaea methanogenic (Bhatt et al. 2022). Biogas production also allows the evaluation of integrated biofuel production approaches since residual biomass can be obtained sequentially. For example, biodiesel and biogas were produced from *Chlorella* sp. after the microalgae were used to treat wastewater from agricultural runoff, demonstrating a vital potential to make the technology viable (Rana et al. 2024). Another integrated strategy was using *Arthrospira* (*Spirulina*) *platensis* biomass for ethanol production, followed by pelleting and combustion of the residual biomass for thermal energy. In this scenario, the generated ash was used to treat effluent, which was returned as a culture medium for the cyanobacterium *A. platensis* (Serrà et al. 2020).

5.2 PRODUCTS AIMED AT THE AGRICULTURAL SECTOR

In addition to its use to obtain biofuels, microalgal biomass from the bioremediation processes can be used to get products aimed at agriculture, consolidating its advantages as a raw material in a context of circular bioeconomy, since microalgae are mainly used for the removal of chemical compounds such as pesticides from use in agriculture.

Among the main products obtained from microalgal biomass are biochar, biofertilizers, and biomass as supplementation in animal feed. Biochar is one of the by-products obtained from microalgal biomass and is widely applied in the environment. Its production occurs through pyrolysis, a process in which biomass is thermally decomposed by limiting the presence of oxygen. It can be used as an adsorbent catalyst to improve soil conditions and create a CO_2 reservoir in carbon capture. However, the use of biomass from the bioremediation process to obtain this bioproduct is still little reported in the literature, and biomass of the genus *Chlorella* is a potential for securing biochar, as it is the most used in the removal of pollutants (Nie et al. 2020; Amaral et al. 2023).

Microalgal biomass from bioremediation processes can be an alternative to the use of chemical fertilizers; due to the presence of vitamins, minerals, peptides, and amino acids in its composition, biomass can act as a biostimulant in plants, improving germination, nutrient absorption, and growth rates (Moreira et al. 2023). The preparation of microalgae-based biofertilizers takes place through aerobic or anaerobic digestion processes. Aerobic digestion

can be carried out using solid or liquid compounds, whereas in solid composting, biomass, CO_2, and water are responsible for the generation of biofertilizers. In liquid composting, heterotrophic microorganisms degrade organic matter. In anaerobic digestion through hydrolysis, acidogenesis, acetogenesis, and methanogenesis processes carried out by bactéria that use microalgal biomass as a substrate, biofertilizer and biogas are produced (Magro et al. 2021). *Monoraphidium* sp. is one of the microalgae that have the potential to be used as a biofertilizer after being used in bioremediation processes. Jimenez et al. (2020) found through their study the removal of approximately 65% of PO_4 from liquids from the digestate of a biogas plant through these microalgae, a compound present in the vast majority of chemical inputs. When applied to tomato seedlings, the authors found its biostimulant potential with the improvement of plant growth and number of leaves.

Also, among the applications aimed at the agricultural sector, microalgal biomass is used as a supplement in animal feed. Studies evaluating this application began in 1950, and according to Kusmayadi et al. (2021), using this biomass as feed brings advantages to animal physiology, such as improved immune response, antibacterial and antiviral action, and resistance to diseases. Microalgae of the genera *Spirulina* and *Chlorella* are reported in the literature for their use as feed; however, when it comes to the use of biomass from pollutant bioremediation processes, the toxicity of the compound must be evaluated since there may be an accumulation of these substances in the biomass (Kusmayadi et al. 2021).

5.3 BIOPOLYMERS

Another application for microalgal biomass grown in alternative media, such as contaminants in water, is in the production of biopolymers. It is possible to obtain oil-based polymers such as polyhydroxyalkanoates (PHAs) and polylactic acid (PLA) through fermentation. Therefore, the stages of conversion of biomass to polymers consist of (i) plasticization, organic and non-volatile molecules are added to the medium to improve characteristics such as flexibility and durability; (ii) mixture: compatible polymers such as polyethylene or vinyl alcohol are added to the mixture; and finally (iii) compatibility: stage in which the bioplastic is stabilized to be then molded and prepared for application in the market. In this stage, the interfacial properties of the polymers added in stage (ii) are modified (Morais Junior et al. 2020). Among the microalgae with potential for use in the production of biopolymers are *Chlorella* sp. and *Spirulina* sp. due to the proteins present in their composition and the carbohydrate content (Morais Junior et al. 2020; Zeller et al. 2013).

6 CHALLENGES AND LIMITATIONS

The removal of contaminants through the use of microalgae and the use of biomass from these processes for conversion into valuable compounds is promising and environmentally sustainable; however, it still presents some challenges and limitations. Bioremediation with microalgae has been applied, and its efficiency in the process has been demonstrated. Still, for this to be possible, it is necessary to know the composition of the wastewater to which it will be exposed to choose the microalgae to be used because some compounds can inhibit growth, and therefore, there is no removal of contaminants or even the intercropping of microalgae with other microorganisms is necessary to be efficient in bioremediation (Yadav et al. 2021; Goswami, Agrawal, and Verma 2022).

Furthermore, most of the studies found in the literature are processes carried out on a laboratory scale due to the high cost of obtaining photobioreactors on an industrial scale (Bhatt et al. 2022) and having, as a consequence, the low yield in obtaining biomass for conversion into valuable compounds. Another factor that must be considered is the toxicity of the compounds obtained from the bioconversion of biomass from bioremediation processes due to the lack of studies investigating how these contaminants are stored in microalgae cells (Yadav et al. 2021).

As seen, there are still many factors to be investigated when it comes to the removal of xenobiotic contaminants based on the use of microalgae, from their application as a bioremediation agent to the pollutants that are evaluated for the use of this process due to the presence of several compounds that have not yet been investigated for their removal using microalgae.

7 CONCLUSION

The presence of xenobiotic contaminants in water is one of the many consequences of anthropogenic actions that affect aquatic ecosystems and human health. The search for sustainable alternatives, such as using microalgae to remove these contaminants from polluting sources, such as wastewater treatment plants, is a way to reduce the negative impacts caused by these actions. However, as observed, there are still many limitations when employing bioremediation, mainly aiming at the bioconversion of biomass into valuable compounds, and studies are still needed to fill these gaps.

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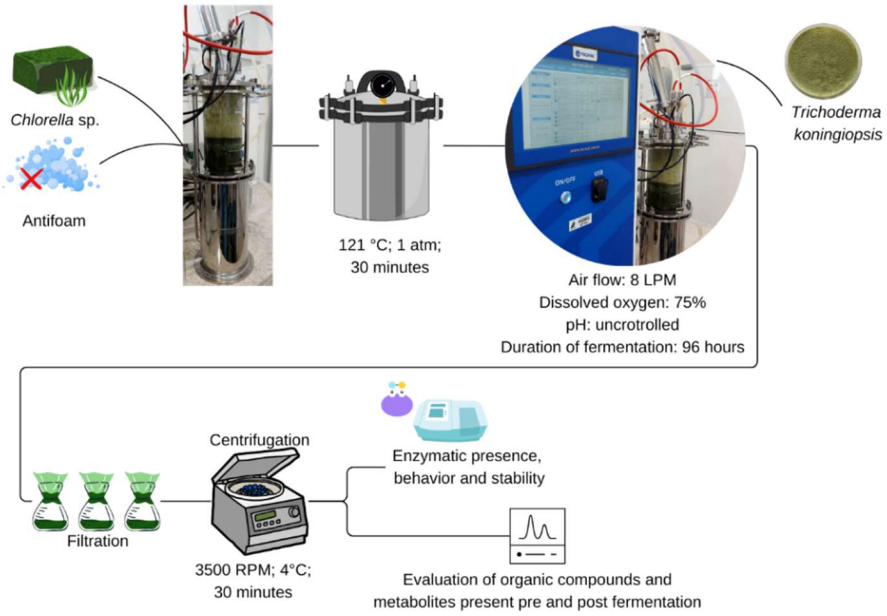
CAPÍTULO 02: Characterization enzymatic and metabolic of a biocomposite based on *Trichoderma koningiopsis* and *Chlorella* biomass

ABSTRACT

The gradual concern with promoting sustainability has encouraged the search for new methodologies for obtaining bioproducts. This study, therefore, analyzed the generation of a fungal biocomposite using *Trichoderma koningiopsis* and biomass of the microalgae *Chlorella* sp. from swine wastewater, aiming at a product with potential for environmental and agronomic application. The process involved submerged fermentation in an Airlift bioreactor, using microalgal biomass as the substrate and *Trichoderma koningiopsis,* such as fermentative microorganisms. Afterward, the enzymatic activity and stability were evaluated, and the stability was monitored for 90 days at different storage temperatures (20°C, 4°C, and -80°C). The results demonstrated considerable enzymatic activity of the biocomposite, mainly for catalase (1274,79 μmol/min.mL), peroxidase (52,08 U/mL), and protease (97,50 U/mL), with good stability over time**.** By analyzing the organic compounds before and after fermentation using UHPLC-ESI-qTOF-MS/MS, the production of metabolites such as tyrosine and leucine which have herbicidal potential, koningins a, b, and d, trichodermin, which are considered antifungal agents and of relevance in the health area, in addition to primary metabolites such as fructose, organic acids, and ethanol, was observed. Therefore, through this study, it was possible to highlight the potential of the biocomposite obtained as a matrix of products aimed at the environmental and agricultural are through enzymes and metabolites of interest for producing bioinputs and removing contaminants in effluents.

Keywords: Antioxidant activity; Catalase; Enzyme; Metabolites; Tyrosine; Agriculture; Environmental.

Graphical abstract



1. INTRODUCTION

Concern about the inconsistent use of natural resources has been considered important in developing new products and techniques for different industrial and agricultural sectors. Given this concern, the member states of the United Nations have adopted a set of global goals known as the Sustainable Development Goals, which include reducing the generation of solid and chemical waste, ensuring sustainable patterns of production and consumption, and ensuring systems for preserving food production through the implementation of practices that do not affect the qualities of ecosystems and that can strengthen adaptation to climate change and improve the quality of land and soil (Merlo et al., 2021). In this sense, searching for raw materials and a green/sustainable technological approach for their use is convenient. Numerous residues from industrial and agricultural sectors can serve as raw materials in microbial processes, reducing the use of chemicals and avoiding costs (López-Hernández et al., 2022). Among these residues, microalgal biomass stands out, which can be obtained from wastewater from biogas production and, due to its rich composition, serve as raw material for various applications in different areas (Brasil et al., 2017; Matthiensen and Michelon, 2022).

Microalgae are unicellular and photosynthetic organisms; that is, they can convert solar energy into chemical energy and, due to the process carried out by these organisms, microalgae have in their composition several bioactives, amino acids, vitamins, lipids, proteins, and sugars that make them an attractive raw material (López-Hernández et al., 2022; Matthiensen and Michelon, 2022; Priyadarshani and Rath, 2012). In addition, microalgae can be cultivated in

different media, such as fresh seawater, brackish water, and wastewater collected continuously throughout the year (Brasil et al., 2017; Chisti, 2007).

Due to its composition, microalgal biomass brings numerous opportunities for use, such as the production of biofuels, wastewater treatment, organic compounds, and bio inputs, driving sustainable development (Oliveira et al., 2022). However, the use of this raw material requires processes that facilitate access to the components present in the biomass, such as the use of commercial enzymes, which involve a custodial process, requiring economic technical means aimed at the production of these enzymes and which at the same time allow easier access to the biomass components (Klein-Marcuschamer et al., 2012). Several commercially available enzymes can be obtained from filamentous fungal microorganisms, such as strains of the *Trichoderma* spp., which are recognized for being excellent enzyme producers because, when using the available substrate, they secrete metabolites and enzymes such as amylases, cellulase, oxidoreductases, proteases, phytohormones, organic acids and alcohols, and are therefore considered an alternative to facilitate access to the components present in the biomass through fermentation processes (Bader et al., 2020; Camargo et al., 2023a, 2024; Ferreira Filho et al., 2017; Macías-Rodríguez et al., 2020; Sutaoney et al., 2024; Sperandio and Ferreira Filho, 2021; Lima et al., 2022; Goorue et al., 2022).

In addition to being less costly, microbial fermentation processes have as final product several organic and metabolic compounds; therefore, a biocomposite obtained from fermentation processes with enzyme-secreting microorganisms, such as *Trichoderma* spp., can be used for application in several fields, such as energy production, basic sanitation, wastewater, and agricultural water treatment, and as a bioherbicide and bioinsecticide (Camargo et al., 2024, 2023; El-Dalatony et al., 2016; Orejuela-Escobar et al., 2021). Therefore, this study aimed to evaluate the presence and behavior of different enzymes, organic compounds, and metabolites in fungal biocomposite fermented with *Trichoderma koningiopsis* in an *Airlift* bioreactor.

2. MATERIAL AND METHODS

2.1. OBTAINED FERMENTED FUNGAL BIOCOMPOSITE

The fermented fungal biocomposite was obtained according to the methodology of Camargo et al. (2024) through submerged fermentation in an *Airlift* bioreactor. Therefore, the fermentation medium consisted of 200g of microalgal biomass composed mainly of *Chlorella* sp., 400 mL of antifoam, 1400 mL of distilled water, and 10^7 cells per milliliter of inoculum of fermenting microorganism. The microalgal biomass used in this study comes from the

phycoremediation of swine wastewater, implemented at EMBRAPA Swine and Poultry (Concórdia, SC, Brazil) (Michelon et al., 2016). The microalgal biomass comprises 56,1% protein, 34,7% carbohydrate, 1,7% lipid, and 7,8% minerals (Stefanski et al., 2020). The microorganism used in the fermentation process is the *Trichoderma koningiopsis* (identification code in GenBank MK860714) and was isolated from the weed *Digitaria ciliares* (Reichert Júnior et al., 2019).

Fermentation occurred in a benchtop *Airlift* bioreactor, model Bio-Tec-Pro-II (Tecnal, Brazil), with a practical volume of 3.0 L. The fermentation in this study occurred with a final volume of 2 L. The fermentation conditions establish dissolved oxygen at 75% for the best performance of the microorganism, air flow equal to 8 liters per minute, and uncontrolled pH. The fermentation process lasted 96 hours, and at the end, the biocomposite was filtered by manual pressing in synthetic fabric, the retained solid was sterilized and discarded, and the liquid permeate was centrifuged (NT 815-NovaTecnica, Brazil) at 2000 rpm, 04°C for 30 min. The centrifugation supernatant was used for this study (Camargo et al., 2024).

2.2. PRESENCE AND BEHAVIOR OF ENZYMES IN THE FUNGIC BIOCOMPOSITE IN DIFFERENT PH AND TEMPERATURE RANGES

Different enzymes in the fermented fungal biocomposite were evaluated, including:

- Amylase

  The activity of the amylase enzyme was determined by the methodology of Fuwa (1954) and Pongsawasdi and Yagisawa (1987) using soluble starch as substrate. The starch was diluted in the buffer by 1:100 (m:v) proportion. Afterward, 01 mL of this solution was mixed with 01 mL of the fungal biocomposite and kept in a thermostatic bath for 10 minutes. At the end of this time, the enzymatic activity was quantified by the release of total reducing sugars by the DNS method (3,5-dinitrosalysic acid) according to Miller (1959), in which 0.5 mL of the initial reaction solution was mixed with 0.5 mL of DNS and kept in a thermostatic bath at 100°C for 10 minutes. Then, the tubes containing the reaction medium were kept in an ice bath, and the reaction was stopped with sodium tartrate. Absorbance was determined in a spectrophotometer at a wavelength of 540 nm. One unit of amylase activity is defined as the amount of enzyme capable of releasing 01 μmol of glucose per minute under the reaction conditions.

- Cellulase

The quantification of the cellulase enzyme was performed according to the methodology adapted from Ghose (1987) using cellulose as a standard substrate. Therefore, the reaction medium was composed of 50 mg of Whatman filter paper n° 01, 02 mL of buffer, and 01 mL of fungal biocomposite and kept in a thermostatic bath for 01 hours. At the end of the reaction, the cellulase activity was determined by the release of total reducing sugars by the DNS method (3,5-dinitrosalysic acid) (Miller, 1959), and the absorbance was determined in the spectrophotometer using a wavelength of 540 nm. The results were expressed as cellulase activity of the filter paper (FPase). Thus, one FPase activity (U) unit is the enzyme capable of releasing 01 µmol of glucose per minute under current conditions.

- Laccase

Hou et al. (2004) determined laccase activity using 2,2′-azino-di-3-ethylbenzothialozin-6-sulfonic acid (ABTS) as a standard substrate. The reaction medium comprised 0.4 mL of ABTS 0.01M, 0.2 mL of fungal biocomposite, and 3.4 mL of buffer. The test tubes containing the reaction medium were kept in a thermostatic bath for 04 minutes. Ultimately, the absorbance was quantified using a spectrophotometer at a wavelength of 420 nm. One unit of laccase activity (U) is defined as an amount of enzyme capable of forming 01 µmol of $ABTS^{+}$ per minute under the conditions of occurrence.

- Lipase

The activity of the lipase enzyme was determined according to the methodology of Treichel et al. (2016), in which an emulsion of 10% (m:v) olive oil and 5% (m:v) gum arabic diluted in 90% (v:v) buffer was used. The reaction medium comprised 01 mL of fungal biocomposite and 09 mL of emulsion, which was incubated in an orbital shaker at different temperatures at 165 RPM for 32 minutes. The reaction was stopped by adding 10 mL of acetone/ethanol solution (1:1 v/v). Subsequently, the medium was subjected to the titration process, adding 0.049M NaOH until the medium reached pH 11. The determination of lipase activity was calculated using equation 01.

$$At = \left(\frac{(Va- \quad)*M*1000}{t*Vc}\right) * 1000 \quad \text{Equation 01}$$

Where:

Va = The average volume of NaOH used in the titration of the samples (mL);

Vb = The average volume of NaOH used in the titration of the reaction blank (mL);

M = The molarity of NaOH;

t = Reaction time (min);

Vc = The volume of fungal biocomposite in the reaction (mL).

A unit of lipase activity (U) is defined as the amount of enzyme capable of hydrolyzing 01 µmol of substrate per minute under the reaction conditions.

- Protease

  The activity of the protease enzyme was determined according to the methodology adapted from Waghmare et al. (2015). The reaction medium consisted of 01 mL of casein, 01 mL of fungal biocomposite, and 0,5 mL of buffer. The reaction medium was kept in an ultra thermostatic bath for 30 minutes. Then, the reaction was stopped by adding 0.5 mL of trichloroacetic acid, and a 0.5 mL aliquot of the initial reaction medium was collected and mixed with 2.5 mL of 0.05M sodium carbonate buffer and 0.5 mL of 01M Foulin reagent. The reaction mixture was kept at room temperature (20°C) for 20 to 30 minutes, and then absorbance was determined using a spectrophotometer at a wavelength of 660 nm. One unit of enzymatic activity is defined as the amount of enzyme required to release 01 µg of tyrosine residue per minute under the test conditions (Waghmare et al., 2015).

- Peroxidase

  The activity of the peroxidase enzyme was determined by the methodology of Khan and Robinson (1994) and Devaiah and Shetty (2009), in which the reaction medium consisted of 1.5 mL of buffer, 02 mL of distilled water, 0.5 mL of 1% guaiacol, 01 mL of 8% hydrogen peroxide, and 01 fungal biocomposite. The reaction medium was kept in an ultrathermostatic bath for 20 minutes, and then the absorbance was determined using a spectrophotometer at a wavelength of 470 nm. One unit of peroxidase activity (U) is defined as the amount of enzyme capable of causing an increase in the absorbance unit by 0.001 per minute under the reaction conditions.

- Catalase

  The activity of the catalase enzyme was determined according to the methodology of Havir and Mchale (1987) and Hasan et al. (2022), in which the reaction medium was composed of 01.5 mL of buffer, 0.9 mL of distilled water, 0.1 mL of fungal biocomposite and 0.5 of 0.0125M hydrogen peroxide. The reaction medium was kept in an ultrathermostatic bath for 02 minutes. Then, the absorbance

of the oxidized compounds was quantified in a spectrophotometer at 240 nm for 03 minutes, taking readings every 30 seconds.

- Ascorbate peroxidase

  The activity of the ascorbate peroxidase enzyme was determined according to the methodology of Nakano and Asada (1981) and Fal et al. (2022), in which the reaction medium was composed of 01.5 mL of buffer, 0.86 of distilled water, 0.24 mL of 0.008M ascorbic acid, 0.1 mL of fungal biocomposite, and 0.3 mL of 0.001M hydrogen peroxide. The reaction medium was kept in an ultrathermostatic bath for 02 minutes. Then, the absorbance was quantified in a spectrophotometer at a wavelength of 290 nm for 01 minute, taking readings every 15 seconds.

- Superoxide dismutase

  The activity of the superoxide dismutase enzyme was determined according to the methodology of Hasan et al. (2022), in which the reaction medium was composed of 01.5 mL of buffer, 0.78 mL of 0.013M methionine, 0.225 mL of 75µM of NBT, 0.06 mL of 0.001M EDTA, 0.06 mL of 02µM riboflavin, 0.345 mL of distilled water, and 30 µL of fungal biocomposite. The reaction occurred with exposure to light, using a 15W fluorescent lamp, and the absorbance was quantified in a spectrophotometer at a wavelength equal to 560 nm with readings taken every 15 seconds within 01 minute. The reaction blank was performed without exposure to light. The activity of the superoxide dismutase is given by the enzyme required to inhibit 50% of the photoreduction of NBT.

Activity determinations were performed according to each enzyme's standard methodology (cited above), and the reaction medium's pH and temperature were varied to analyze the behavior of enzymatic activities, as shown in Table 01. For the enzyme superoxide dismutase, the pH range of the reaction medium and the exposure time to light was varied, as indicated in Table 02, since the enzymatic determination reaction occurs with exposure to light.

Table 01: Variables analyzed in the experimental design.

Value coded	pH	Temperature (ºC)
-1	4.0	20.0
+1	10.0	80.0

0	7.0	50.0
+1.41	11.24	92.43
-1.41	2.76	7.57

For the enzyme experimental design tests, the following buffers were used: acetate (pH 4.0), sodium phosphate (pH 7.0), Na_2HPO_4 + NaOH (pH 10.0), Na_2HPO_4 + citric acid (pH 2.76), and Na_2HPO_4 + NaOH (pH 11.24) with a concentration of 0.1 M. For the standard methodology tests of enzyme activity, the buffers were used: 0.1 acetate buffer (pH 5.5) for cellulase, 0.05 M acetate buffer (pH 5.0) for laccase, 0.1M sodium phosphate buffer (pH 6.0) for lipase, 0.05 M glycine NaOH buffer (pH 9.0) for protease, 0.005 M phosphate buffer (pH 5.0) for peroxidase, 0.05 M sodium phosphate buffer (pH 7.8) for superoxide dismutase, 0.05M potassium phosphate buffer (pH 6.8) for catalase, and 0.05M potassium phosphate buffer (pH 6.0) for ascorbate peroxidase.

Table 02: Variables analyzed in the enzymatic determination reactions of superoxide dismutase.

Value coded	**pH**	**Time of exposition (minutes)**
-1	4.0	2
+1	10.0	10
0	7.0	6
+1.41	11.24	11.66
-1.41	2.76	0.34

2.3.EVALUATION OF ENZYME ACTIVITY STABILITY

After obtaining the best reaction condition for determining the enzymes present in the fungal biocomposite, the stability of their activity was evaluated. Therefore, the stability of the enzymes present in the fungal biocomposite was performed every 30 days, with the biocomposite being stored at different temperatures (20°C, 04°C, and -80°C) for 90 days.

2.4.EVALUATION OF COMPOUNDS AND METABOLITES PRESENT PRE AND POST-FERMENTATION IN THE FUNGAL BIOCOMPOSITE

The presence of organic compounds pre and post-fermentation was analyzed by high-performance liquid chromatography – HPLC (Shimadzu chromatograph), equipped with a RID 10-A refractive index detector and AMINEX® BIORAD HPX87H column. The samples were chromatographed at 45°C, using H_2SO_4 0.005 M as mobile phase and a flow rate of 0.6 mL/min, and the concentrations of the compounds were determined by calibration curves (Bazoti et al., 2017).

The presence of metabolites and other compounds was performed using a Nexera X2 UHPLC system (Shimadzu) and the Impact II MS (Bruker Corporation Massachusetts) with electrospray ionization (ESI) source and quadrupole-time of flight (Q-TOF). Samples were injected into an ACQUITY UPLC$^{@}$ column T3 (50 × 2.1 mm, 1.8 μm particle size). The mobile phase comprised a gradient of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. A linear gradient was applied: 0-0.5 min, 0% B; 0.5-8 min, 100% B; 8-9 min isocratic at 100% B; 9-10 min, 0% B. The mobile phase flow rate was 0.5 mL min^{-1}, and the temperature was 40 °C. The analysis was performed with an injection volume of 5 μL per sample. UHPLC was coupled to QTOF-MS with an ESI source operated in positive and negative ionization modes to enable comprehensive coverage of the metabolite profile. The settings of the mass spectrometer (MS) were as follows: gas temperature of 310 °C, gas flow of 8 L min^{-1}, nebulizer of 4.5 bar, and mass range of 50–1000 *m/z*. The MS/MS was performed automatically with a limit of 1500 counts and literature data. The annotation of the compounds present was carried out by comparing the fragmentation data of the compounds found in the samples using spectral libraries, fragmentation data obtained from the literature, GNPS2 online platform (https://gnps2.org/) and *SIRIUS software* (https://bio.informatik.uni-jena.de/software/sirius/).

3. RESULTS AND DISCUSSION

3.1. ASSESSMENT OF THE PRESENCE AND BEHAVIOR OF ENZYMES IN THE BIOCOMPOSITE

In this study, it was possible to observe the presence of several enzymes in the obtained biocomposite through fermentation and the activation of some of them when subjected to reaction media with different pH ranges and temperatures concerning their standard quantification methodology. The production of this range of enzymes in the biocomposite is due to the action of the microorganism used in this study; *Trichoderma* is known to use the substrate present in the medium efficiently, successfully colonizing the habitat in which it is inserted, resulting in the secretion of metabolites and enzymes, for example (Schuster and Schmoll, 2010). Therefore, from the results obtained regarding the production of enzymes, it is because *Trichoderma koningiopsis* efficiently uses microalgal biomass as a source of nutrients, which has already been observed in other studies (Camargo et al., 2024; Stefanski et al., 2020).

By observing the enzymatic activities determined by the standard methodology of quantification and by being subjected to a reaction medium to different pH and temperature ranges, it was possible to verify that enzymes such as amylase, cellulase, superoxide dismutase, and ascorbate peroxidase are only activated when exposed to certain pHs and temperatures different from that used for quantification in the standard methodology, as shown in Table 03 and Table 04.

Table 03: Enzymes quantified according to the standard methodology.

Standard methodology			
Enzyme	**pH**	**Temperature (°C)**	**Enzymatic activity**
Amylase	5.0	38	0.00 U/mL
Cellulase	5.5	50	0.69 U/mL
Lipase	5.0	40	0.00 U/mL
Laccase	6.0	35	0.00 U/mL
Protease	9.0	40	97.50 U/mL
Peroxidase	5.0	35	52.08 U/mL
Catalase	6.8	25	216.80 µmol/min.ml
Ascorbate peroxidase	6.0	25	4.95 U/mL
Enzyme	**pH**	**Time of Exposition (minutes)**	**Enzymatic activity**
Superoxide dismutase	7.8	05	0.00 U/mL

Table 04: Enzymes present in biocomposite and their behavior in reaction media with different pHs and temperatures.

Assay	pH	Temperature (°C)	Amylase (U/mL)	Cellulase (U/mL)	Lipase (U/mL)	Laccase (U/mL)	Protease (U/mL)	Peroxidase (U/mL)	Catalase (µmol/min.ml)	Ascorbate Peroxidase (U/mL)
1	4 (-1)	20 (-1)	13.09	0.05	0.29	0.02	0.00	8.17	198.67	0.00
2	10 (1)	20 (-1)	0.00	0.15	0.79	0.01	2.89	8.83	168.52	3.32
3	4 (-1)	80 (1)	0.00	0.61	0.00	0.00	2.33	5.00	215.05	0.00
4	10 (1)	80 (1)	40.52	1.28	0.25	0.01	2.00	3.00	204.78	0.00
5	2.76 (-1.41)	50 (0)	2.91	0.00	0.89	0.00	0.56	7.67	1274.79	6.31
6	11.24 (+1.41)	50 (0)	3.30	0.19	1.22	0.01	4.67	6.33	154.33	6.28
7	7 (0)	7.57 (-1.41)	11.15	0.24	0.00	0.00	4.00	1.50	168.09	18.20
8	7 (0)	92.43 (+1.41)	3.39	0.10	0.00	0.04	0.00	6.83	195.06	8.79
9	7 (0)	50 (0)	8.53	0.12	0.75	0.00	0.11	5.83	153.78	10.86
10	7 (0)	50 (0)	7,37	0.12	0.00	0.00	0.00	5.83	160.55	10.64
11	7 (0)	50 (0)	11.73	0.24	0.29	0.00	0.56	6.17	162.35	10.37

When analyzing the behavior of amylase enzymatic activity and given its way of obtaining, the enzyme present in this biocomposite is an α-amylase. The α-amylase is stable in a wide temperature range, from 30 to 90°C, in addition to performing its optimal function at alkaline pH (Farooq et al., 2021), which corroborates what was obtained in this study, since the standard methodology in which the reaction pH was 5. Initially, the temperature was equal to 38°C, and there was no enzymatic activity. For pH 10 and reaction temperature of 80°C, activity equal to 40.52 U/mL was obtained.

Regarding the fermentation process, the production of this enzyme is due to the fact that the microorganisms of the genus *Trichoderma* produce high amounts of extracellular amylase when they have available starch (Abdulaal, 2018). Considering that the microalgal biomass used in this study has about 37% carbohydrates, it is found that starch is also available to the microorganism (Bader et al., 2023). Also, it is necessary to highlight that the production of this enzyme by *Trichoderma koningiopsis* and microalgal biomass is still little reported in the literature.

Cellulase also demonstrated its best behavior in assay 04, in an alkaline reaction medium and high temperature, reaching 1.28 U/mL, representing a relative activity of 185,51% concerning the use of the standard methodology of quantification of the enzyme, where the reaction medium occurred at 50°C and pH 5.5. Therefore, when considering that both enzymes (amylase and cellulase) obtained high activities in the same assay, it can be concluded that among the variables analyzed in the assay, the one that influenced the increase in activity and enzymatic activation is the reaction temperature. Furthermore, cellulose in the fermentation medium is necessary for producing this enzyme by the microorganism Trichoderma, acting as a carbon source for the microorganism. Among the substrate alternatives containing this composition, there is microalgal biomass because cellulose is one of the main components of the cell walls of this organism (Yan et al., 2021; Zanchetta et al., 2021). In this study, it was also possible to obtain this enzyme by *Trichoderma koningiopsis*, which is little reported in the literature for the production of cellulase, resulting in activities very close to those reported by the studies of Shokrkar et al. (2022) and Shokrkar and Zamani (2023), who use microalgae as a nitrogen source together with other nutrients in the fermentation medium, and T. reesei as fermenting microorganism and resulted in cellulolytic activities equal to 1.34 U/mL and 2.29 U/mL, respectively.

For protease, differently amylase, and cellulase, the highest enzymatic activity occurred using the standard methodology in which the reaction occurred at 40°C and pH 9.0 and resulted in 97.50 U/mL of activity. This result demonstrates the efficiency of the microorganism in using

the microalgae as a substrate and its ability to produce this enzyme. In addition to the production of cellulolytic enzymes, microorganisms of the genus *Trichoderma* are also producers of proteolytic enzymes, especially the filamentous fungus *Trichoderma reesei*, which is considered a source of proteases, as its genome has a variety of genes that encode extracellular proteases (Haab et al., 1990; Sun et al., 2021). The production of this enzyme by *Trichoderma reesei* using different substrates, mainly agro-industrial residues, is widely reported in the literature (Lizardi-Jiménez et al., 2019; Qian et al., 2019; Sharma et al., 2016; Sun et al., 2021). However, using *Trichoderma koningiopsis* and microalgae as the substrate is still rarely reported, so this study bring as new way to obtain this enzyme.

For lipase and laccase enzymes, the activity values obtained in most tests were less than 1 U/mL and, therefore, through the evaluation of the presence and behavior of both enzymes carried out in this study, it can be verified that there is no presence in the fungal biocomposite.

Regarding the antioxidant activity of the fermented composite, the presence of four antioxidant enzymes was evaluated, namely peroxidase, catalase, ascorbate peroxidase, and superoxide dismutase. The peroxidases present in the biocomposite are stable at various pH and temperature ranges, as described in Table 03, with their highest activity value in the standard methodology, equal to 52.08 U/mL, in which the reaction occurs at pH 5.0 and 35°C. Peroxidases require H_2O_2 or organic hydroperoxides as a cosubstrate to catalyze oxidative reactions, in addition to having different classifications due to their form of production (Demarche et al., 2012; Passardi et al., 2007). The family of plant peroxidases includes those considered heme peroxidases of fungal and bacterial origin, and this class is subdivided into three other classes. In the case of the study and based on the fermentation medium, the peroxidase present in the composite belongs to class II of the family of plant peroxidases, which are secreting fungal peroxidases, which can be lignin (LiP) or manganese (MnP) peroxidases (Si and Cui, 2013).

Catalase, responsible for the degradation or reduction of hydrogen peroxide into water and oxygen (Gauthier et al., 2020; Ighodaro and Akinloye, 2018), showed activity in all the experimental designs trials and the standard methodology, highlighting assay 05, in which the reaction occurred at pH 2.76 and 50°C and resulted in 1274.79 μmol/min.mL. The increase in activity in assay 05 is due to the enzyme's affinity for more acidic pHs and high temperatures, corroborating what is described in the literature in which fungal catalases present their best behavior under these conditions (Isobe et al., 2006). In addition, it is necessary to highlight the activity values in the other assays of the planning and in the standard methodology in which it is possible to observe the performance of the enzyme in a wide range of pH and temperature,

this production being high due to its abundance in the biocomposite and the ability to break millions of hydrogen peroxide molecules in just one second (Ighodaro and Akinloye, 2018).

The enzyme ascorbate peroxidase, which also acts in the reduction of H_2O_2 with the aid of ascorbate and which has at the end of the reaction water and monodehydroascorbate (MDHA) as products (Gauthier et al., 2020; Noctor and Foyer, 1998), had its highest activity values in the experimental design assays, as described in Table 03, highlighting the 07 assay that resulted in 18.20 U/mL of activity. The activity resulting from the test at pH 7.0 and 7.57°C was due to the decrease in temperature since the other assays that occurred at pH 7.0 resulted in lower activity values.

Finally, when evaluating the behavior of the enzyme superoxide dismutase (SOD), results were obtained only in assays 04, 05, and 07, as described in Table 05. Superoxide dismutase acts in the detoxification of reactive oxygens species; that is, it catalyzes the dismutation of superoxide anion molecules into hydrogen peroxide and molecular oxygen (Gauthier et al., 2020; Ighodaro and Akinloye, 2018) in this study its highest activity value was in assay 04, in which the reaction occurred at pH 10 with a duration of exposure to light of 10 minutes, resulting in 12.51 U/mL of activity, and this result was obtained due to the longer time of exposure to light. An activity value similar to that of this study was obtained by Ismaiel et al. (2016) only for the microalgae *Spirulina* (*Arthrospira*) *platensis*, in which its behavior was evaluated in different pH ranges, and the authors obtained activity equal to 12.53 U/mg of protein at pH 10.5.

Table 05: Behavior of SOD enzymatic activity at different pHs and reaction times.

Essay	pH	Time of exposition (minutes)	Superoxide dismutase (U/mL)
1	4 (-1)	2 (-1)	0.00
2	10 (1)	2 (-1)	0.00
3	4 (-1)	10 (1)	0.00
4	10 (1)	10 (1)	12.51
5	2.76 (-1.41)	6 (0)	1.33
6	11.24 (+1.41)	6 (0)	0.00
7	7 (0)	0.34 (-1.41)	7.98
8	7 (0)	11.66 (+1.41)	0.00
9	7 (0)	6 (0)	0.00
10	7 (0)	6 (0)	0.00

11	7 (0)	6 (0)	0.00
	Standard method		0.00

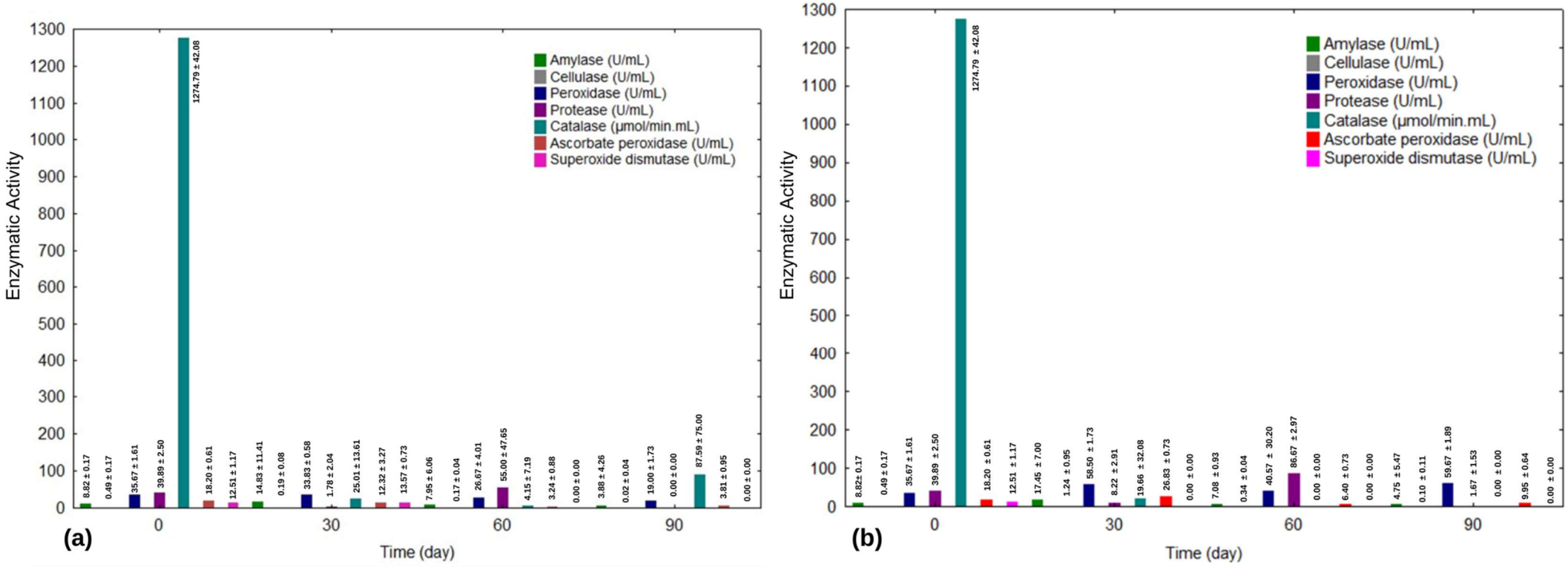
The presence of this enzyme *pool* in the biocomposite obtained in this study brings the possibility of uses for effluent treatment and agriculture, mainly due to the activity of antioxidant enzymes. According to studies found in the literature, the presence of these enzymes allows the removal of textile dyes in effluents due to the redox potential and in the control of weeds due to the increased concentration of peroxide radicals and hydrogen superoxide that damage the structure of weeds (Camargo et al., 2023; Klanovicz et al., 2022). Regarding the amylase, cellulase, and protease enzymes obtained in this study, their use can be to acquire products in the biorefinery context

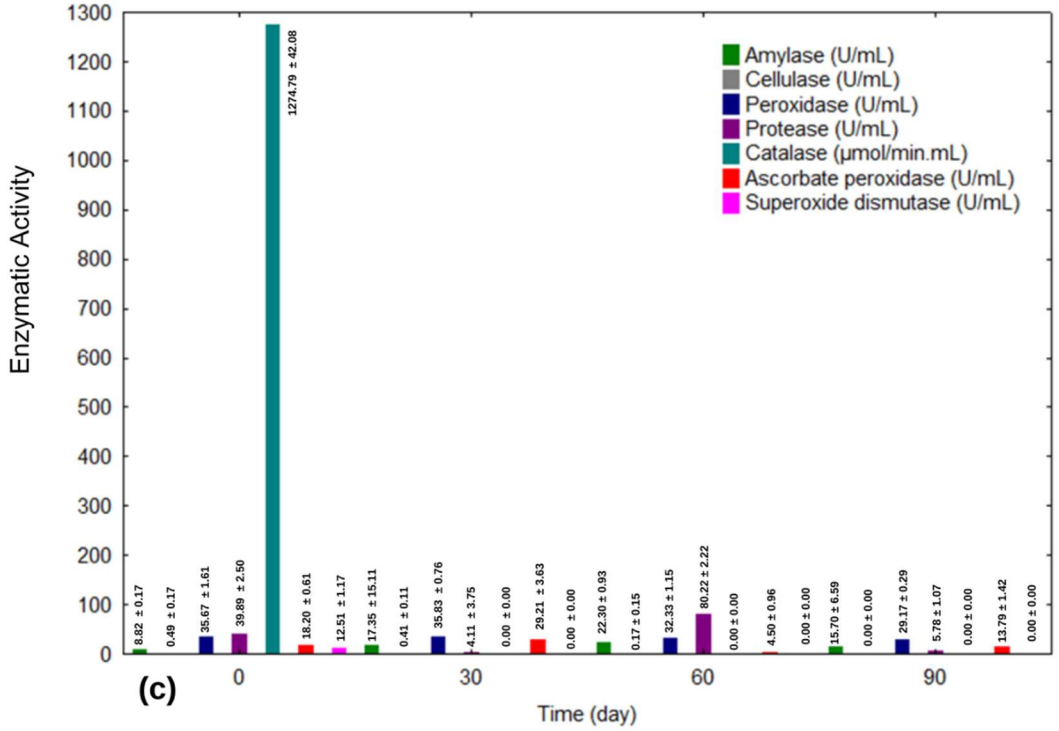
3.2. EVALUATION OF ENZYME STABILITY

From the best conditions of the enzymatic reaction, the stability of the enzymes present in the biocomposite was evaluated when stored at 20°, 04°, and -80°C for 90 days. Enzyme quantifications were performed under the assay conditions that presented the highest enzymatic activity values. Therefore, amylase and cellulase activity were conducted under the conditions of assay 04 (pH 10.0 and temperature of 80°C), catalase activity was performed under the conditions of assay 05 (pH 2.76 and temperature of 50°C), ascorbate peroxidase activity was determined under the conditions of assay 07 (pH 7.0 and temperature equal to 7.57°C), superoxide dismutase under the conditions of assay 05 (pH 10 and light exposure time of 10 minutes) of the experimental design. Peroxidase and protease enzymes were performed under the established conditions by the standard methodology with reaction media for enzymatic determination at pH 5.0, temperature of 35°C, pH 9.0, and temperature of 40°C, respectively.

As described in Figure 01, storage at different temperatures for 90 days did not result in the loss of activity for the enzymes amylase, peroxidase, protease, and ascorbate peroxidase; however, their activities changed during the period with variations every 30 days.

Figure 01: Stability of enzymatic activities (a) stored at 20°C; (b) stored at 04°C; (c) stored at -80°C.




Enzymatic Activity
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
Amylase (U/mL)
Cellulase (U/mL)
Peroxidase (U/mL)
Protease (U/mL)
Catalase (µmol/min.mL)
Ascorbate peroxidase (U/mL)
Superoxide dismutase (U/mL)
8.82 ± 0.17
0.49 ± 0.17
35.67 ± 1.61
39.89 ± 2.50
1274.79 ± 42.08
18.20 ± 0.61
12.51 ± 1.17
17.35 ± 15.11
0.41 ± 0.11
35.83 ± 0.76
4.11 ± 3.75
0.00 ± 0.00
29.21 ± 3.63
0.00 ± 0.00
22.30 ± 0.93
0.17 ± 0.15
32.33 ± 1.15
80.22 ± 2.22
0.00 ± 0.00
4.50 ± 0.96
0.00 ± 0.00
15.70 ± 6.59
0.00 ± 0.00
29.17 ± 0.29
5.78 ± 1.07
0.00 ± 0.00
13.79 ± 1.42
0.00 ± 0.00
(c)
0
30
60
90
Time (day)

The enzyme peroxidase maintained its activity values very closely in the three storage conditions during the 90 days without significant changes in its activity, and the amylase remained active throughout the evaluated period. For protease, the activity values changed mainly after 60 days of storage, resulting in activities equal to 55.00, 86.67, and 80.22 U/mL at 20°C, 04°C, and -80°C, with an increase in this period and at 90 days a decrease reaching 0.00, 1.67, and 5.78 U/mL for the respective temperatures mentioned above. A behavior similar to that of protease was observed for ascorbate peroxidase, with an increase in activity after 30 days of storage, while for the other antioxidant enzymes, there was a decrease in activity from 30 days for the assays at 04 and -80°C.

3.3. EVALUATION OF COMPOUNDS AND METABOLITES PRESENT PRE AND POST-FERMENTATION IN FUNGAL BIOCOMPOSITE

At first, when evaluating the organic compounds at time zero and after fermentation via HPLC, it was possible to verify that there was the consumption of cellobiose (0.01 g/L), glucose (0.45 g/L), and glycerol (0.01 g/L) by *Trichoderma koningiopsis* during the fermentation process. After fermentation, small concentrations of fructose (0.38 g/L), citric acid (0.02 g/L), acetic acid (0.14 g/L), and ethanol (0.02 g/L) were quantified in the biocomposite. Considering its use to obtain products aimed at the biorefinery for better efficacy and increased ethanol fields, it is necessary to use biomass pretreatments (Bader et al., 2020).

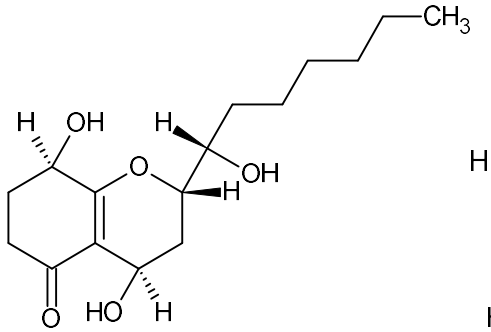
By performing a complete analysis of the biocomposite via UHPLC-ESI-qTOF-MS/MS, it was possible to verify the presence of metabolites of interest for the use of the compound obtained as a bioproduct for agriculture, as shown in Tables 06 and 07. At times zero and 96 hours, the amino acids leucine and tyrosine were observed , which have herbicidal action, especially against *Striga hermonthica*. The amino acids produced by *Fusarium oxysporum* demonstrated their action in controlling weeds and, in this study, there is also the presence of both amino acids being produced by *Trichoderma koningiopsis* and present in the microalgal biomass (Nzioki et al., 2016; Ocán-Torres et al., 2024). In addition to the amino acids already reported, there is also the presence of phenylalanine, which is a compound responsible for inducing resistance in plants and naturally occurring in their structure. Phenylalanine can increase resistance to pathogenic fungi such as *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, and when there are low levels of this amino acid in plants, applications of this compound can be made to increase it availability in plants

and, therefore, the biocomposite obtained in this study can be used for this purpose (Patel et al., 2020; Kumari et al., 2023).

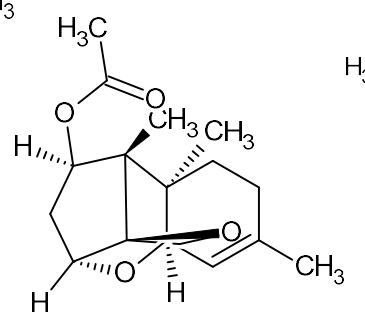
Another metabolite of interest in agriculture and present in the biocomposite is vitamin B3, which has a biostimulant effect on plant growth because it is essential for the formation of coenzymes NAD^+/NADH and $NADP^+$/NADPH that act in the photosynthetic process of plants. In addition, vitamin B3 also promotes plant resistance to environmental stresses, protecting plant cells (Berglund et al., 2017; Ferreira et al., 2024; Hussein et al., 2014; Ramos et al., 2023). In addition to the metabolites already mentioned, d-gluconic and 10-hydroxy decanoic acids are also widely used in various sectors. D-gluconic acid and its derivatives are used in the construction, food, medicine industries, and agriculture, as it is a versatile, slightly acid, non-toxic, and easily degraded organic compound (Ma et al., 2022). In agriculture, the antifungal potential of d-gluconic acid occurs against pathogens that attack plant roots, acting to reduce host's pH, followed by the production of an acidic environment that favors the degradation of the cell wall and ultimately causes the accumulation of toxins (De Werra et al., 2009; Fugaban et al., 2023; Hadas et al., 2007; Kaur et al., 2006; Vilanova et al., 2014; Wu et al., 2023). 10-hydroxydecanoic acid, like d-gluconic acid, also it is an antimicrobial agent. Present in royal jelly, its potential as an antibacterial agent has been studied mainly in the health area, in the combating against bacterial infections and protozoa that cause diseases such as malaria and leishmaniasis (Alkhaibari and Alanazi, 2022; Hong et al., 2025)

Furthermore, other compounds such as trichodermin, koninginin D, B, and A, described in Figure 02, which have high added value, were also observed. Koninginins are secondary metabolites produced by *Trichoderma koningiopsis* and act against *G. graminis* var. *tritici* and fungal pathogens such as *F. oxysporum, Bipolaris sorokiniana, P. cinnamomi* and *Pythium middletonii* (Almassi et al., 1991; Ghisalberti and Rowland, 1993; Khan et al., 2020). Koninginins are also widely used in research in the field of medicine and pharmacology as pharmaceutical agents in the treatment of fungal infections and as an antitumor agent, with an inhibitory effect observed when applied to gastric cancer models (Peng et al., 2024; Ramos et al., 2024).

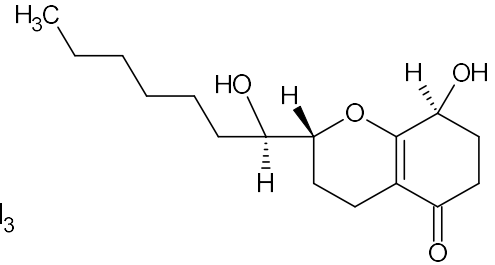
Figure 02: Chemical structures of metabolites at 96 h.



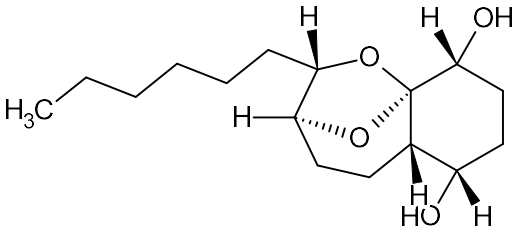
Koninginin D



Trichodermin



Koninginin B



Koninginin A

Trichodermin is also a compound produced by *Trichoderma* genus microorganisms, isolated from the microorganism for the first time in 1960. It is a naturally occurring sesquiterpene antibiotic that can potentially inhibit squamous cell carcinoma cells and antitumor activity in tumors such as chondrosarcoma (Chen et al., 2022; Su et al., 2013). The action of these compounds present in the fungal biocomposite has been investigated through preliminary tests with tumor and non-tumor cells and is showing promising results.

Table 06: Annotated Metabolites at 0 h presents in the biocomposite.

Compound	t_R (min) [a]	Molecular Formula	Positive mode		Negative mode		Level [b]
			Experimental [M+H]	Error (ppm)	Experimental [M-H]	Error (ppm)	
Glucose	0.40	$C_6H_{12}O_6$	n.d.	n.c.	179.0565	-2.0	3
VitaminB-3 [nicotinicacid]	0.40	$C_6H_5NO_2$	124.0393	0	n.d.	n.c.	3
Leucine	0.80	$C_6H_{13}NO_2$	132.1021	-1.6	n.d.	n.c.	3
Allopurinol	0.50	$C_5H_4N_4O$	137.0459	-0.6	135.0318	-4.5	3
Phenylalanine	2.2	$C_9H_{11}NO_2$	166.0863	-0.5	164.0720	-1.5	3
Citric acid	2.2	$C_6H_8O_7$	n.d.	n.c.	191.0202	-2.8	3
1-Acetoxy-2-hydroxy-16 heptadecyn-4-one	3.6	$C_{14}H_{24}N_6O_3$	325.1991	-2.6	n.d.	n.d.	3
1-[9Z,12Z-octadecadienoyl]-sn glycero-3-phosphocholine	2.9	$C_{26}H_{50}NO_7P$	520.3342	26	n.d.	n.d.	3

[a] Retention time on the acquity UPLC HSS T3 column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.

[b] Identification confidence level according to Schymanski et al. (2014). n.d.: not detected. n.c.: not calculated.

Table 07: Annotated Metabolites at 96 h presents in the fungal biocomposite.

Compound	t_R (min) [a]	Molecular Formula	Positive mode		Negative mode		Level [b]
			Experimental [M+H]	Error (ppm)	Experimental $[M-H]^-$	Error (ppm)	
D-Gluconic acid	0.3	$C_6H_{12}O_7$	n.d.	n.c.	195.0510	-1.1	3
Vitamin B-3 [nicotinicacid]	0.40	$C_6H_5NO_2$	124,0393	0,4	n.d.	n.c.	3
Allopurinol	0.7	$C_5H_4N_4O$	137,0458	0.0	135.0316	-2,4	3
Leucine	0.8	$C_6H_{13}NO_2$	132,1018	0,7	130,0876	-2.2	3
Tyrosine	0,9	$C_9H_{11}NO_3$	n.d.	n.c.	180,0363	1,8	3
Citric acid	2.2	C_6H8O_7	n.d.	n.c.	191.0198	-0.6	3
10-hydroxydecanoic acid	4.5	$C_{10}H_{20}O_3$	n.d.	n.c.	189,1339	0.1	3
Koninginin D	3.7	$C_{16}H_{26}O_5$	321.1672 [M+Na]	0.2	297.1701	2.0	3
Trichodermin	3.9	$C_{17}H_{24}O_4$	n.d.	n.c.	291.1598	1.5	3
Koninginin B	4.5	$C_{16}H_{26}O_4$	305.1723 [M+Na]	-0.1	281.1752	2.3	3
Koninginin A	4.7	$C_{16}H_{28}O_4$	n.d.	n.c.	283.1909	2.0	3

[a] Retention time on the acquity UPLC HSS T3 column and solvent: gradient of 0.1% formic acid in water and acetonitrile with 0.1% formic acid.

[b] Identification confidence level according to Schymanski et al. (2014). n.d.: not detected. n.c.: not calculated.

4. CONCLUSION

Based on the results of enzymatic activity and composition of the fungal biocomposite obtained in this study, it is possible to prove its potential as a bioproduct aimed at agriculture and the environment. The high activity values, mainly for antioxidant enzymes, allow using the biocomposite to remove contaminants in effluents and control weeds. Furthermore, metabolites such as tyrosine, leucine, d-gluconic acid, and phenylalanine give this biocomposite the potential for antimicrobial and bioherbicide use. However, studies investigating application concentrations are still necessary to ensure no negative impacts on ecosystems, even though it is a biological compound with antitumor compounds such as koninginins in its composition.

Finally, based on the results of this study, some research can still be carried out, such as investigating the protection mechanism of the enzymes when exposed to low temperatures and the application of the biocomposite in the agricultural sector in plant selectivity tests, investigating the herbicidal effect given by metabolites present in the biocomposite.

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CRediT authorship contribution statement

Simone Kubeneck and Helen Treichel conceived and designed the study. Simone Kubeneck, Aline Frumi Camargo, Vitória Dassoler Longo, Larissa Capeletti Romani, Júlia Pieper Nerling, and Suzana Fátima Bazoti analyzed the data, drafted, wrote, and carefully revised the manuscript. Carlos Henrique Pagno, Eliseu Rodrigues, and Helen Treichel critically reviewed and supervised the development of the paper. All authors reviewed and approved the final manuscript.

Declaration of Competing Interest

I have nothing to declare.

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4 CONCLUSÃO GERAL

Através do estudo desta dissertação, pode-se concluir que as microalgas desempenham um papel importante no setor ambiental, promovendo a economia circular ao fazer sua utilização na remediação de contaminantes ambientais de forma eficiente e servindo como matéria-prima na obtenção de produtos de valor agregado quando utilizadas em processos de fermentação.

Além disso, nos processos de fermentação, foi comprovada a importância do microrganismo ideal para se ter um produto rico em compostos orgânicos de interesse e *pools* enzimáticos, através do uso de *Trichoderma koningiopsis* como microrganismo fermentador.

Por fim, foi possível caracterizar um biocompósito obtido de forma sustentável, sem o uso de produtos nocivos ao meio ambiente e que, devido à sua rica composição, pode ser utilizado no setor ambiental e na agricultura, auxiliando no cumprimento de metas globais que buscam a redução do uso de produtos químicos e a preservação dos recursos naturais.

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APÊNDICE A: Publicações referentes a dissertação de mestrado.

Capítulo de livro intitulado “Linking microalgae-based processes and conversion of water pollutants for valuable compounds obtaining” de autoria de Simone Kubeneck, Aline Frumi Camargo, Júlia Pieper Nerling, Loisleini Fontoura Saldanha, Thamarys Scapini, Natalia Klanovicz e Helen Treichel.

Linking microalgae-based processes and conversion of water pollutants for valuable compounds obtaining

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ABSTRACT

The use of microalgae in the bioremediation of contaminants has been widely discussed as a viable alternative for reducing environmental impacts due to their metabolic versatility and ability to grow in different environments. Therefore, when applied in effluent treatment and using the nutrients in the environment for cell growth, microalgal biomass acts in biosorption and bioadsorption processes on pollutants, such as xenobiotics. The presence of these compounds in the aquatic environment causes accumulation in invertebrate organisms, passing them to other levels of the food chain. In this sense, this chapter will address the state of the art regarding water remediation processes of xenobiotic pollutants based on the use of microalgae, such as pesticides and pharmaceutical products, and also explore the value-added products that can be obtained through this biomass.

Keywords: wastewater; bioremediation; nutrients; biomass; organisms.

Artigo científico intitulado “Characterization of enzymatic and metabolic of a biocomposite based on *Trichoderma koningiopsis* and *Chlorella* biomass” aceito para publicação no periódico internacional Biocatalysis and Agricultural Biotechnology e de autoria de Simone Kubeneck, Aline Frumi Camargo, Vitória Dassoler Longo, Larissa Capeletti Romani, Júlia Pieper Nerling, Suzana Fátima Bazoti, Carlos Henrique Pagno, Eliseu Rodrigues e Helen Treichel.

Characterization of enzymatic and metabolic of a biocomposite based on *Trichoderma koningiopsis* and *Chlorella* biomass

Simone Kubeneck[1,3], Aline Frumi Camargo[2,3], Vitória Dassoler Longo[3], Larissa Capeletti Romani[3], Júlia Pieper Nerling[3], Suzana Fátima Bazoti[3], Carlos Henrique Pagno[4], Eliseu Rodrigues[4], and Helen Treichel[1,3*]

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Manuscript Number: **BAB-D-24-03551R2**

Characterization of a biocomposite based on Trichoderma koningiopsis and Chlorella biomass

Dear Dr Treichel,

Thank you for submitting your manuscript to Biocatalysis and Agricultural Biotechnology.

I am pleased to inform you that your manuscript has been accepted for publication.

Resumo simples intitulado “Caracterização de extrato enzimático obtido a partir de microalgas produzidas em águas residuárias” publicado nos Anais do X Simpósio em Ciência e Tecnologia Ambiental e VI Encontro Multidisciplinar em Ciências Ambientais da Fronteira Sul, e de autoria de Simone Kubeneck, Aline Frumi Camargo, Júlia Pieper Nerling, Charline Bonatto, Suzana Fátima Bazoti e Helen Treichel.

ANAIS DO X SIMPÓSIO EM CIÊNCIA E TECNOLOGIA AMBIENTAL
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CARACTERIZAÇÃO DE EXTRATO ENZIMÁTICO OBTIDO A PARTIR DE MICROALGAS PRODUZIDAS EM ÁGUAS RESIDUÁRIAS

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As microalgas são organismos unicelulares fotossintéticos que possuem uma rica composição em aminoácidos, bioativos, proteínas e lipídios, além de sintetizar uma gama de enzimas, que as tornam uma fonte de biomassa valiosa para diferentes usos. No entanto, para isso são necessários processos que facilitem o acesso aos seus componentes, como processos fermentativos microbianos que tem como produto um extrato rico em enzimas e outras substâncias. A presença de enzimas como amilase, celulase, lacase, lipase, protease, xilanase e β-glucosidade tornam esse extrato uma alternativa para a produção de biocombustíveis, composto orgânicos e bioinsumos agrícolas. Outra classe de enzimas cuja presença no extrato microalgal é interessante são as enzimas antioxidantes, consideradas mecanismos de defesa das microalgas. A sua produção se dá principalmente em situações de estresse ambiental, como a exposição a metais pesados, elevadas temperaturas e meios salinos, onde são formadas espécies reativas de oxigênio que são tóxicas as células das microalgas e assim são acionados seus mecanismos de defesa que incluem a produção dessas enzimas. Algumas enzimas pertencentes a esta classe são as peroxidases, superóxido dismutases, ascorbato peroxidases, catalases e glutationa redutases, as quais permitem o uso do extrato na produção de bioinsumos e no tratamento de efluentes industriais com a presença de metais pesados. Entretanto a produção de enzimas como amilase, celulase, lipase, lacase, protease e de enzimas antioxidantes em processos fermentativos que envolvam o uso da biomassa microalgal e microrganismos do gênero *Trichoderma* sp. são escassos na literatura. O objetivo deste projeto será caracterizar o extrato microalgal obtido a partir de fermentação microbiana em biorreator *Airlift* envolvendo biomassa microalgal proveniente de águas residuárias e o microrganismo *Trichoderma koningiopsis*. O extrato microalgal será avaliado quanto a presença das enzimas amilase, celulase, lacase, lipase, protease, xilanase, β-glucosidase, peroxidase, superóxido dismutase, catalase, ascorbato peroxidase e glutationa redutase, e o comportamento deste conjunto de enzimas à exposição do extrato a diferentes faixas de pH e temperatura por meio de planejamento experimental do tipo Delineamento Composto Central (DCC). Ainda será avaliado a estabilidade enzimática por meio do armazenamento do extrato em diferentes temperaturas e determinando a atividade enzimática a cada 30, 60 e 90 dias .Também será realizada a caracterização física da biomassa microalgal bruta e após o processo fermentativo, em que será determinado sólidos totais, teor de umidade, peso seco e cinzas e por fim será determinada a presença de compostos como glicose, frutose, celobiose, ácido cítrico, ácido acético e etanol via HPLC. Como resultados se espera que este projeto demonstre o potencial do uso deste biocomposto em diferentes áreas por meio da presença do conjunto de enzimas em questão, visando o uso de processos economicamente e ambientalmente amigáveis e assim promover a sustentabilidade.

Palavras-chave: *Trichoderma koningiopsis*, biomassa, antioxidantes, microrganismo.

Resumo intitulado “Characterization of enzyme extract obtained from microalgae produced in wastewater” aceito para apresentação no I Congresso Brasileiro de Tecnologia Industrial – I COBBIND que ocorreu no mês de Agosto. O resumo é de autoria de Simone Kubeneck, Aline Frumi Camargo, Júlia Pieper Nerling e Helen Treichel.

CHARACTERIZATION OF ENZYME EXTRACT OBTAINED FROM MICROALGAE PRODUCED IN WASTEWATER

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ABSTRACT

Reducing the generation of solid and chemical waste and more sustainable practices aimed at agriculture are among the global goals for achieving the 2030 agenda. Because of this, enzymatic extracts obtained from fermentations with fungi and microalgae are an excellent choice for their use in agriculture and treating industrial effluents. Therefore, this study aimed to characterize the extract obtained from fermentation in an Airlift bioreactor using microalgae and *Trichoderma koningiopsis* as the fermentative medium. The presence of the enzymes amylase, cellulase, peroxidase, laccase, lipase, and protease and their behavior in reaction media at different pH and temperature ranges were evaluated. As a result, if obtained, high peroxidase (52.083 U/mL) and protease (97.500 U/mL) activity and an increase in amylase activity (40.52 U/mL) were observed when having an alkaline reaction medium and high reaction temperature. The results demonstrate the possibility of using the extract obtained for different environmental applications, contributing to achieving global goals and the reduction of chemical compounds harmful to the environment.

.**Keywords:** *Trichoderma koningiopsis*. Peroxidase. Activities. Fermentation. Reaction medium.

1 INTRODUCTION

The establishment of the 2030 Agenda, along with the Objectives of Sustainable Development, has led to the search for the use of environmentally friendly processes that reduce the generation of solid and chemical waste, intending to ensure sustainable production and consumption standards and guarantee sustainable food production systems and that do not harm ecosystems.

Microalgae are considered promising organisms for their use in the most diverse sectors, from agriculture to effluent treatment, due to their rich composition of amino acids, bioactive lipids, and other components [1,2,3]. Furthermore, the microalgae can synthesize several enzymes, and microorganisms such as *Trichoderma* can produce an enzymatic pool, enabling fermented microalgal extract in several areas of environmental interest.

That said, the objective of this study is to evaluate the presence of different enzymes in the microalgal extract fermented with *Trichoderma koningiopsis* and the behavior of the reaction medium at various pH and temperature ranges.

2 MATERIAL & METHODS

The microalgae used in this study were supplied by Embrapa Swine and Poultry (Concórdia, SC, Brazil), and the microorganism used was the *Trichoderma koningiopsis* (GenBank MK860714), isolated from weed *Digitaria ciliares* [4].

The fermentative process occurred in Airlift Bioreactor, the fermentative medium composed of 200g of microalgae, 400 mL of antifoam, 1400 mL of distilled water, and 10^7 cells per mL of inoculum of *Trichoderma koningiopsis*.

The microalgae extract obtained after 96 hours of fermentation was evaluated for the enzymes in its composition. Therefore, cellulase, amylase, peroxidity, laccase, lipase, and protease activities were determined [5-12]. Afterward, the behavior of enzymatic activities was evaluated based on the change in pH and temperature of the reaction medium to determine enzymatic activity. Experimental designs were carried out for this evaluation using a central rotational composite design 2^2.

3 RESULTS & DISCUSSION

When evaluating the presence of the enzymes cellulase, amylase, peroxidase, laccase, lipase, and protease, only cellulase, peroxidase, and protease activities were obtained, being equal to 0.691 U/mL, 52.083 U/mL, and 97.500 U/ml, respectively.

However, to confirm whether the enzymes amylase, laccase, and lipase are present and their absence may be linked to the pH and temperature of the reaction medium, experimental planning was carried out for all enzymes, and the results are described in Table 1.

Table 1 Results of pH and temperature changes from enzymatic reactions.

Essay	pH	Temperature (ºC)	Amylase (U/mL)	Cellulase (U/mL)	Lipase (U/mL)	Laccase (U/mL)	Peroxidase (U/mL)	Protease (U/mL)
1	4 (-1)	20 (-1)	13.09	0.05	0.29	0.02	8.17	0.00
2	10 (1)	20 (-1)	0.00	0.15	0.79	0.01	8.83	2.89
3	4 (-1)	80 (1)	0.00	0.61	0.00	0.00	5.00	2.33
4	10 (1)	80 (1)	40.52	1.28	0.25	0.01	3.00	2.00
5	2.76 (-1.41)	50 (0)	2.91	0.00	0.89	0.00	7.67	0.56
6	11.24 (+1.41)	50 (0)	3.30	0.19	1.22	0.01	6.33	4.67
7	7 (0)	7.57 (-1.41)	11.15	0.24	0.00	0.00	1.50	4.00
8	7 (0)	92.43 (+1.41)	3.39	0.10	0.00	0.04	6.83	0.00
9	7 (0)	0 (50)	8.53	0.12	0.75	0.00	5.83	0.11
10	7 (0)	0 (50)	0.00	0.12	0.00	0.00	5.83	0.00
11	7 (0)	0 (50)	11.73	0.24	0.29	0.00	6.17	5.44
Traditional methodology			0.00	0.691	0.00	0.00	52.083	97.500

By changing the pH and temperature of the reaction medium, amylase (40.52 U/mL) and lipase (1.22 U/mL) activities were obtained. Cellulase resulted in a higher activity value, equal to 1.28 U/mL. The amylolytic activity obtained in test 4 may be due to its affinity for more alkaline pH levels; however, this is a new behavior since most fungal amylases have maximum activity in pH ranges between 4 and 6 and at temperatures between 30 and 70°C, most of which are produced by fungi from other genera and not *Trichoderma* sp.[13]. Cellulase and lipase activities have increased but are still considered low values. Peroxidase obtained its highest activity using the traditional methodology, as did protease, which resulted in activities equal to 52.083 U/mL and 97.500 U/mL, respectively.

The presence of these three enzymes in the fermented extract makes it possible to use them in obtaining agricultural bio inputs, treating effluents, and biorefinery products. The high presence of peroxidase mainly highlights its use as a bioherbicide. For example, how this enzyme acts when applied to plants increases the concentration of peroxide radicals and hydrogen superoxide, thus damaging the structure of weeds[1]. Furthermore, due to its redox potential, peroxidases in this extract make it possible to use it to remove dyes from effluents from the textile industry[2].

4 CONCLUSION

The enzymatic characterization of microalgae extract ferment by *Trichoderma koningiopsis* demonstrated that the change in pH and temperature causes the enzyme present in the extract to become active, as was the case with amylase. In addition to its presence, the activity values obtained for peroxidase and protease also make the use of this extract promising for the various environmental and economic sectors, enabling the reduction of waste generation and sustainable agriculture practices, since due to the presence of peroxidase it has potential for bioherbicidal action on specific plants.

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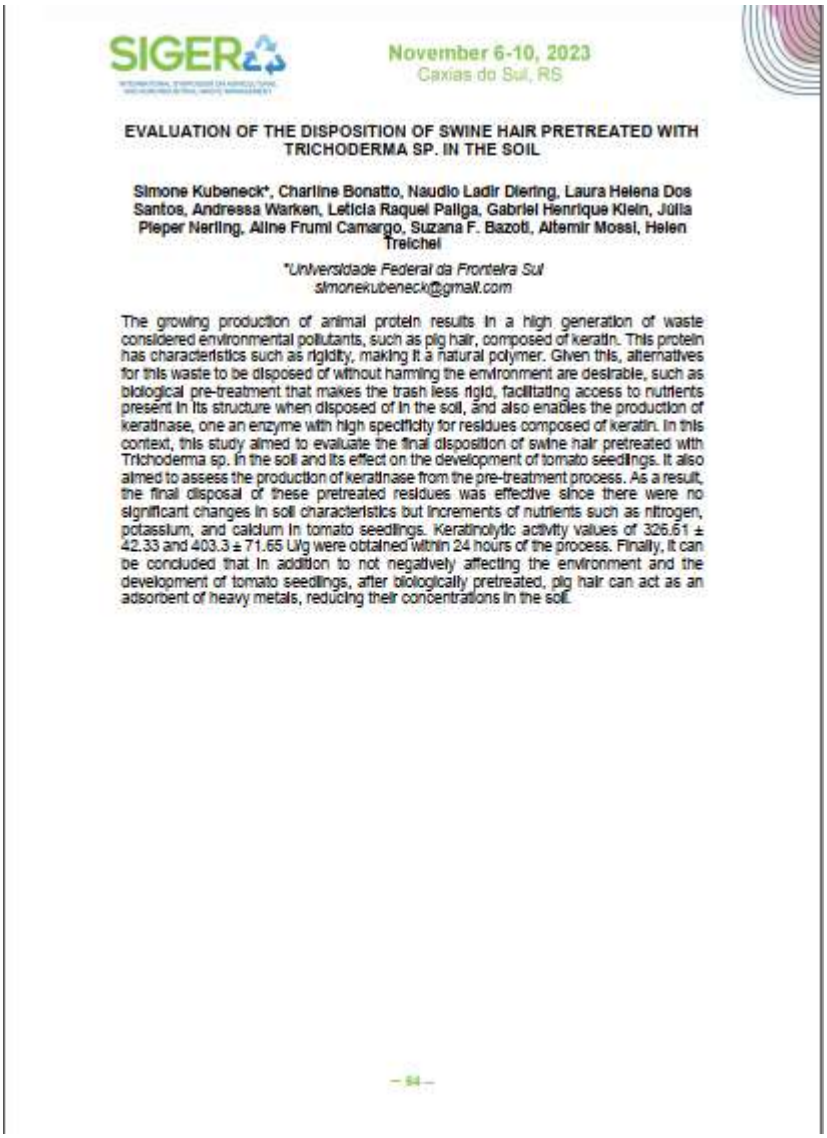
APÊNDICE B: Outras publicações realizadas durante o mestrado.

Resumo intitulado “Evaluation of the disposition of swine hair pretreated with *Trichoderma* sp. in the soil” publicado em Proceedings of the VIII International Symposium of Agricultural and Agroindustrial Waste Management e de autoria de Simone Kubeneck, Charline Bonatto, Naudio Ladir Diering, Laura Helena dos Santos, Andressa Warken, Letícia Raquel Paliga, Gabriel Henrique Klein, Júlia Pieper Nerling, Aline Frumi Camargo, Suzana Fatima Bazoti, Altemir José Mossi e Helen Treichel.



SIGER

November 6-10, 2023
Caxias do Sul, RS

EVALUATION OF THE DISPOSITION OF SWINE HAIR PRETREATED WITH TRICHODERMA SP. IN THE SOIL

Simone Kubeneck*, Charline Bonatto, Naudio Ladir Diering, Laura Helena Dos Santos, Andressa Warken, Leticia Raquel Paliga, Gabriel Henrique Klein, Júlia Pieper Nerling, Aline Frumi Camargo, Suzana F. Bazoti, Altemir Mossi, Helen Treichel

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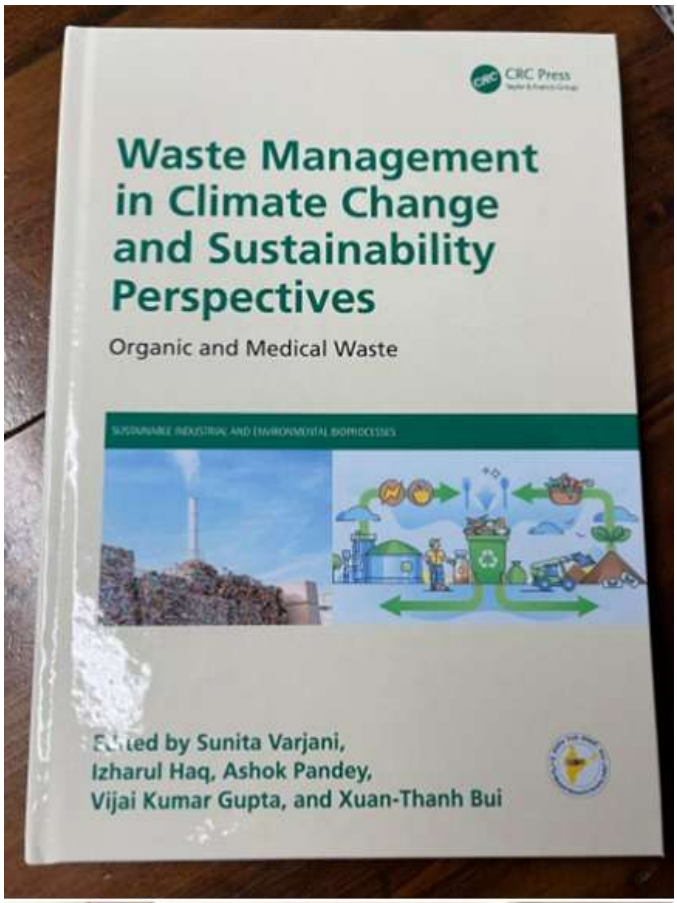
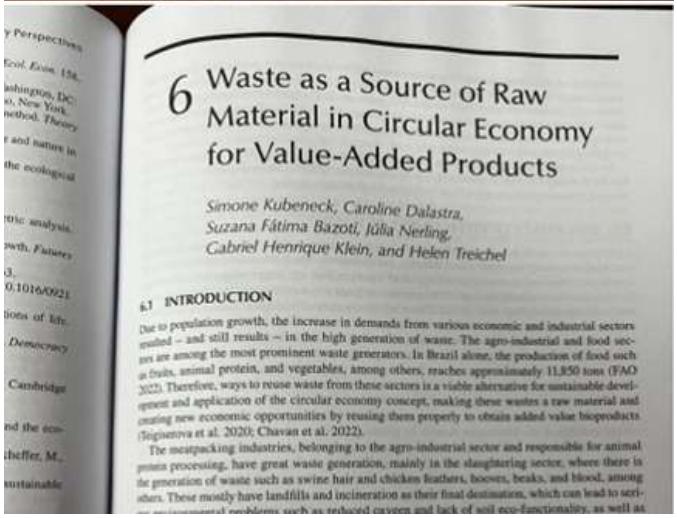
The growing production of animal protein results in a high generation of waste considered environmental pollutants, such as pig hair, composed of keratin. This protein has characteristics such as rigidity, making it a natural polymer. Given this, alternatives for this waste to be disposed of without harming the environment are desirable, such as biological pre-treatment that makes the trash less rigid, facilitating access to nutrients present in its structure when disposed of in the soil, and also enables the production of keratinase, one an enzyme with high specificity for residues composed of keratin. In this context, this study aimed to evaluate the final disposition of swine hair pretreated with Trichoderma sp. in the soil and its effect on the development of tomato seedlings. It also aimed to assess the production of keratinase from the pre-treatment process. As a result, the final disposal of these pretreated residues was effective since there were no significant changes in soil characteristics but increments of nutrients such as nitrogen, potassium, and calcium in tomato seedlings. Keratinolytic activity values of 326.61 ± 42.33 and 403.3 ± 71.65 U/g were obtained within 24 hours of the process. Finally, it can be concluded that in addition to not negatively affecting the environment and the development of tomato seedlings, after biologically pretreated, pig hair can act as an adsorbent of heavy metals, reducing their concentrations in the soil.

Capítulo de livro intitulado “Waste as a source of raw material in circular economy for value-added product” de autoria de Simone Kubeneck, Caroline Dalastra, Suzana Fátima Bazoti, Júlia Pieper Nerling, Gabriel Henrique Klein e Helen Treichel. O capítulo faz parte do livro Waste management in climate change and Sustainability perspectives e tem como editores Sunita Varjani, Izharul Haq, Ashok Pandey, Vijai Kumar Gupta e Xuan-Thanh Bui. O capítulo pode ser acessado pelo DOI: doi.org/10.1201/9781003386902-8.




6 Waste as a Source of Raw Material in Circular Economy for Value-Added Products

Simone Kubeneck, Caroline Dalastra, Suzana Fátima Bazoti, Júlia Nerling, Gabriel Henrique Klein, and Helen Treichel

6.1 INTRODUCTION

Artigo científico intitulado “Evaluation of the disposition of swine hair pre-treated with *Trichoderma* sp. in the soil” publicado no periódico internacional Water, Air and Soil Pollution e de autoria de Simone Kubeneck, Charline Bonatto, Naudio Ladir Diering, Aline Frumi Camargo, Laura Helena dos Santos, Andressa Janaína Warken , Letícia Raquel Paliga, Gabriel Henrique Klein, Júlia Pieper Nerling, Altemir José Mossi e Helen Treichel. O artigo pode ser acessado pelo DOI: https://doi.org/10.1007/s11270-024-06923-x.

Water Air Soil Pollut (2024) 235:107
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Evaluation of the Disposition Of Swine Hair Pre-treated with *Trichoderma* sp. in the Soil

Simone Kubeneck · Charline Bonatto · Naudio Ladir Diering · Aline Frumi Camargo · Laura Helena dos Santos · Andressa Janaína Warken · Letícia Raquel Paliga · Gabriel Henrique Klein · Júlia Pieper Nerling · Altemir José Mossi · Helen Treichel

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Abstract The growing animal protein production results in a high generation of waste considered environmental pollutants, such as swine hair, which is composed of keratin. This protein has characteristics such as rigidity, making it a natural polymer. Given this, alternatives for this waste to be disposed of without harming the environment are desirable, such as biological pre-treatment that makes the waste less rigid, facilitating access to nutrients present in its structure when disposed of in the soil and also enabling the production of keratinase, one an enzyme with high specificity for residues composed of keratin. In this context, this study aimed to evaluate the final disposition of swine hair pre-treated with *Trichoderma* sp. in the soil and its effect on the development of tomato seedlings and to evaluate the production of keratinase from the pre-treatment process. As a result, the final disposal of these pre-treated residues was effective since there were no significant changes in soil characteristics but increased nutrients such as nitrogen, potassium, and calcium in tomato seedlings. Also, keratinolytic activity values of 326.61 ± 42.33 and 403.3 ± 71.65 U/g were obtained within 24 h of the process. Finally, it can be concluded that in addition to not negatively affecting the environment and the development of tomato seedlings, after biologically pre-treated, swine hair can act as an adsorbent of heavy metals, reducing their concentrations in the soil. So, the significance of this study could be synthesized in biological pre-treatment enabled the use of swine hair for multivariate purposes; the fermentation process resulted in elevated keratinolytic activity; the treated swine hair decreased concentrations of heavy metals in the soil; and swine hair pre-treated biologically provide the increment of nutrients in the soil.

Keywords Tomato seedlings · Nitrogen · Keratinase · Nutrients

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1 Introduction

The production of animal protein is constantly growing. In Latin America alone, it is estimated that the livestock sector will grow by around 28%, and pork production is expected to grow by about 16% by 2031 (FAO, 2022). However, the production process of the slaughterhouse sector results in a high generation of waste, such as blood, hooves, and swine hair, which are considered environmental pollutants because they are

Artigo de revisão intitulado “ICMS Ecológico como instrumento de política ambiental: um estudo comparativo entre estados do Sul e Centro-Oeste” publicado na Revista Gestão & Sustentabilidade e de autoria de Simone Kubeneck, Helen Treichel e Darlan Christiano Kroth. O artigo pode ser acessado pelo DOI: https://doi.org/10.36661/2596-142X.2023v5n1.14230.

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ICMS Ecológico como instrumento de política ambiental: um estudo comparativo entre estados do Sul e Centro-Oeste

Simone Kubeneck[1]
Helen Treichel[2]
Darlan Christiano Kroth[3]

Resumo
O aumento das atividades econômicas requerentes de recursos naturais culminou em uma série de consequências ao meio ambiente. Diante desse cenário, a criação de políticas voltadas ao meio ambiente se fez necessária. No Brasil a inserção de políticas ambientais se iniciou em 1981 com a criação da Política Nacional do Meio Ambiente e se intensificou ainda mais com a nova Constituição Federal de 1988, a qual garante o direito ao meio ambiente ecologicamente equilibrado e sua preservação para as presentes e futuras gerações, tornando a tributação ambiental possível e apta para seu uso em políticas públicas. Nesse contexto, e devido à restrição quanto ao uso da terra para atividades econômicas, teve-se a criação do ICMS Ecológico como forma de compensar os municípios que possuíam áreas de conservação de uso restrito. Tendo em vista esse contexto, este estudo visa à realização de uma revisão integrativa do ICMS Ecológico desde sua implantação. Além disso, apresenta a realização de um estudo de caso sobre a atual situação dos estados da região Sul e Centro-Oeste do País, quanto à implantação dessa política. Como resultados, observou-se a efetividade da política em questão nos estados, entretanto algumas melhorias na redação da lei são necessárias em alguns casos.

Palavras-chave: Tributos; Conservação; Políticas públicas; Biodiversidade.

Ecological ICMS as an environmental policy instrument: a comparative study between southern and central-western states

Abstract
The increase in economic activities demanding natural resources culminated in a series of consequences for the environment. Given this scenario, the creation of policies aimed at the environment became necessary. In Brazil, the insertion of environmental policies began in 1981 with the creation of the National Environmental Policy and intensified even further with

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APÊNDICE C: Publicações como autor colaborador

Resumo intitulado “Bioherbicida fúngico produzido a partir de biomassa microalgal e isolados fúngicos” publicado em Anais do X Simpósio em Ciência e Tecnologia Ambiental e VI Encontro Multidisciplinar em Ciências Ambientais da Fronteira Sul e de autoria de Júlia Pieper Nerling, Larissa Romani, Simone Kubeneck, Aline Frumi Camargo, Altemir José Mossi e Helen Treichel.

Erechim - RS

ANAIS DO X SIMPÓSIO EM CIÊNCIA E TECNOLOGIA AMBIENTAL
e VI Encontro Multidisciplinar em Ciências Ambientais da Fronteira Sul
8 a 10 de Novembro de 2023

ISSN 2594-4061

BIOHERBICIDA FÚNGICO PRODUZIDO A PARTIR DE BIOMASSA MICROALGAL E ISOLADOS FÚNGICOS

Júlia Pieper Nerling[1*], Larissa Romani[1], Simone Kubeneck[1], Aline Frumi Camargo[2], Altemir José Mossi[1], Helen Treichel[1]

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A otimização de métodos de controle de plantas daninhas é crucial para a produtividade agrícola. Nesse contexto, os fungos têm ganhado destaque na produção de bioherbicidas devido às vantagens sobre compostos sintéticos. Assim, o potencial fitotóxico desses bioherbicidas está relacionado à presença de enzimas que afetam processos fisiológicos nas plantas-alvo, impactando a absorção de nutrientes, fotossíntese e permeabilidade da membrana. Além disso, microalgas oferecem substrato rico em carboidratos e proteínas, promovendo uso viável em fermentação. Dessa forma, o estudo visou formular um bioherbicida por processos fermentativos utilizando isolados fúngicos e biomassa microalgal cultivada em digestato de produção de biogás. Avaliou-se a ampliação de escala do processo em biorreator airlift, empregando microalgas como substrato. As enzimas amilase, celulase, lacase, lipase e peroxidase, foram quantificadas nos extratos fermentados com a finalidade de parametrizar sua atividade e eficácia quando aplicado. A biomassa microalgal foi obtida em cooperação com Embrapa Suínos e Aves, proveniente do tratamento de digestato de biogás usando reator com manta de lodo, sendo utilizada especificamente a linhagem *Chlorella* spp. Já na questão fúngica, três isolados foram estudados, sendo o *Trichoderma koningiopsis* (isolado de *Digitaria ciliares*) o com maior potencial para esta finalidade, mostrando destaque em estudos prévios. Por fim, foram efetuadas duas fermentações, uma com agitação mecânica em incubadora e outra no biorreator airlift, nas quais amostras dos extratos fermentados foram coletadas em 72 horas para avaliação de esporos e enzimas ambientais. A alteração de escala é marcada principalmente pela alteração do método de agitação, passando de mecânica em Erlenmeyers para aerada no biorreator airlift. A partir dos resultados obtidos, observou-se potencial na produção de algumas enzimas, corroborando com estudos anteriores realizados pelo grupo, e ainda, mostrando que os testes de aplicação (em andamento), devem resultar em atividade bioherbicida do composto produzido. A pesquisa destaca a aplicação da economia circular na agricultura, enfatizando a relevância de bioherbicidas, uma vez que a redução de herbicidas comerciais é um forte indicador de melhoria na qualidade de vida e sustentabilidade.

Palavras-chave: biorreator airlift, *Trichoderma koningiopsis*, enzimas, bioprocessos.

Resumo intitulado “Seletividade de bio-herbicida a base de *T. koningiopsis* para trigo” publicado nos Anais do X Simpósio em Ciência e Tecnologia Ambiental e VI Encontro Multidisciplinar em Ciências Ambientais da Fronteira Sul e de autoria de Eduardo José Pedroso Pritsch, Aline Frumi Camargo, Júlia Pieper Nerling, Cauê Betiato Bieniek, Simone Kubeneck, Altemir José Mossi, Sérgio Luiz Alves Júnior e Helen Treichel.

Erechim - RS

ANAIS DO X SIMPÓSIO EM CIÊNCIA E TECNOLOGIA AMBIENTAL
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8 a 10 de Novembro de 2023

ISSN 2594-4061

SELETIVIDADE DE BIO-HERBICIDA A BASE DE *T. koningiopsis* PARA TRIGO

Eduardo José Pedroso Pritsch[1*], Aline Frumi Camargo[2], Júlia Pieper Nerling[1], Cauê Betiato Bieniek[1], Simone Kubeneck[1], Altemir José Mossi[1], Sérgio Luiz Alves Júnior[3], Helen Treichel[1]

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A produção de alimentos é mundialmente baseada na agricultura convencional, fazendo uso de quantidades cada vez mais exorbitantes de produtos sintéticos, como os agrotóxicos, para controlar as pragas presentes no campo, sejam elas insetos, doenças ou plantas daninhas. Essa prática causa, além da contaminação de alimentos, a exposição do agricultor aos riscos do contato com tais produtos. Pensando no risco ao consumir esses alimentos, há a busca crescente por alimentos mais saudáveis e livres desses contaminantes. A substituição ou redução do aporte de agrotóxicos sintéticos na produção agrícola é necessária para reduzir esse problema, nesse sentido, produtos que possam substituir os agrotóxicos sintéticos tornam-se de grande importância para a melhoria no ambiente de produção e também na garantia de alimentos sem contaminantes, como é o caso do bio-herbicida à base do fungo *Trichoderma koningiopsis* que mostra-se como potencial herbicida para plantas daninhas e podendo ser utilizado em algumas culturas. Este trabalho tem como objetivo investigar a seletividade do bio-herbicida à base de *T. koningiopsis* para a cultura do trigo (*Triticum aestivum*), avaliando a mortalidade e altura de plantas de trigo tratadas com o bio-herbicida. O bio-herbicida foi produzido no Laboratório de Microbiologia e Bioprocessos (LAMIBI) da Universidade Federal da Fronteira Sul, Erechim, RS, Brasil, através da fermentação do fungo *T. koningiopsis* com microalgas em biorreator, foi centrifugado e posteriormente armazenado sob refrigeração até o seu uso. As sementes de trigo cultivar BRS 374 foram semeadas no dia 20 de junho de 2023 com uma população de 300 plantas por hectare, a área das parcelas foi de 3 x 4 m, totalizando 12 m² e a área útil de 2,5 x 3,5 m, totalizando 8,75 m². O herbicida foi pulverizado sobre a cultura no sétimo dia após a emergência utilizando um pulverizador costal pressurizado a CO_2, o volume de calda utilizado foi de 120 L/ha de produto bruto e 2% do volume de calda de óleo mineral. Os tratamentos consistem em: 0 aplicações, 1 aplicação e 2 aplicações. Foram avaliadas as alturas das plantas aos 14 dias após aplicação, além da mortalidade do trigo. Os resultados foram submetidos a teste Tukey a 5% de significância utilizando software estatístico SISVAR®. Não houve mortes ou danos foliares nas plantas de trigo tratadas com o bio-herbicida, do mesmo modo, não houve diferença estatística na altura de plantas entre os tratamentos aplicados no trigo. As plantas não tratadas com o bio-herbicida, as tratadas com 1 aplicação e 2 aplicações do bio-herbicida apresentaram médias de 20,2 cm, 21,5 cm e 21,5 cm de altura, respectivamente, sendo iguais estatisticamente pelo teste de Tukey a 5% de significância. Tais dados nos mostram que o herbicida à base de *T. koningiopsis* além de não causar a morte da cultura de interesse, também não causou interferência no desenvolvimento inicial do trigo, demonstrando a seletividade para a cultura do trigo.

Palavras-chave: *Triticum aestivum*, *Trichoderma koningiopsis*, bio-herbicida, daninhas.

Resumo intitulado “Bioethanol production from raw orange industrial waste using seawater-based fermentation media” publicado em Proceedings of the VIII International Symposium of Agricultural and Agroindustrial Waste Management e de autoria de Charline Bonatto, Thamarys Scapini, Jessica Zanivan, Caroline Dalastra, Laura Helena dos Santos, Gabriel Henrique Klein, Aline Frumi Camargo, Simone Kubeneck, Suzana Fatima Bazoti, Sérgio Luiz Alvez Júnior, Débora de Oliveira e Helen Treichel.

SIGER
INTERNATIONAL SYMPOSIUM ON AGRICULTURAL AND AGROINDUSTRIAL WASTE MANAGEMENT

November 6-10, 2023
Caxias do Sul, RS

BIOETHANOL PRODUCTION FROM RAW ORANGE INDUSTRIAL WASTE USING SEAWATER-BASED FERMENTATION MEDIA

Charline Bonatto*, Thamarys Scapini, Jessica Zanivan, Caroline Dalastra, Laura Helena Dos Santos, Gabriel Henrique Klein, Aline Frumi Camargo, Simone Kubeneck, Suzana F. Bazoti, Sérgio Luiz Alves Júnior, Débora De Oliveira, Helen Treichel

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Citrus fruits, predominantly represented by oranges, are among the most produced and consumed crops globally, constituting a vital processing sector. After processing and extraction of the fruit juice, the residues generated (peel, internal tissues or bagasse, and seeds) are rich in soluble, fermentable sugars, lignin, proteins, essential oils, and polysaccharides such as pectin, cellulose, and hemicellulose. These characteristics make orange residue an ideal source for producing and recovering different individual compounds, such as ethanol, which can be produced from soluble sugars or by subjecting the residue to a treatment step for disintegrating polysaccharides into simple sugars for further fermentation. Given this, this study aims to critically evaluate the challenges of applying industrial orange waste directly, i.e., without removing interfering compounds such as essential oils and galacturonic acid, resulting from the solubilization of pectin, for ethanol production by the yeast Wickerhamomyces sp. UFFS-CE-3.1.2 uses seawater and ultrapure water as a solvent.

The orange residue was suspended in seawater and ultrapure water to solubilize free sugars and treated with sulfuric acid diluted in seawater to release available sugars in the lignocellulosic and pectin fraction. The acid treatment was evaluated by experimental planning (DCCR 2^3), and the influence of the variables acid concentration, solid-liquid ratio, and temperature on sugar release was studied. 8.35±0.10 g L-1 of free sugars were extracted from orange residues, producing ethanol production of 0.61±0.11 g L-1 and 0.57±0.08 g L-1 using seawater and ultrapure water, respectively. The broth resulting from the acid treatment showed high amounts of sugars (51.61±3.67g L-1), including 13.02±1.04 g L-1 of galacturonic acid, but they were not fermented. The presence of essential oils and galacturonic acid in the fermentation broths in citrus residues hydrolysates is a challenge to overcome to enable the direct application of these residues in ethanol production since these compounds can inhibit cellular functions and consequently negatively affect the fermentation process.

Resumo intitulado “Use of antifoam in na airlift bioreactor for the production of bioherbicide” aprovado para apresentação no I Congresso Brasileiro de Tecnologia Industrial – I COBBIND que ocorreu em Agosto. O resumo é de autoria de Aline Frumi Camargo, Simone Kubeneck, Larissa Capeletti Romani, Júlia Pieper Nerling, Gislaine Fongaro e Helen Treichel.

USE OF ANTIFOAM IN AN AIRLIFT BIOREACTOR FOR THE PRODUCTION OF BIOHERBICIDE

Aline Frumi Camargo[1,2], Simone Kubeneck[2,3*], Larissa Capeletti Romani[2], Júlia Pieper Nerling[2], Gislaine Fongaro[1] & Helen Treichel[1,2,3]

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ABSTRACT

In the development of bioprocesses that involve scaling up fermentations, choosing a bioreactor and studying parameters (such as temperature, agitation, aeration, among others) are fundamental to ensure the product quality. This study evaluates the use of antifoam and its effect on parameters of the fermentation process of a fungal extract using microalgal biomass as a substrate in an a Airlift bioreactor, aiming to produce bioherbicide. For that, we quantified the production of enzymes involved in the bioherbicide activity (amylase, cellulase, laccase, lipase, and peroxidase). We identified a fermentation condition (412 mL of antifoam; 28°C; pH of 8.3; 8 LPM of aeration) in which antifoam allowed the production of an enzyme cocktail and did not negatively affect fungal development and enzyme production.

Keywords: Bioprocesses. Fermentative strategies. Process optimization. Bioinputs. Scale-up.

1 INTRODUCTION

The use of bioherbicides can bring a series of benefits to agriculture. This technology effectively reduces food production costs and crop yields and can be added to integrated strategies for managing weeds. The quality of a bioherbicide directly depends on how the fermentation process is conducted, in which the microorganism needs specific nutrients and appropriate conditions for its development[1].

One of the current challenges for popularizing bioherbicides is to make their production method economically viable. To do so, it is necessary to understand how parameters (i.e., temperature, agitation, pH, oxygen availability) affect the production of secondary metabolites, which are responsible for the phytotoxic effects caused in target plants[2]. The fermentation conditions can be adjust according to expectations, directing the composition of the final product[3].

Foam formation may occur during fermentation, resulting from protein-protein interaction or interaction with other molecules present in the medium. Sometimes the foam demonstrates stability and persistence, in these cases, the use of antifoam is essential to preserve the bioprocess and the equipment[4,5]. Furthermore, in airlift bioreactors, the agitation of the medium occurs with the upward entry of air, which can favor the formation of foam in the fermentation medium, if it already has characteristics that predispose the occurrence of foam, such as interaction with proteins[6].

Therefore, this study aims to study the use of antifoam and its effect in the fermentatios of a fungal extract using microalgal biomass as a substrate in an Airlift bioreactor. In response, we quantified the production of enzymes involved in bioherbicidal activity (amylase, cellulase, laccase, lipase, and peroxidase)[7,8] in extracts obtained by different fermentations.

2 MATERIAL & METHODS

The microalgae used as fermentative substrate belong to the genus *Chlorella* spp. and come from the phytoremediation of wastewater from biogas production (digestate), implemented at EMBRAPA Swine and Poultry (Concordia, SC, Brazil)[9]. The fungus used in this study was *Trichoderma koningiopsis* (identification code in GenBank MK860714).

The fermentations occurred in an benchtop airlift bioreactor, model Bio-Tec-Pro-II (Tecnal, Brazil), with a working volume of 3.0L. Five different fermentations were done, with a 2.0 L of medium, in which adjustments in the operational parameters were made until an adequate condition for the development of the bioherbicide potential was obtained. The medium comprised 200 g of wet microalgal biomass, and the remainder of the liquid portion was divided between distilled water, inoculum, and antifoam (see Table 1).

As a control, F1 was performed without inoculum, only with the substrate. In F2, an antifoam called Fermax, provided by the Centro de Tecnologia Canavieira (CTC) was used. In testes F3, F4, and F5 was used the antifoam AFP 320 (FAXON Química). Table 1 also shows the total amounts of antifoam used in the fermentations, and the values are arranged as follows: $V_i+V_f=$total. F3, F4, and F5 were performed with antifoam diluted in the fermentation medium, represented by the initial volume values (V_i). In addition, the antifoam was added during the process (V_f). In F4 and F5, at each sample withdrawal, the same volume of microalgal biomass diluted in distilled water (10% concentration) was added to the bioreactor to evaluate how the process would behave

with the reintroduction of the substrate, in fed-batch mode. So that, a volume of fresh substrate equal to the volume of the withdrawn sample.

Table 1 Operating conditions used in the benchtop airlift bioreactor to optimize the fermentation process to obtain bioherbicide potential.

Fermentation	Airflow (LPM*)	Temperature (ºC)	pH**	Antifoam (mL) (Vi+Vf=total)***
F1	2	28	7.0	-
F2	2	28	8.0	0+50 = 50
F3	5	28	7.9	20+131 = 151
F4	5	28	7.8	200+34 = 234
F5	8	28	8.3	400+12 = 412

*Liters per minute
**pH was not controlled. The values refer to the end fermentation process, in 168 h.
*** Antifoam diluted in the fermentation medium, represented by the initial volume values (V_i). Antifoam add during the process (V_f).

The bioreactor was autoclaved at 120°C for 30 min. After sterilization, they were inoculated with 10^6 spores/mL of *Trichoderma koningiopsis* suspension. During fermentation, samples (approximately 20 mL) were taken every 24 h for enzymatic verification (amylase, cellulose, laccase, lipase, and peroxidase)[10-16]. One unit of enzymatic activity (U) corresponds to the formation of one micromol of product per minute by the enzyme, under test conditions.

3 RESULTS & DISCUSSION

Between 72 and 120 h of fermentation, was observed an intensification of foam formation in the medium. This may be linked to the more excellent metabolic activity of the fungus *Trichoderma koningiopsis*, a period in which the fungus assimilates nutrients from the medium[6].

The foam demonstrated stability and persistence, which may indicate the presence of saponins, possibly originating from microalgae. The main characteristic of the existence of saponins is the formation of foam when in contact with water and agitation, which was the situation observed when fermentations occurred in the Airlift bioreactor. Furthermore, as saponins are considered secondary metabolites, they can produce various agro-industrial products, including biopesticides[4]. Foam can also originate from protein-protein interaction, and interaction with enzymes produced during fermentation[5]. Antifoam did not negatively affect fungal development and enzyme production; however, it enabled fermentations to occur in this bioreactor.

Fermentations continued for 168 hours until the enzymatic activities in the culture broth reached constant values. The highest enzymatic activities occurred between 120 and 168 h, may be related the fungus's more excellent metabolic activity, followed by enzymatic activities and foam formation. Fermentations F4 and F5 gave the highest enzyme activities, which can be linked to the reintroduction of substrate during the fermentation.

During F5, dissolved oxygen remained close to 100% throughout the process. The aeration of 8 LPM, the reintroduction of a substrate, and the volume of defoamer enabled an optimized system, which benefited an excellent adaptation of the microorganism to the process, also providing a favorable environment for the production of enzymes (Figure 1).

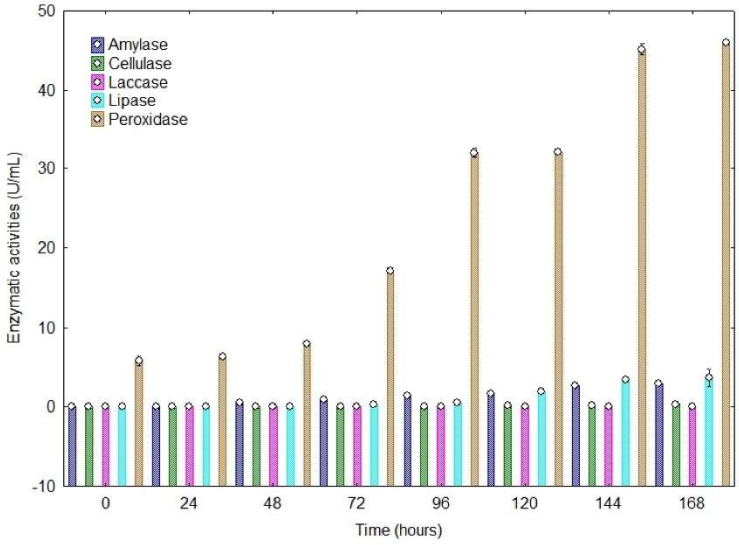

Figure 1 Results of production of amylase, cellulase, laccase, lipase, and peroxidase, in fermentation F5 (optimized condition), carried out in the airlift bioreactor, during 168 h of fermentation.

The pH of the medium increased with the addition of antifoam, which may be due to the accumulation of protein particles and changes in surface hydrophobicity[17]. The pH values of antifoam vary between 6.5 and 8.3, which corroborates the medium's pH values, which increase with the addition of antifoam[18]. Furthermore, other studies that investigated the enzymatic production of *Trichoderma koningiopsis* using microalgae biomass as substrate found that at pH 8.5, it was possible to obtain an enzymatic pool (amylase, cellulase, lipase, laccase, and peroxidase) and an ideal value of fungal biomass for application as a potential bioherbicides[8].

4 CONCLUSION

The results obtained in this study expand our knowledge about antifoam's beneficial effects in bioherbicide production using airlift bioreactors. Also, we can associate the production of enzymes with fermentative conditions such as aeration, dissolved oxygen, antifoam, pH, microorganism, and substrate.

The results suggest that use of antifoam allowed fermentation to occur, taking into account its characteristics of fermentative medium, microorganism and type of bioreactor, presenting the possibility of large-scale production of a potential bioinput, providing scientists in this field with innovative and more sustainable management tools.

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RESEARCH PAPER

Trichoderma koningiopsis fermentation in airlift bioreactor for bioherbicide production

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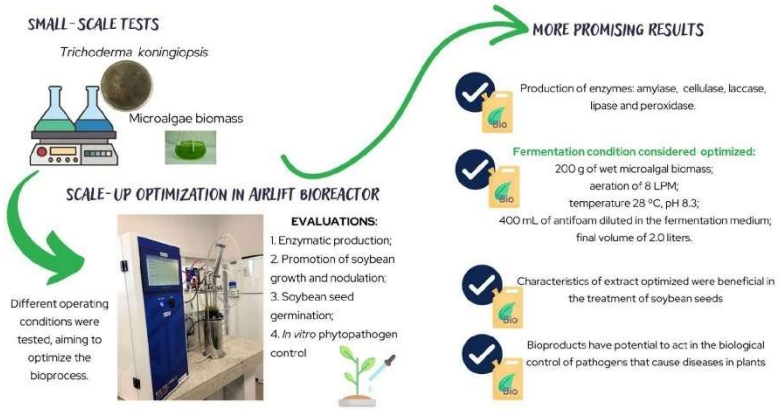
Abstract
During scaling of fermentations, choosing a bioreactor is fundamental to ensure the product's quality. This study aims to produce bioherbicides using *Trichoderma koningiopsis* fermentation, evaluating process parameters in an Airlift bioreactor. As a response, we quantified the production of enzymes involved in the bioherbicide activity (amylase, cellulase, laccase, lipase, and peroxidase). In addition, it evaluated the agronomic efficiency of the fermented extract optimized through tests that promoted soybean growth and nodulation, soybean seed germination, and in vitro phytopathogen control. As a result of optimizing the scaling bioprocess, it was possible to obtain an adequate fermentation condition, which, when applied to soybean seeds, had beneficial effects on their growth. It allowed the production of an enzyme cocktail. These results add a crucial biotechnological potential factor for the success of the optimized formulation in the Airlift bioreactor, in addition to presenting relevant results for the scientific community.

Graphical Abstract



Keywords Bioprocesses · Fermentative strategies · Process optimization · Agronomic validation

Extended author information available on the last page of the article

Artigo científico intitulado “Fermented Beverage Based on Lupines (*Lupines luteus*) Using Water Kefir” publicado no periódico Food Science and Engineering e de autoria de Claudia Moreira Santa Catharina Weis, Luan Gabriel Techi Diniz, Gessica Suiany Andrade, Luciane Mendes Monteiro, Jane Manfron, Aline Frumi Camargo, Simone Kubeneck, Gabriel Henrique Klein, Larissa Capeletti Romani, Vitoria Dassoler Longo, Julia Pieper Nerling, Luciano Tormen, Catia Tavares Dos Passos Francisco, Helen Treichel e Larissa Canhadas Bertan. O artigo pode ser acessado pelo DOI: https://doi.org/10.37256/fse.5120243694 .

Food Science and Engineering
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UNIVERSAL WISER PUBLISHER

Research Article

Fermented Beverage Based on Lupines (*Lupinus luteus*) Using Water Kefir

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Abstract: This research aimed to elaborate and characterize a fermented lupine vegan drink from water kefir grains. Firstly, the standard protocols were carried out to extract and identify alkaloids in lupine. The second stage consisted of elaborating the water-soluble lupine vegetable extract (WLVE), and the effect of sucrose, inulin, and xanthan gum concentration on the extract was evaluated. The formulations were characterized for cell growth of kefir grains, beverage yield, soluble solids, and lactic acid. In the third step, the optimized drink (fermented and non-fermented) was characterized for moisture content, ash, total lipids, crude protein, and determination of total carbohydrates. The elaboration and characterization of a fermented vegan drink from water kefir grains using lupine was conducted. The optimized fermented drink was the formulation given by the central point. The lupine seed characterization revealed a high protein content, and the optimized beverage characterization showed a considerable carbohydrate content. The optimized drink has enzymatic activity with an emphasis on lipases. The drink's development as a new product using water-soluble lupine plant extract brought an exciting application for this legume and an additional food option for vegan consumers or consumers with dietary restrictions related to dairy products.

Keywords: vegan market, experimental design, product development, dietary restrictions

1. Introduction

In recent years, the dietary profile of consumers has shown a trend towards healthier, more natural, accessible, low-cost, safe, and convenient products [1-2]. People are increasingly concerned about protecting natural resources and ethical issues in their lifestyles, and, together with this, the population consuming vegan food has grown. The option for a vegan diet can come from several reasons, such as nutrition and health, allergies and/or intolerance to dairy products, and concern for animal welfare [3].

Artigo científico intitulado “Study and characterization of a product based on a vegetable extract of quinoa fermented with water kefir grains” publicado no periódico internacional World Journal of Microbiology and Biotechnology e de autoria de Flavia Leticia Sanches, Cláudia Moreira Santa Catharina Weis, Giovanna Camile Vaz Gonçalves, Gessica Suiany Andrade, Luan Gabriel Techi Diniz, Aline Frumi Camargo, Simone Kubeneck, Gabriel Henrique Klein, Larissa Capeletti Romani, Vitoria Dassoler Longo, Monize Bürck, Luciano Tormen, Anna Rafaela Cavalcante Braga, Catia Tavares Dos Passos Francisco, Helen Treichel e Larissa Canhadas Bertan. O artigo pode ser acessado pelo DOI: https://doi.org/10.1007/s11274-024-03943-x.

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RESEARCH

Study and characterization of a product based on a vegetable extract of quinoa fermented with water kefir grains

Flavia Leticia Sanches[1] · Cláudia Moreira Santa Catharina Weis[1] · Giovanna Camile Vaz Gonçalves[1] · Gessica Suiany Andrade[1] · Luan Gabriel Techi Diniz[1] · Aline Frumi Camargo[2] · Simone Kubeneck[2] · Gabriel Henrique Klein[2] · Larissa Capeletti Romani[2] · Vitoria Dassoler Longo[2] · Monize Bürck[3,4] · Luciano Tormen[1] · Anna Rafaela Cavalcante Braga[3,4,5] · Catia Tavares Dos Passos Francisco[1] · Helen Treichel[1,2] · Larissa Canhadas Bertan[1]

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Abstract
This work aimed to study and characterize a product based on vegetable extract of quinoa (WVEQ) fermented with water kefir grains. The effect of sucrose concentration (SC), inulin concentration (IC), and xanthan gum (XG) concentration were evaluated using a central composite design (CCD) 2^3. They were subsequently characterized regarding cellular growth of the grains, beverage yield, pH, soluble solids, carbon dioxide (CO_2) production, lactic acid, and ethanol production. Therefore, for the final stage, two formulations (F1 and F8) of the CCD were chosen to be characterized in terms of proximate composition, microbiological composition of the kefir culture, analysis of organic compounds, sensory analysis, and enzymatic and microbiological characterization before and after simulation of in vitro gastrointestinal digestion. In the two chosen products, one can see that fermentation optimized the bioavailability of proteins due to the high proteolytic activity of the microorganisms in kefir and the increase in lipid content. In identifying microorganisms, there was a prevalence of *Saccharomyces* sp. yeasts. In the sensory analysis, the F8 formulation showed better results than the F1 formulation. In vitro, gastrointestinal digestion showed reduced lactic acid bacteria and yeast and increased acetic acid bacteria in the liquid phase for both formulations. In the enzymatic profile, there was a reduction in all enzymes analyzed for both formulations, except for amylase in F1, which went from 14.05 U/mL to 39.41 U/mL. Therefore, it is concluded that using WVEQ as a substrate for the product appears to be a viable alternative with nutritional and technological advantages for serving a specific market niche.

Keywords Formulations · Pseudocereal · Enzymes · Simulated digestion · Probiotics

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Introduction

A change in the dietary profile of consumers is observed concerning the growing trend towards plant-based diets. This is consistent with the increase in vegan people or people who have a food allergy that restricts milk intake (Diniz et al. 2020; Mosso et al. 2020; Väkeväinen et al. 2020). The increased consumption of plant-based foods may also be associated with physiological factors such as lactose intolerance or allergies to milk proteins (α-lactalbumin, β-lactoglobulin, and casein) (Sharma et al. 2001; Vanga and Raghavan 2018).

The vegan market has been consequently increasing its space, where global sales in 2019 reached around 55 billion dollars, 25 times more than in 2015, and in 2023, there is a prospect of getting more than 60 billion dollars (Statisca

Artigo científico intitulado “Water-soluble vegetable extract of cashew nut (*Anacardium occidentale* L.) fermented with water kefir: development and characterization” publicado no periódico Food and Humanity e de autoria de Cláudia Moreira Santa Catharina Weis, Giovana Camile Vaz Gonçalves, Flavia Leticia Sanches, Milena Cia Retcheski, Aline Frumi Camargo, Simone Kubeneck, Gabriel Henrique Klein, Larissa Capeletti Romani, Vitoria Dassoler Longo, Luciano Tormen, Catia Tavares Dos Passos Francisco, Helen Treichel e Larissa Canhadas Bertan. O artigo pode ser acessado pelo DOI: https://doi.org/10.1016/j.foohum.2024.100307.

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Water-soluble vegetable extract of cashew nut (*Anacardium occidentale* L.) fermented with water kefir: development and characterization

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ABSTRACT

Cashew nuts from the *Anacardium occidentale* L. tree stand out as a promising raw material for developing innovative food products due to their rich nutrients and versatile applications. This work aimed to study and characterize a product based on a vegetable extract of cashew nut (CNWSE) fermented with water kefir grains. A 2^3 central composite design (DCC) was applied, with independent variables: sucrose (S), inulin (I), and xanthan gum (GX), and the dependent variables were grain cell growth (Δx), beverage yield (R), soluble solids (SS) and lactic acid (LA). Proximate analysis, organic compounds, and enzymatic activity characterized the optimized drink (fermented and non-fermented). Among the 11 formulations studied, F2 was said to be optimized based on the statistical results generated and by the researchers' choice after evaluating sensory attributes. High levels of proteins, lipids, and carbohydrates determined in cashew nuts indicate that this raw material is an excellent energy source and protein of vegetable origin. In the enzymatic analysis of the optimized drink, the majority of enzyme activity was amylase, followed by cellulose, proteases, peroxidase, lipases, and laccases. The approach of this work contributes to the diversification of vegetable protein-based food options on the market while promoting principles of sustainability and inclusion.

1. Introduction

The vegan audience has increased around the world in recent years. Asia has 19% of the vegetarian population; in Africa and the Middle East, the average is 16%; South and Central America around 8%; North America 6%; and Europe 5% (Hargreaves et al., 2021). In Brazil, between 2012 and 2018, there was an increase of 75% in the population that considers themselves vegetarian. This represents 30 million Brazilians, of which 7 million are probably considered vegans, according to the Brazilian Society of Vegetarians (SBV), following a global trend (SBV, 2018). This may be due to the population's concern about protecting natural resources and lifestyle changes for ethical reasons. Therefore, the option for a vegan diet is probably multifactorial, as, in addition to what has been mentioned, the option may be due to health issues, such as allergies and intolerance to milk protein, nutrition, and health, and concern for animal welfare (Moretti et al., 2022). The industry is soon aware of this trend; however, there is a need for more significant investment in the area so that new products are created for the vegan public and for people who have restrictions on milk consumption (Wolf et al., 2020). This fact can be proven when researching the market. The American vegan food market was worth $15.4 billion in 2020 and is expected to increase to $22.7 billion by 2025 (Martinelli & De Canio, 2022). Additionally, global brands such as McDonald's (McPlant burger) and PepsiCo (The PLANeT Partnership) are attracted to this market and have created alternatives to animal products (Ghaffari et al., 2021).

Fermented drinks represent complex solutions containing various chemical compounds derived from the extract used, whether of

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Research Article

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Levulinic acid as a strategy for control of postharvest citrus blue mold by a newly isolated *Penicillium italicum*

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Abstract

BACKGROUND: Levulinic acid (LA) is a multifunctional compound that is relevant to the economy of bio-based chemical products. *Citrus sinensis*, the Valencia variety, is one of the world's most consumed citrus fruit varieties but suffers significant losses as a result of blue mold infestation. This research evaluated the inhibitory action of unpurified levulinic acid obtained from watermelon residues against the fungus *Penicillium italicum*, proposing a new use as a fungal inhibitor.

RESULTS: LA was evaluated in *in vitro* tests and inhibited the mycelial growth of the fungus. In *in vivo* experiments with oranges, fungal proliferation in fruits was investigated by applying a crude mixture containing LA (43 mmol L^{-1}) and formic acid (FA, 28 mmol L^{-1}), and comparing the effects with FA alone and negative control (without inhibitory agent). The weight loss and disease incidence results decreased when LA was used as an inhibitory agent, with no negative impacts on fruit quality being observed. Its inhibitory effect was confirmed by determining the activities of the antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidase (POD), where there was no increase in activities owing to inhibition of the fungus before its proliferation in the fruits.

CONCLUSION: This study provides relevant data on the new use of raw LA as an antifungal agent, an effect still unexplored for this compound in recent literature, offering a practical and innovative solution to combat blue mold.

Keywords: fungal inhibition; 4-oxopentanoic acid; citrus fruits; blue mold

INTRODUCTION

Citrus fruits are the most widely sold worldwide, commanding high commercial value. Notable varieties include orange, lemon, lime and tangerine. Citrus ranks as the 26th largest crop globally in terms of cultivated area, primarily produced by China, Brazil, India and the USA.[1,2] Citrus fruits boast high nutritional value; however, postharvest, untreated fruits become susceptible to fungal infections caused by mechanical damage or inadequate storage and transportation conditions. The high rate of generated waste leads not only to economic and environmental losses, but also additional public health concern. Aiming to promote the circular economy and contribute to reducing waste generation, citrus fruits were chosen for this study.[3-5]

Among the diseases affecting citrus fruits, blue mold caused by the fungus *Penicillium italicum* is the most prominent. This necrotrophic fungus induces citrus rot and produces mycotoxins, posing a potential threat to human health. Moreover, it can attack healthy fruits directly, regardless of lesions. It is responsible for significant losses in fruit production, impacting the economies of producing countries such as Brazil, the largest exporter of this biomass.[6] Typically, control of blue mold involves the application of synthetic fungicides, which can contribute to environmental pollution and the development of resistant fungal strains. Therefore, searching for new compounds capable of effectively combating *P. italicum*, ensuring sanitation and preservation is necessary to guarantee the supply of citrus fruits with reduced processing, enhanced safety and high quality.

Levulinic acid (LA), or 4-oxopentanoic acid, is an organic acid obtained from the degradation of cellulose or the acid-catalyzed synthesis of hexoses present in biomass. It can also be derived from residues with high levels of glucose and fructose, such as fruit residues.[7] Like most organic acids, the Food and Drug Administration (FDA) recognizes LA as safe (GRAS). It exhibits

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Hydrothermal and microwave-assisted synthesis of levulinic acid from watermelon residue

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Abstract: Levulinic acid (LA) is considered a versatile chemical building block and has emerged as one of the leading platforms for products derived from biomass. It is regarded as important from an economic perspective. The main route of synthesis to obtain LA is from the degradation of cellulose by acid catalysis, with lignocellulosic biomass being a promising and sustainable way to obtain it. In this study, for the first time, the use of watermelon residues was proposed for the synthesis of levulinic acid, adding value to a residual biomass that has still been little explored for this purpose.
Watermelon residues were subjected to acid hydrolysis using H_2SO_4 or HCl. Two reactors were analyzed for this purpose – autoclave and microwave assisted. The experiments were optimized through a central composite design, where temperature, biomass load, and acid concentration in the microwave were evaluated as variables. In the autoclave, the variables investigated were catalyst concentration and biomass loading.
The highest yields, 14.8% and 17% by weight, were obtained with solid fraction (SF) in micro wave (MW). In the best condition obtained in the central composite design, the liquid fraction (LF) and SF were analyzed. It was observed that a clean product, containing only levulinic acid and formic acid, was in the liquid fraction. The MW was more efficient and is a promising alternative for reactions requiring energy. This study contributes to an energy-saving strategy, using waste and low-cost catalysts,

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CRITICAL REVIEW

Fungus-based bioherbicides on circular economy

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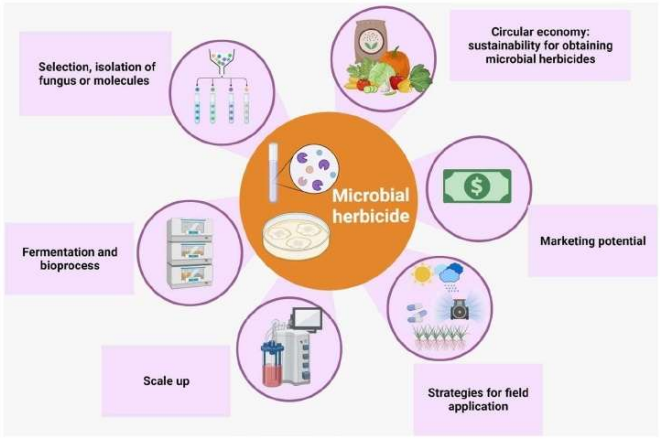
Abstract
This review aimed to show that bioherbicides are possible in organic agriculture as natural compounds from fungi and metabolites produced by them. It is discussed that new formulations must be developed to improve field stability and enable the commercialization of microbial herbicides. Due to these bottlenecks, it is crucial to advance the bioprocesses behind the formulation and fermentation of bio-based herbicides, scaling up, strategies for field application, and the potential of bio-herbicides in the global market. In this sense, it proposed insights for modern agriculture based on sustainable development and circular economy, precisely the formulation, scale-up, and field application of microbial bioherbicides.

Graphical abstract



Keywords Bioherbicides · Secondary metabolites · Bioprocess · Production safety

Extended author information available on the last page of the article

Artigo científico intitulado “Production and concentration of queratinases and application of fermentation residual in removing hexavalent chromium” publicado no periódico internacional Bioprocess and Biosystem Engineering e de autoria de Andressa Janaína Warken, Simone Kubeneck, Aline Frumi Camargo, Vitória Dassoler Longo, Larissa Capeletti Romani, Gabriel Henrique Klein, Sérgio L. Alves Jr, Maulin P. Shah e Helen Treichel. O artigo pode ser acessado pelo DOI: https://doi.org/10.1007/s00449-024-03087-0.

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RESEARCH PAPER

Production and concentration of keratinases and application of fermentation residual in removing hexavalent chromium

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Abstract
The production of keratinases was evaluated in submerged fermentation with *Aspergillus niger* and by pigs' swine hair in a batch bioreactor. Experimental planning was performed to assess the interaction between different variables. The enzyme extract produced was characterized at various pH and temperatures and subjected to enzyme concentration using a biphasic aqueous system and salt/solvent precipitation techniques. In addition, the substrate's potential in reducing hexavalent chromium from synthetic potassium dichromate effluent with an initial concentration of 20 mg L^{-1} of chromium was evaluated. The resulting enzyme extract showed 89 ± 2 U mL^{-1} of keratinase. The enzyme concentration resulted in a purification factor of 1.3, while sodium chloride/acetone and ammonium sulfate/acetone resulted in a purification factor of 1.9 and 1.4, respectively. Still using the residual substrate of swine hair from the fermentation, a 94% reduction of hexavalent chromium concentration occurred after 9 h of reaction. Thus, the study proved relevant for producing keratinases, with further environmental applicability and the possibility of concentrating the extract via low-cost processes.

Keywords By-products · Keratinase · Swine hair · Chromium (VI)

Introduction

Brazil is the 4th largest pork producer in the world and, in 2020, produced more than 4.4 million tons. In this sense, this sector's waste is significant, causing environmental concern. Swine hair, feathers, beaks, wool, nails, and hooves are sources of keratin, a rigid and fibrous protein widely found in waste from mammalian and poultry processing [1].

These residues are usually sent to landfills but are essential substrates for producing enzymes such as keratinases and could be used for applications such as reducing heavy metals in industrial effluents [2–4].

Keratinases (EC3.4.21/24/99.11) are a category of metalloproteases that hydrolyze keratins present in the keratin substrate due to their high specificity for this type of substrate [5, 6]. These enzymes can be obtained commercially via chemical or biotechnological methods, using low-cost and environmentally appropriate fermentative processes by a series of microorganisms to hydrolyze the keratin in peptide bonds [7].

The production of this enzyme is relatively low, and consequently, its commercial value is high [6, 8]. For this reason, evaluating simple and low-cost enzyme-concentration techniques becomes relevant after fermentative processes aiming to improve the results obtained for the specific enzyme activity.

Before applying a homemade enzyme, it was interesting to think about its concentration, using a methodology with a low cost, too. The Biphasic Aqueous System (BAS) involves a salt and one or two polymers, forming a solution in two immiscible liquid phases. This strategy is suitable for application in the purification and concentration of enzymes, replacing other techniques, such as chromatography and

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Artigo de revisão intitulado “Application of mushrooms as a pollutant remediator”, publicado no periódico Brazilian Applied Science Review e de autoria de Guiliherm Cabral Wancura, Maria Leticia Engel Leite, Laísa Prestes, Breno de Paiva Magalhães Simone Kubeneck, Aline Frumi Camargo, Altemir José Mossi e Helen Treichel. O artigo pode ser acessado pelo DOI: https://doi.org/10.34115/basrv8n1-012.

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Aplicação de cogumelos como remediador de poluentes

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ABSTRACT
From the Industrial Revolution, at the end of the 19th century and the beginning of the 20th century, there was a significant impact on the environment due to increased emission of environmental pollutants. This problem has seen considerable growth in recent years due to population growth, resulting in further ecosystem changes due to human action. Furthermore, demands for water, electricity, and food have led to increasingly large concentrations of waste being emitted. In this context, bioremediation is an up-and-coming biotechnological alternative to combat the growing emission of pollutants into the environment. From this perspective, basidiomycetes, popularly known as mushrooms, are an effective tool for remediating environmental pollutants. These fungi use different mechanisms in bioremediation processes, such as biodegradation, biosorption, bioaccumulation, and bioconversion. Currently, the remediation potential of mushrooms has been increasingly studied. Therefore, this bibliographic review aims to analyze the literature on the potential of mushrooms in pollutant remediation. At the end of this analysis, it is concluded that macrofungi are a very effective and promising tool for suppressing the emission of environmental pollutants.

Keywords: mycoremediation, heavy metals, dyes, pharmaceutical residues, biodegradation, biosorption.

RESUMO
Desde a Revolução Industrial, no final do século XIX e início do século XX, houve um impacto significativo no meio ambiente devido ao aumento da emissão de poluentes ambientais. Esse problema teve um aumento considerável nos últimos anos devido ao crescimento populacional, resultando em mais mudanças no ecossistema devido à ação humana. Além disso, as demandas por água, eletricidade e alimentos levaram à emissão de concentrações cada vez maiores de resíduos. Nesse contexto, a biorremediação é uma alternativa biotecnológica emergente para combater a crescente emissão de poluentes no meio ambiente. Sob essa perspectiva, os basidiomicetos, popularmente conhecidos como cogumelos, são uma ferramenta eficaz para a remediação de poluentes ambientais. Esses fungos utilizam diferentes mecanismos nos processos de biorremediação, como biodegradação, biossorção, bioacumulação e bioconversão. Atualmente, o potencial de remediação dos cogumelos tem sido cada vez mais estudado. Portanto, esta revisão bibliográfica tem como objetivo analisar a literatura sobre o potencial dos cogumelos na remediação de poluentes. Ao final dessa análise, conclui-se que os macrofungos são uma ferramenta muito eficaz e promissora para suprimir a emissão de poluentes ambientais.

Capítulo de livro intitulado “Controle de plantas espontâneas mediado pelo uso de bioherbicidas” de autoria de Aline Frumi Camargo, Simone Kubeneck, Júlia Pieper Nerling, Cauê Betiato Bieniek, Larissa Capeletti Romani, Altemir José Mossi, Gislaine Fongaro e Helen Treichel. O capítulo faz parte do livro Tendências Biotecnológicas Sustentáveis Para Fins de Saúde Única e tem como editora Dra. Gislaine Fongaro, O capítulo pode ser acessado pelo DOI: https://doi.org/10.56041/9786599841859-3

Capítulo 03

Controle de plantas espontâneas mediado pelo uso de bioherbicidas

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Capítulo de livro intitulado “Carbohydrate-based economy: Perspectives and challenges” de autoria de Caroline Dalastra, Natalia Klanovicz, Simone Kubeneck, Fábio Spitza Stefanski, Debora Fretes Argenta, Gabriela Schneider Rauber, Thiago Caon, Rafael Dorighello Cadamuro, Gislaine Fongaro e Helen Treichel. O capítulo faz parte do livro Polysaccharide Degrading Biocatalyst, organizado por Rosana Goldbeck e Patricia Poletto. O capítulo pode ser acessado pelo DOI: https://doi.org/10.1016/B978-0-323-99986-1.00014-4.

CHAPTER

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Carbohydrate-based economy: Perspectives and challenges

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1. Introduction

Following the population's growing demand for efficient and affordable products, the bioeconomy and the circular economy have emerged in research and the market in recent years. While the circular economy is more comprehensive and is concerned with all productive sectors and the valuation of their waste, the bioeconomy focuses on bio-based products and processes. It covers the development of fully or partially bio-based products, not necessarily applying the circular economy concept.

In this sense, the carbohydrate-based economy is contained in the bioeconomy context but not necessarily inserted in the circular economy. Linking both concepts can be advantageous in creating market opportunities for carbohydrate-based products and attracting consumers and industries. Fig. 1 provides an overview of the context in which the carbohydrate-based economy is inserted to better understand the interactions between concepts.

Capítulo de livro intitulado “Wastewater as a feasible feedstock for biorefineries” de autoria de Caroline Dalastra, Thamarys Scapini, Simone Kubeneck, Aline Frumi Camargo, Natalia Klanovicz, Sérgio Luiz Alves Júnior, Maulin P. Shah e Helen Treichel. O capítulo faz parte do livro Biorefinery for Water and Wastewater Treatment, organizado por Maulin P. Shah. O capítulo pode ser acessado pelo DOI: https://doi.org/10.1007/978-3-031-20822-5_1.

Wastewater as a Feasible Feedstock for Biorefineries

Caroline Dalastra, Thamarys Scapini, Simone Kubeneck, Aline Frumi Camargo, Natalia Klanovicz, Sérgio Luiz Alves Júnior, Maulin P. Shah, and Helen Treichel

Abstract Large volumes of wastewater are generated by industrial and urban activities and can be effectively used in other processes to reduce dependence on freshwater. To prevent risks to human and environmental health, the treatment of effluents before discharge is imperative. In conventional biofuel production, large volumes of fresh water are needed for the entire process. The development of technologies and strategies based on non-potable water resources is fundamental for optimizing biofuel biorefineries and value-added products in the market. Thus, wastewater application is an alternative to freshwater resources in a circular economy context. In addition to promoting wastewater management and treatment, energy recovery, value-added products, and reduced competition for freshwater, naturally occurring compounds in wastewater can function as nutrient sources for cell growth and maintenance of microorganisms. To carry out a comprehensive review on the alternative use of water for bioproduct purposes, in this chapter, we will address the most recent studies

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