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Tesis doctoral:

DECOLORATION OF DYE FROM THE EFLUENTS OF THE TEXTILE INDUSTRY BY MACROMYCETES

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DECOLORATION OF DYE FROM THE EFLUENTS OF THE TEXTILE INDUSTRY BY MACROMYCETES

Linha de Pesquisa: Tecnologias e gestão de resíduos

Projeto de Pesquisa apresentado ao Programa de Pós-Graduação em Engenharia Ambiental da Universidade Regional de Blumenau como requisito parcial para a obtenção o título de Doutor em Engenharia Ambiental.

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I dedicate this thesis to my husband Luiz Henrique da Silva

for all support and unconditional love.

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RESUMO

O tratamento de efluentes proveniente da indústria textil constitui um importante desafio tecnológico. A descoloração de águas residuais por métodos químicos, físicos e biológicos tradicionais apresenta como principais desvantagens os custos elevados, pouca eficiência e aplicabilidade limitada. Por estas razões, o propósito desta tese é, ter um enfoque interdisciplinar, com soluções inovadoras para a resolução destes problemas. Além disso, há a valorização de um importante resíduo agroindustrial gerado amplamente na região sul do Brasil, o resíduo da palmeira pupunha (*Bactris gasipaes*). Para isso, realizou-se ensaios de degradação de corantes sintéticos através de fermentação em estado sólido (FES) em presença de fungos de podridão branca no residuo de pupunha. O cultivo no estado sólido foi realizado em diferentes condições de operação: quantidades de resíduo, volume de fase líquida e concentrações de corante. O estudo experimental de dados obtidos mostra que a enzima ligninolítica produzida em maior quantidade por *G. lucidum* foi a lacase. Os experimentos de controle realizados com o corante Remazol Brilliant Blue R (RBBR) mostraram que a condição sob a qual a maior porcentagem de remoção de cor (97%) foi conduzida pela atividade de lacase. Para as experiências realizadas com efluentes industriais, as maiores atividades de lacase foram obtidas em baixas concentrações de efluentes, sugerindo a existência de um efeito inibitório no metabolismo fúngico. Por outro lado, foram realizados testes com diferentes tipos de fungos de podridão branca para avaliar a eficácia da fermentação em estado sólido contra a fermentação submersa (FS) e a adsorção como estratégias para a descoloração de efluentes têxteis. A maior porcentagem de descoloração (80%) foi alcançada após 10 dias de fermentação em estado sólido usando o fungo *G. lucidum* EF 31, não havendo diferenças significativas em relação aos outros dois fungos. Por conseguinte, conclui-se que a adição de resíduos de pupunha e o uso do sistema FES proporcionam um habitat adequado para o crescimento do fungo e a conseqüente produção da enzima lacase, necessária para a decoloração do efluente. Estes resultados foram tomados como base para a escala do sistema, utilizando primeiro um reator de 2 L de capacidade e, finalmente, um de 15 L. Esses experimentos, de 50 dias, foram realizados com o fungo *G. lucidum* e o corante Remazol Brilhant Blue R (RBBR) e a eficácia da descoloração, atividade lacase e pH dentro do biorreator foram analisados. O processo de adsorção foi estudado caracterizando as interações entre os grupos funcionais presentes na superfície do resíduo e as moléculas de corante usando a espectroscopia de transformação de Fourier infravermelho (FTIR). No biorreator de 2 L, a descoloração atingiu quase 80% e a atividade de lacase foi de 847,2 $UI.mL^{-1}$. No biorreator 15 L, a descoloração atingiu 91%. Nos estudos de adsorção realizados com o resíduo de pupuna, 50% da remoção de cor foi alcançada nas primeiras 24 horas, porém a diminuição do pH registrada nesta experiência pode ser devido ao crescimento de outros microorganismos, o que contribuiria para a descoloração. Os resultados do teste de fermentação em estado sólido, independentemente do tamanho do biorreator utilizado, indicam claramente que o fungo *G. lucidum* que cresce no resíduo de pupuna

tem a capacidade de descolorir os efluentes têxteis, particularmente aqueles que contêm o corante RBBR.

Palavras-chave: Efluente têxtil; Resíduo agroindustrial; Fungo de podridão branca; Fermentação em estado sólido; Ampliação de escala.

ABSTRACT

The treatment of effluent from the textile industry is an important technological challenge. Decoloration of wastewater by traditional chemical, physical and biological methods presents as major drawbacks: the high costs, low efficiency and limited applicability. For these reasons, the purpose of this thesis is to have an interdisciplinary approach with innovative solutions to solve these problems. In addition, there is a valorization of an important agroindustrial residue generated largely in the southern region of Brazil, the residue of the pupunha palm (*Bactris gasipaes*). In these sence, degradation tests of synthetic dyes were carried out through solid state fermentation (SSF) in the presence of white rot fungi with pupunha residue. Solid state fermentation was performed under different operating conditions: amount of residue, volume of liquid phase and concentrations of dye. The experimental study of the data shows that the ligninolytic enzyme produced in greater quantity by *G. lucidum* the laccase. The control experiments performed with Remazol Brilliant Blue R (RBBR) showed that the condition with the highest percentage of color removal (97%) was conducted by laccase activity. For the experiments carried out with industrial effluents, the major activities of laccase were obtained in low concentrations of effluents, suggesting the existence of an inhibitory effect in the fungal metabolism. On the other hand, tests were carried out with different types of white rot fungi to evaluate the efficacy of solid state fermentation against submerged fermentation (SF) and adsorption as strategies for the decoloration of textile effluents. The highest percentage of decoloration (80%) was reached after 10 days of solid state fermentation using the fungus *G. lucidum* EF 31, and there were no significant differences in relation to the other two fungi. Therefore, it was concluded that the addition of pupunha residues and the use of the SSF system provide a suitable habitat for the growth of the fungus and the consequent production of the lacase enzyme, necessary for the effluent decoloration. These results were taken as a basis for the scale of the system, using first a reactor of 2 L of capacity and finally one of 15 L. These experiments, of 50 days, were realized with the fungus *G. lucidum* and the dye Remazol Brilhant Blue R (RBBR) and the efficacy of decoloration, laccase activity and pH inside the bioreactor were analyzed. The adsorption process was studied by characterizing the interactions between the functional groups present on the surface of the residue and the dye molecules using infrared Fourier transform (FTIR) spectroscopy. In the 2 L bioreactor, decoloration reached almost 80% and the laccase activity was 847.2 $IU.mL^{-1}$. In the 15 L bioreactor, the decoloration reached 91%. In the adsorption studies performed with the pupunha residue, 50% of the color removal was achieved in the first 24 hours, but the decrease in the pH recorded in this experiment may be due to the growth of other microorganisms, which would contribute to the decoloration. The results of the solid state fermentation test, regardless of the size of the bioreactor used, clearly indicate that the *G. lucidum* fungus that grows on the pupunha residue has the ability to decolor the textile effluents, particularly those containing the RBBR dye.

Keywords: Textile effluent; Agro-industrial residue; White-rot Fungi; Solid-state fermentation; Scale-up.

RESUMEN

El tratamiento de los efluentes provenientes de la indústria textil constituye un importante desafío tecnológico. La descoloración de dichas aguas residuales mediante métodos químicos, físicos y biológicos tradicionales presenta como principales desventajas los elevados costos, bajas eficiencias y aplicabilidad limitada. Por estas razones, el propósito de esta tesis es proporcionar, desde un enfoque interdisciplinar, soluciones inovadoras para la resolucion de dichos problemas. Asimismo, también se plantea en esta tesis la valorización de un importante resíduo agroindustrial generado ampliamente en la región sur de Brasil, el residuo de la palmera pupuña (*Bactris gasipaes*). Para ello, se han realizado ensayos de degradación de colorantes sintéticos mediante fermentación en estado sólido (FES) en presencia del hongo de podredumbre blanca *Ganoderma lucidum* EF 31 cultivado en el residuo de pupuña. El cultivo en estado sólido se realizó bajo diferentes condiciones de operación, es decir, usando diferentes cantidades del residuo de pupuña, volúmenes de fase líquida y concentraciones de colorante. El análisis experimental de los datos obtenidos muestra que la enzima ligninolítica producida en mayor cantidad por *G. lucidum* fue la lacasa. Experimentos de control realizados con el colorante Remazol Brilliant Blue R (RBBR) mostraron que la condición bajo la que se conduce el mayor porcentaje de eliminación de color (97%) conduce igualmente a la mayor actividad lacasa. Para los experimentos realizados con efluentes industriales, las mayores actividades lacasa fueron obtenidas a bajas concentraciones de efluentes, sugiriendo la existencia de un efecto inhibitorio en el metabolismo fúngico al aumentar la dosis de colorantes. Por otra parte, se realizaron ensayos con diferentes tipos de hongos de podredumbre blanca para evaluar la eficacia de la fermentación de estado sólido frente a la fermentación sumergida (FS) y la adsorción como estrategias para la decoloración de los efluentes textiles. El mayor porcentaje de decoloración (80%) se alcanzó a los 10 días de fermentación en estado solido usando el hongo *G. lucidum* EF 31. Por tanto, se conluye que la adición del residuo de pupuña y el empleo del sistema FES proporciona un hábitat adecuado para el crecimiento del hongo y la consiguiente producción de la enzima lacasa, necesária para la decoloración del efluente. Estos resultados se tomaron como base para el escalado del sistema, primero usando un reactor de 2 L de capacidad, y finalmente uno de 15 L. Estos experimentos, de 50 días de duración, se realizaron con el hongo *G. lucidum* y el colorante Remazol Brilhant Blue R (RBBR) y se analizó la eficacia de decoloración, la actividad lacasa y el pH en el interior del biorreactor. El proceso de adsorción se estudió mediante la caracterización de las interacciones entre los grupos funcionales presentes en la superficie del residuo y las moléculas de colorante usando espectroscopía infrarroja por transformada de Fourier (FTIR). En el biorreactor de 2 L, la descoloración alcanzó casi el 80% y la actividad lacasa fue de hasta 847,2 $UI \cdot mL^{-1}$. En el biorreactor de 15 L, la descoloración alcanzó el 91%. En los estudios de adsorción realizados con el residuo de pupuña, se alcanzó el 50% de remoción de color en las primeras 24 horas, sin embargo, el descenso del pH registrado en estos experimentos podría deberse al

crecimiento de otros microorganismos, lo que contribuiría a la decoloración observada. Los resultados del ensayo de fermentación en estado sólido, independentemente del tamaño del biorreactor utilizado, indican claramente que el hongo *G. lucidum* creciendo sobre el residuo de pupuña tiene la capacidad de decolorar efluentes textiles, particularmente aquellos que contienen el colorante RBBR.

Palabras clave: Efluente textil; Residuos agroindustriales; Hongo de pudrición blanca; Fermentación en estado sólido; Bioreactores.

SUMMARY

LIST OF TABLES

LIST OF CHART

LIST OF FIGURES

LIST OF ABBREVIATIONS

WRF	White Rot Fungi
G. lucidum	*Ganoderma lucidum*
T. versicolo	*Trametes versicolor*
PDA	Potato Dextrose Agar
RBBR	Remazol Brilliant Blue R
RDB	Residual Dyebath
FE	Final Effluent
q_t	Adsorbate Concentration
SSF	Solid state fermentation
SF	Submerge fermentation
C_o	Initial Concentration of the compound
C_e	Equilibrium Concentration in solution
M	Mass of Adsorbent
V	Solution Volume
Q_e	Equilibrium State
C_t	Concentration at time t
ED	Experimental designs
R	Quantity of Residue of peach palm
V	Volume of Liquid Phase
C	Concentration of Dye
De	Efficiency of Decolorization
La	Laccase activity
MnP	Manganese peroxidase activity
LE	Liquid Extract
ABTS	3-ethylbenzthiazoline-6-sulfonic acid
$U.L^{-1}$	One Unit of Enzyme Activity
M_c	Moisture Content
K_1	Constants of Pseudo First Order
K_2	Constants of Pseudo Second Order
K_{id}	Constant Rate of Intraparticle Diffusion
C	Constant Indicating the Effect of the Boundary layer
q_{max}	Maximum Amount of Adsorbate per Unit of Mass
B	Adsorption Equilibrium Constant
K_f	Freundlich Constants

I BACKGROUND

1.1 Introduction and Justification

Studies on urban environmental issues are gaining prominence, especially since the development of urban centers and the continued population growth led to the emergence of some forms of pollution which have reached catastrophic proportions and thereby, can be observed through changes in soil quality, air and water (Mansour et al., 2016; Souza and Rosado, 2009; Kunz et al., 2002).

The textile industry is a main creator of effluent wastewater due to a more consumption of water for its different wet processing operations. This effluent contains chemicals like acids, alkalis, dyes, hydrogen peroxide, starch, surfactants, dispersing agents and soaps of metals (Paul et al., 2012). So, in terms of its environmental impact, the textile industry is estimated to use more water than any other industry, globally and almost all wastewater discharged is highly polluted (Holkar et al., 2016). At present, aromatic and heterocyclic dyes are used in textile industry. The complicated and stable structure of dye is posing a greater difficulty in degradation when present not only in textile wastewater but also in any kind of complex matrix (Swami et al., 2012).

The Remazol Brilliant Blue R (RBBR) is one of the most important synthetic dyes in the textile industry and is frequently used as a starting material in the production of polymeric dyes. It has an anthraquinone structure and represents an important class of toxic and recalcitrant organo-pollutants (Javaid et al., 2016). The state of Santa Catarina in Brazil has one of the most advanced textile manufacturing facilities in Latin America and the second largest in the country. The industrial complex of the state is located mainly in the Itajaí Valley, in this sense stands out for being the largest center of Santa Catarina textile industries (Gazzoni, 2013), having 9,264 textile and clothing industries, and is responsible for 1.9% of SC exports, reaching in 2011, US $ 176 million (FIESC, 2013).

Nevertheless, conventional treatments of these effluents do not appear effective in removing dyes compounds (El-Rahim et al., 2017) actually has several drawbacks such the inability to remove dyes completely, the high cost of the chemicals used,

generation of hazardous secondary wastes and its disposal problem (Sen et al., 2016; (Lade et al., 2015).

A fungal culture has an ability to acclimate its metabolism to changing environmental conditions. This ability is a vital for their existence. Here, intra and extracellular enzymes help in metabolic activity. These enzymes have ability to degrade various dyes present in the textile wastewater. Due to these enzymes, fungal cultures seem to be suitable for the degradation of dyes in textile wastewater.

Therefore, there is a great interest in studying alternative treatment processes, which are able to reduce the environmental impact of these wastewaters and which allow its reuse.

The wastewater treatment technology using microorganisms is considered an option for the degradation of emerging contaminants, for it constitutes an environmentally cleaner and low cost technology, compared to conventional procedures (Mansour et al., 2016). The biodegradation process implies the breackdown of the structure of pollutant compounds, what generally reduces its toxicity. Among the great diversity of microorganisms that can be used in these processes, macromycetes are highlighted because of it ability to degradade a wide variety of toxic substances, leading in some cases to complete mineralization of recalcitrant substances, such us organochlorine compounds, aromatic hydrocarbon s, pesticides, synthetic dyes, polymers and other emerging pollutants.

In recent years, researches have been carried out as intent to develop dye biodegradation processes by the use of white-rot-fungi (WRF). Despite of that, concerted efforts are still required to establish biological systems able to use for industrial scale bioremediation of industrial effluents (Asgher et al., 2012). According to Lee et al. (2011), the ultimate target of basic research for industrial applications of these technologies is to reduce the processes costs by optimizing the process parameters.

From this point of view, studies of decoloration of the textile effluents and flexography are being made by professors and students of the Programa de Pós-graduação em Engenharia Ambiental – FURB (Fundação Universidade Regional de Blumenau, SC, Brazil). The results reported in this Thesis are included in a project involving studies of wastewater decoloration approved by the Ministry of Science,

Technology, Innovation and Communication (notice n.28/2013) and CNPq / Biotec (Brazil).

1.2 The textile industry

The textile industry has great relevance worldwide. In 2014, Brazil occupied the sixth position. In the world textile production, earning US $ 55.4 billion and generated 1.6 million jobs, with an estimated textile production at 2.1 million tons (ABIT, 2014). This sector is the second largest employer in the Brazilian manufacturing industry, providing 16.4% of jobs, just behind food and beverage industry (TEXBRASIL, 2014).

The state of Santa Catarina (Brazil) has one of the most advanced textile manufacturing facilities in Latin America and the second largest in the country. The industrial complex in this state is located mainly in the Itajaí Valley. The city of Blumenau stands out for being the largest center of Santa Catarina's textile industries (Gazzoni, 2013), having 9.264 textile and clothing industries, and being responsible for 1.9% of exportations, reaching in 2011, US $ 176 million (FIESC, 2013).

However, the major environmental problem associated with the textile sector, which is the contamination of natural waters, is not a regional but rather global concern. A significant part of the synthetic dyes and other chemicals products, called auxiliary in the activities of processing and finishing of fabrics, unfixed on the fibers during the dyeing process are found in the wastewaters generated (Barcellos et al., 2009).

In the period prior to the half of XIX century, the dyes were often extracted from natural sources, mainly of animal or vegetable origins. Naturally, the properties of many of these substances were far from ideal (poor fixing, high biodegradability, etc.) and this fact, together with the limited commercial availability of sources of supply, encouraged the search for synthetic dyes with superior properties (Arslan-Alaton, 2003). Viable discoveries came quickly and the natural dyes have been almost completely replaced by synthetic in the early twentieth century. The first synthetic dye was discovered only in 1856 in England, and the monopoly of synthetic dye production belonged to Germany from 1915 until the Second World War (Isenmann, 2013).

Nowadays, except of some important inorganic pigments, all the dyes and pigments commercially available are synthetic substances. According to Guarantini;

Zanoni (2000), due to the increasingly demanding market, millions of colorful chemical compounds have been synthesized in the last 100 years. About 10.000 different dyes are used industrially, representing an annual consumption of approximately 8x105 tons on the planet, with 26.500 tons only in Brazil (Silveira-Neta et al., 2012).

The wide number of colorants commercially available is justified by the diversity of fibers, which requires well defined dyeing characteristics, and to the great demand of new colors and dyes with greater binding capacity and specificity related to the fibers (Isenmann, 2013).

The dyes have large structural diversity that comes from different chromophore groups and different application technologies. They are aromatic and heterocyclic compounds, being in most of cases are difficult in degradation (Barcellos et al., 2009; Rodríguez, 2013). They can be classified according to their chemical structure or fixing method in textile fiber (Guarantini and Zanoni, 2000).

In addition to the dyes, the textile effluents present extremely heterogeneous composition and large amounts of toxic and recalcitrant material which makes its treatment a complex task. Besides the strong coloring, large amounts of suspended solids, highly fluctuating pH, temperature, and concentrations of chemical oxygen demand (COD) and trace elements (Cr, Ni and / or Cu), as well as chlorinated organic compounds and surfactants (Jerônimo, 2012).

According Dellamatrice et al. (2008), given the physicochemical characteristics of the textile effluent highlighted to pH typically between 8 and 11 total solids between 1.000 and 1.600 mg. L^{-1} and solids content of the suspension between 30 50 mg.L^{-1}. Such characteristics are subject to variation according to the type and stage of the process in progress within each industry. But generally, is composed of a vast array of complex components, including synthetic dyes, dispersants, bases, acids, detergents, salts, surfactants, grease, and oil, among other compounds (Pelosi et al., 2014).

The characteristics of industrial effluents are variable about the color tones and concentrations, which makes it difficult to quantify the color of an industrial effluent. The residual wastewater from the dyebath used is derived from cotton dyeing process and has as a component only, yellow dye, Remazol red and blue R, calcium carbonate, caustic soda and neutralizer. The final effluent is composed of the entire effluent collected at the treatment station from the company after the physical and chemical process and prior to biological treatment. Anthraquinone-based dyes, e.g., Remazol

Brilliant Blue R (RBBR), constitute the second largest class after the azo dyes (Gregory, 1990) and are considered the most resistant to degradation (Jaonani et al., 2003).

The dye concentrations in the textile processing wastewaters are in the range of 10-200 mg. L^{-1} (Kadam, et al., 2011), which interferes in the penetration of sunlight into waters, retards photosynthesis, inhibits the growth of aquatic biota and affects gas solubility in water bodies (Banat et al., 1996). Furthermore, many dyes are believed to be carcinogenic or are synthesized from known carcinogens (Clarke and Anliker, 1980). In addition, effluents from the textile industry contain many toxic compounds (Szalinska et al., 2010).

Since the formation of the dye to its deposition in water course, the contaminants may be associated with some particles becoming bioavailable to the ecosystem; suffer biotransformation, causing toxic substances or migrate from sediment to other environmental compartments via trophic chain (Zagatto and Bertoletti, 2006). They can also be assimilated and retained in the organisms, both directly, by direct contact in the environment, or indirectly, through predation contaminated organisms. In the indirect way, the metals are accumulated faster than excreted or detoxified, which may lead to bio-magnification, i.e., toxic substances are passed from a trophic level to another, leading to increased concentration along the trophic chain (Khan et al., 2012).

The decolorization of this industrial waste represents a considerable challenging because certain dyes are resistant to degradation. Chemical, physical and biological methods are used for the removal of dyes from wastewater (Kadam et al., 2013), however, several of them have disadvantages such as high costs and/or limited applicability. Also, due to the low efficacy of the traditional approaches to treatment, the development of new processes is required. The improvement and integration of different treatment methods are needed convert the treatment wastewater a sustainable process (Manavalan et al., 2013).

1.3 Treatment of the effluent from textiles industries

In a general way, after a pollution event, the balance between the different ecosystem communities is firstly affected, resulting in an initial disorganization. However, after certain adaptation time and under determinate conditions, these systems

have a tendency to further reorganize themselves (Sperling, 1996). Besides of that, there is a limit from which the aquatic ecosystem become saturated and cannot be restored.

Therefore, the low efficacy on the elimination of toxic products has forced government agencies to establish restrict environmental regulations to protect the natural ecosystems (Mansour et al., 2016). Some of the measurements proposed include strategies for the reuse of water and the disposal of effluents, changes on the limits of emission standards in the course of water, as well as increasing the costs associates with the treatment and disposal of waste and by products from the wastewater (Faria, 2004). All of these practices stimulate the use of cleaner production technologies (Peixoto, 2011).

Several environment protection agencies worldwide have imposed rules entrusted with the protection of human health and guarding the environment from pollution caused by the textile industry. These agencies imposed certain limits on the disposal of effluents into the environment. Some of the regulations imposed by several countries are presented in Chart 1. The disposal limits are found to differ from country to country. However, a constant check is to be kept on these discharge limits every now and then to maintain a safe and a healthy environment (Ghaly et al., 2014).

Chart 1. Discharge limits of some countries (Ghaly et al., 2014).

Parameter	CCME	China	BIS	Hong Kong	FEPA	Mexico	Thailand	Philippines	Indonesia	Bangladesh	SL
pH	6.5-8.5	6-9	5.5-9	6-10	6-9	6-8.5	5-9	6-9	6-9	6.5-9	6-8.5
Temperature (°C)	30	-	50	43	40	-	-	40	-	40-45	40
Colour (Pt-Co)	100	80	None	1(Lovibond)	7(Lovibond)	-	-	100-200	-	-	30
TDS mg/L	2000	-	2100	-	2000	-	2000-5000	1200	-	2100	2100
TSS mg/L	40	150	100	800	30	-	30-150	90	60	100	500
Sulphide µg/L	200	1000	2000	1000	200	-	-	-	-	1000	2000
Free Chlorine µg/L	1000	-	1000	-	1000	-	-	1000	-	-	-
COD mg/L	80	200	250	2000	80	< 125	120-400	200-300	250	200	600
BOD_5 mg/L	50	60	30	800	50	< 30	20-60	30-200	85	150	200
Oil & Grease mg/L	-	-	10	20	10	-	300	5-15	5	10	30
Dissolved Oxygen µg/L	6000	-	-	≥ 4000	-	-	-	1000-2000	-	4500-8000	-
Nitrate µg/L	13000	-	10000	-	20000	10000	-	-	-	10000	45000
Ammonia µg/L	0.1	-	-	500	0.2	-	-	-	-	5000	60
Phosphate µg/L	<4000	1000	5000	5000	5000	-	-	-	2000	-	2000
Calcium µg/L	-	-	-	-	200000	-	-	200000	-	-	240000
Magnesium µg/L	200000	-	-	-	200000	-	-	-	-	-	150000
Chromium µg/L	1	-	100	100	<100	50	500	50-500	500	2000	50
Aluminium µg/L	5	-	-	-	<1000	5000	-	-	-	-	-
Copper µg/L	<1000	1000	3000	1000	<1000	1000	1000	1000	2000	500	3000
Manganese µg/L	5	2000	2000	500	5.0	200	5000	1000-5000	-	5000	500
Iron µg/L	300	-	3000	1500	20000	1000	-	1000-20000	5000	2000	1000
Zinc µg/L	30	5000	5000	600	<10000	10000	-	5000-10000	5000	5000	10000
Mercury µg/L	0.026	-	0.01	1	0.05	-	5	5	-	10	1

CCME - Canadian Council of Ministers of the Environment
BIS - Bureau of Indian Standards
FEPA - Federal Environmental Protection Agency (United States)
SL - Sri Lanka

In Brazil, the Resolution of "*Conselho Nacional do Meio Ambiente* (CONAMA)" no. 430 of 13 May 2011 brings the conditions of effluent standards release, which supplements the amends no. 357 of the Resolution of March, 2005. Establishing in its Article 3, that the effluent from any source of pollution can only be discarded directly into water body after proper treatment and if they satisfy the conditions standards and requirements laid out in this resolution and other applicable standards (Brazil, 2011). Some of these requirements are: the effluent must have a pH between 5-9, the temperature should be less than 40ºC, with no particulate materials and dyes. In addition to not causing intoxication or having toxic effects to aquatic organisms and cannot modify the chemical characteristics of the water body and derail supply to the water network. The treatment of textile effluents should be carried out on site, prior to discharge into the water body to mitigate possible environmental impacts.

The water is mainly used for application of chemicals onto the fibres and rinsing of the final products. The wastewater produced during this process contains large amount of dyes and chemicals containing trace metals such as Cr, As, Cu and Zn which are capable of harming the environment and human health. The textile waste water can cause haemorrhage, ulceration of skin, nausea, skin irritation and dermatitis. The chemicals present in the water block the sunlight and increase the biological oxygen demand thereby inhibiting photosynthesis and reoxygenation process. The effluent water discharged from the textile industries undergoes various physio-chemical processes such as flocculation, coagulation and ozonation followed by biological treatments for the removal of nitrogen, organics, phosphorous and metal. The whole treatment process involves three steps (Figure 1): primary treatment, secondary treatment and tertiary treatment. The primary treatment involves removal of suspended solids, most of the oil and grease and gritty materials. The secondary treatment is carried out using microorganisms under aerobic or anaerobic conditions and involves the reduction of BOD, phenol and remaining oil in the water and control of color. The tertiary treatment involves the use of electrodialysis, reverse osmosis and ion exchange to remove the final contaminants in the wastewater (Ghaly et al., 2014).

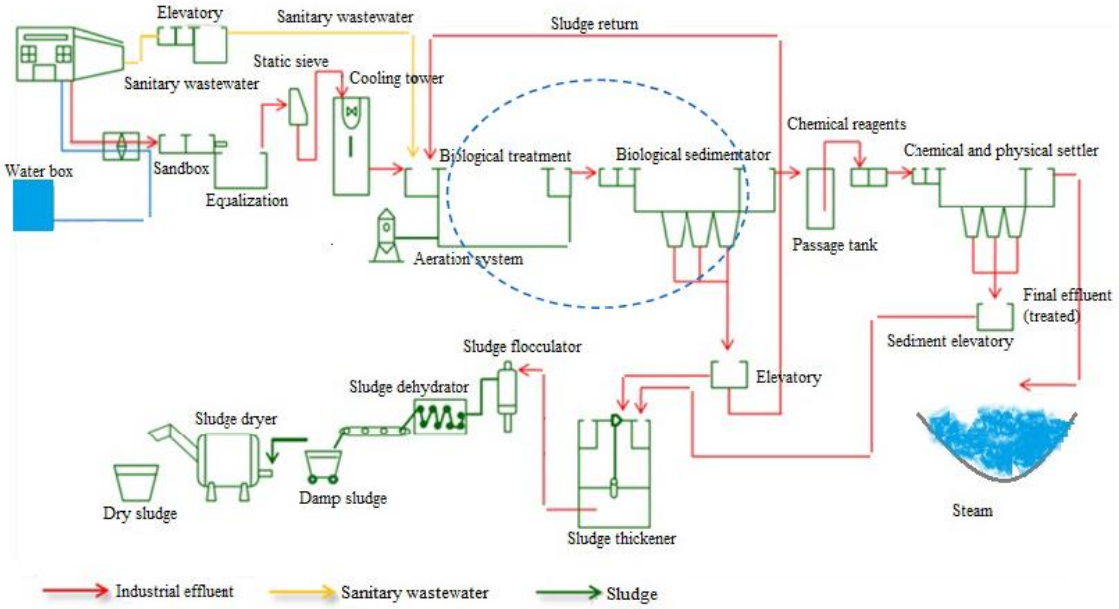

Figure 1. Diagram of effluent treatment plant using activated sludge, as show in the circle, typically used for the treatment of textile effluent.

A widely used commercial chemical method of dye removal is coagulation which rapidly transfers dyes from the liquid to the solid phase but has several drawbacks such the inability to remove dyes completely, the high cost of the chemicals used, generation of hazardous secondary wastes and its disposal problem (Saratele et al., 2009).

Due to these drawbacks, there is a certain predilection for the use of processes that can actually degrade the species of interest. Within the destructive processes context, it is for biological processes a prominent place, mainly due to the relative ease encountered in the implementation of systems that operate on a large scale. The most frequently used biological processes are represented by activated sludge systems. This process consists in mixing the effluent in the presence of microorganisms and oxygen during the time required to metabolize a large part of the organic matter (Bitton, 1994). Unfortunately, there is a major downside which is being susceptible to the effluent composition (shock loads), and produces a large volume of sludge.

Frequently, the industrial textile wastewater treatment starts with use of the precipitation-coagulation process, followed by activated sludge system. The combination of the physicochemical and biological operations presents a relatively high efficiency, enabling the removal of about 80 % of dyes load. Unfortunately, one

important inconvenient of this process is the accumulation of dye into the sludge, which is frequently quite high, preventing any possibility of reutilization (Kunz et al., 2002).

1.4 The fungi as microorganisms decomposers

All fungi are chemoheterotrophic, and adsorption of nutrients occurs due to enzyme secreted extracellularly. Some fungi can obtain energy by fermentation, such as yeast. Fungi are present in all continents, occupying different niches and are predominantly saprophytes. Decomposition is the main ecological function exercised by them, being fundamental in the equilibrium of the different ecosystems. There are two forms of growth, multicellular filamentous (filamentous fungi) and unicellular. The filaments are called hyphae, and hyphae set are called mycelium (Raven et al., 2007). These are the unique microorganisms having exclusive complex enzymatic machinery for the degradation of as lignin and holocellulose components as a source of carbon and energy along with the removal of polysaccharides and hence total biomass breakdown usually occurs (Madhavi et al., 2009).

It is well known that, in any ecosystem, the fungi are among the main microorganisms decomposing organic matter such as cellulose, hemicellulose and lignin. Fungi can mineralize and bio accumulate toxic materials as well as storing and releasing elements and ions (Singh, 2006). The WRF are the best organisms to degrade lignin, cellulose and hemicellulose into smaller molecules to CO_2 and water (so they are called lignocellulolytic fungi), and are consequently used in bioremediation of pollutants.

The lignin macromolecule is responsible for the rigidity of the cell wall of plants and trunk thickening (Figure 2). Furthermore, it is a recalcitrant and chemically complex polymer, considering the heterogeneity of functional groups present in structure (Cohen et al., 2002). The WRF are the only micro-organisms able to hydrolyze lignin to CO_2 and H_2O. These micro-organisms have been widely studied since the last century to take advantage of their degrading ability and find biotechnological applications, especially in the treatment of emerging contaminants (Rodríguez, 2013).

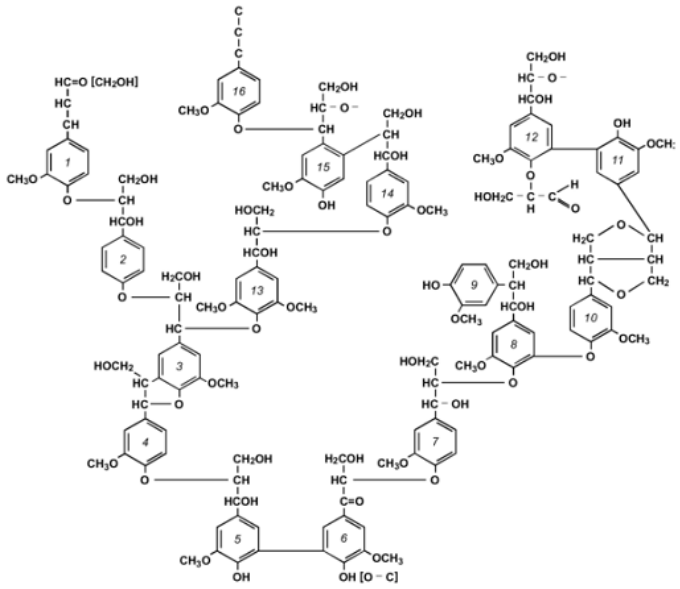

Figure 2. Fragment of lignin (Rodríguez, 2013).

The capacity of the microorganisms to degrade organic compounds is scientifically recognized and has been explored in biological treatment processes for wastewater, as well as processes involving decoloration and metabolism of recalcitrant compounds. No doubt, basidiomycetes fungi with their large batteries of ligninolytic enzymes, among the different types of phenol oxidases with their different broad substrate ranges, and with their expanded but barely exploited genomes present an excellent "green" potential for handling of many problematical types of pollution (Kües, 2015).

Enzymes in lignocellulose degradation are commonly extracellular, which is compulsory by the large molecule sizes of the envisaged substrates. Larger polymers are broken down into smaller fragments and finally into individual molecule units that might be taken up into the cells for eventual metabolic use or for further detoxification by the xenome, that is the protein machineries for detection, transport and metabolism of xenobiotics (Kües, 2015; Morel et al., 2013).

There are four main means of usage of ligninolytic enzymes in environmental management with partial overlaps: (i) Enzymes might be used to purify pollutions in contaminated water or solid materials prior to release into an environment; (ii) Enzymes might be used in bioremediation within environments; (iii) The environment might be manipulated in favor of organisms producing enzymes of environmental benefit; (iv)

Enzymes might be used in biosensors and as bioindicators to monitor pollution in the environment (Rao et al., 2014).

The ligninolytic enzymes have very low substrate specificity, enabling them to mineralize a wide variety of recalcitrant xenobiotic compounds and organopollutants having structural similarity with the lignin (Hofrichter et al., 2002). Lignin-modifying enzymes are potential industrial enzymes for several applications. These include bio-bleaching, bio-pulping, the functionalization of lignocellulosic materials, the modification of wood fibers, the remediation of contaminated soil and effluents, as well as improvement of the enzymatic hydrolysis of lignocellulosic substrates (Moilanen et al., 2011). One of the main challenges in the development of industrially relevant applications is to produce these enzymes cost-effectively in sufficient amounts to prove the attractiveness of the biochemical approach as an alternative to the more traditional processes. One solution for the feasible commercial production of lignin-modifying enzymes could be the use of solid-state fermentation (SSF) techniques in the production process (Mansour et al., 2016).

Laccases belong to the multicopper oxidase family of enzymes that catalyze the oxidation of various substrates with the simultaneous reduction of molecular oxygen to water, through a radical-catalyzed reaction mechanism. They are mainly of fungal or plant origin, although a few representatives have been identified and isolated in bacteria and insects. The most studied laccases are fungal in origin, mainly in phyla Ascomycota, Zygomycota, and Basidiomycota. The most biotechnologically useful laccases are also of fungal origin (Giardina et al., 2010). Physiologically, the functions of laccases are diverse, ranging from lignolysis, pigment formation, detoxification, to pathogenesis. All these functions are attributed to the enzymes ability to oxidize a wide range of aromatic substrates such as polyphenols and diamines and even some inorganic compounds. Compared with fungal laccases, bacterial laccases are generally more stable at high pH and temperatures. Although fungal laccases can be both intra and extracellular, bacterial laccases are predominantly intracellular (Cho et al., 2011). Laccase produced using agroindustry wastes are proved as an effective synthetic dye degrader (Das et al., 2016). Most basidiomycetes produce laccase and manganese peroxidase, while lignin peroxidase seems to be rare distribution (Silva et al., 2008).

The manganese peroxidase (hydrogen peroxide oxidoreductase, EC 1.11.1.13), is the most common ligninolytic peroxidase produced by almost all basidiomycetes

(Liers et al., 2014; Hofrichter et al., 2010). Manganese peroxidase is a glycoprotein with a heme (ferric protoporphyrin) group that shares the mechanistic properties of other peroxidases and the formation of oxidized intermediates, compound I and compound II, in the presence of H_2O_2 for aromatic and nonphenolic substrates oxidation (Mester et al., 2000).

The biodegradation process can be defined as the biological decomposition or breakdown of a chemical compound which occurs by the action of an enzyme system consisting of different enzymes and mediator compound. It is process known as mineralization and uses microorganisms to metabolize toxic waste in the environment, degrading it and transforming it into little toxic or non-toxic elements, such as carbon dioxide (CO_2), water (H_2O) and inorganic salts (Buratini, 2008).

The breakdown of synthetic dyes using different fungi is becoming a promising in the treatment of effluents with dyes approach. The biodegradability of these micro-organisms can be gradually increased by exposure to higher concentrations of organic or synthetic chemicals. The adaptation of a microbial community by toxic and recalcitrant components is very favorable for the biological process of decolorization. This process has been suggested as a promising method which not only potentially mineralizes dye molecules into CO_2 and H_2O, but also generates low amounts of sludge. A wide variety of basidiomycetes, particularly WRF, have been used in experimental works of bio-treatment of wastewater dyes, such as strategies to decolorization, mineralization, processing or degradation of various natural or synthetic compounds. Table 1 shows some processes and organisms used by various authors.

Table 1. Application of fungi degradation in experimental trials dye bleaching bio-treatment and wastewater.

Fungal species	**Application**	**Reference**
Agaricus blazei	Decolorization of reactive dyes	Santos et al. (2004)
Bjerkandera sp.	Decolorization of textile dyes	Anastasi et al. (2011)
Capinus plicatilis	Decolorization of reactive dyes	Akdogan; Canpogat (2014)
Cerrena unicolor	Decolorization of textile dyes	Michniewcz et al. (2008)
Coriolopsis sp. (1c3)	Decoloration of Triphenylmethane dyes	Chen; Yien Ting 2015)
Coriolus rigida	Decolorization of textile dyes	Saparrat et al. (2010)
Coriolus versicolor	Decolorization of textile dyes	Asgher et al. (2009)
Dichomitus squalens	Decolorization of dyes	Susla et al. (2008)

Ganoderma sp.	Decolorization of synthetic dyes	Sadaf et al. (2013)
Ganoderma lucidum	Degradation and decoloration of dyes	Manavalan et al. (2013); Sharma et al. (2015)
Ganoderma australe	Decolorization of dyes	Rigas; Dritsa (2006)
Lentinula edodes	Decolorization of dyes	Boer et al. (2004)
Phanerochaete chrysosporium	Degradation and Decolorization of dyes	Akdogan; Canpogat (2014)
Pleurotus calyptratus	Decolorization of dyes	Eichlerová et al.(2005)
Pleurotus citrinopileatus	Decolorization of dyes	Santos et al. (2004)
Pleurotus cornucopiae	Decolorization of dyes	Eichlerová et al. (2006)
Pleurotus cystidiosus	Decolorization of dyes	Eichlerová et al. (2005 e 2006)
Pleurotus dryinus	Decolorization of dyes	Eichlerová et al. (2006)
Pleurotus eous	Decolorization of reactive dyes	Santos et al. (2004)
Pleurotus eryngii	Degradation and decoloration of dyes	Eichlerová et al. (2006)
Pleurotus flabellatus	Decolorization of textile effluent	Nilsson et al. (2006)
Pleurotus ostreatus	Degradation and decoloration of dyes	Parenti et al. (2013)
Pleurotus sajor-caju	Decolorization of dyes	Munari et al. (2008)
Pycnoporus sp.	Decolorization of textile dyes	Anastasi et al. (2012)
Pycnoporus sanguineus	Decolorization of azo and anthraquinone dyes	Ramirez-Cavazos et al. (2014)
Trametes sp.	Decolorization and detoxification of dyes	Yan et al. (2014)
Trametes trogii	Decolorization and detoxification of dyes and effluents	Pazarbasi et al. (2012)
Trametes versicolor	Degradation and decoloration of dyes	Champagne; Ramsay (2010); Silverio et al. (2013)
Trametes gibbosa	Decolorization of reactive dyes	Adnan et al. (2014)
Trichoderma asperellum	Decolorization of Leucocrystal violet	Shanmugam et al. (2017)

The literature is replete with reports that show the excellent ability of fungi to degrade dyes. Its potential so far, however, has not found industrial application, mainly

due to the difficulty in selecting the organisms able to grow and degrade the widely varying conditions and restrictive of the textile industry effluents.

The textile effluents are one of the most difficult wastes to treat because of the considerable number of suspended solids and the massive presence of dyes, salts, additives, detergents and surfactants in it. They vary widely in terms of quantity and pollution load, pH and temperature, depending on the type of textile materials (Vanhulle et al., 2008).

Furthermore, most studies on dyes degradation is carried out in Erlenmeyer scale. However, before an industrial application, it is necessary to scale-up the process using bioreactors that can be operated under industrial conditions. In these sense, there are only a few studies which report the treatment of industrial textile wastewaters by macromycetes in bioreactors operated under continuous mode and under non-sterile conditions.

Blanquez et al. (2008) showed that the fungi Trametes versicolor was able to promote the decolorization of textile wastewater (40 to 60 %) in a bioreactor operated under 15 days in non-sterile conditions. Anastasi et al. (2010) also reported the decolorization of effluent from textile dyeing with *Bjerkandera adusta* fungi in a fixed bed bioreactor. The fungus was effective for four cycles of decolorization and remained active for a longer period (70 days) under non-sterile conditions and without addition of nutrients. Furthermore, treatment of fungi has greatly reduced toxicity of the effluent. In this case, dilution of the effluent, nutrient addition and control of chemical parameters were not done, however, the experiment showed the applicability of the developed system.

Later, the same authors tested the ability of the same fungus to degrade the effluent of a textile industry after a secondary treatment. They found that yeast treatment caused a 40 % of color removal in 24 h of treatment (Anastasi et al., 2011). The same authors, using wastewater from a dyeing cotton industry, demonstrated that treatment by fungi, especially with the *Trametes pubescens* fungus tested in led to very good results in terms of decoloration (over 60 %) with toxicity removal (Anastasi et al., 2012).

Selvakumar et al. (2012) studied textile effluent in a batch reactor with *Ganoderma lucidum* under optimized conditions of pH (6.6); temperature (26.5ºC);

stirring speed (200 rpm); effluent concentrations of colorant (1:2), found the decolorization of 81.4 %.

Wastewater discharged by the textile processing industry is a complex mixture of several substances that accompany a huge variety of dyes with diverse chemical structures. Application of fungal to raw textile wastewater can be effective for achieving a large decrease in the dye content of wastewater despite the enormous amounts of salts, metals and other contaminants. However, in most cases, the microorganisms that have been tested for decolorizing dyes have been studied using wastewater with relatively simple chemical compositions under laboratory conditions and with SmF. As this technology goes to scaled up, it will be necessary to evaluate the true potential for use of microorganisms to decolorize real textile wastewaters in bioreactor systems with SSF, built at industrial outlets that receive water directly from the dyeing units.

Also, the metabolic pathways involved in biodecolorization of textile effluent have not yet been fully elucidated. It is necessary to target the underlying processes and metabolic pathways by identifying genes and metabolites in the decolorization processes.

1.5 Solid-state fermentation and agroindustry's waste

The two main strategies for the cultivation and therefore enzyme production are submerged fermentation (SF) and solid-state fermentation (SSF), which differ about their environmental conditions and driving ways. Unquestionably one of the most exalted parameters in the differentiation of two types of processes is the water content present in the reaction medium (Pandey, 2003).

Solid-state fermentation is a process that occurs under complete absence of free-flowing water contents in the growth media. Some of the advantages of solid cultivation compared to SmF are: higher enzyme production, low production costs, extended stability of products, among others. With increasing progress and application of rational methods in engineering, solid cultivation has achieved higher levels in standardization and reproducibility (Iqbal et al., 2011). SSF has a good cost-effective relation since agroindustry waste materials can be used directly as culture media without additional pre-processing.

Bioconversion of lignocellulosic wastes to higher value products through fungal fermentation has economic and ecological benefits. The degradation of methylene blue by SSF of agricultural residues rice straw with *Phanerochaete chrysosporium* was investigated by Zeng et al. (2015). A maximum decolorization was found with 84.8% for an initial dye concentration of 0.4 g/L. Li et al. (2015) Also used this strategy to stimulated production of manganese peroxidase (MnP) from cassava residue by *Phanerochaete chrysosporium* in SSF. The decolorization *in vitro* of indigo carmine by the crude MnP attaining the ratio of 90.18% after 6 h of incubation. Das et al. (2016) used co-substrates of paddy straw and corn husk to produce laccase for decoloration of Congo red dye from *Pleurotus ostreatus* MTCC 142.

Up to now, there are no studies reporting the use of large-scale reactors operated in a SSF for the treatment of textile effluents. The drawbacks of SSF in larger scale production could be due to some engineering problems, such as moisture and temperature control together with agitation systems to provide a homogenous mixing. While high lignin-modifying enzyme production levels have been reported in many small-scale laboratory experiments (Gassara et al., 2010), scaling up the process is rather challenging.

A fungal culture has an ability to acclimate its metabolism to changing environmental conditions. This ability is a vital for their existence. Here, intra and extracellular enzymes help in metabolic activity. These enzymes have ability to degrade various dyes present in the textile wastewater. Due to these enzymes, fungal cultures seem to be suitable for the degradation of dyes in textile wastewater. These enzymes are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Chen et al., 2015; Li et al., 2015). The key issue, in practice, for applying the white-rot technology is to design and establish a suitable reactor. Different configurations such as a stirred-tank reactor (Rodarte-Morales et al., 2012), an airlift (Zhou et al., 2006), bubble column (Cerrone et al., 2011), fluidized bed reactor (Badia-Fabregat et al., 2017; Mir-Tutusaus et al., 2017; Cruz-Morató et al., 2014), trickling packed-bed bioreactor (Ehlers and Rose, 2005) and packed bed bioreactor (Li et al., 2015) have been used for wastewater treatment. Despite the massive work already done by the scientific community, the use of fungi in the field of wastewater treatment still find poor application.

For this reason, the interest of this study is to present a scaling-up of the Remazol Brilhant Blue R biodegradation by white-rot fungi Ganoderma lucidum in

solid state fermentation. First preliminary studies were in small bioreactor with 2 L presented in order to study the biodegradation abilities of the selected WRF (the fungal *G. lucidum* was selected in a previous study by Chicatto et al., 2018a), in different conditions (attached on biodegradable and inert support). The same processes were done at a scaled-up into batch treatment using packed bed bioreactors (PBRs) with the capacity of 15 L working with peach palm residue.

Finding alternative for the reutilize of these wastes is an aim that has been strongly taken into account by countries around the world, considering environmental and economic aspects (Ergun et al., 2017).

Several crop biomasses such as rice bran, sugarcane bagasse, wheat bran, leaf sheath of peach palm, etc. are generated every year in million-ton scale as "agricultural waste", easily available at low cost and they are known to contain high amounts of carbon and nitrogen (Kadam et al., 2011; Singh et al., 2012). As an example, the native palm tree from the Amazon region, but currently disseminated by Central and South Americas. The palm tree which was domesticated and widespread in these regions by indigenous people is characterized by having an erect stipe, with diameter ranging from 15 to 30 cm, height between 15 and 20 m, with varieties that may or may not be covered by the stem thorns. Some features are considered attractive for palm production such as earliness, tillering, yield and quality of palm (Chaimsohn, 2000).

Palm trees can be divided into three layers: outer, medium and internal sheaths. The outer layer that surrounds the heart of palm is fibrous and its function is to protect the leaves still to be formed, those are not used in heart of palm processing, and represent on average, 30% of the weight of the plant, depending on the species (Tonini, 2004). The second layer is formed by the median sheath and it is used to protect the heart of palm during transportation through processing, then being discarded thereafter. This layer is 25 to 30% of plant weight. The last layer is formed by the kernel or heart of palm, which contains low-fiber and represents only 2% of the weight of the plant and is the plant's part with the greatest economic value (Ramos and Heck, 2003).

The peach palm (*Bactris gasipaes* Kunth) is a palm tree widespread in Brazil and is one of the major producers of the heart-of-palm (locally known as "palmito") (Helm et al., 2014) with a harvested area of 22 537 ha and a production of 109409 tons in 2015 (IBGE, 2015). The residue (leaf sheath) of the stem generated by the industry during the processing of canned heart-of-palm corresponds to about 85-95% of the

weight of the palm depending on the species, and currently has no economic value, and important environmental impact (Helm et al., 2014). In the beneficiation process of the heart of palm it is estimated that for every kg of processed heart of palm another 3 kg sheaths that are not used are generated and discarded. It is estimated that during the heart of palm processing, 90 % of the plant is useless waste (Pupo, 2012).

According Helm et al., (2014), leaf sheath of peach palm residue has potential as a food supplement in the form of fiber, and also potential as an inducer of the enzymatic activity due to chemical characteristics such as the presence of lignin and cellulose. Along with the increased availability of this residue from the production of palm, it might thus become a source of unexplored resources as well (Pupo, 2012).

The use of agro-industrial residues as the culture medium for WRF could offer some advantages: they are easily available at low cost, can act as a selective carbon source for fungi, represent a physical support, serve as an adsorption agent for further biodegradation, can provide a habitat for these fungi, can also, enhance the expression of ligninolytic enzymes, and are a source of natural mediators (Palli et al., 2015), which are often necessary for the degradation of non-phenolic compounds by laccases (Guiza et al., 2014). In addition, this technique allows the utilization of diverse agro-industrial wastes as support-substrate, making the process more economical and eco-friendly (Kadam et al., 2013).

In this context the aim of this interdisciplinary approach was to provide innovative solutions to environmental problems, in particular, finding a use for the residual biomass generated from peach palm extraction and improving the treatment of effluents generated by the textile industry.

II RESEARCH PROBLEM AND OBJECTIVES

2.1 Research problem

Is it possible for macromycetes to decolorize textile effluents in a biological reactor using biomass from peach palm (*Bactris gasipaes*) waste as a solid matrix?

2.2 Hypothesis

- Basidiomycetes are able to produce oxidative enzymes that can hydrolyze aromatic compounds existing in textile effluents efficiently.

- The agroindustrial solid waste from peach palm (*Bactris gasipaes*) can be successfully used as a fixed bed in a biological reactor to promote the adsorption of the colorant and subsequent degradation.

2.3 Objectives

This work aims to study the decoloration process of textile effluents by solid-state fermentation in the presence of white rot fungi and the peach palm residue from *Bactris gasipaes*.

The specific objectives are:

- To study the production of oxidative enzymes by a group of white-rot fungi in a solid-state fermentation system in presence of dyes and textile effluents and of the agroindustrial waste from *Bactris gasipaes*;
- To determine the effect of the amount of peach palm residue, liquid phase volume, and dye concentration on the process of decolorization of textile effluents under solid cultivation;

- To determine the decoloration kinetics in solid-state fermentations with different white-rot fungi;
- To evaluate the adsorption capacity of palm tree residue and its application for dye removal;
- To establish the best strategy for the treatment of textile effluents (solid-state fermentation, submerged fermentation or adsorption);
- To study the Remazol Brilhant Blue R biodegradation by white-rot fungi *Ganoderma lucidum* in pilot-scale bioreactors.

III STRUCTURE OF THESIS

This thesis is divided into 6 chapters: (I) Background, (II) Research problem and objectives, (III) Structure of thesis, (IV) Material and methods, (V) Results and discussion, (VI) Conclusions. A list of references used in this work is provided at the end of thesis.

Here follows a brief description of Chapter 5:

- Subsection 5.1 - Study 1: "Biological Process for Decolorization of Textile Industry Wastewater with *Bactris gasipaes* Residue as a Solid Matrix". In this part of thesis, we evaluated for first time the decolorization capacity of the white rot fungus *Ganoderma lucidum* EF 31 grown on peach palm residue (*Bactris gasipaes*) moistened with textile industrial effluents. Laccase was the most dominant ligninolytic enzyme produced by *G. lucidum*. Control assays with Remazol Brilliant Blue R showed that the highest percentage of color removal 97 % while with the final effluents the highest decolorization values reached 76 %. The results of this work were accepted for publication in Brazilian Journal of Biology, which has an impact factor JCR (2016) of 0.479.

- Subsection 5.2 – Study 2: "Strategies for Decoloration of Textile Industry effluents by White-Rot-Fungi with Peach Palm Residue". In this work, three different white-rot fungi were used for the dye removal, applying different experimental strategies: i) solid-state fermentation (SSF), ii) submerged fermentation (SF), and iii) adsorption. In all cases it was used the peach-palm residue. In the SSF strategy, both decolorization process and laccase production was enhanced by increasing the concentration of final effluent. Even though the highest decolorization percentage (80%) was attained after 10 days of fermentation with *G. lucidum* EF 31 applied in the treatment of a final effluent, no significant differences were found in relation to the other two fungi. The decolorization efficiency obtained

in the SF was lower compared with SSF, however, the presence of final effluent in the SF process improved the laccase activity. It was found that the addition of peach-palm residue in this system provides a habitat for the fungus as well as a suitable source material for laccase production with the consequent decolorization of the effluent. This work was accepted for publication in Acta Scientiarum Technology, which has an impact factor JCR (2016) of 0.259

- Subsection 5.3 – Study 3: "Biodegradation of a textile dye by *Ganoderma lucidum*: Scale-up into packed bed bioreactors", which will be submitted to Bioresource Technology, Impact Factor JCR (2016) of 5.651. A first scale-up of a solid-state fermentation process into fungal packed-bed bioreactor is presented. The white-rot fungi *Ganoderma lucidum* was able to decolorize the anthraquinone dye Remazol Brillant Blue R in a bioreactor with 15 liters of capacity working in a continuous mode for fifty days and achieved more than 90 % of decoloration. The use of peach palm residue as a carbon source simulates the natural habitat of the fungus and provides a suitable source for laccase production.

The experimental tests carried out in this work were developed at the Biomass Engineering Labs (LEBIO) I and II (reactors) of the "Programa de Pós-Graduação em Engenharia Ambiental", Universidade Regional de Blumenau (FURB), with financial contribution from the MCTI/CNPq/CTBiotec 28/2013. During the period of Interuniversity exchange doctorate, the experiments were also carried out in the Surfactant Tecnology Laboratory of the Chemical Engineering Department of the Granada University, Spain.

IV MATERIAL AND METHODS

4.1 Fungal Strains and Culture Conditions

Three white-rot fungi (WRF) were used in this Thesis. The fungus *Ganoderma lucidum* EF31, which was kindly provided by the National Research Center for Forestry/EMBRAPA FORESTS (Colombo, PR, Brazil), was used in the studies 1 and 3. The *Ganoderma lucidum* DSM 9621, from the German Collection of Microorganisms and Cell Cultures and *Trametes versicolor* CECT 20817, acquired from the Spanish Type Culture Collection were used in the "Study 2". The WRF were cultivated in Petri dishes containing potato dextrose agar (PDA) medium for seven to ten days at 25°C ± 1°C in the absence of light. The cultures were then stored at 4°C for a maximum of 2 weeks.

4.2 Dye Solution and Industrial Wastewater

The dye used in the studies 1 and 3 was Remazol Brilliant Blue R (RBBR, molecular formula $C_{22}H_{16}N_2Na_2O_{11}S_3$, molar weight of 626.5 g. mol^{-1} and λmax: 592 nm) was supplied by a local textile company. The concentration of the solution depends on the study. For the "Study 1" it was used a range from 0 to 200 mg. L^{-1} and for the "Study 3", 150 mg. L^{-1}.

At the studies 1 and 2 were used also two different industrial wastewaters: (a) Residual dyebath effluent (RDB), which was the wastewater produced during the washing process of dyed material; and (b) the final effluent (FE), collected at the end of the primary physiochemical treatment and prior to biological treatment. Both wastewaters were provided from a textile industry that processes cellulose textile fibers in Santa Catarina, Brazil.

4.3 Peach Palm Residue

The peach-palm (*Bactris gasipaes*) residue was employed in all tests. The residue is an abundant agro-industrial waste generated from palm tree extraction, and it

was kindly provided by an agribusiness company (Santa Catarina State, Brazil). Briefly, the residue was milled in a knife mill to give a particle size of 2 mm and dried at 60ºC for 24 h prior to use.

4.4 Solid-state fermentation assays

Solid-state fermentation at the "Study 1" was carried out in 1 L flasks, leaf sheaths was weighted (10, 20 or 30 g) and transferred to the flasks with 20 mL of a mineral medium (Leung and Pointing, 2002). For the "Study 2" it was used only 10 grams and for the "Study 3" was used 20 grams of the residue. After that, the flasks were autoclaved (121 °C for 15 minutes). The dye solution was also previously autoclaved. Then, 1/6 petri dish of fungal inoculum were introduced with the dye in the flasks, cultivation took place at 28 ± 2 °C in the dark.

After 14 days of processing (or 30 days in the case of the "Study 2"), the entire content of the flasks was manually homogenized and a liquid extract (LE) was prepared by mixing 8 g sample with 50 mL of distilled water for 3 hours (100 rpm, 20 ± 1 °C). The solids were then separated from the LE by vacuum filtration followed by centrifugation for 15 min at 4 ºC (5,000 rpm). LEs were used to evaluate the efficiency of the decolorization process, enzymatic activity, and the total concentration of phenolic compounds.

4.5 Submerged fermentation assays

Submerged fermentation assays were carried out only in the "Study 2". Were used erlenmeyer flasks (250 mL capacity) on a rotary shaker at 28ºC and 150 rpm for 14 days. The culture medium was composed of 12 mL of final effluent, 48 mL of mineral medium (Zhou et al., 2011) and 2 g of peach-palm residue. The culture medium was autoclaved and 0.5 cm micellium PDA agar plugs were inoculated with *G. lucidum* EF 31. After 14 days of incubation, the biomasses were filtered and the remaining solids were separated by centrifugation at 9000 rpm for 15 min at 4ºC. A control assay was performed with distilled water, i.e., without the addition of the final effluent.

4.6 Experimental Design

To study the dye solution decolorization process and the production of oxidative enzymes, at the "Study 1", four, full-factorial experimental designs were carried out (ED1 – ED4) using the same explanatory factors: peach palm sheath quantity (R), liquid phase (wastewater) volume (V), and the concentration of dye in the liquid phase (C). In all cases, the response variables were the efficiency of decolorization (De), laccase activity (La), and manganese peroxidase activity (MnP). ED1 and ED2 were conducted using RBBR solution, ED3 was conducted with residual dyebath effluent, and ED4 with final effluent.

The experimental matrix used for the different factorial designs is shown in Tables 2 and 3. The experimental results were used to fit a linear model which includes the main effects and interactions, as follows (Equation 1):

$$Re = a_0 + a_1X + a_2Y + a_3Z + a_4X * Y + a_5X * Z + a_6Y * Z \quad (1)$$

where: X, Y and Z correspond to the explanatory factors, a_i represents the model parameters, and Re represents the response variable.

Analysis of variance (ANOVA) and lack-of-fit tests were used to verify the adequacy of the model proposed for each response variable. Statistical analyses were performed using the Modde Software Package (Umetrics, Umea, Sweden).

Table 2. Factors and levels of the experimental designs.

Factors	**Level**		
	-1	0	+1
ED1			
RBBR solution (mg/L)	0	50	100
Volume of the RBBR solution (mL)	20	30	40
Peach palm sheath (g)	20	30	40
ED2			
RBBR solution (mg/L)	0	100	200
Volume of the RBBR solution (mL)	20	40	60
Peach palm sheath (g)	10	20	30
ED3 and ED4			
RDB (ED3, %) and FE (ED4, %)	0	50	100
Volume of the wastewater (mL)	20	40	60
Peach palm sheath (g)	10	20	30

Table 3. Experimental matrix for the full factorial designs ED1 to ED4.

Assay number	Levels of the codified factors		
	Volume of liquid phase (V)	Dye concentration (C)	Quantity of peach palm residue (R)
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	0	0	0
10	0	0	0
11	0	0	0

In the "Study 2", three different strategies for the decolorization of dyeing wastewaters were tested: (1) solid-state fermentation (SSF); (2) submerged fermentation (SF); and (3) adsorption onto peach-palm residue. In all cases, peach-palm residue was used in the treatment process. Table 4 shows the experimental design for the solid-state fermentation.

Table 4. Solid-state fermentation by WRF with textile effluent and peach-palm residue.

Assay	**WRF**	**Type of effluent**	**Effluent concentration (%)**	**Volume of liquid (mL)**	**Fermentation time (d)**
1	*G. lucidum* EF 31	FE	0, 25, 50, 75, 100	60	14
2	*G. lucidum* EF 31	RDB	0, 25, 50, 75, 100	60	14
3	*G. lucidum* EF 31	FE	100	20	30
4	*G. lucidum* EF 31	FE	100	40	30
5	*G. lucidum* EF 31	FE	100	60	30
6	*G. lucidum* DSM 9621	FE	100	40	30
7	*T. versicolor*	FE	100	40	30

4.7 Adsorption assays

The kinetic of the adsorption process and the adsorption isotherm of the dyes were studied in the "Study 1". The experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml of dye solution (50 mg. L^{-1}) and 2.0 g of adsorbent. The mixture was stirred in a shaker (Tecnal TE-421) for 48 h at 28 °C at a constant speed of 50 rpm (solution pH: 6.20). Each vial was removed from the shaker at predetermined time intervals and then the sample was immediately filtered using a vacuum pump filter. The concentration of each sample was determined by reading the absorbance in a spectrophotometer (Shimadzu UV-1650) at 590 nm. The quantity of adsorbate adsorbed in time t, q_t (mg. g^{-1}) was calculated using equation (1).

$$q_t = \frac{(C_0 - C_e) * V}{m} \qquad (1)$$

where q_t is the adsorbate concentration at time t adsorbed by the adsorbent (mg. g^{-1}) at equilibrium conditions, C_o is the initial concentration of the compound (mg. L^{-1}), C_e is the equilibrium concentration in solution (mg. L^{-1}), m is the mass of adsorbent (g), and V is the solution volume (L).

Adsorption equilibrium studies were conducted in 250 ml Erlenmeyer flasks containing 100 mL of dye solution and 2.0 g of adsorbent. Assays were done in triplicate. The mixture was stirred in a shaker at 28 °C and 45 °C at a constant speed of 50 rpm for 8 h at levels of 20-100 mg. L^{-1} (pH 6.2). After equilibration, the mixture was then immediately filtered using a vacuum pump filter. The RBBR concentration of each sample was determined by reading the absorbance in a spectrophotometer at 590 nm.

The quantity of adsorbate adsorbed in the equilibrium state, Q_e (mg. g^{-1}) and the dye removal efficiency (%) were determined using equations (2) and (3), respectively.

$$q_e = \frac{(C_0 - C_e) * V}{m} \qquad (2)$$

where, q is the concentration of adsorbate taken up by the adsorbent (mg. g^{-1}) at equilibrium conditions, C_o is the initial concentration of the compound (mg. L^{-1}), C_e is the equilibrium concentration of the solution (mg. L^{-1}), m is the mass of the adsorbent (g), and V is the solution volume (L).

$$\%\ Removal = \frac{(C_0 - C_t)}{C_0}\ X\ 100 \qquad (3)$$

where % removal is the initial concentration of the compound (mg. L^{-1}), and Ct is the concentration at time t (mg. L^{-1}).

In the "Study 2", the adsorption experiments were performed with 60 mL of the RDB or FE (25, 50, 75 and 100 %) for 14 days. In these tests, the experimental conditions described in the section on the solid-state fermentation were applied, but without the addition of the inoculum. A control assay was carried out with distilled water, i.e., without the addition of an effluent.

Adsorption test was done in the "Study 3", to further the decoloration process in the bioreactor with 15 liters under the same conditions of the culture but without inoculation of the fungus. The dye solution was circulated in 3 batch of 7 days, totaling 21 days of experiment.

4.8 Dye degradation experiments in packed-bed bioreactor

In the "Study 3", after the solid-state fermentation methodology (were used 10 flasks) and subsequently of the complete growth of the fungus, all 10 flasks contents are transferred to the bioreactor of 2 liters. After five days of adaptation, the day solution was circulated in the system by batch, once a day, manually. Where there was enough moisture for fungal growth, but without presence of free water, characterizing of a solid state fermentation system. Samples were taken every day and evaluate the efficiency of the decoloration process, the enzymatic activities of laccases and manganese peroxidase and the pH. After that, were used the packed-bed bioreactor with 15 L.

Packed-bed bioreactor consists of three cylindrical tubes made of acrylic. The first cylinder (30 cm in diameter and 20 cm in height) is the dispersal location of the dye solution, which can handle 15 liters. The second cylinder (30 cm in diameter and 20 cm each) is fixed-bed solid cultivation. This site was established the residue of peach palm with the fungus previously inoculated and grown. The latter is the site of collection of the dye when is in the circulating process and storage when is not circulating (overnight period). In this study, 6 batch were performed, each batch being 7 days of circulating of remazol dye solution in the form of batch flow. The recirculation was made automatically, with a flow of 36 $L.h^{-1}$ being turned on at 8 o'clock A.M. and turned off at 5 o'clock P.M., accumulating 9 hours per day. To make the scale-up of this system,

we used the entire system adopted for the 2 L bioreactor. Where initially the fungus was grow in 500 mL flasks, in this case we used 40 flasks, and after growth were passed to the packed-bed bioreactor. The Figure 3 shows the schematic overview of the bioreactor.

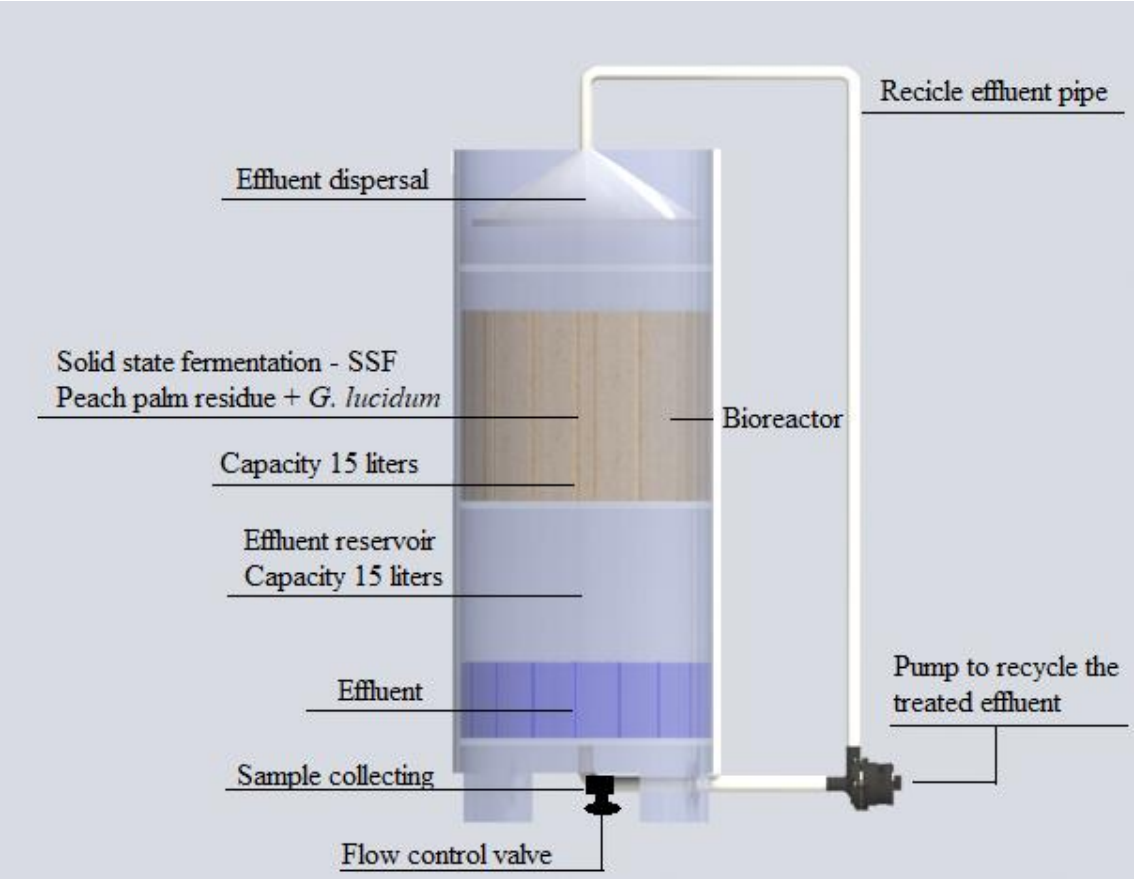

Figure 3. Schematic overview of the packed bed bioreactor.

4.9 Evaluation of the decolorization process

The color removal efficiency (E_D, %) was analyzed in all experiments and determined by comparing the initial absorbance value for the dyeing wastewaters with the value obtained at the end of the all fermentations. Initially, a swept-wavelength absorbance value was obtained for the Remazol Brillant Blue R dye (Pereira, et al., 2010), the maximum absorption of the dye was 592 nm.

4.10 Laccase and Manganese Peroxidase Activities

Laccase activity (A_{la}) was determined in all studies, from samples of the liquid extracts, as described by Hou, et al. (2004). Briefly, 0.1 mL of the LE preparations were transferred to test tubes with 0.8 mL of 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 0.1 mL acetate buffer (pH 4.0) and 0.1 mL of water. Control assays used 0.8 mL of ABTS with 0.1 mL of acetate buffer. Tubes were placed in the oven for 20 minutes at 30 °C. ABTS oxidation in the experimental and control samples was monitored by measuring absorbance at 420 nm (ε = 36 mM^{-1} cm^{-1}) every 15 seconds for 2 minutes.

Manganese peroxidase activity, analyzed in the studies 1 and 2, was carried out as reported by Wariishi et al (1992). Briefly, 0.4 mL hydrogen peroxide [0.5 mM] was added to a mixture composed of 0.4 mL of LE, 2.8 mL of sodium malonate buffer [50 mM] at pH 4.5, and 0.4 mL [10 mM] manganese sulfate. The oxidation reaction of MnSO4 in sodium malonate buffer in the presence of H_2O_2 forms a complex with manganic ions (Mn^{3+}) and malonate, which show absorbance at 270 nm (ε = 11.59 mM^{-1} cm^{-1}).

For both enzymes, one unit of enzyme activity (U. L^{-1} or UI. mL^{-1}) was defined as the amount of enzyme oxidizing 1 µmol of substrate per min^{-1}. All experiments were carried out in triplicate.

4.11 Concentration of total phenolic compounds

The concentration of total phenolic compounds was determined only in the "Study 2", by the Folin-Ciocalteu method, as described by Anagnostopoulou et al. (2006). Briefly, 0.5 mL of sample, 5 mL of distilled water and 0.25 mL of Folin-Ciocalteu's reagent were mixed in a test tube. After 3 min, 1 mL of saturated Na_2CO_3 was transferred to the tubes and the mixture was left to stand for 1 h in the dark. The absorbance of the samples was measured at 725 nm. A blank with distilled water was also analyzed. The determination of total phenols was performed from a standard curve constructed with solutions of gallic acid (0 and 500 µg. mL^{-1}).

4.12 Total organic carbon concentration

The concentration of total organic carbon (TOC) analyzed in the "Study 2", obtained by determining the total carbon (TC) and total inorganic carbon (TIC) contents separately in a TOC analyzer, model TOC-V CHS (Shimadzu, Kyoto, Japan). The measurements were carried out at a least three times.

4.13 Fourier transform infrared (FTIR)

The changes in the functional groups of the lignocellulosic biomass during the bioremediation experiments with Remazol Brilliant Blue R (Study 3) were analized by Fourie-transform infrared spectroscopy (FTIR). The measuremts were conducted in a Bruker spectrometer (model Vertex 70 Platinum ATR).

4.14 Moisture Content and pH

Moisture content of the cultured samples from the Studies 1 and 2 was determined by a gravimetric method proposed by Hermann et al. (2013). Briefly, three samples (5 grams each) from the solid-state fermentation were weighted and dried at 60 ºC until constant weight. The moisture content (MC) was calculated by the following equation 4.

$$MC(\%) = \frac{MC_i - MC_f}{MC_i} \cdot 100 \qquad (4)$$

where MC_i and MC_f correspond to the initial and final humidity of the samples in flasks (initial - beginning of fermentation; final - after 14 days of solid-state fermentation).

The pH values were measured at time zero and at the end of fermentations, being the liquid extracts were prepared as described in the solid-state fermentation section.

V RESULTS AND DISCUSION

5.1 Study 1: Biological Process for Decolorization of Textile Industry Wastewater with Bactris gasipaes Residue as a Solid Matrix

The results of this study were submitted and accepted by the Brazilian Journal of Biology with the title: "Biological Process for Decolorization of Textile Industry Wastewater with *Bactris gasipaes* Residue as a Solid Matrix", v. 78, n. 4, 2018. Impact Factor JCR (2016) 0,479 and Category: BIOLOGY (75/85) - Tercil 3.

5.1.1 Effect of Potential Adsorbent of Peach Palm Residue

The adsorption occurred quickly initially and then gradually decreased over time until reaching equilibrium at 8 hours. The experimental data based on adsorption kinetics expressions (Figure 4), both, first and second order kinetics was in agreement with the experimental data. However, the t/qt versus t plot best fit the second order kinetic model (Table 5), and Pearson (correlation coefficient) and R^2 values (determination coefficient) for the pseudo second-order model were higher than in the pseudo first-order model.

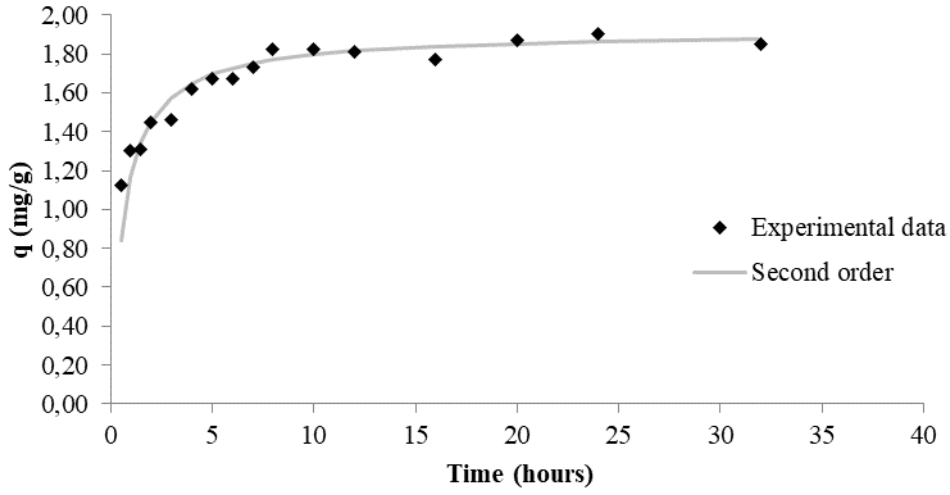

Figure 4. Experimental data based on adsorption kinetics expressions.

The percentage of dye removal by peach palm sheath had a direct relationship with dye concentration, and a linear relationship with temperature. At 28°C decolorization efficiency along the gradient from the lowest to the highest dye concentrations ranged from 78 % to 8 5%. At 45 °C, decolorization efficiency from lowest to highest dye concentrations ranged from 78-80 %. Thus, increasing the dye concentration and decreasing temperature increases the efficiency of the adsorption process.

Table 5. Kinetic and Isothermal models for adsorption of RBBR dye (Remazol Brilliant Blue R) on peach palm sheath.

Kinetic models	**Variables**	**Peach palm**	
Pseudo 1ª Order $\log(qeq - qt) = \log qeq \frac{K1xt}{2,303}$	K_1 (min^{-1})	0,150	
	Q_{eq}(mg g^{-1})	1,538	
	Q_{eqExp} (mg g-¹)	1,920	
	R^2	0,946	
	Pearson r	0,973	
Pseudo 2ª Order $\frac{t}{qt} = \frac{1}{k_2 q_{eq}^2} + \frac{t}{q_{eq}}$	K_2 (min^{-1})	0,817	
	Q_{eq}(mg g^{-1})	1,912	
	Q_{eqExp} (mg g^{-1})	1,920	
	R^2	0,998	
	Pearson r	0,999	
Intraparticle diffusion $qt = K_{id} t^{1/2} + Ci$	K_{id} (mg g^{-1} $min^{-1/2}$)	0,173	
	C_i (mg g^{-1})	1,178	
	R^2	0,838	
	Pearson r	0,916	
Isothermal	**Variables**	**28°C**	**45°C**
Langmuir $\frac{C_{eq}}{q_{eq}} = \frac{1}{q_m b} + \frac{C_{eq}}{q_m}$	$q_{max.}$(mg g^{-1})	5,757	25,641
	B (L mg^{-1})	0,030	0,007
	R^2	0,841	0,891
	Pearson r	0,970	0,944
Freundlich $\log q_{eq} = \log K_f + \left(\frac{1}{n}\right) \log C_{eq}$	K_f (mg g^{-1})	0,114	0,160
	1/n	1,356	1,079
	R^2	0,996	0,999
	Pearson r	0,998	1,000

q_t e q_{eq} = quantity adsorbed at time t and the balance of time respectively, K_1 and K_2 = constants of pseudo first and second order, respectively; K_{id} = constant rate of intraparticle diffusion; C = constant indicating the effect of the boundary layer.[2] $q_{max.}$ = maximum amount of adsorbate per unit mass of the biosorbents, b = adsorption equilibrium constant k_f = Freundlich constants.

Isotherm equilibrium is an important factor in the development of adsorption processes, and is used to describe how the adsorbate molecules interact with active sites on the adsorbent. The dye equilibrium data for RBBR were assembled using the Langmuir (Rahchamani et al., 2011) and Freundlich (Chiang and Wu, 2010) isotherms. The Pearson r (correlation coefficient) and R^2 values are shown in Table 6. The experimental data are consistent with both isothermal models. The Langmuir isotherm is specific for monolayer adsorption, wherein the adsorbent consists of active surface sites with equal energies available for adsorption. According to the RL parameter, the isotherm can be considered to be favorable at 0<RL<1 to the dye. On the other hand, the Freundlich isotherm is best applied to heterogeneous adsorption sites (Isah et al., 2015).

5.1.2 Solid-State Fermentation Experiments with Remazol Brilliant Blue R

The effect of RBBR concentration (0 – 100 %), liquid phase volume (20 – 60 mL), and palm sheath quantitiy (10 – 30 g) on decolorization percentage is shown in Figure 5.

(a) (b)

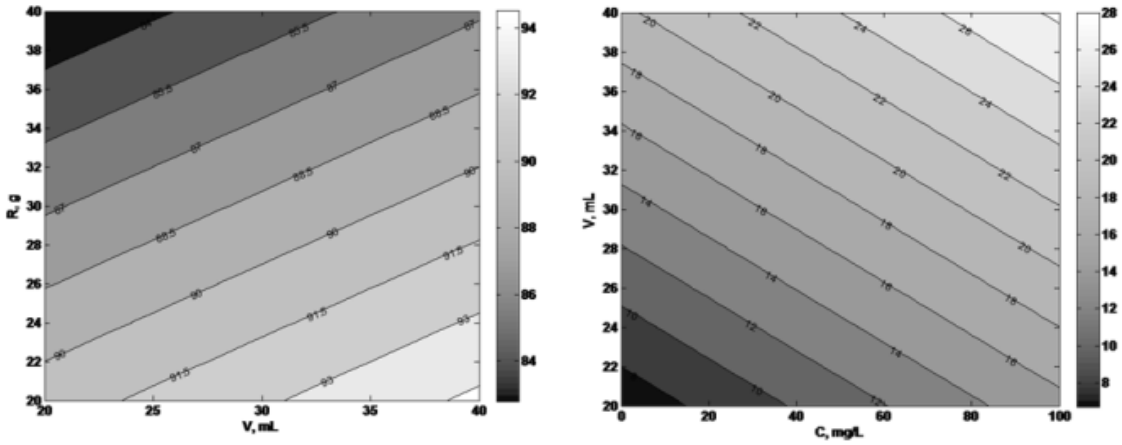

Figure 5. Contour plots for ED1: (a) Predicted De values at different liquid volumes of the dye (mL) and peach palm sheath quantities (g) (C = 100 %); (b) Predicted La values at different liquid volumes of the dye (mL) and RBBR concentrations (%) (R = 30 g).

RBBR was efficiently decolorized by *G. lucidum*, reaching efficiency values near 97%. The contour curves for ED1 were generated using equations 6 and 7, which were obtained from the fitting of experimental data.

$$De\ (\%) = 2.180 * 10^{-5} - 8.177 * 10^{-7}\ V + 0.948C + 4.237 * 10^{-8} R + 0.002\ V * C - 0.004\ C * R \quad (6)$$

$$La\ (U\ L^{-1}) = -21.508 + 2.232\ V + 0.414C + 0.50827R - 0.0528\ V * R - 0.0109\ C * R \quad (7)$$

Both ANOVA and the lack-of-fit test confirmed the validity of both mathematical models. The significance of the model parameters from Equations 6 and 7 are listed in Table 6. If the p-value of the coefficient was lower than 0.05, it was considered statistically significant and was retained in the mathematical model. Factors with p-values higher than 0.05, were removed from the model. Although V, R, and C all had significant effects on decolorization percentages, the most important factor for dye removal was RBBR concentration, followed by liquid phase volume and peach palm sheath quantity. The V*C and C*R interactions were significant, and experimental data indicate that in the range of 0 to 100 mg. L^{-1} RBBR increased dye concentration leads to higher De values.

Table 6. ED1: Model parameters and corresponding p-values.

ED1	Coeff. SC	Std. Err.	p-value	Conf. Int. (±)
Decolorization efficiency (R^2 = 0.999)				
Constant	44.5097	0.330774	3.44259e-023	0.709445
V	0.752497	0.339278	0.0436077	0.727686
C	44.6309	0.338942	4.66201e-023	0.726965
R	-1.5725	0.339278	0.000386031	0.727686
V*C	0.752499	0.339278	0.0436072	0.727686
V*R	0.0289979	0.332423	0.931722	0.712983
C*R	-1.5725	0.339278	0.000386027	0.727686

Laccase activity (R^2 = 0.925)				
Constant	17.4062	1.02717	1.64739e-012	2.158
V	6.33277	1.14569	3.00839e-005	2.40701
C	4.14236	1.14569	0.00197738	2.40701
R	-16.1646	1.15477	4.07447e-011	2.42608
V*C	-1.03864	1.1239	0.36764	2.36123
V*R	-5.13527	1.14569	0.000288063	2.40701
C*R	-5.32819	1.14569	0.000198854	2.40701
Manganese peroxidase activity (R^2 = 0.912)				
Constant	2.44257	0.21775	2.21214e-008	0.467032
V	-1.85731	0.229301	1.18488e-006	0.491806
C	0.803526	0.227196	0.00328655	0.487291
R	-1.30894	0.229301	5.4054e-005	0.491806
V*C	-0.971897	0.229301	0.000826337	0.491806
V*R	1.16899	0.229085	0.000160849	0.491343
C*R	0.0731471	0.229301	0.754436	0.491806

Laccase and manganese peroxidase enzyme production was also significantly affected by the three main factors, with some significant interactions among factors. Optimal response conditions were obtained through the regression model. Table 7 summarizes the experimental conditions which resulted in the highest production of enzymes and efficacy of decolorization. There is a clear relationship between the degradation of the dye and enzymatic activity with RBBR concentrations between 0 to 100 mg. L^{-1}. The highest De values and greatest enzyme production were obtained with 100 mg. L^{-1} of dye solution (level 0) and 20 g of peach palm sheath (level -1). The greatest decolorization values (predicted as 93.97 %) also coincide with the highest laccase activity (53.94 U. L^{-1}).

The ED2 results are in strong agreement with those of ED1. The highest De value (96.1 %) was achieved when 10 g of peach palm sheath was moistened with 60 mL of 200 mg L^{-1} RBBR solution. Hence, no inhibitory effect on the color removal was

observed at concentrations in the range of 100 to 200 mg. L^{-1}. Compared to ED1, laccase production was more affected by dye concentration.

Table 7. Highest values of decolorization, and laccase and manganese peroxidase activity in Remazol Brilliant Blue R solutions.

ED	Dye	Factors	Responses	Highest predicted value	Factors (values decodified)		
					V (mL)	C (mg L^{-1})	R (g)
ED1	RBBR	V, C and R	De (%)	95.6	40.0	100.0	20.0
			La ($U.L^{-1}$)	53.5	40.0	100.0	20.0
			MnP ($U.L^{-1}$)	8.7	20.0	100.0	20.0
ED2	RBBR	V, C and R	De (%)	96.1	60.0	200.0	10.0
			La ($U.L^{-1}$)	38.6	60.0	200.0	10.0
			MnP ($U.L^{-1}$)	Lof	---	---	---
ED3	Dyebath effluent	V, C and R	De (%)	Lof	---	---	---
			La ($U.L^{-1}$)	38.2	60.0	0.0	10.0
			MnP ($U.L^{-1}$)	Lof	---	---	---
ED4	Final effluent	V, C and R	De (%)	Lof	---	---	---
			La ($U.L^{-1}$)	43.8	60.0	0.0	10.0
			MnP ($U.L^{-1}$)	Lof	---	---	---

Our results indicate that RBBR concentration exerts a significant and positive effect on laccase production. MnP activities cannot be satisfactorily fitted by the mathematical model proposed in Equation 4, which yielded the highest experimental value (0.24 ± 0.9 U. L^{-1}) using 200 mg. L^{-1}.

5.1.3 Solid-State Fermentation Carried out with Industrial Wastewaters

The highest decolorization values (76% and 73%) from solid state fermentation were obtained using 60 mL of the final effluent (100%) and 10 grams of peach palm sheath. De values for the final effluent are thus at least 20 – 30% lower than those found with RBBR solutions (ED1).

The experimental De values for ED3 and ED4 cannot be adequately described by the mathematical model presented in Equation 4. In both cases, the lack-of-fit test was significant at the 95% confidence level. Equations 6 and 7, however, were suitable

to describe the experimental values of laccase activity in ED3 and ED4, respectively. Factors that did not affect laccase activity (non-significant terms) were excluded from the models. The adequacy of both models was verified by an analysis of variance (ANOVA) and a lack-of-fit test, and hence, the models are adequate for use in predicting outcomes within the experimental intervals tested. It is worth noting that: (i) all terms presented in Equations 8 and 9 significantly affected laccase production and, (ii) the parameters which exhibit a significant effect on La are the same in both experimental designs.

$$La_{DOE3} = 7.836 + 0.550\,H + 0.139\,W - 0.2618\,R - 0.008\,H * R \quad (8)$$

$$La_{DOE4} = 23.3325 + 0.412\,H - 0.148\,W - 0.429\,R - 0.004H * R \quad (9)$$

For both the final effluent and the residual dyebath wastewater (ED3 and ED4, Table 8), the highest predicted values of La were reached using volumes of wastewater or dye solution near 60 mL, 10 g of peach palm sheath, and wastewater concentrations less than or equal to 11%.

Table 8 - Experimental results from RDB (ED3) and FE (ED4) assays. R1, R2 and R3 indicate replicate numbers.

Assay	Factors			De-exp (%)			La-exp ($U.L^{-1}$)			MnP-exp ($U.L^{-1}$)		
	V (mL)	**C (%)**	**R (g)**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
ED3 (pHi = 11.2 ± 0.4)												
1	20	0	10	0	0	ND	13.54	17.29	ND	0	0	ND
2	60	0	10	0	0	ND	41.76	31.88	15.43	0	0	ND
3	20	100	10	0	0	ND	14.06	ND	ND	0	0	ND
4	60	100	10	0	0	ND	0.00	ND	ND	0	0	ND
5	20	0	30	0	0	ND	13.01	16.05	18.00	0	0	ND
6	60	0	30	0	0	ND	32.10	29.82	39.07	0	0	ND
7	20	100	30	0	0	ND	0.00	ND	ND	0	0	ND
8	60	100	30	0	0	ND	0.00	ND	ND	0	0	ND
9	40	50	20	0	0	0	15.10	16.66	15.92	0	0	ND
ED4 (pHi = 9.7 ± 0.0)												
1	20	0	10	0	0	ND	29.24	27.01	ND	0.000	0.56	ND
2	60	0	10	0	0	ND	40.68	56.19	ND	0.09	ND	ND
3	20	100	10	65.78	63.21	ND	1.94	3.89	ND	0.34	0.30	ND
4	60	100	10	76.05	72.91	ND	2.64	2.08	5.63	0	0	ND
5	20	0	30	0	0	ND	14.79	24.24	21.67	0	0	ND
6	60	0	30	0	0	ND	29.03	31.67	ND	0	0	ND
7	20	100	30	0	0	ND	0.00	ND	ND	0	0	ND
8	60	100	30	68.06	66.92	ND	0.00	ND	ND	0	0	ND
9	40	50	20	0	0	0	16.81	11.02	10,18	0.04	0	ND

It is remarkable that MnP production in the industrial wastewaters was either scarce or non-existent (Table 4). The ANOVA for multiple regression showed no significant relationships (p-value > 0.05) between the main factors and the MnP activity. Low production of MnP was previously observed in the RBBR assays.

Moisture content for SSF is usually defined during the initial substrate preparation prior the inoculation with white-rot fungi, and its optimum value depends on the microorganism and the substrate employed (Isroi et al., 2011). After our initial determination, the experimental MC values were used to fit Equation 4 for each of the experimental designs. In all cases, MC values were significantly affected by liquid phase volume and peach palm residue quantity. Interestingly, the experimental conditions that lead to higher moisture content (for example, 83 % in ED3) coincide with the higher production of laccase.

5.1.4 Discussion

The experimental data suggests that the solid substrate not only provides a similar environment to the natural habitat of the fungi (Boran and Yesilada, 2011), but also constitutes a quality source for production of laccase. The interactions between dyes and lignocellulose fibers, has received considerable attention. Peach palm sheaths are composed of 21.65 % lignin, and 73.51 % holocellulose (hemicellulose and cellulose). Cationic dyes no doubt have a higher affinity for the lignin-containing fibers than for the anionic dyes, and the presence of positive charge is undoubtedly an importance factor for dye affinity for lignin (Drnovsek and Perdih, 2005). In addition, RBBR dyes have an electrophilic reactive group which is capable of forming covalent bonds with hydroxyl groups from the cellulose fibers.

Laccases have a broad substrate range and act with low specificity on o-phenols and p-phenols via transfer of four electrons from the organic substrate to molecular oxygen. Such behavior may be due to laccase abstraction of a phenol electron as a function of reducing Cu^{2+} to Cu^{1+}. That in turn reduces O_2 to H_2O, allowing the enzyme to act cyclically if phenols are present and conditions are suitable (e.g., acceptable pH). Solid-state fermentation is thus a stable process. The equilibrium of the system occurs through biochemical mechanisms of aromatic molecule degradation by dye enzymes, which develop oxidation-reduction reactions in fungal cells such as laccases (Kües, 2015; Sarkar et al., 2014; Özcan et al., 2005).

Hence, no inhibitory effect on the color removal was observed at concentrations in the range of 100 to 200 mg L^{-1} with the RBBR (ED2). But, when is compared to ED1, laccase production was more affected by dye concentration. This behavior as also found in a study by (Mohan et al., 2005) in which the aqueous phase substrate concentration significantly influences the reaction mediated by the enzyme. If the enzyme concentration is held constant and the concentration of substrate is gradually increased, the reaction rate will increase until reaching the maximum value. After reaching balance, any added substrate will not change the rate of reaction.

Murugesan et al. (2007) evaluated dye decolorization by *G. lucidum* KMK2, and found that when using pure laccase, De increased for RBBR concentrations up to 300 mg L^{-1}, then decreased at higher RBBR concentrations. Our results indicate that RBBR concentration exerts a significant and positive effect on laccase production.

De values for the final effluent are thus at least 20 – 30 % lower than those found with RBBR solutions (ED1). This is likely because the final effluents contain organic compounds, metals, and salts that directly affect water color, have chemical and biochemical oxygen demand (COD/BOD), have high quantity of total dissolved solid (TDS) and total suspended solid (TSS), and basic pH (Kabra et al., 2013). Unexpectedly, decolorization did not occur in residual dyebath effluent regardless of experimental conditions. This suggests that treating the whole textile effluent is better than treating effluent produced exclusively by the dyeing operation. This could be because the final wastewater comes from all of the industrial processes that utilize water, and it is therefore much more dilute than the residual dyebath.

Several studies report decolorization by laccases, but also indicate that the pH and moisture conditions dictate the success of the process. Our data on final and residual dyebath effluent show that the extract pH remained constant after 14 days of solid state fermentation. However, in the RBRR treatments the pH of the extracts varied from 2.4 ± 0.1 (time zero) to 4.3 ± 0.2 regardless of experimental conditions. The phenomenon of the pH elevation has been previously reported at the literature and might be related to ability of some white rot fungi to reduce substrate acidity under certain conditions (Zandrazil and Brunnet, 1981).

The literature is replete with reports demonstrating the excellent capacity of fungi to degrade dyes, but their potential use in real industrial applications has been limited for various reasons. First, it has proven difficult to select organisms that are capable of growth and degradation in the highly variable and restrictive conditions of textile industry wastewaters. Second, most studies have focused on single model dyes at low concentrations, and these conditions cannot accurately predict or represent the decolorization efficiency of real effluents in which dyes are usually present as mixtures and often, at high concentrations. Moreover, biotransformation of model dyes cannot always be extrapolated to industrial dyes (Lucas et al., 2008), and most studies on dye degradation are conducted at Erlenmeyer scale. Before an industrial application can be implemented, fungal bioreactors which can be operated under industrial conditions using real wastewaters must be developed (Anastasi et al., 2010). In this sense, this is the first report on laccase production in *G. lucidum* on peach palm sheaths moistened with industrial dye wastewater. This study will provide the basis for the solid-state fermentation reactor in fixed bed (Packed bed reactors, Patent BR 20201200192) for

decolorization of textile wastewater, given the capacity of the peach palm sheath act as an adsorbent of dyes and concomitantly, as a substrate for basidiomycetes fungal hyphae which degrade xenobiotic compounds.

This study provides new perspectives for up-scaling the process of textile dye decolorization using white-rot fungi and peach palm sheaths. The peach palm sheath functions well in solid-state fermentation, supporting the development of fungal hyphae and production of oxidative enzymes. Our results suggest that palm sheaths have considerable potential in the process of RBBR dye removal via degradation through *G. lucidum* enzymes. In particular, *G. lucidum* EF 31 was able to grow in restrictive conditions, and proved to be effective in decolorization of the final effluent.

5.2 Study 2: Strategies for Decoloration of Textile Industry effluents by White-Rot-Fungi with Peach Palm Residue

The results of this study were submitted and accepted by the Acta Scientiarum Technology with the title: "Strategies for Decolorization of Textile Industry Effluents by White-Rot-Fungi with Peach Palm Residue", v. 40, n. 4, 2018. Impact Factor JCR (2016) 0,259 and Category: MULTIDISCIPLINARY SCIENCES (61/64) – Tercil 3.

5.2.1 Physicochemical compositions of the industrial wastewaters

The physicochemical compositions of the industrial wastewaters are shown in Table 9. The two effluents were obtained from different locations of an industrial plant: (i) The residual dyebath effluent (RDB) was collected from the location where the dyed material is washed; (ii) The final effluent (FE) was collected at the end of the primary physicochemical treatment and prior to biological treatment at the treatment plant for the entire industrial process. Thus, the two effluents are very distinct with different compositions in terms of organic compounds, metals, salts, dyes, chemical and biochemical oxygen demand (COD/BOD), total dissolved solid (TDS), total suspended solid (TSS), and pH (Kabra et al., 2013). Furthermore, the final effluent originates from all areas involved in the industrial process where water is used, and it is therefore much more dilute than the residual dyebath effluent.

Table 9. Physicochemical characterization of the two textile effluents.

Parameter	Residual dyebath effluent	Final effluent
pH	11.34	10.19
BOD (mg L^{-1})	992.00	239.00
COD (mg L^{-1})	2600.00	789.00
Turbidity (FTU)	58.20	113.00
Color (Pt Co)	5500.0	903.0
Total solids (mg L^{-1})	30456.00	1368.00
Suspended solids (mg L^{-1})	153.33	227.50
Volatile solids (mg L^{-1})	123.33	2.69
Nitrogen (mg L^{-1})	88.60	<0.01
Total phenols (mg L^{-1})	0.01	0.020
Cu (mg L^{-1})	0.367	0.024
Fe (mg L^{-1})	0.19	0.10
Ni (mg L^{-1})	<0.01	<0.01
Cd (mg L^{-1})	<0.001	<0.001
As (mg L^{-1})	0.02	<0.01
Ba (mg L^{-1})	<0.20	<0.20
Pb (mg L^{-1})	<0.01	<0.01
Cr (mg L^{-1})	<0.01	0.01
P (mg L^{-1})	7.46	2.54
Mn (mg L^{-1})	<0.05	<0.05
Hg (mg L^{-1})	<0.0002	<0.0002
Ni (mg L^{-1})	<0.01	<0.01
Zn (mg L^{-1})	0.05	0.05
Sulfides (mg L^{-1})	0.81	0.14

5.2.2 Influence of the industrial wastewater concentration

To study the influence of the industrial wastewater concentration on the decolorization efficiency and laccase production, SSF experiments were carried with *G. lucidum* EF31 using several dilutions of the FE and RDB effluents (0, 25, 50, 75 and 100 %). The corresponding results are shown in Figures 6 and 7. Independently of the initial pH, no significant differences were found in the pH values at end of tests (4.8 ± 0.1, at 14 days) conducted with FE and RDB.

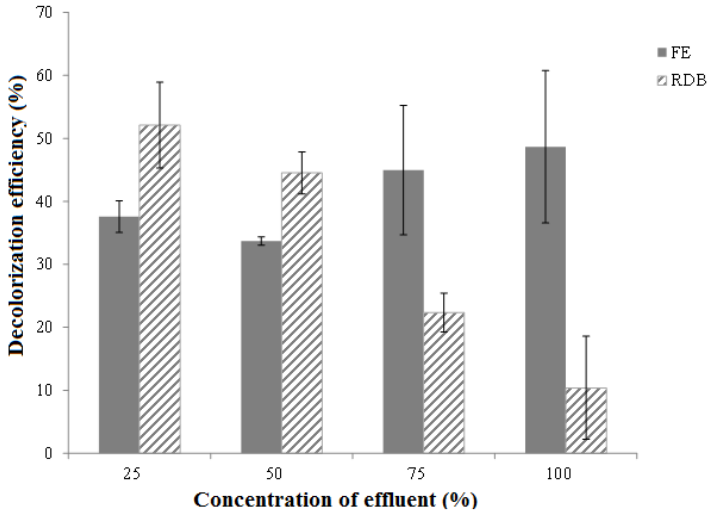

Figure 6. Decolorization efficiency observed in the SSF assays carried out with 60 mL of FE or RDB.

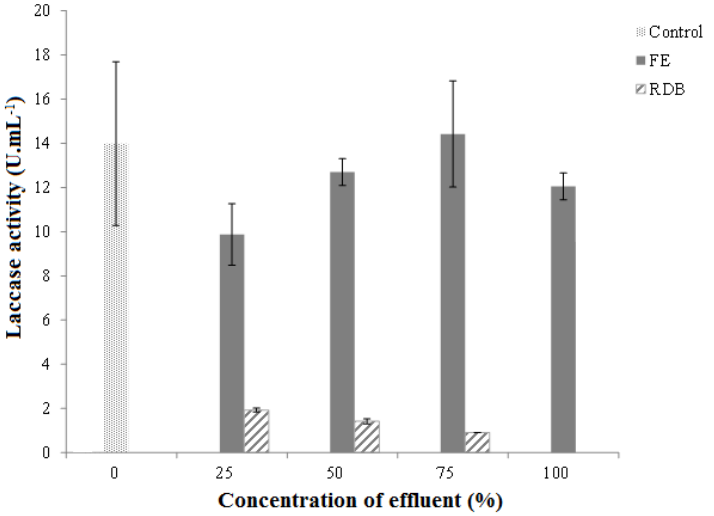

Figure 7. Laccase activity found in SSF assays carried out with FE and RDB.

As can be observed in Figure 6, an increase in the RDB concentration negatively affected the color removal efficiency, which varied from 52.1 % (25 % effluent) to 10.4 % (100 % effluent). Thus, the experimental data clearly indicate an inhibitory effect of this effluent on the fungus metabolism, which varies according to the RDB concentration. Similarly, the laccase production was negatively influenced by increases in the RDB concentration (Figure 7).

On the other hand, these findings were not observed in the assays performed with the final effluent. In this case, the decolorization efficiency and laccase production

tended to increase with increasing FE concentration. In addition, the values for the laccase activity obtained with the final effluent and the control are nearly the same. Regarding the manganese peroxidase, its production was negligible under all experimental conditions assayed. Similar results have been reported by Mota et al. (2015) who observed that *G. lucidum* did not produce MnP.

It is well known that some industrial effluents are toxic toward white-rot fungi and in these cases the enzyme production and decolorization process are inhibited. The differences in the results obtained with EF and RDB can be attributed to the compositions of the two wastewaters, which differ particularly in terms of the dye concentration (Table 10). Under the experimental conditions, it was observed that on diluting the RDB effluent to at least 50 % the E_D values were similar to those obtained with FE. However, since the E_D values are still low (around 50 %), it is necessary to optimize the SSF conditions to enhance the decolorization process.

Regarding the laccase activity, there were no significant differences between the results obtained with FE and the control. In contrast, in the tests performed with RDB, the laccase activity was at least 5 times lower than that obtained at different concentrations of FE.

5.2.3 Solid-state fermentation with different white rot fungi

Regardless of the phenolic compounds content on the liquid extracts carried out with 100 % of FE and RDB, the values decreased over the 14 days of fermentation to 0.02 µg. mL^{-1} and 0.09 µg. mL^{-1}, respectively. These values represent reductions in the phenolic compounds content of 66.4 % and 53.3 %, respectively. Thus, the treatment process which provides the greatest reductions in both the phenolic compounds content and the laccase production was SSF carried out with FE.

With the aim to improving the efficacy of the process, new SSF assays were carried out with lower moisture content and also with another two strains of WRF, *G. lucidum* DSM 9621 and *Trametes versicolor* CECT 20817. Figure 8 shows the decolorization efficacy and Figure 9 the laccase production during these experiments. Although the highest decolorization percentage (80 %) was attained after 10 days of fermentation with *G. lucidum* EF 31, no significant differences were found in relation to

the other two fungi. Furthermore, except for *T. versicolor*, the decolorization efficiency remained constant ($p > 0.05$) throughout 30 days of fermentation.

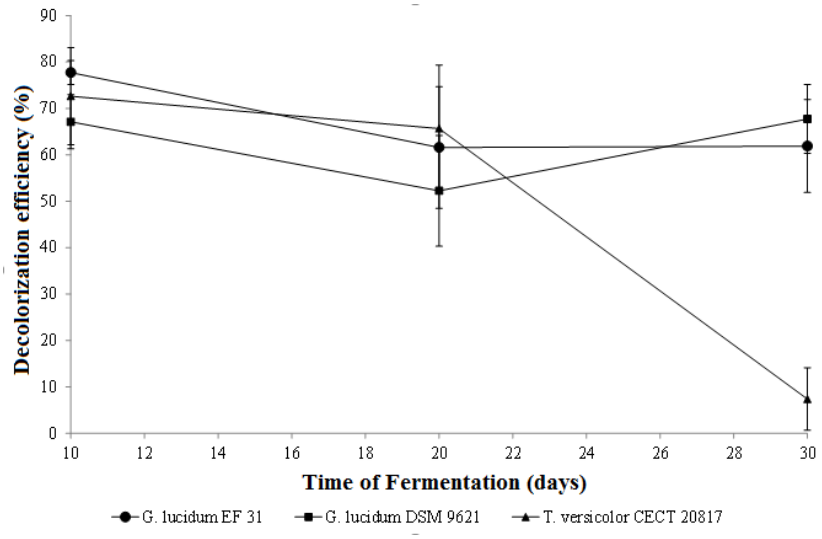

Figure 8. Time course in SSF experiments carried out with 40 mL of final effluent (100%) and three different WRF for decolorization efficiency $^{-1}$).

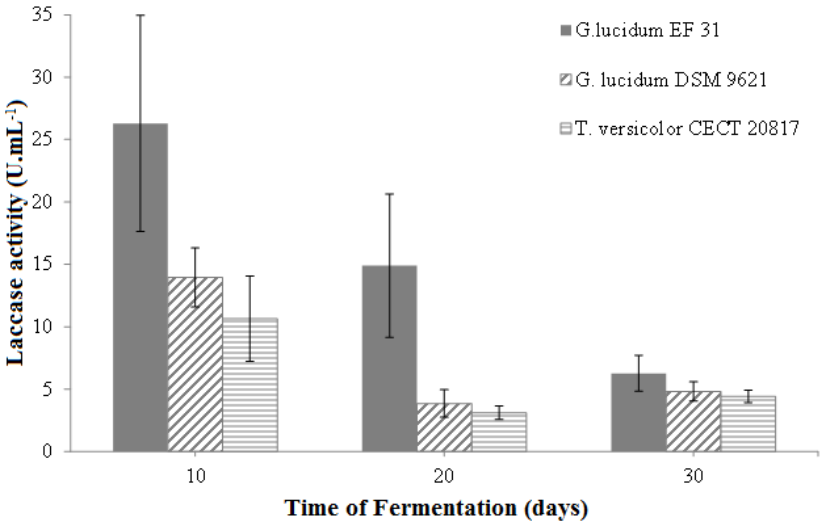

Figure 9. Time course in SSF experiments carried out with 40 mL of final effluent (100%) and three different WRF for Laccase activity.

Interestingly, independently of the WRF used, the laccase activity decreased over time during the solid cultivation tests. The values for the laccase activity obtained in the assays conducted with the final effluent are higher than those reported by Elisashvili et al. (2008). The cited authors performed SF assays with *T. versicolor* using

a culture medium supplemented with 4 g. L^{-1} of yeast extract and 50 g. L^{-1} of different lignocellulosic biomasses and the laccase activity varied from 0.54 to 3.01 U. mL^{-1}.

The reduction in the total organic carbon (TOCr) in the liquid extracts was also evaluated as a function of the fermentation time. The highest TOCr values were obtained with *G. lucidum* EF 31. *Trametes versicolor* also demonstrated good potential for reducing the TOC, presenting values close to those observed with *G. lucidum* EF 31. Regarding the efficacy of *G. lucidum* DSM 9621, its ability to degrade the FE presented a linear increase over time. Remarkably, under all assay conditions, it was found that the concentration of inorganic carbon in the samples was negligible. On comparing the data on the decolorization efficacy with the TOC_r values, it can be verified that TOC_r is not directly related to the color removal. This finding could be attributed to the complex composition of the final effluent, the dyes being only one of the components.

The TOCr values for the SSF assays carried out with *G. lucidum* EF 31 and different volumes of final effluent (20 and 60 mL). Interestingly, when the moisture content was low (20 ml), the TOC reduction decreased over time from 62.3 % (10 days) to 10.9% (30 days). In contrast, at a volume of 60 mL of FE, the TOC reduction increased over time, reaching a maximum of 65.4 % after 30 days.

Regarding to the pH of the liquid extracts, it was verified that with the *Ganoderma lucidum* strains the pH was slightly higher than that observed in the assays conducted with *T. versicolor*, i.e., 5.1 ± 0.1 and 4.9 ± 0.1, respectively. This pH behavior has been previously reported in the literature (Murugesan et al., 2007; Zadrazil and Brunnet, 1981) and it is associated to the capacity of some white rot fungi to establish the optimum pH of the substrate for growth.

5.2.4 Submerge fermentation

Table 10 summarizes the values obtained for the decolorization efficiency, laccase activity, pH and surface tension of the solid-free supernatant after 14 days of submerged fermentation. No statistical differences were found between the values for the pH, ST and phenolic compounds concentration obtained in assays performed with the final effluent and the control (distilled water). It can be observed that the decolorization efficacy values obtained in these tests were much lower than those

observed in the SSF assays. Additionally, although the presence of FE in the culture medium improved the laccase activity (from 0.5 to 1.4 U. mL^{-1}), the values obtained are considered low when compared to data published in the literature. Elisashvili and Kachlishvili (2009) reported values for laccase activity of 20.8 to 75.4 U. mL^{-1} in submerged cultivation tests with strains of *G. lucidum* using mandarin peelings in the medium. No MnP production was detected. Hence, under our experimental conditions, SF did not represent an appropriate alternative to treat these effluents. Compared to SSF, the decolorization process and laccase production are not favored in the SF process.

Table 10 - Experimental results for the SF assays carried out with *G. lucidum* EF 31.

	pH		**TS ($mN.m^{-1}$)**		**E_D (%)**		**A_{la} (U.mL)**		**Phenolic compounds**	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Final effluent	3.82	0.07	52.0	1.56	40.97	5.11	1.39	0.35	0.02	0.00
Control	3.81	0.05	57.65	3.18	---	---	0.55	0.05	0.02	0.00

5.2.5 Adsorption experiments

The adsorption experiments (Figure 10) were performed under the same conditions applied in the SSF assays. The efficacy of the decolorization process was 67.2 ± 3.6 % in the tests carried out with the final effluent at a concentration of 75 %. In the solid-state fermentation experiments carried out with *G. lucidum* EF 31, the highest decolorization percentage was 80 % with 100 % of final effluent. Waghmare et al. (2014) obtained an absorption of 60 % for Reactive Blue 172 onto a residue of sugar cane bagasse and *Providensia staurti* in solid-state fermentation with bagasse as a dye adsorbent they achieved more than 99 % of decolorization. Concerning the experiments with RDB, there was clearly no relationship between the adsorption and the dye concentration, the decolorization efficiency being 17.73 ± 8.2 % with 25 % of effluent.

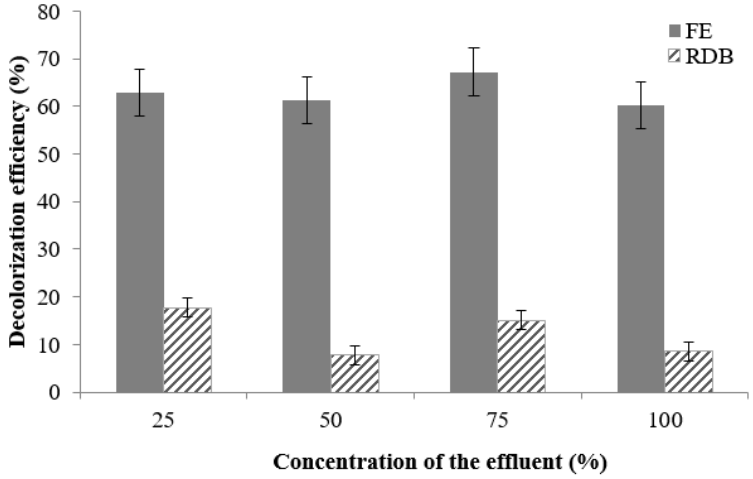

Figure 10. Decolorization efficiency obtained in the adsorption assays carried out with 60 mL of FE and RDB.

The interactions between the dyes and these fibers have received considerable interest. The structure of peach palm sheaths is comprised of 21.65 % lignin and 73.51 % holocellulose (hemicellulose and cellulose). Cationic dyes clearly have a higher affinity for lignin-containing fibers, and the presence of a positive charge plays an important role in the dye affinity for lignin (Drnovsek and Perdih, 2014).

Agricultural wastes are known to be the best low cost adsorbents for textile dyes and adsorption is an economically viable approach to the treatment for effluents containing dyes (Gupta and Suhas, 2009). On the other hand, the removal of dyes from textile effluent using exclusively the adsorption technique does not ensure the complete removal of the dye from the environment (Nigam et al., 2000), which leads to other serious environmental challenges. However, after the removal of the textile dyes, adsorbed onto agricultural wastes, the solid-state fermentation ensures the complete removal of the textile dye (Sarkar et al., 2014). In addition, the biomass generated after the bioremediation applying solid-state fermentation could be used as agricultural manure.

This study on the decolorization of textile dyes using white-rot fungi and peach-palm sheaths opens new perspectives for up-scaling. Solid-state fermentation was found to be a suitable system for treating the final effluent, comprised of wastewaters from all stages of the industrial process. In addition, it was verified that peach-palm sheath is a very useful agent offering support to the system, stimulating the development of fungal

hyphae and the production of laccases. All three fungi studied degraded the effluent by producing enzymes. In particular, *G. lucidum* EF 31 was found to be able to grow under restrictive conditions and proved to be more effective in the decolorization of the final effluent.

5.3 Study 3: Biodegradation of a textile dye by Ganoderma lucidum: Scale-up into packed bed bioreactors

The results of this study will be submitted to BioResources Technology with the title: "Biodegradation of a textile dye by *Ganoderma lucidum*: Scale-up into packed bed bioreactors". Impact Factor JCR (2016) 5,651.

5.3.1 Degradation experiments in packed-bed bioreactor with 2L

The experiments carried out in the small bioreactor were conducted with the white-rot fungus *Ganoderma lucidum* and the agricultural residue of peach palm tree (*Bactris gasipaes*). During 7 days the enzymatic activity and decoloration percentage were daily evaluated. The circulation process started on the first day of the Remazol dye solution. It was verified that the color removal was almost 80 %.

Figure 11, shows the laccase activity profile and decoloration in a small-scale trial in a two-liter graduated cylinder. The amount of laccase produced was up to 847.2 IU. mL^{-1}. Cantele et al., 2017 worked with the ability of *Marasmiellus palmivorus* to decolorize synthetic dyes to demonstrate its potential industrial application. The results for Reactive Blue 220 in 24 h showed decolorization percentages above 90 %. However, it is possible to observed that the laccase activity reaches this percentage in less time: 30 min for 4000 U. mL^{-1} and 2 h for 1000 U. mL^{-1}. Therefore, the greater the enzymatic activity of laccases, the faster was the decolorization. The remazol dye degradation in this study started from the first day of operation and increased during time. Of course it has sorption on the biomass, as already observed (Palli et al., 2014) nevertheless, as we can see also at the Figure 11b, the amounts of laccase are very correlated (R^2 = 0,9042) with higher remazol dye degradation. As the enzyme

manganese peroxidase was not found, this observation suggests that the laccases are possibly the main factor in the bio-decoloration process. Similar results have been observed in solid-state fermentation (Das et al., 2016; Zeng et al., 2015).

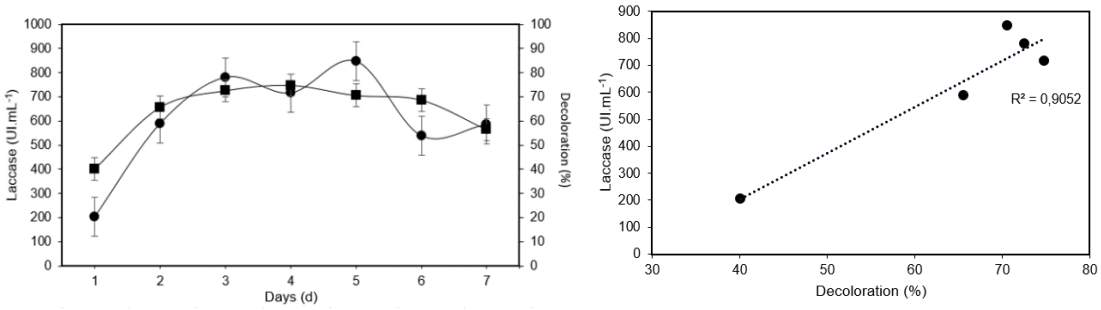

Figure 11. a) Time course of decoloration (■) and laccases production (●) in small scale inoculated with *G. lucidum* attached on peach palm residue. b) Relationship between the percentage of decoloration and laccase activity in a small scale.

This study provided new perspectives for scaling-up the process of textile dye decoloration using WRF and peach palm residue, given the capacity of this waste act as an adsorbent of dyes and as a substrate for fungal hyphae producing oxidative enzymes which degrade xenobiotic compounds.

5.3.2 Degradation experiments in packed-bed bioreactor 15 L

After the preliminary experiments carried out in a small-scale bioreactor with 200 g total of the residue, the color removal was evaluated under a batch flow bioreactor configuration. Scaling-up tests were conducted filling the reactor with *G. lucidum* on peach palm residue, following the method described in Materials and Methods section.

In the first batch (Figure 12), the decoloration reached 91 % and laccase production reached 220 IU. mL^{-1}. When analyzing the relationship graph (Figure 13) it is possible to observe the co-relation between them was of R^2 = 0.928. As in the previous study, manganese peroxidase was not found in any batch. In the second batch, the maximum decoloration was 50%, and laccase production was even higher than that of batch 1. The linear correspondent in this assay was R^2 = 0.9956. So it is probably that

in the first batch the adsorption process occurred between the dye solution and the peach palm fibers.

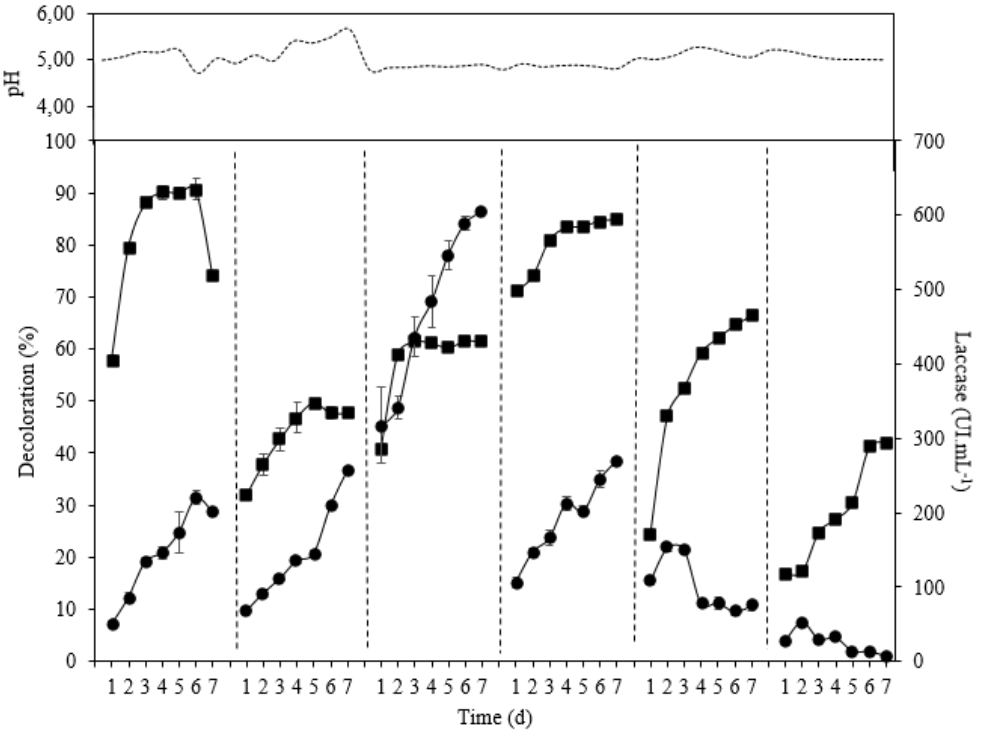

Figure 12. Time course of decoloration (■), laccases production (●) and pH values (---) inoculated with *G. lucidum* attached on peach palm residue.

In order not to drop the production of laccase enzymes the reactor was fed with the mineral medium with 1 hour of circulation. In batch 3 is possible to observe the production of laccase increases significantly, however the decoloration does not exceed 61 %. That is, even though this relationship is linear (R^2 = 0.9072) there is a limit of decoloration of the enzyme against RBBR dye solution, in other words, it can be produced in large quantity that will not occur decoloration or either the action of the enzyme laccase was late and only reflected in batch 4 (where the decoloration was up to 85 %). In batch 5, there is a decline in laccase production. Following batch 6, where it is possible to correlate the decline of laccase with decoloration (R^2 = 0.9413). Thus, showing the deterioration and exhaustion of the whole system. The pH varied little allowing conclude that bacterial growth was limited inside this bioreactor.

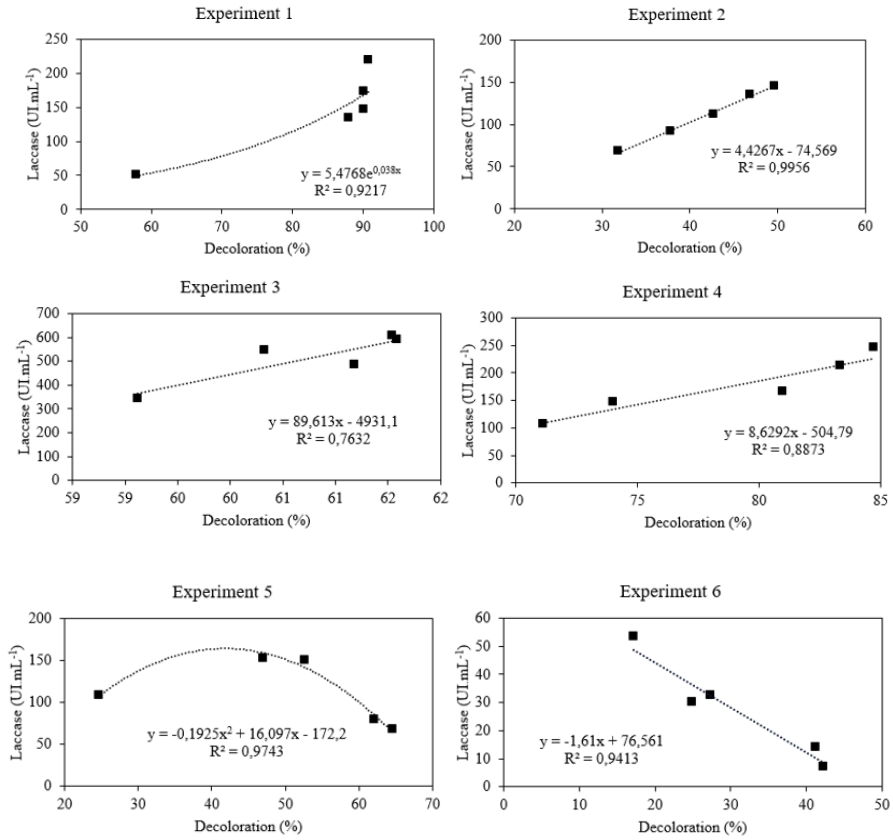

Figure 13. Relationship between the percentage of decoloration and laccase activity in a scaling up reactor.

FTIR technique was used to examine the surface groups of adsorbent (peach palm residue) and to identify the groups responsible for dye biodegradation. Infrared spectra of the dye biodegradation samples, before and after the biodegradation process, were recorded in the range 4000–500 cm^{-1} (Cardoso et al., 2011). The presence of functional groups is of fundamental importance since many of them act as active sites for different types of adsorbates. The interaction between the functional groups and the colorant generates a decrease in the wavelength. This occurs when the dye electron withdraws a functional group from the adsorbent (Rainert et al., 2017). It can be observed that the three components of biomass (cellulose, hemicellulose and lignin) are most likely consisted of alkene, esters, aromatics, ketone and alcohol, with different oxygen-containing functional groups observed, e.g., OH (3400–3200 cm^{-1}), C=O (1765–1715 cm^{-1}), and C–O–(H) (1050 cm^{-1}) (Yang et al., 2007).

The bands at 3311 and 332927 cm^{-1} are assigned to O–H bond stretching, before (Figure 14a) and after biodegradation (Figure 14b), respectively, indicating that

this group plays a role on the biodegradation of the RBBR dye. The CH_2 stretching band at 2917 cm^{-1} are assigned to asymmetric stretching of CH_2 groups which present the same wavenumbers before and after the biodegradation, indicating that these groups did not participate in the biodegradation process (Cardoso et al., 2011).

The region 1700–1730 cm^{-1} is associated with the aromatic, which cannot be seen in the Figure 14b (bioreactor after fifty days of cultivation), evidenced by the fact that the peak 1726 cm^{-1} of the FTIR bands disappear from the spectrum. This indicates that the oxidation processes by the enzymes were efficient and lead to the oxidation of the functional group. The peak of 1632 cm^{-1} (C=C) shows the presence of the Benzene stretching ring (Yang et al., 2007). The strong peak in the region 1100–1000 cm^{-1} can be assigned to the stretching vibrations of the lignin C–O bond (Calvete et al., 2010).

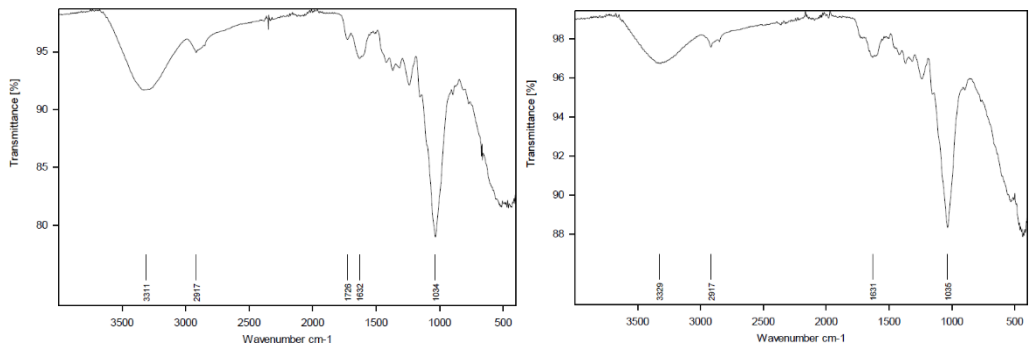

Figure 14. Bioreactor FTIR spectrometry. a) Time zero; b) after fifty days of cultivation.

To further study the decoloration process through adsorption, the circulation of the dye solution without inoculation of the fungus was also carried out in the same reactor under the same culture conditions. It is possible to divide this study into three phases (Figure 15):

- Phase 1: as Chicatto et al., 2018b studies, the maximum adsorption occurs after 8 hours of contact so in the present study we observed that during the first 24 hours of the residue exposure with the dye solution it occurs for at least 50 % color removal. This decoloration tended to 0 (saturation of the fiber to the dye solution) and the pH became acid until it reached 1.36.

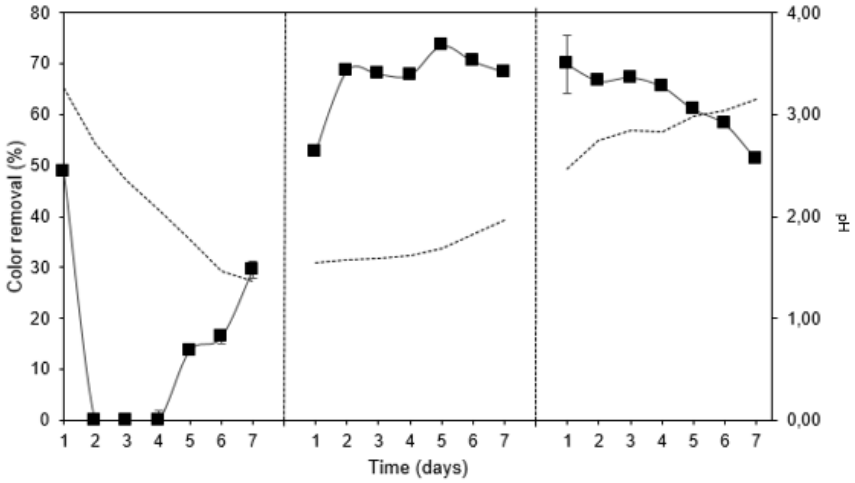

Figure 15. Time course of decoloration (■) and pH values (---) on peach palm residue.

- Phase 2: The proliferation of bacteria that has found a friendly environment to develop and reproduce without competition with no other microorganism, decrease the pH, decoloring up to 80 %.
- Phase 3: at the last week of the test, the pH increases, probably bacteria's died and the basidiomicetos fungi grown, the mycelium could be visualized which cannot so effectively decolor the dye.

Recent advances obtained in studies on the decontamination of polluted environments have encouraged the search for new alternatives for the treatment of industrial effluents. The use of basidiomycetes fungi in the decolorization of dyes is one such alternative treatment (after physical-chemical treatment), as demonstrated by the results reported herein. An extracellular laccase was produced by *Ganoderma lucidum* on peach palm residue under solid-state fermentation. In the scale of 2 liters the decoloration reached 80 %, moreover in the scaling of 15 liters the decoloration reached 91 %. The results presented here show an approach to dye removal using white-rot fungus, cheap agricultural residues and also provide a first step to cycling utilization of cultivated residue in up-scale solid-state fermentation, never described in the literature.

VI CONCLUSIONS

In this thesis the degradation of textile effluents and the Remazol Brilliant Blue R (RBBR) dye was studied by solid-state fermentation. For this, tests have been carried out in the presence of macromycetes using the residue of the palm of pupuna (*Bactris gasipaes*) as a solid matrix. Next, we will summarize the main conclusions obtained in these experiments:

- For the first time, the ability to decolorise the fungus *Ganoderma lucidum* EF 31 grown in palm residues of pupunha (*Bactris gasipaes*) and moistened with industrial textile effluents has been evaluated. Both the decoloration process and the production of lignolytic enzymes have been significantly affected by the amount of pupunha residue, the volume of the liquid phase and the concentration of the dye. In all the tests carried out, the laccase was the ligninolytic enzyme predominantly produced by *G. lucidum*. The highest percentage of color removal of RBBR (97%) and laccase production (53.94 U L^{-1}) was achieved in solid culture assays with 100 mg L^{-1} of dye and 20 g of pupunha residue.

- Different behaviors were observed in the decolorization tests of EF and RDB industrial textile effluents. In the solid-state fermentation tests carried out with the RDB effluent, the increase in its concentration inhibited the growth of the *G. lucidum* fungus, which has been reflected in a reduction in the decoloration efficiency, which varied from 52.1% (25 % effluent) to 10.4% (100% effluent), as well as in laccase production. On the other hand, in the case of FE effluent, the efficiency of decoloration and the production of enzymes increased with the concentration of the FE. These differences in the results obtained can be attributed to the composition of the effluents, and in particular, to the difference in the concentration of dyes in them.

- Regarding the RBBR adsorption experiments carried out with the pupunha residue, it was verified that the dye concentration and the temperature affect the percentage of dye removal. The efficiency of this process has been increased with the increase of RBBR concentration and with the reduction of temperature. Additionally, it is also

concluded that both first and second order kinetic models are suitable to represent experimental RBRR adsorption data.

- Among the decoloration strategies evaluated (submerged fermentation, solid-state fermentation and adsorption), the potential of solid-state fermentation for the treatment of these effluents has been demonstrated. The efficiency values of decoloration and laccase production obtained in the submerged fermentation tests with the FE effluent were lower than those obtained in the solid culture. In the same way, the efficiency of color removal by adsorption (in the absence of the fungus) has also been lower than that achieved by solid culture and fungus.

- Studies of biodegradation of RBBR carried out in bioreactors on pilot scale with *G. lucidum EF31* corroborate the results obtained in flasks. Decoloration efficiency values of 80 and 91% were achieved in bioreactors of 2 and 15 L capacity, respectively. On the other hand, in the case of the 15 L reactor, the efficiency of the process varied according to time, from 91% (batch 1), 50% (batch 2), 61% (batch 3), 85% (batch 4), 65% (batch 5) and up to 43% (batch 6).

- Control tests carried out in the 15 L capacity bioreactor and in the absence of the fungus (in conditions identical to those carried out with RBBR), indicate that the decoloration process takes place in three differentiated phases: (i) Physical adsorption on the waste of pupunha, how predominant mechanism; (ii) Decrease in the pH of the medium, adsorption accompanied by the development of microorganisms (bacteria, mainly) and biodegradation; (iii) Increase in the pH of the medium, accompanied by the growth of basidiomycete fungi (mycelial display).

Based on the results obtained and in order to give continuity to the work already developed, it is proposed to carry out solid-state fermentation tests using the bioreactors on a pilot scale, varying the hydraulic retention times, the composition of the effluent (mixtures of dyes well-known textiles), as well as the effluent recirculation regime.

CONCLUSIONES

En la presente tesis doctoral se ha estudiado la degradación de efluentes textiles y del colorante Remazol Brilliant Blue R (RBBR) mediante fermentación en estado sólido. Para ello, se han llevado a cabo ensayos en presencia de hongos macromicetos usando el residuo de la palma de pupuña (*Bactris gasipaes*) como matriz sólida. A continuación, se pasa a resumir las principales conclusiones obtenidas en dichos experimentos:

- Por primera vez la capacidad de decoloración del hongo *Ganoderma lucidum* EF 31 cultivado en residuos de palma de pupuña (*Bactris gasipaes*) y humedecido con efluentes industriales textiles ha sido evaluada. Tanto el processo de descoloración, como la producción de enzimas lignolíticas, han sido significativamente afectados por la cantidad de residuo de pupuña, el volúmen de fase líquida y la concentración del colorante. En todos los ensayos realizados, la lacasa fue la enzima ligninolítica predominantemente producida por *G. lucidum.* El mayor porcentaje de eliminación de color del colorante RBBR (97%) y de producción de lacasa (53.94 U L^{-1}) fueron alcanzados en ensayos de cultivo sólido con 100 mg L^{-1} de colorante y 20 g de residuos de pupuña.

- Diferentes comportamientos fueron observados en los ensayos de descoloración de los efluentes textiles industriales EF y RDB. En los ensayos de fermentación en estado sólido realizados con el efluente RDB, el aumento de su concentración inhibió el crecimiento del hongo *G. lucidum*, lo que se ha visto reflejado en una reducción en la eficiencia de descoloración, que varió de 52.1 % (25 % efluente) a 10.4 % (100 % efluente), así como en la producción de lacasa. Por otra parte, en el caso del efluente FE, la eficacia de descoloración y la producción de enzimas aumentaron con la concentración del mismo. Dichas diferencias en los resultados obtenidos pueden ser atribuidos a la composición de los efluentes, y en particular, a la diferencia de concentración de colorantes en los mismos.

- Respecto a los experimentos de adsorción de RBBR realizados con el residuo de pupuña, se verificó que tanto la concentración de colorante como la temperatura afectan el porcentaje de remocion del colorante. La eficiencia de dicho processo se ha visto incrementado con el aumento de concentración de RBBR y con la reducción de la temperatura. Adicionalmente, también se concluye que ambos modelos

cinéticos de primer y segundo orden son adecuados para representar los datos experimentales de adsorción de RBRR.

- Entre las estrategias de descoloración evaluadas (fermentación submergida, fermentación en estado sólido y adsorción), se ha puesto de manifiesto el potencial de la fermentación en estado sólido para el tratamiento de dichos efluentes. Los valores eficiencia de descoloración y de producción de lacasa obtenidos en los ensayos de fermentación sumergida con el efluente FE han sido inferiores a los obtenidos en el cultivo sólido. Del mismo modo, la eficiencia de remoción del color mediante adsorción (en ausencia del hongo) también ha sido inferior a la alcanzada mediante cultivo sólido.
- Estudios de biodegradación de RBBR llevados a cabo en biorreactores en escala piloto con *G. lucidum* EF31 (ambiente no ésteril) corroboran los resultados obtenidos en frascos. Valores de eficiencia de descoloración de 80 y 91% fueron alcanzados en biorreactores de 2 y 15 L de capacidad, respectivamente. Por otra parte, en el caso del reactor de 15 L, la eficacia del proceso varió en función del tiempo, desde 91% (batch 1), 50% (batch 2), 61% (bach 3), 85% (batch 4), 65% (batch 5) hasta 43% (batch 6).
- Ensayos control realizados en el biorreactor de 15 L de capacidad y en ausencia del hongo (en condiciones identicas a de los realizados con RBBR), indican que el proceso de descoloración se desarrolla en tres fases diferenciadas: (i) Adsorción física sobre el residuo de pupuña, cómo mecanismo predominante; (ii) Disminución del pH del medio, adsorción acompañada por desarrollo de microorganismos (bacterias, principalmente) y biodegradación; (iii) Subida del pH del medio, acompañada por el crscimiento de hongos basidiomicetos (visualización de micelio).

En base a los resultados obtenidos y con vistas a dar continuidad al trabajo ya desarrollado, se propone la realización de ensayos de fermentación en estado sólido usando los biorreactores en escala piloto, variándose los tiempos de retención hidrálica, la composición del efluente (mezclas de colorantes textiles conocidos), así como el régimen de recirculación de efluente.

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